GRADUATE STUDENT SYMPOSIUM

The Graduate School of Biomedical Sciences
Baylor College of Medicine
Thursday, October 17, 2013
THE 25th ANNUAL GRADUATE STUDENT RESEARCH SYMPOSIUM

The Graduate School of Biomedical Sciences
Baylor College of Medicine
Houston, Texas

October 17, 2013

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Cover Legend:
The Johnston’s organ is the auditory organ of Drosophila located in the second segment of the antennae. It consists of hundreds of mechanosensory neurons with specialized sensilla surrounded by tube-shape cells, scolopale cells. This image is a confocal section of a pupal Johnston’s organ stained with fluorescently labeled Phalloidin (cyan) and NompA antibody (red), showing the specific morphology of scolopale cells. Phalloidin labels the actin bundles within scolopale cells, which maintain the specific morphology of the cells. NompA is originally produced in the cell bodies of scolopale cells (red signals on top of the actin bundles). It is then secreted by scolopale cells and localizes to the apical junctions, where it forms filamentous structures (red signals bellow the actin bundles) to connect the tip of the sensilla of neurons to the cuticle of the antennae. This allows the neurons to transduce mechanical stimulation. Developmental Biology student Tongchao Li from Drs. Hugo Bellen and Andy Groves
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<td>9:15 – 9:30</td>
<td>Breakfast (Coffee &amp; Pastries provided)</td>
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<td>Introductions</td>
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<td>Announce Poster Finalist</td>
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<td>9:45 – 10:00</td>
<td>Kuang-Yui Michael Chen, Biochemistry &amp; Molecular Biology</td>
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<td>10:00 – 10:15</td>
<td>Caterina Clementi, Developmental Biology</td>
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<td>Jennifer Mamrosh, Molecular &amp; Cellular Biology</td>
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<td>10:30 – 10:45</td>
<td>Break (Coffee &amp; Snacks provided)</td>
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<td>10:45 – 11:00</td>
<td>Abhisek Bhattacharya, Immunology</td>
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<td>11:00 – 11:15</td>
<td>Ryan Ash, Neuroscience</td>
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<td>3:00 – 4:00</td>
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Awards Ceremony & Reception  
Thursday, October 17, 2013  
4:00 – 5:00 PM  

8-Stranded Beta-Barrel Jelly Roll Awards  
Professor John J. Trentin Scholarship Awards  
Deborah K. Martin Achievement Award in Biomedical Sciences  
Marc Dresden Excellence in Graduate Education Award  
Milton Gregory Poster Awards  
Beckman Poster Awards  
Mavis P. Kelsey Student Speaker Awards  
Beckman Platform Award  

Abstract Book can be located at http://www.bcm.edu/osa/gsc/
Welcome to Baylor College of Medicine’s 25th Annual Graduate Student Symposium! Thank you for joining us as we take time to honor all graduate students at BCM and to showcase some of the exceptional biomedical research being performed at our institution.

This year’s symposium features student poster presentations and platform talks. Students chosen to give talks represent the best of their respective departments, having won awards at scientific retreats. These talks and poster presentations provide an invaluable opportunity for students to discuss their research with a diverse group of faculty and peers. Year after year, the Symposium showcases excellent student research; we think you’ll agree this year promises to be no exception. In addition to the outstanding student work, we are pleased to have Dr. Deborah Johnson, the Dean of the Graduate School of Biomedical Sciences here at BCM, culminate the day’s events by delivering the Joseph L. Melnick Distinguished Lecture.

The events of today would not have been possible without the help of many dedicated students and faculty. Members of the Graduate Student Council have graciously volunteered their time and efforts all year. For their hard work and their indispensable help with the symposium, we extend our sincerest thank you.

A special thanks is also owed to Melissa Houghton and Dr. Gayle Slaughter for their leadership roles in planning and organizing all aspects of today’s symposium. We also thank Dr. Hiram Gilbert for his work in organizing the Symposium and his continued mentorship of the GSC. Additionally, our sincere gratitude goes to each and every faculty and staff member at BCM who work tirelessly day after day to ensure students receive the best educational experience possible. We would especially like to recognize Donna Otwell, Dr. Gad Shaulsky, Dr. Carolyn Smith, Dr. Rick Sifers and all of our faculty mentors and program administrators for their dedication. Finally, we thank our poster and presentation judges who volunteered their time to help make this a successful symposium, and of course we thank Dr. Deborah Johnson for joining us at BCM to deliver the Distinguished Lecture.

It has been our pleasure serving you over the last year and we hope you enjoy today’s Symposium.

Christopher Nobles, GSC President
Peter Tu, GSC Vice President
From left to right:
Front row – Sarah Hein, Daisy Lu, Caterina Clementi, Christopher Nobles, Timothy Dosey, Eiffel Manzano, Tiffany Fleet
Back Row – Shawn Badal, Amritha Nair, Justin Anglin, Peter Tu, Aaron Kelly, Trace Stay

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<td>Timothy Dosey</td>
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Each year, the Graduate Student Symposium of the Graduate School of Biomedical Sciences at Baylor College of Medicine gives a glimpse into the future of biomedical science while demonstrating excellence in two of the College’s important missions -- education and research. The achievements of the Graduate School’s student scientists reflect not only their own abilities and the mentoring provided by some of the best scientists in the College but also the promise of such research for patients around the globe. Each year, the School’s leadership and I are honored to be part of this important event and amazed at the intellect and ingenuity that various projects represent.

I have been President of Baylor College of Medicine for three years, and one of the most enjoyable things about my tenure has been interacting with the graduate students. Coming from different backgrounds and even different countries, they join together in the laboratories of Baylor researchers with international reputations to push the boundaries of our current knowledge of biomedical science. They attack this challenge with passion and attention to detail that result in new findings about disease and biology that often appear in the most prestigious professional journals.

Fostering the education and growth of such students is the essence of the Baylor Graduate School of Biomedical Sciences, and this research symposium is a graphic demonstration of the accomplishments of its students.

On behalf of the Baylor College of Medicine faculty, I would like to congratulate the students, their faculty mentors and the leadership of the Graduate School for creating an outstanding Graduate Student Symposium for 2013. We are committed to their continued success, which represents the best of Baylor College of Medicine.

Paul Klotman, M.D.
President & CEO
Baylor College of Medicine
The Graduate Student Research Symposium is a culminating academic event that showcases the enormous research contributions from our graduate students at Baylor College of Medicine. It celebrates those individuals that drive our research enterprise and highlights the impressive quality of their work. The stimulating discussion that ensues during the Symposium not only enhances our students’ ability to articulate their work but also presents opportunities for networking and fostering new collaborations.

I would like to gratefully acknowledge those individuals that made this Symposium possible. I would like to specifically thank Gayle Slaughter who solicited judges for critiquing the platform presentations and posters, Melissa Houghton for assembling the abstract book, and the Graduate Student Council who was instrumental in planning the scientific program.

As the new Dean of the Graduate School of Biomedical Sciences, this Symposium will be my first opportunity to become acquainted with the breath and diversity of research conducted by our graduate students. I am delighted to be part of this special event and hope you will take the time to celebrate the tremendous efforts and productivity of our students and their mentors.

Debbie Johnson, Ph.D.
Dean
Graduate School of Biomedical Sciences
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Schizophrenia is a devastating psychiatric disorder and is associated with dysregulation of the dopamine (DA) system. Older generation antipsychotics selectively target the DA system, but newer generation antipsychotics that also target the serotonin (5-HT) system are being used for their added therapeutic efficacy. In particular, altering the activity of the 5-HT2C receptor may modulate DA release differently between the dorsal striatum (d. striatum) and the nucleus accumbens (NAc), key DA system targets, to offer additional therapeutic benefit. Previous microdialysis studies have suggested that inhibiting 5-HT2C receptor activity increases DA release, but more insight into how this receptor modulates DA release will provide rationale about whether or not this receptor should continue to be a pharmaceutical target. I used fast scan cyclic voltammetry (FSCV) to measure dynamic DA release differences in brain slices from wild-type and 5-HT2C receptor knockout mice. I compared the pulse number dependent DA release and the phasic-to-tonic DA release ratio between wild-type and knockout animals. Phasic DA release is high frequency DA release thought to correspond to behaviorally relevant stimuli and tonic release is low frequency release that corresponds to basal DA levels. 5-HT2C receptor knockout animals showed significantly reduced pulse number dependent facilitation in the d. striatum, but this facilitation was largely intact in the NAc. Additionally, the phasic-to-tonic ratio was significantly decreased in the d. striatum but not significantly altered in NAc. These data suggest that the 5-HT2C receptor facilitates phasic DA release to a greater extent in the d. striatum than in the NAc.
The prediction and manipulation of protein-protein interactions remains a difficult task. Model systems such as the β-lactamase inhibitory protein II (BLIP-II) and β-lactamases have been used to investigate the principles of protein-protein interactions. Previous studies focused on the determinants of binding affinity and specificity between BLIP-II and class A β-lactamases; however, interactions between BLIP-II and other proteins have yet to be explored. In this study, we characterized the novel interaction between BLIP-II and penicillin binding protein 2a (PBP2a) from methicillin resistant Staphylococcus aureus (MRSA). Mutagenesis and surface plasmon resonance experiments were used to determine how specificity is achieved between BLIP-II and its binding partners. The results suggest that an outer ring of residues on the BLIP-II interface plays a critical role in binding PBP2a while an inner ring of residues on the binding surface is primarily responsible for the binding of β-lactamases. Interestingly, changes in BLIP-II binding affinity for PBP2a were found to be largely due to changes in both $k_{on}$ and $k_{off}$ while changes in binding affinity for β-lactamases were primarily mediated by $k_{off}$. In summary, the results of the study indicate BLIP-II binds PBP2a in addition to β-lactamases and provide insights into how BLIP-II binds a wide range of target proteins.
ACTIVE CASE FINDING: A COMPARISON OF HOME-BASED TESTING AND HEALTH CENTER BASED TESTING FOR IDENTIFYING HIV-INFECTED CHILDREN IN LILONGWE, MALAWI

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Clinical Scientist Training Program
Advisor: Thomas Giordano, M.D.-Department of Medicine
Elizabeth Chiao, M.D./M.P.H.-Department of Medicine

Background: Studies estimate that less than 10% of children overall and 20% of children of adult ART patients have been HIV-tested. Home-based HIV testing may improve early identification and enrollment into care of HIV-infected children. The objective of this study is to compare the effectiveness of home versus health center based HIV testing in identifying HIV-infected children.

Methods: The Tingathe community outreach program conducts both health center and home based HIV testing. Health center testing included both patient and provider initiated testing. Home testing included both routine door-to-door testing as well as solicited visits of family members of current ART patients. Children were generally offered testing only if the mother was infected. We evaluated testing data from March 2008 to March 2011.

Results: Of 37,984 HIV tests performed, 14,358 (37.8%) were conducted in patient homes. A total of 4501 (11.9%) new positive persons were identified, 948 (21.1%) of whom were identified through home-based testing.

Health center based testing demonstrated a significantly higher prevalence than home based testing (12.9% vs 2.9%, p<.001). However, four times more children were able to be tested through the home based strategy, resulting in roughly equivalent numbers of total children being identified (170 vs 157, p=.467).

Conclusions: Our study demonstrates that though a higher prevalence was seen in health center based testing, the overall yield of the home versus health center testing strategies were comparable. Both strategies will likely be important for a comprehensive approach to identification and enrollment of HIV-infected children. The children identified through the home based strategy may have been found earlier in their disease course, but further studies are necessary to compare clinical characteristics and outcomes of children identified through these differing strategies. Contributors: Ahmed, Saeed; Kim, Maria; Kazembe, Peter
THE JAK/STAT PATHWAY REGULATES THE EXPRESSION OF GENES DELIVERED BY ADENOVIRAL VECTORS

Patricia Joy Akinfenwa
Program in Translational Biology & Molecular Medicine
Advisor: Richard Hurwitz, M.D.-Department of Pediatrics
Donald Parsons, M.D./Ph.D.-Department of Pediatrics

Understanding host pathways regulating the expression of transgenes delivered by adenoviral vectors (AdV) could result in improved gene therapy strategies. Gene therapy protocols have been particularly successful in the eye. AdV transgene expression (TGE) is increased in the presence of vitreous, the gelatinous material that serves a major structural component of the posterior eye. Vitreous treatment has no effect on the efficiency of vector internalization but results in increased transgene mRNA levels. We have also determined that the interaction of hyaluronan (HA), a major vitreous component, with CD44, a cell-surface glycoprotein, contributes to enhancement of AdV TGE. Transduction in the presence of ACHN supernatant derived from a renal carcinoma cell line cultured in SF media and concentrated for proteins <100kDa in size, also results in enhancement of AdV TGE. Dasatanib and PP2, two inhibitors of Src kinase, a known transcriptional activator, resulted in the unexpected enhancement of AdV TGE. This enhancement was abrogated by inhibitors of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway. We hypothesize that the JAK/STAT signaling pathway is responsible for regulating AdV TGE enhancement.

To assess the role of JAK in AdV TGE, cancer cell lines were transduced with an AdV in the presence of enhancers of TGE (vitreous or ACHN supernatant) and ruxolitinib, a small molecule JAK inhibitor. Likewise, the relevance of STAT molecules was tested using C188-9, a small molecule STAT inhibitor. Cells transduced with AdV in the presence of 5% vitreous or ACHN supernatant resulted in a 4-fold enhancement of TGE. Upon treatment with 1µM ruxolitinib or 10µM C188-9, the enhancement of TGE was decreased, revealing that AdV enhancement mediated by vitreous or ACHN supernatant was lost upon inhibition of JAK and/or STAT. In addition, inhibiting STAT in the absence of TGE enhancers resulted in a decrease in baseline expression levels, suggesting that STAT activation regulates not only enhancement effects but also baseline AdV transgene expression.

Our results support a model in which the extracellular binding of a vitreous and ACHN supernatant component results in JAK and STAT activation, facilitating enhanced expression of adenoviral vector transgenes. Src kinase seems to serve as a negative regulator of JAK/STAT activation, which is relieved upon Src inhibitor treatment. The modulation of these biochemical pathways could serve as a method of regulating AdV TGE in gene therapy protocols.

Future directions include identifying which JAK/STAT molecules are involved in enhancement and determining the component within vitreous eliciting this enhancement effect.

Contributors: Akinfenwa, P; Bond, W; Hurwitz, M; Hurwitz, R.
Increased TGFβ Signaling As a Mechanism In Osteogenesis Imperfecta

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Integrative Program in Molecular and Biomedical Sciences/M.D.-Ph.D. Program
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Osteogenesis imperfecta (OI) is a debilitating genetic osteodysplasia that results in low bone mass, bone deformity, and bone fractures. Most cases of OI are caused by mutations in the structural protein type I collagen (dominant OI) or in protein complexes that post-translationally modify type I collagen (recessive OI); both types of mutations can lead to biochemical overmodification. Type I collagen, while important for structural integrity of bone is also essential for extracellular matrix (ECM) cell signaling. It extensively interacts with ECM components that regulate bioavailability of signaling molecules, such as small leucine-rich proteoglycans (SLRPs), thereby affecting tissuespecific cell behavior. SLRPs regulate signaling by sequestering signaling molecules in the ECM. Due to the phenotypic similarities between OI mouse models and TGFβ overexpression models, we evaluated the contribution of altered TGFβ signaling to OI pathogenesis. Expression levels of TGFβ target genes were used to assess the status of TGFβ signaling in OI bone from a recessive OI model (Crtap-/-) and a dominant OI model (G610C OI) with quantitative PCR. Both OI models demonstrated increased expression of target genes p21, PAI-1, and Col1a1. Additionally, western blot analysis for Smad2 status, indicating TGFβ intracellular downstream signaling, demonstrated an increased ratio of phosphorylated Smad2 to total Smad2 in bone samples from both OI mouse models. Bone marrow stromal cells (BMSCs) from Crtap/-/- mice cultured under osteogenic conditions and measured with a TGFβ reporter cell line secreted more active TGFβ and induced higher TGFβ signaling in vitro. Treatment of Crtap/-/- and G610C OI mice with a TGFβ antibody (1D11) significantly rescued the OI bone phenotype. Together, these experiments demonstrate that increased TGFβ signaling contributes to the pathogenesis of OI. SLRPs were identified as the best-characterized ECM components that can bind to type I collagen and TGFβ to determine how structural mutations could alter cell signaling. Surface plasmon resonance analysis demonstrated that the SLRP decorin (Dcn) binds Crtap/-/- type I collagen 45% less effectively than wildtype type I collagen. This altered binding could modulate TGFβ signaling by preventing proper sequestration to type I collagen. Future studies will investigate the ability of other SLRPs to bind to type I collagen from OI mouse models. In vitro systems will be used to further evaluate the interaction between SLRPs and altered TGFβ signaling in OI. Ultimately, establishing the molecular mechanisms behind altered ECM signaling in OI will allow for development of more specific treatments for this disease.

Contributors: Grafe, Ingo; Homan, Erica; Lietman, Caressa; Bächinger, Hans Peter; Lee, Brendan
The major focus of this study is to uncover emerging functional role of long non-coding RNA (lncRNA) in the development and progression of cancer. With torrent of genomic data, we are learning intricate pattern of structural and epigenetic ‘driver’ alterations, albeit with limited knowledge on mechanistic link between these driver events and heterogeneous downstream gene expression program they coordinate. At the transcription core, such variations are attributed to differential interaction patterns of TF with other TFs, co-regulators or availability of TF DNA-binding site by actions of chromatin remodelers. However, it is unknown whether these TF-co-regulator-DNA interactions at known cancer gene sites are coordinated by an abundance of sequence-specific tethering elements. In this context, our hypothesis underscores both mechanistic and functional role of lncRNAs in cancer. Based on recent research, we hypothesize that abundance of lncRNAs harboring sequence-specific motif or structural motif coordinate TF-DNA interactions at promoter regions of known cancer genes and thus, drive carcinogenesis. Our approach will use both computational and experimental methods to characterize yet unknown lncRNA-DNA-RNA interactions. Using data from the cancer genome atlas melanoma project, we have characterized differential lncRNAs expression in melanoma subtype enriched with BRAF hotspot mutation and PTEN deletion. Also, we observe enrichment of AluYc family transposable elements in DNA coding region of these lncRNAs. Further, using ChIP-seq data on BRAF-V600E PTEN knockout cell lines, we are investigating potential epigenetic regulatory marks in regions of differentially expressed lncRNAs and possible sequence-specific or structural motifs using computational motif discovery algorithms and RNA secondary structure prediction tools. We will describe detailed methods and early results in the presentation. Finally, our approach will be scalable and extensible in understanding mechanistic and functional role of lncRNA in other cancers.
MAPPING THE PATHWAYS OF CELLULAR DIFFERENTIATION FROM THE HUMAN EPIGENOME ATLAS DATA USING THE EPIGENOME TOOLSET WITHIN THE GENBOREEE WORKBENCH

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The NIH Roadmap Epigenomics Consortium has developed the Human Epigenome Atlas (www.epigenomeatlas.org), a compendium of reference epigenomes from human cell lines, primary cells and tissues. Dynamic changes of the epigenome during cellular differentiation may for the first time be reconstructed comprehensively using the Atlas data. We first grouped epigenomes by a clustering algorithm into a dendrogram of major cell lineages such as neuronal, muscle, immune, myoepithelial, intestinal, mesenchymal, and immune. Epigenomic changes along specific branches of the dendrogram are then analyzed to identify (1) patterns of coordinated changes of epigenomic marks over enhancers, other regulatory elements and gene bodies; (2) enhancer, genes and pathways activated in each lineage; (3) lineage-specific transcription factors activity. Our data-driven epigenomic analysis recapitulates vast amounts of knowledge accumulated in the literature over the decades of gene-centric and pathway-centric research. Thousands of lineage-specific enhancers are identified. We discover that surprisingly small subset of epigenomic marks over enhancers, promoters, and gene bodies contains much information required to identify genes and pathways active in specific lineages. In addition to the analysis of cellular differentiation, we have also integrated a number of open-source tools into the Epigenomic Toolset within the Genboree Workbench (www.genboree.org) as cloud-integrated suite for comparative epigenomic analysis.

Contributors: Amin, Viren; Coarfa, Cristian; Harris, Alan; Onuchic, Vitor, Jackson, Andrew; Raghuraman; Sriram; Paithankar, Sameer; Roth, Matthew; Milosavljevic, Aleksandar; and NIH Roadmap Epigenomics Consortium
INHIBITORS OF THE H3K79 METHYLTRANSFERASE DOT1L KILL MLL-REARRANGED LEUKEMIC CELLS

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MLL-translocated leukemias comprise 70% of infant leukemias and 10% of adult leukemias. This leukemia has less than a 40% survival rate and it is therefore imperative that new drugs are needed. DOT1L, a histone3-lysine79 methyltransferase, plays a crucial role in the initiation and maintenance of MLL-translocated leukemia and knocking down DOT1L activity results in the loss of transforming ability of MLL-oncogenes. Therefore, DOT1L represents a target for intervention.

Our lab has developed novel small molecules with the goal of competitively inhibiting DOT1L and inhibiting the growth of MLL-translocated leukemic cell lines. We show that, by retaining the adenosine moiety of SAM but substituting the 5’ position, we can obtain inhibitors with inhibition constants as low as 0.5 nM. A urea moiety in the 5’ substituent is critical for high binding activity. These compounds are specific for DOT1L compared to three other SAM-dependent histone methyltransferases. Also, isothermal titration calorimetry studies show that two representative inhibitors bind to the DOT1L: nucleosome complex and only compete with SAM but not the nucleosome substrate. In addition, potent inhibitors of DOT1L showed selective activity against the proliferation of the MLL-translocated cell lines MV4; 11 and THP1 with EC50 values of 4-11 μM. One selected inhibitor is shown to downregulate expression of the leukemia-relevant genes Hoxa9 and Meis1, induce differentiation, and reduce the population of leukemic stem cells.

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Contributors: Anglin, Justin; Deng, Lisheng; Yao, Yuan; Liu, Zheng; Jiang, Hong; Cheng, Gang; Chen, Pinhong; Dong, Shuo; Song, Yongcheng;
Information flows in the vertebrate nervous system as electrical impulses known as action potential. Action potential is initiated at the axonal initial segment and requires high density of voltage gated ion channels. A diffusion barrier based on actin has been proposed to explain the immobilization of voltage gated ion channels at the axonal initial segment, however the molecular mechanism of the diffusion barrier remain to be elucidated. Our aim is to assess and quantify the nature of membrane cytoskeleton adhesion to gain mechanistic insights into the nature of the diffusion barrier.

We have developed a novel membrane probe based on optical tweezers to pull long membrane tethers from cells in order to detach membrane from the cytoskeleton and make precise measurement of membrane material properties. Polyethylene coated fluorescent beads will be trapped using a continuous wave, tunable Titanium-Sapphire laser pumped by 5 W solid state Nd: YVO₄ laser operated at 830nm. The image of the bead is projected onto a quadrant photodiode (QPD) that will be used for position sensing of the trapped beads. The QPD gives the displacement of the bead from the center of the QPD in volts. The bead displacement (in nm) and QPD output signal (in mV) has linear range proportional to the bead diameter and currently we can resolve displacements as small as 10nm. A restoring force is exerted on the bead that varies linearly (up to 2000nm) with its displacement from the trap center and the tether pulling force will be computed from the bead displacement. Currently we can measure tether forces up to 280pN. We bring a cell membrane in contact with the trapped polystyrene coated bead and attach. The cell is then moved away from the trapped bead by moving the piezo-controlled microscope stage at a preset velocity. Once a tether was formed, the tether is elongated at ~1μm/s for 30s until reaching a peak force. When the tether length reached 25-30μm, movement will be halted and tether will be maintained until the tether force to relax to a non-zero equilibrium force. We measure the tether pulling force from bead displacement.

We pulled membrane tethers from the soma and AIS of pyramidal hippocampal neurons and measured the mechanical force require to form and elongate the tethers. We observed a faster rise and higher peak in the passive tether force profile which indicates the membrane cytoskeleton attachment is stronger in the AIS. Nervous system injury causes cascade of events lead to the disruption of the cytoskeleton at the axon initial segment and cause loss of clustering of the voltage gated ion channels. The outcome of this study will benefit therapeutic strategy aimed at preserving the cytoskeleton following nervous system injury.

Contributors: Mussie Araya, Yuan, Tao, Rajasekharan, Vivek, William E. Brownell
Acquisition and honing of motor skills depends on the iterative fine-tuning of neural circuitry throughout life. In vivo imaging studies have revealed one example of this fine-tuning: With learning of a novel motor skill, new synapses are formed onto the apical dendrites of corticospinal neurons in motor cortex, and if stabilized these synapses appear to represent a durable structural correlate of skill memory.

MECP2 duplication syndrome is a single-gene developmental disorder in which patients demonstrate impairments in motor skill learning, along with intellectual disability, autistic features, and epilepsy. The mouse model for MECP2 duplication recapitulates many of these features, but interestingly early on exhibits enhanced motor learning and memory on the rotarod task.

We hypothesize that the enhanced motor behavior in these mice could stem from abnormal biases in motor learning-associated structural plasticity. Pilot studies bear out an upregulation in dendritic spine formation and loss with motor learning in these mice which exceeds that seen in wild-type animals, and correlates with enhanced learning on the task.
The Gram-negative intracellular bacterium Francisella tularensis (Ft) is the causative agent of tularemia and one of the most infectious and widely dispersed zoonoses. Two subspecies of Ft, ssp. tularensis (type A) and holarctica (type B), are responsible for 100% of tularemia fatalities in the U.S. Type B accounts for most natural cases of tularemia in humans, but type A is responsible for the majority of deaths. The CDC classifies Ft as a Tier One select agent due to its low infectious dose (<15 CFU), high morbidity, ease of dispersal, potential social and economic impacts, and lack of a licensed vaccine. An attenuated Live Vaccine Strain (LVS) was empirically derived in the 1950s from repeated passage of a type B strain. While it was later shown that LVS is less effective against inhalational type A, it is to date the only vaccine for which formal efficacy data in humans exists. However, LVS is not FDA-approved for multiple reasons, one of which is that the mechanism of attenuation is unknown.

Comparative genomics revealed possible explanations for virulence disparities between type A and type B. One such difference is the rearrangement of 51 syntenic blocks in type A compared to type B genomes, thus complicating the ability to make comparisons between LVS and type A. Therefore, in order to increase our understanding of Ft virulence and LVS attenuation, we aligned the LVS genome to virulent type B strains and identified 17 genes that are disrupted in LVS. Along with several proteins of unknown function, this list includes proteins involved in sugar modification, secretion, nutrient acquisition, and intracellular survival within macrophages, the primary reservoir for Ft. These candidate genes are not well described and their respective roles in pathogenesis remain unknown. A type II intron system (TargeTron®) has been adapted for use in Ft that enables the stable, permanent and specific disruption of genes. We therefore propose that the disruption of a subset of these 17 candidate genes in virulent type B will result in attenuation as shown by reduced intracellular replication, increased activation of infected macrophages, and decreased virulence in mice. Our lab has shown proof of concept of the TargeTron system through the disruption of mglA, a transcriptional regulator essential for intracellular growth of Ft, which will serve as an avirulent control for macrophage assays. Characterizing the mechanism of LVS attenuation may lead to an improved version of LVS that is genetically defined and potentially more protective against type A. An approved vaccine would not only lead to a decline of natural tularemia incidence rates, but also greatly reduce the incapacitating illness and treatment costs that would result from intentional release of Ft as a bioweapon.

Contributors: Atkins, Lisa; Petrosino, Joseph.
Different substitutions impact the function of a protein in different ways. While some substitutions cause a large effect, others are neutral or harmless. In light of widespread exome sequencing, it is critical to distinguish deleterious substitutions from harmless ones in a clinical setting. We hypothesize that the impact of a substitution on phenotype depends on two factors: the positional importance of the residue and size of substitution. To test this, we chose bacterial RecA protein that plays a central role in homologous recombination and regulates DNA damage repair. RecA is also a key component of the bacterial SOS response where it controls the expression of many other DNA damage repair genes. We quantitatively measured the impact of a mutation on RecA by assaying for DNA damage repair function and recombination function. The results of these assays reveal a correlation between residue position and the size of substitution on the functions of RecA. These experimental results complement prior correlations in retrospective analyses, including the leading performance in independent evaluations of blind predictions. Together these data shed new light on the relationship between genotype and phenotype variations.

Contributors: Katsonis, Panagiotis; Adikesavan K. Anbu; Lichtarge, Olivier
Microglia have long been known to respond to virtually any insult to the central nervous system. They quickly migrate to the site of the insult, and play many important roles, such as barricading the injury site, phagocytosing debris, and releasing cytokines. The role of microglia in the normal brain, however, has only recently begun to be appreciated. With the advent of in vivo imaging, microglia have been shown to be constantly surveying their environment with rapid extensions and retractions of their processes. Subsequent studies have shown that these ‘resting’ microglia (now known as ‘surveying microglia’) are indeed active throughout development and adulthood. During development, microglia have been shown to be involved in synaptic monitoring and pruning as well as synaptic stripping after injury. Thus far, many studies have focused on the role of microglia at the synapse. However, we report here, for the first time, that in the cortex of normal adult rodents, a small percentage of microglia are specifically associated with the axon initial segment (AIS). The AIS is characterized by a high density of voltage-gated ion channels and plays an important role in initiation of the action potential and maintenance of neuronal polarity. This interaction seems to be limited to the ‘surveying’ phenotype of microglia and much less frequent in ‘activated’ microglia. This overlap of processes appears early in development and continues throughout adulthood although the function of microglia at the AIS is still currently unknown.

Contributors: Baalman, Kelli; Godoy, Marlesa; Cotton, R. James; Rasband, Matthew
Tumor suppressor p53 is a critical protein in the prevention of cancer. It regulates the cell cycle, prevents genome mutation by activating DNA repair pathways, and can initiate apoptosis when DNA damage is irreparable. More than half of all human tumors contain a mutation in or deletion of p53. For a protein with such impact, it is desirable to understand not only p53 itself but also the proteins that interact with it. For example, phosphorylation in response to cellular stress is an important mechanism in the control of p53 activity. Out of all 525 known kinases, 33 have been identified to phosphorylate p53, but there are likely additional kinases that are not yet identified as having p53 activity. This study will show that it is possible to predict novel p53 kinases using protein-protein relationships and global network diffusion, a computational prediction method.

Global network diffusion is an established method for propagating information known about nodes in a network to other nodes based on information describing the similarities between them. In this case, nodes are human proteins, and the information connecting them is based on several diverse information sources, both publicly available ones and those built as part of this work. The resulting predictions were combined using logistic regression, and classifies known kinases in a validation test set with an ROC AUC of .894, showing strong predictive power.

Based on this model, several novel p53 kinases were predicted and are currently being validated experimentally by collaborators. Initial in vitro validation shows enrichment among our positive set and several promising targets for future in vivo activity studies. By starting with a computational approach, the amount of time and money required to identify novel p53 kinases has been drastically reduced. While this work has focused on p53 due to its biological importance, the approach is applicable to any protein that has a partial set of known kinases.

Contributors: Wilkins, Angela; Tajhal Dayaram; Neha Parikh; Donehower, Larry; Lichtarge, Olivier
Conditional Overexpression of MicroRNA-93 In The Kidney Ameliorates Progression of Diabetic Nephropathy

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MicroRNAs (miRNAs) comprise a broad class of small non-coding RNAs that negatively regulate gene expression by base-pairing to partial or perfectly complementary sites in the 3'-untranslated regions (UTR) of specific target mRNAs. Recent studies from our group suggest that miRNA-93 may play a central role in the pathogenesis of diabetic nephropathy (DN). The exact mechanism of its action in vivo, however, is unknown. Using established murine models of diabetic nephropathy; we deployed a dual genetic and pharmacological forced expression of miR-93 to further understand its effect on progression of DN. First, a tissue-specific, tamoxifen-inducible model of miR-93 overexpression was generated to selectively induce miR-93 overexpression only in podocytes (Pod-miR-93). At 24 weeks of age, diabetic db/db mice with forced expression of Pod-miR-93 exhibited improved kidney function as measured by a significant reduction in albuminuria, when compared to diabetic controls. Additionally, these mice exhibited a significant reduction in kidney fibrosis compared to controls as measured by Periodic-Acid-Schiff's (PAS) staining. Importantly, micrographs of kidney cortices prepared for transmission electron microscopy revealed reduced podocyte detachment, and improved glomerular basement membrane thickness in diabetic Pod-miR-93 mice, similar to that of non-diabetic control mice. Second, in order to assess the therapeutic potential of miR-93, a pharmacological approach was employed using the in vivo delivery of modified mimic miR-93 in db/db mice. To this end, 8-week-old db/db mice were intra-peritoneal (IP) injected with 2 mg/kg of a modified miR-93 mimic or a control non-targeting (NT) mimic. Following 10 weeks of biweekly IP injections, we found that diabetic db/db mice allocated to miR-93 mimic injections exhibited improved kidney function as measured by a significant decrease in albuminuria at 16 weeks of age. This decrease was statistically significant when compared to diabetic animals treated with control mimics. Consistent with these findings, histological analysis revealed significantly attenuated kidney fibrosis. Importantly, the effect of miR-93 mimic on albuminuria was at least partially reversible since when mimic injections were withdrawn after 20 weeks, albuminuria worsened in db/db mice, suggesting that sustained miR-93 expression is essential for the observed improvement in diabetic kidney function. Finally, to further explore the effect of VEGF in vivo, a target previously identified by our group and critical to DN pathogenesis, we utilized a constitutively active VEGF-LacZ reporter mouse, and assessed the expression of VEGF in response to exogenous overexpression of miR-93 using modified mimics. We found that miR-93 mimics dramatically reduced VEGF-LacZ expression as assessed by β-galactosidase staining compared to controls, confirming the effect of miR-93 on VEGF in vivo. In summary, our findings provide evidence for a central regulatory role of miR-93 in the progression of DN, and identify miR-93 as a promising therapeutic target in patients with DN.

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MTORC2 MAY CONTRIBUTE TO SPECIFIC ASPECTS OF THE PTEN-MUTANT BEHAVIORAL PHENOTYPE

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Autism spectrum disorder (ASD) is a heterogeneous group of diseases collectively characterized by impaired social interaction and communication, repetitive behaviors, and cognitive deficits. Although the etiology of the disease is unknown, several genes have been identified as mutated in individuals with ASD, including the phosphatase and tensin homolog gene (PTEN). PTEN encodes for the eponymous PTEN protein, a tumor suppressor and inhibitor of the PI3K pathway. PTEN directly antagonizes the actions of PI3K, dephosphorylating PIP3, thereby preventing activation of the pathway. Mutation of PTEN causes overactivation of the pathway, leading to an increase in mTOR Complex 1 (mTORC1) activity and also, through an unknown mechanism, increased mTOR Complex 2 (mTORC2) activity. Pharmacological reduction of both complexes can rescue many abnormalities associated with PTEN mutation; however, to date it is unclear which abnormalities are associated with which complex. We have generated forebrain-specific knockout mice with loss of PTEN and loss of rictor (a key mTORC2 component) or raptor (a key mTORC1 component). Our preliminary data show that single copy deletion of rictor may be able to rescue select social deficits and mildly rescue memory deficits associated with loss of functional PTEN. These results suggest that mTORC2 affects specific behaviors that are altered with PTEN mutation.

Contributors: Baete, Dillon; Huang, Wei; Costa-Mattioli, Mauro
Influenza A virus (IAV) is a pathogenic, negative-sense, single-stranded RNA virus that replicates in the mammalian nucleus rather than the cytoplasm, in contrast to nearly every other RNA virus. This replication strategy has been highly successful for IAV despite the localization of numerous ribonucleases and RNA-binding proteins in the nucleus. We hypothesized that the IAV genome, itself made up of small, noncoding ssRNA segments, may have adapted to this harsh environment by conserving stem-loop hairpin folds with the potential to directly enter the RNAi pathway, similar to mammalian pri-miRNA molecules. Computational prediction of vRNA secondary structure by VIENNA-RNA was performed on each of the eight vRNA segments of the 2009 pandemic swine-origin H1N1 influenza (pdH1N1). These predictions revealed microRNA-like stem-loop structures. In order to test whether these structures could be cleaved by DICER, vRNA extracted from purified pdH1N1 was treated with the recombinant human DICER enzyme. Full-length vRNA segments, subgenomic RNAs, and small RNA products from DICER cleavage reactions were separated by denaturing PAGE (4-16% gradient, 6M Urea). Gels were analyzed by fluorescent laser scanning after staining with ssRNA-stain Sybr-GREEN II. A 27-nt DICER-cleavable positive control and a DICER-resistant negative control RNA duplex ensured that the reaction conditions only promoted the canonical 18-21 nt RNA-duplex cleavage products. Indeed, DICER produced ~19 nt RNAs as cleavage products from an ATP-specific cleavage activity. We additionally created an A549 cell line (A549-IAVmIR) that stably overexpresses four computationally predicted RNA hairpins from both vRNA and cRNA sequences. Preliminary data suggest that overexpression of these isolated, untranslated stem-loop RNAs causes alterations in cell morphology, including increased cell size and development of multinucleated giant cells. Our data suggest that significant secondary structure of negative-sense vRNA, the strand found in excess during IAV infection, may underlie a second role for negative-sense RNA genomes beyond a template for its protein-coding sister strand, potentially contributing to viral pathogenesis.

Contributors: Bakkalbasi, Erol; Kaelber, Jason; Yu, Zhifeng; Catanese, Jamie; Koire, Amanda; Machado, Annette; Matzuk, Martin M.; Gilbert, Brian E.; Zechiedrich, Lynn.
HAL IS A BACILLUS ANTHRACIS HEME ACQUISITION PROTEIN

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The mammalian ionic iron level (10-18 M) is far too low to sustain normal bacterial growth. Thus, successful bacterial pathogens have evolved specific systems to attain host iron. B. anthracis, the causative agent of anthrax and a potential weapon of bioterrorism, grows rapidly in mammalian hosts, which suggests it efficiently acquires iron during infection. Recent studies have uncovered both heme (isd) and siderophore-mediated (asb) iron transport pathways in this pathogen. Whereas deletion of the asb genes results in reduced virulence, the loss of three surface components from isd had no effect, thereby leaving open the question of what additional factors in B. anthracis are responsible for iron uptake from the most abundant iron source in mammals, that of heme. Here, we describe the first functional characterization of hal (heme-acquisition leucine-rich repeat protein), a gene recently implicated in anthrax disease progression. Hal encodes a single near-iron transporter (NEAT) domain and several leucine-rich repeats. The NEAT domain binds heme, despite lacking a stabilizing tyrosine common to the NEAT superfamily of hemoproteins. The NEAT domain also binds hemoglobin and can acquire heme from hemoglobin in solution. Deletion of hal resulted in bacilli unable to grow efficiently on heme or hemoglobin as an iron source and yielded the most significant phenotype relative to other putative heme uptake systems, a result that suggests this protein plays a prominent role in the replication of B. anthracis in hematogenous environments. Finally, preliminary studies using a novel vaccine, composed of the conserved protein NEAT domains of B. anthracis, suggest these subunits confer resistance and provide protective immunity to anthrax disease in a murine model of infection. These studies advance our understanding of heme acquisition by this dangerous pathogen and justify efforts to determine the mechanistic function of this novel protein for vaccine or inhibitor development.

Contributors: Balderas, Miriam; Maresso, Anthony W.
IMMUNOGENICITY AND EFFICACY OF A THERAPEUTIC TC24 NANOPARTICLE VACCINE AGAINST CHAGAS DISEASE

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Chagas disease is a neglected tropical disease of great importance in Latin America, with over 8 million people infected. The causative agent is Trypanosoma cruzi (T. cruzi), and results in an acute febrile illness that progresses to chronic chagasic cardiomyopathy in approximately 30% of patients. Current treatments are plagued by significant side effects, long duration, poor efficacy, and are contraindicated in pregnancy. There is an urgent need for new therapeutics. Our long-term goal is to produce a therapeutic vaccine for Chagas disease. Potential advantages of a therapeutic vaccine include a shorter course of treatment and the induction of multiple effector mechanisms against T. cruzi that will increase efficacy and decrease the possibility of resistance. Our objective is to identify a candidate vaccine using a murine model of Chagas disease and to better understand the protective immunogenicity. Prior work in mice using a DNA vaccine platform has identified a protective T. cruzi antigen, Tc24. DNA vaccines have historically performed poorly in human studies, therefore we propose utilizing Tc24 in a recombinant protein vaccine. Multiple studies have demonstrated that a TH1-mediated CD8+ T cell response is required for protective immunity to T. cruzi. To elicit a cell-mediated immune response from a recombinant protein, we propose using a nanoparticle delivery system in conjunction with an immunomodulatory adjuvant that stimulates appropriate toll-like receptors (TLR), such as CpG motif-containing oligodeoxynucleotides. We hypothesize that the recombinant Tc24, when delivered in the nanoparticle delivery system with a TLR agonist, will produce the desired CD8+ T lymphocyte response for vaccine efficacy as measured by an appropriate TH1-mediated CD8+ T cell immune response, improved cardiac outcomes, decreased parasitemia, and increased survival. To test our central hypothesis, the following specific aims will be completed:

Determine the immunogenicity of a Tc24 nanoparticle vaccine. We hypothesize that the inclusion of an immunomodulatory TLR agonist adjuvant in a nanoparticle delivery system will produce a robust TH1-mediated CD8+ T cell immune response to the Tc24 protein.

Evaluate the efficacy of a Tc24 nanoparticle vaccine in a mouse challenge model. We postulate that the vaccine formulation that shows the best TH1-mediated CD8+ T cell immune response will also demonstrate protective efficacy in a mouse T. cruzi challenge model as measured by decreased parasitemia and increased survival.

Correlate an immune response to Tc24 with decreased Chagas disease progression in humans. Our working hypothesis is that in patients who have significant disease progression there will be a minimal TH1-mediated CD8+ T cell immune response to Tc24, in comparison to a more robust immune response in patients who have no clinical manifestations of disease.

Contributors: Barry, Meagan; Wang, Qian; Beaumier, Coreen; Heffernan, Michael; Hotez, Peter.
Within cardiomyocytes, junctophilin-2 (JPH2) is a structural protein involved in forming junctional membrane complexes (JMCs) approximating voltage-gated L-type calcium (Ca2+) channels on the plasma membrane and type 2 ryanodine receptors (RyR2) on the sarcoplasmic reticulum (SR). JPH2 has been shown to be downregulated in various animal models of cardiomyopathies as well as cardiomyopathy patients. The loss of JPH2 coincides with disruption of JMC structure and abnormal cardiac Ca2+ handling. These molecular disturbances can precipitate disease progression to heart failure. We hypothesize that overexpression of JPH2 in the heart will stabilize RyR2 and prevent the disruption of JMC architecture following pathological stress and preserve cardiac function.

In order to study the potential protective effects of JPH2, a transgenic mouse model was generated with cardiac specific JPH2 overexpression (JPH2-Tg). At baseline these mice were shown to have normal cardiac function, dimensions, and T-tubule structure compared to non-transgenic (NTg) littermates. However, JPH2-Tg mice had decreased size and frequency of Ca2+ sparks resulting in an increased intracellular SR Ca2+ load, and suggests hyperstabilization of the RyR2 channel compared to NTg mice. Next, JPH2-Tg and NTg mice were subjected to pressure-overload stress using transaortic constriction (TAC). Following one month of TAC, JPH2-Tg mice had preserved cardiac function while NTg mice had decreased levels of JPH2 and progressed to heart failure. Additionally, isolated cardiomyocytes from JPH2-Tg mice showed preserved T-tubule structure while NTg mice had severe disturbances in T-tubule architecture. These preliminary studies suggest that JPH2 is not only critical for JMC maintenance, but overexpression of JPH2 is protective against pathological remodeling associated with cardiomyopathies. Future studies will focus on Ca2+ handling in JPH2-Tg cardiomyocytes following pathological stress and to test whether restoration of JPH2 can have therapeutic effects following the onset of heart failure.

Contributors: Beavers, DL; Wang, W; Reynolds, JO; Respress, JL.
Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect involving the invasion of the thoracic cavity by abdominal organs. The presence of these organs in the thoracic cavity can place pressure on the developing lungs, leading to pulmonary complications and hypoxemia after birth which can result in significant morbidity or even death. The incidence of CDH has been estimated at ~1 in 3000 live births, and CDH accounts for ~8% of all major congenital anomalies. Learning more about the etiology of CDH could lead to improvements in care and early detection. To that end, the goal of the research presented here is to identify and characterize novel genes and mutations in known genes which cause CDH. We show that recessive mutations in FREM1 can cause isolated CDH in humans and we explore this phenotype in mice using two novel Frem1 mutant mouse strains. We also expand on the phenotypic spectrum of Frem1 mutant mice by identifying lung lobe fusions and anorectal malformations in these mice. Finally, we analyze whole-exome sequencing data from a large cohort of CDH patients. In doing so, we identify novel genes and mutations which may contribute to this defect and we provide, for the first time, a molecular diagnosis for several families and individuals in this cohort. These studies further our understanding of CDH in both humans and mice and may lead to novel therapeutics for patients with CDH.
Novel cell-type specific roles of autophagy in mediating EAE, a mouse model of multiple sclerosis

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Autoimmunity affects about 5% of the world population. Multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS), affects more than a million people worldwide. Similar to most autoimmune diseases, insufficient understanding of pathogenesis has precluded curative therapy for MS. Autophagy is a conserved bulk degradation process involved in infection, immune cell homeostasis, antigen processing & presentation and tolerance. Though all these processes are critical for triggering or exacerbating autoimmunity, potential roles of autophagy in regulating autoimmune diseases are poorly understood.

To test our hypothesis that autophagy in innate immune cells is required for mediating autoimmunity, we generated conditional autophagy deficient mice where Atg7 or Atg5, essential autophagy genes, were deleted from dendritic cells (DCs) or myeloid cells (including macrophages and neutrophils) and induced experimental autoimmune encephalomyelitits (EAE), a mouse model of MS.

We found that loss of autophagy in myeloid cells or DCs ameliorated EAE by two distinct mechanisms. Atg7-deficient DCs showed reduced antigen presentation during EAE, resulting into reduced activation of antigen-specific T cells which, in turn, led to reduction in severity and incidence of EAE. In contrast, autophagy deficiency in neutrophils resulted in reduced secretory functions leading to reduced breakdown of blood-CNS barrier and reduced infiltration of inflammatory cells, thereby reducing severity of EAE. Administration of Chloroquine, an autophagy-lysosomal inhibitor, before EAE onset delayed disease progression, and when administered after the onset, reduced disease severity. Other neutrophil-mediated inflammatory processes were also reduced in absence of autophagy.

Our data identified novel cell-type specific roles of autophagy in mediating autoimmune processes and uncovered this pathway as a potential therapeutic target for treating autoimmune diseases such as MS. We also identified novel roles of autophagy in mediating degranulation from neutrophils. Our findings have the potential to open up a novel angle concerning the role of autophagy in neutrophils and how that might affect related diseases, particularly pulmonary diseases, within and outside the domains of autoimmunity.

Contributors: Bhattacharya, Abhisek; Eissa, N Tony
Fibroblast growth factor receptor 1 (FGFR1) is one of the top ten genes amplified in all cancers. Our previous study using an inducible MMTV-iFGFR1 X MMTV-Wnt1 genetically engineered mouse model of luminal B breast cancer showed that activation of FGFR1 signaling accelerated tumor growth by enhanced translation of Wnt1 target mRNAs. To dissect the role of FGFR1 in tumorigenesis, we have utilized the drug ribavirin suggested to inhibit cap-dependent protein translation, and BJG398, an FGFR tyrosine kinase signaling inhibitor. The results show that tumor growth was suppressed by ribavirin, due primarily to reduced proliferation as shown by IHC and IF. In contrast, in the BJG398 treatment group, a rapid and marked reduction in tumor volume was observed. Palpable tumors were no longer detectable after several days due to suppression of proliferation as well as a marked increase in apoptosis. However, after ten days the treatment was stopped, and the tumors recurred but initially only after a lag of 4 weeks. Recurrent tumor growth rates were enhanced, but they were still sensitive to a second and third cycle of treatment with BLG398. These results suggest that a residual population of cancer stem cells perhaps maintained by Wnt signaling might be responsible for tumor recurrence.
Foxi3 is a forhead transcription factor that is expressed early in chick and mouse in the preplacodal region. This region gives rise to multiple craniofacial placodes, such as lens, trigeminal or epibranchial in addition to otic placode, the precursor to the inner ear. Inner ear induction is triggered by FGF signaling, and competence to induce otic placode markers in response to FGFs correlates with the expression pre-placodal markers such as Foxi3. Our lab has knocked out Foxi3 in mice and Foxi3 mutants completely lack all ear structures and also have additional craniofacial defects. Ear induction appears to fail at the very earliest stages, as we see no markers of the otic placode, such as Pax2, induced in Foxi3 mutants. Foxi3, like other Forkhead transcription factors, has a DNA binding domain that resembles H1 and H5 linker histones and it is believed that due to this structural resemblance it may serve as a pioneer factor that can bind to condensed chromatin and help to make it transcriptionally accessible. This in turn may induce competence for the cell to respond to future inducing signals.

At one extreme, Foxi3 might simply confer competence for the expression of inner ear genes after pre-placodal ectoderm receives FGF signals. Foxi3 might also provide competence to pre-placodal ectoderm by regulating genes (such as FGF receptors) that to respond to FGF signals. At the other extreme, Foxi3 might be required to induce pre-placodal genes in the first place. We will analyze Foxi3 mutants together with gain- and loss-of-function experiments in chick embryos to test the necessity and sufficiency of Foxi3 in each of these stages of ear induction. By using both mouse knockouts and chicken embryos, we will be able to test the role of Foxi3 at multiple steps in chick, even if the induction of the inner ear is blocked at the very first step in Foxi3 null mice. We will also analyze the mutant mice to pinpoint, where exactly the Foxi3 plays role in FGF signaling since the mutant mice lack Pax2 induction, which is one of the first responsive genes in ear.

Moreover, Foxi3 is expressed early embryonically in some neural tissue and later in PPR region that is very close to the presumptive neural crest tissues. We are not sure whether these expressions are biologically relevant. In some Foxi3 mutant mice, the embryos exhibit exencephaly phenotype. Furthermore, the branchial arches that are normally filled with neural crest cells fail to form. Considering all these, there is a high we hypothesize that Foxi3 is significant for some neural tissue and neural crest cells as well. In order to investigate this part, we plan to perform fate mapping and tissue specific knock out studies.
Aberrant regulation of nuclear receptor coregulators is associated with a wide variety of human diseases. We recently determined that the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) coregulator interacts with steroid receptor coactivator-3 (SRC-3) a known oncogene, increasing its intrinsic transcriptional activity and association with an estrogen receptor- (ER) target gene, cyclin D1 (Mol Endocrinology 24:1187, 2010). Expression of SRC-3 and SMRT were also positively correlated in a small cohort of breast tumors. We therefore wanted to examine the impact of SMRT using control versus SMRT-specific siRNA and short hairpin RNA (shRNA) in ER(-positive (MCF-7 and ZR-75-1) and ER(-negative (MDA-MB-231) breast cancer cell lines. Depletion of SMRT reduced proliferation of MCF-7 cells grown as a monolayer on plastic and grown in colonies in soft agar under basal, estradiol (E2) or 4-hydroxytamoxifen (4HT) treatment conditions. Growth of ZR-75-1 cells but not MDA-MB-231 cells was inhibited when siRNA technology was used to knock-down SMRT. Treatment of control and SMRT depleted MCF-7 cells with ICI 182,780 determined that there is an ER independent effect of SMRT knockdown on growth. Flow cytometric analysis of cell cycle distribution revealed the E2-induced shift in synchronized populations from the G1 to S/G2/M phase was compromised in SMRT-depleted MCF-7 cells, consistent with a loss of estrogen induction of cyclin D1 expression in these cells. Moreover, a hormone independent increase in the percentage of SMRT-depleted MCF-7 cells in the subG0 population was detected suggesting an increase in apoptosis in SMRT depleted cells; this was confirmed by Cell Death ELISA, annexin V staining and PARP cleavage. Additionally cell cycle analysis revealed that the rate of re-entry of SMRT depleted cells, in comparison to control cells, into the G1 phase of the cell cycle after treatment with the mitotic block nocodazole is reduced. Microarray and RT-qPCR analyses demonstrated that SMRT knock-down results in changes to the expression of genes involved in mitosis and apoptosis. Collectively, these data reveal that SMRT impacts breast tumorigenesis through multiple pathways that inhibit apoptosis, promote cell proliferation at multiple points within the cell cycle and promote anchorage-independent cell growth, and together these data suggest SMRT is a potential therapeutic target for breast cancer.

Contributors: Karmakar, Sudipan; Chaubal, Vaishali; Wang, Liguo; Li, Wei
Background: AD HIES is a primary immunodeficiency caused by mutations of STAT3. The molecular basis for defective STAT3 function, however, is unclear, and treatment is supportive. We hypothesize that AD HIES mutations decrease STAT3 stability. Also, since STAT3 depends on chaperones, such as tailless-complex polypeptide-1 ring complex (TRiC) and heat shock proteins (HSP) 70 and 90, for biogenesis and optimal function, we further hypothesize that AD HIES STAT3 function can be improved through enhanced STAT3 proteostasis.

Methods: Mutations were characterized using computer modeling (Evolutionary Trace, PoPMuSiC, I-mutant, MUpro, SDM, DFIRE). STAT3 protein half-life (t1/2), a surrogate marker of stability, was measured in EBV-transformed B cell lines (EBLs) from AD HIES and control patients. EBLs were treated with proteostasis modulators HSF1A, a small molecule that upregulates TRiC (3uM x 48h), and geranylgeranylacetone (GGA), an anti-ulcer drug that upregulates HSP70 and 90 (3uM x 48h). After IL-21 stimulation, total and phosphorylated (pY) STAT3 were measured using Luminex bead-based assays, and expression of the STAT3 target gene SOCS3 was measured using Q-RT-PCR.

Results: Seventy-three mutations were characterized using computer modeling; 78% were predicted to impair structure and destabilize STAT3. Consistent with this, STAT3 t1/2 in EBLs containing mutations predicted to impair stability (R423Q, V463del, S611N, T622I, N647D, Y657S, V637M) was significantly reduced (24-80%, p<0.01) compared with STAT3 t1/2 in EBLs with mutations predicted not to affect stability (R382W) or controls. Treatment of EBLs containing mutations predicted to impair stability with HSF1A and GGA each normalized STAT3 protein t1/2 (p<0.01), increased pY-STAT3 levels from 10-80% to 40-150% of wild type (WT; p<0.01), and increased SOCS3 mRNA levels from 10-20% to 40-80% of WT (p<0.01). HSF1A, but not GGA, increased total STAT3 levels from 40-90% to 110-200% of WT (p<0.05). Conclusion: Thus, most STAT3 mutations identified in AD HIES patients are destabilizing and their function can be improved dramatically with the use of proteostasis modulators, which provide a novel treatment approach to improve STAT3 function and prevent infection in AD HIES patients.
Estrogen receptor-α (ERα) is a central transcription factor that regulates mammary gland physiology and a key driver in breast cancer. In the present study, we aimed to identify novel modulators of ERα-mediated transcriptional regulation via a custom built siRNA library screen. This screen was directed against a variety of coregulators, transcription modifiers, signaling molecules, and DNA damage response proteins. By utilizing a microscopy-based, multi-endpoint, estrogen responsive biosensor cell line platform, the primary screen identified a wide range of factors that altered ERα protein levels, chromatin remodeling, and mRNA output. We then focused on UBR5, a ubiquitin ligase and known oncogene that modulates ERα protein levels and transcriptional output. Finally, we demonstrated that UBR5 also affects endogenous ERα target genes and E2-mediated cell proliferation in breast cancer cells. In conclusion, our multi-endpoint RNAi screen identified novel modulators of ERα levels and activity, and provides a robust systems level view of factors involved in mechanisms of nuclear receptor action and pathophysiology.

Contributors: Bolt, Michael; Stossi, Fabio; Callison, Austin; Mancini, Maureen; Mancini, Michael
Spinocerebellar Ataxia type 1 (SCA1) is a dominantly inherited fatal neurodegenerative disorder for which there is no available treatment. It is caused by the expansion of polyglutamine (polyQ) tract in ATAXIN-1 (ATXN1), rendering the protein more stable and resulting in toxic accumulation of mutant ATXN1. We hypothesize that reduction of ATXN1 protein levels will suppress SCA1 pathogenesis. Thus, we aim to identify modulators of ATXN1 protein levels. To monitor ATXN1 levels we engineered a human medulloblastoma-derived (DAOY) cell line with a transgene encoding a glutamine expanded ATXN1 fused with red fluorescent protein (mRFP-ATXN1[82Q]). To distinguish suppressers that affect ATXN1 protein levels from those that regulate transgene transcription, internal ribosomal entry site (IRES) followed by yellow fluorescent protein (YFP) is inserted, resulting in mRFP-ATXN1[82Q]-IRES-YFP transgene. A forward genetics screening approach is chosen to identify suppressors of mutant ATXN1 using shRNA library screen targeting over 2,500 potentially druggable human genes. shRNA screen was performed, where cells are sorted based on lowest five percent fluorescent ratio of mRFP/YFP. In parallel, an independent screen is performed to identify suppressors of ATXN1 in fruit fly, in vivo. Twenty six candidates were selected based on several criteria such as known fly modifiers and lowest mRFP/YFP ratio. A secondary screen in cells further prioritized the gene candidates obtained from the shRNA screen by analyzing mRFP-ATXN1[82Q] levels with flow cytometry when knock down with multiple siRNAs per candidate. We further narrowed down the list by knocking down each candidate in wild type and transgenic DAOY cells using western blotting. We selected five genes that when inhibited lead to significant reduction of ATXN1. Currently we are elucidating molecular mechanisms by which selected modifiers suppress ATXN1 levels.
APP overexpression, not Aβ overproduction, underlies epileptiform activity in a mouse model of Alzheimer’s disease

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Alzheimer’s disease patients show an elevated risk for seizures compared to the general population. Aberrant network activity may contribute to cognitive dysfunction, a critical part of AD progression. Consistent with this observation, many AD mouse models exhibit abnormal cortical discharges and seizures. We used tet-off amyloid precursor protein (APP) overexpressing mice that display abnormal electrical discharge events to understand how neuronal excitability is regulated by APP and amyloid beta (Aβ). APP suppression progressively decreases epileptiform activity to levels not significantly different from control mice after four weeks of treatment. Past studies have suggested that Aβ accumulation contributes to neuronal excitability and disruption of normal negative feedback. However, we found that treating tet-off APP mice with a γ-secretase inhibitor to selectively decrease Aβ levels while maintaining APP overexpression had no effect on sharp wave discharge frequency. To better understand the effect of APP overexpression on cortical network excitability, we tested the effect of APP suppression during the critical time period of post-natal development. Delaying APP overexpression until mice have matured delays onset of epileptiform activity, which suggests an interaction between APP and cortical development. We are currently working to understand the relationship of APP overexpression to disruption of the neuronal network excitatory: inhibitory balance at synapses.

Contributors: Born, Heather; Das, Pritam; Guo, Qinxi; Golde, Todd; Zheng, Hui; Noebels, Jeffrey Noebels; Jankowsky, Joanna
Autism spectrum disorder (ASD) comprises a set of neurodevelopmental conditions manifesting as repetitive behaviors and social and communication impairments. Genes affecting synaptic function have been found to be causative in a subset of ASD patients. Early exposure to environmental factors either by themselves or in conjunction with genetic factors has also been implicated in ASD, and in combination may play a role in ASD penetrance. To minimize confounding factors, our lab uses mouse models to answer this question. The overall goal of my project is to investigate how gene and prenatal environment interact (GXE) to influence ASD-like behavior in a Shank3 mutant mouse model. I chose this mouse model because Shank3 null mice exhibit ASD-like behaviors and SHANK3 heterozygous humans have Phelan-McDermid Syndrome, whose features may include ASD. Our overarching hypothesis is that ASD-associated risk alleles combined with certain common prenatal exposures can modulate neurodevelopment by altering maternal care and offspring behavior. This combination might allow an individual to reach a “phenotypic threshold” resulting in increased ASD penetrance. We will address this hypothesis through two specific aims. SPECIFIC AIM 1: To assess the effects of prenatal CUMS exposure on maternal care in Shank3 heterozygous dams. Preliminary data from our lab indicates that mutant male offspring of heterozygous Shank3 dams exposed to gestational chronic unpredictable mild stressors (CUMS) exhibit an atypical response when compared to non-stressed gender and genotype-matched littermates in the three-chamber social behavior test. Because studies indicate that gestational stress can alter postpartum maternal care and offspring development, I am now examining the effects of gestational stress on maternal care in WT and mutant Shank3 dams. We are currently measuring levels of stress hormones. In the future, we will employ behavior assays to examine whether CUMS exposure influences maternal care in Shank3 heterozygous dams. We will assess brain morphology and histology in dams. We will also measure gene and protein expression in these dams. We will also conduct Diffusion Tensor MRI (DTI) on brains from dams. Proposed experiments will likely answer how stress combined with genetic susceptibility contributes to observed phenotypes. SPECIFIC AIM 2: To assess the effects of prenatal FLX exposure of Shank3 heterozygous dams on offspring neurodevelopment. For the second aim of my project, I am investigating if any other prenatal stressor-genotype combinations will result in altered social behavior in offspring. I am focusing on Fluoxetine (FLX), a Selective Serotonin Reuptake Inhibitor, a regularly prescribed during pregnancy, and because maternal intake of (FLX) in the first trimester is associated with increased incidence of ASD. Therefore, we will also examine whether FLX exposure in Shank3-mutant dams affects penetrance of ASD features in offspring. Dam plasma is being assayed for route and dose optimization by mass spectrometry (LC/MS-MS) to aid future study design.
Epigenetic control is a key process involved in liver regulation. Recent studies have shown that several age-related liver dysfunctions are associated with the change of chromatin structure. These dysfunctions include development of hepatic steatosis (fatty liver), impaired liver proliferation and age-associated liver cancer. Previous studies in Dr. Timchenko’s lab revealed that two members of C/EBP family, C/EBP and C/EBP, are altered by age and are involved in alteration of chromatin structure in the liver. These observations were obtained in several animal models of aging liver including C/EBP-S193D knockin mice and recently generated transgenic mice expressing dominant negative p300, dnp300 mice. We have found that histone acetyltransferase p300 co-operates with C/EBP proteins in the epigenetic control of liver functions and that age dramatically changes p300-C/EBP pathways in the liver. The dnp300 mice have altered expression of C/EBP proteins compared to WT and higher basal proliferation. Upon challenges to the liver using several methods including Carbon Tetrachloride treatment and Partial Hepatectomy, we observe that dnp300 mice have higher rates of proliferation and are more resistant to liver injury. Therefore, the main goal of my project is to determine the role of p300-dependent epigenetic control in the key functions of the liver.

Contributors: Breaux, Meghan; Jin, Jingling
GENETIC VARIANTS OF THE DOPAMINE TRANSPORTER (DAT1) MEDIATE THE ACUTE SUBJECTIVE RESPONSES TO COCAINE IN COCAINE-DEPENDENT VOLUNTEERS

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AIMS: The dopamine transporter (DAT) has been implicated in the subjective and reinforcing effects produced by cocaine, and polymorphisms within the DAT gene (DAT1, or SLC6A3) have also been linked to variations in the response to cocaine. The aim of the present study was to identify candidate gene variants of DAT1 that modulate subjective responses to cocaine in cocaine-dependent volunteers.

METHODS: Non-treatment seeking, cocaine-dependent volunteers received a single bolus infusion of saline and cocaine (40 mg, IV) in randomized order. Subjective effects questionnaires (visual analogue scales: VAS) were administered before (-15 min) and up to 20 min after infusion. VAS scales ranged from zero (no effect) to 100 (greatest effect). Subjective effects ratings were normalized to baseline by subtracting saline infusion values. Data was analyzed using repeated measures ANOVA. DNA from subjects was genotyped for the DAT1 intron 8 and 3’ UTR VNTRs.

RESULTS: Participants (N=47) had a mean age of 43.5 ± 6.6 (mean ± standard deviation) years, were mostly black (78%) and mostly males (87%), and had a mean of 12.8 ± 1.8 years of education. The primary route of cocaine use was smoking (94%), and the participants used a mean of 2.1 ± 2.3 grams of cocaine per day, and 16.8 ± 7.7 days out of the last 30. Carriers of the 9-allele of the DAT1 3’ UTR (9/9 and 9/10) exhibited significantly greater responses to cocaine for “High” (p = .0002), “Any Drug Effect” (p = .00005), and “Stimulated” (p = .0004). In addition, individuals with the 6/6 genotype for the DAT1 intron 8 polymorphism exhibited significantly greater responses to cocaine for “Desire” (p = .0005).

CONCLUSIONS: The data presented here support the hypothesis that individual genetic differences of DAT1 contribute to variation of individual responses to cocaine among dependent participants.

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The presence of paralogs within the genome provides the potential for functional redundancy. If one paralog is lost, another paralog can compensate to potentially maintain normal function. Compensation between paralogs often becomes evident with knockouts such that knockouts of the individual paralogs have substantially less phenotypic effect than a double knockout of two paralogs. In many cases, this compensation is accomplished by increased protein expression of the compensating paralog. Although we understand the importance for compensation, the mechanisms by which the loss of expression of one paralog is detected and results in the compensatory up-regulation of the second paralog is not understood. CUG-binding protein Elav-like family (CELF) and Muscleblind-like (MBNL) proteins are RNA-binding proteins that have six and three highly conserved paralogs in mammals, respectively (CELF1-6 and MBNL1-3). CELF1, CELF2, MBNL1, and MBNL2 are the paralogs expressed in heart and skeletal muscle tissues. These proteins are of importance because both CELF and MBNL proteins regulate alternative splicing during heart and skeletal muscle development. Previous work in the lab has shown reciprocal up-regulation of Celf or Mbnl paralogs following knock down in the mouse myoblast cell line C2C12. In addition, Celf1 or Mbnl1 knockout mice exhibit some level of reciprocal up-regulation of Celf2 or Mbnl2, respectively, in heart and skeletal muscle tissues. We plan to elucidate the mechanism of compensatory up-regulation between paralogs. Since the same reciprocal up-regulation is seen in both in vitro and in vivo, C2C12 cells can be exploited to determine the mechanism, which will be validated and further investigated in heart and skeletal muscle tissues. We will first determine the protein and mRNA levels to establish if this a transcriptional or post-transcriptional mechanism. Then, we can continue to investigate specific events of transcription, mRNA stability, or protein stability. These studies will define the relationships between CELF and MBNL paralogs and potentially reveal a novel mechanism of paralog compensation.

Contributors: Brinegar, Amy; Cooper, Thomas
INNER, OUTER, I, AND II: HOW HAIR CELLS ACQUIRE THEIR IDENTITY

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The inner ear is a complex and specialized vertebrate organ deeply embedded in the function of the auditory sensory pathway as well as in the detection of linear and angular acceleration. The ability of the inner ear to do so is dependent on the six sensory organs contained within the structure. In general, these sensory organs consist of apically ciliated sensory epithelial cells called hair cells and the supporting cells that surround them. There is much that is known about the function of these hair cells, as well as their morphology, but there is less that is known about the developmental cascade by which hair cells arise and differentiate.

Of particular interest is the differentiation of different types of hair cells between and within hair cell populations. In the sensory organs of the vestibular section of the inner ear there are type I and type II hair cells of the cristae and maculae and in the Organ of Corti of the cochlea there are inner hair cells (IHCs) and outer hair cells (OHCs). There is much that is known about the functions and characteristics of these different types of hair cells, but it is unclear what occurs to differentiate these types of hair cells from one another during development. The identification of some of the key players in defining these populations and the role they take in this process is the area I am pursuing.

In order to better ascertain what defines certain types of hair cells during development, I am going to look for genes, specifically for those with some transcriptional regulatory role, that are expressed differentially in the IHC and OHC of the cochlea and in type I and type II hair cells of the cristae and maculae by performing a RNA in situ hybridization screen. The gene candidates will be assembled from the combined sources of a previously performed RNAseq experiment of flow cytometry sorted hair cells, as well as a microarray comparing the gene expression of an IHC enriched population of hair cells to a non-enriched population, with cross referencing to an online database for inner ear gene expression. With the isolation of differentially expressed genes, these can then be characterized for their specific role in the mammalian inner ear using mouse models.

Contributors: Cai, Tiantian; Jen, H-sini; Basch, Martin; Groves, Andrew
Rett Syndrome is a severe X-linked disorder with a prevalence of approximately one per 10,000 live female births. It presents with developmental regression, including loss of speech, motor impairments and autistic features. Loss-of-function mutations in methyl CpG binding protein 2 (MECP2) cause more than ninety-five percent of cases. Mecp2-null mouse models recapitulate many aspects of the human disease, and have been used to show that symptom reversal by restoration of Mecp2 function is possible. Unfortunately, as a widespread epigenetic factor, MECP2 levels are extremely dosage sensitive, making direct manipulation a difficult treatment option. Furthermore, MECP2 has multiple binding partners and its mutation impacts many biological pathways in Rett Syndrome, but it is unclear which are relevant to symptom progression.

Our use of a dominant ENU mutagenesis screen in the Mecp2tm1.1Bird deletion mouse model allowed us to dispense with a priori beliefs about MECP2 function. Five suppressors that ameliorate symptoms of Mecp2 loss were isolated through a combination of SNP linkage mapping and whole exome sequencing strategies. One is a loss-of-function mutation in squalene epoxidase (Sqle), a rate-limiting enzyme in committed cholesterol biosynthesis. This mutation increased longevity and improved motor functioning, activity levels and overall health in Mecp2-null mice. Based on the biochemical role of Sqle, we examined cholesterol and lipid metabolism in the Mecp2-null male mice and found perturbations in both the brain and liver. Similar, but delayed perturbations were found in Mecp2 heterozygous females. Accordingly, we treated Mecp2 mutant mice with cholesterol-lowering statin drugs and found that they also alleviate motor symptoms and confer increased longevity in both males and females.

The discovery of a Rett Syndrome suppressing mutation in the cholesterol biosynthesis pathway was unexpected and unlikely to have been found using the reverse genetics approach that is more common in mouse research. Cholesterol metabolism represents a potential pathway for new therapeutic targets to treat the syndrome. Our data add to a growing body of evidence that cholesterol plays an important role in many neurological diseases. More broadly, the results of this study emphasize the utility of a forward genetic approach in the mouse to identify targetable downstream pathways involved in disease pathogenesis.

Contributors: Buchovecky, Christie; Turley, Stephen; Brown, Hannah; Kyle, Stephanie; McDonald, Jeffery; Liu, Benny; Pieper, Andrew; Huang, Wenhui; Katz, David; Russell, David; Shendure, Jay; Justice, Monica
DESIGNING A TANDEM CHIMERIC ANTIGEN RECEPTOR THAT TARGETS BOTH THE TUMOR AND ITS ASSOCIATED VASCULATURE

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Glioblastoma (GBM) is the most common and most aggressive primary brain tumor in humans. The standard of care is multimodality therapy, namely: surgery, radiation and chemotherapy. Despite multimodal therapy the prognosis for patients with glioblastoma is poor.

We are investigating whether T cells can be used to induce an anti-tumor response by targeting two elements of the tumor microenvironment simultaneously, the tumor and its endothelium. We have chosen the tumor antigen, Human Epidermal Growth Factor Receptor 2 (HER2) and Tumor Endothelium Marker 8 (TEM8).

To do so we have designed a tandem chimeric antigen receptor (TanCAR) that is specific for HER2 and TEM8 to be expressed on T cells. A chimeric antigen receptor (CAR) is a synthetic molecule consisting of a single chain variable fragment (ScFv) of an antibody paired with the intracellular signaling domain of a T cell. A TanCAR consists of two or more CARs placed in-line (in tandem).

We have successfully cloned a TEM8-HER2 TanCAR construct and are able to detect expression of TEM8-HER2 TanCAR on the surface of T cells.

We also show that the glioblastoma cell lines express HER2, while our tumor endothelium cell lines express TEM8 on the surface. Primary cultures from freshly excised glioblastoma samples have been shown to express both HER2 and TEM8, thus serving as a useful model for conducting in-vitro functionality testing of our TEM8-HER2 TanCAR T cells.

Should our TEM8-HER2 TanCAR T cells prove efficacious in targeting both the tumor and its associated vasculature in a xenogeneic model of glioblastoma the result could have great therapeutic applications for glioblastoma and potentially other solid tumors.
Defects in the establishment of cortical networks during development may have long-lasting effects on how the brain processes information and could underlie neuropsychiatric disorders such as autism and schizophrenia. Recent studies suggest that cell lineage influences the connectivity and the functional properties of pyramidal neurons in the neocortex. Clonally related cells derived from a common progenitor are more likely to be synaptically connected and, in primary visual cortex, have similar tuning preferences compared to nearby, unrelated cells. Here, we describe a novel method for lineage tracing that allows precise manipulation of time of progenitor labeling. Using this method we are testing the hypothesis that closely related “sister” cells are more similarly tuned than distantly related “cousin” cells using multiphoton calcium imaging in vivo. These experiments will shed light on how functional networks are established during development and could provide a circuit level foundation to study neurodevelopmental disorders.

Contributors: Cadwell, Cathryn; Yatsenko, Dimitri; Morgan, Amy; Tolias, Andreas
The perception of duration can be biased by the physical properties of a sensory stimulus. For example, visual stimuli with higher temporal frequency are perceived as longer (Kanai et al., 2006). Objects of different temporal frequencies often appear simultaneously in the environment, providing conflicting information about duration. Does the brain keep separate duration representations for each object, or form a single representation? If a single duration representation is kept, how is it formed? One possibility is by Bayesian cue integration (Ahrens & Sahani, 2011); another is by reading out the total neural energy for encoding all the stimuli (Eagleman & Pariyadath 2009, 2012). Human participants estimated the duration of Gabor patterns drifting at 1Hz and 6Hz (denoted by L for low and H for high frequency, and LH when the two were simultaneously presented. In Experiment 1, participants compared the duration of LH against H. Psychometric functions revealed no bias between them. This suggests observers might overweight the dominant frequency channel (every stimulus includes an H), or were able to keep separate duration representations for each frequency channel and only use the H channel for judgments. In Experiment 2, LH was always presented first, followed by LH, H, or L. Duration of H was perceived longer than LH, consistent with a Bayesian cue integration model. Relative to LH, the judgments to H and L were significantly different, ruling out the model of separate duration representations. The precision of judging LH was better than H and L for the majority of participants. Experiment 3 used a static Gabor pattern (S) as the standard stimulus, and showed a compatible result. These data suggest observers weight duration information from multiple stimuli to form a single estimate.

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Atonal homolog1 (Atoh1) encodes a basic helix-loop-helix protein that is the first transcription factor to be expressed in differentiating hair cells. Previous work suggests that expression of Atoh1 in prosensory precursors is necessary for the differentiation and survival of hair cells, but it is not clear whether Atoh1 is required exclusively for these processes, or whether it regulates other functions later during hair cell maturation. We used EGFP-tagged Atoh1 knock-in mice to demonstrate for the first time that Atoh1 protein is expressed in hair cell precursors several days before the appearance of differentiated markers, but not in the broad pattern expected of a proneural gene.

We conditionally deleted Atoh1 at different points in hair cell development and observe a rapid onset of hair cell defects, suggesting that the Atoh1 protein is unstable in differentiating hair cells and is necessary through an extended phase of their differentiation. Conditional deletion of Atoh1 reveals multiple functions in hair cell survival, maturation of stereociliary bundles, and auditory function. We show the presence of distinct critical periods for Atoh1 in each of these functions, suggesting that Atoh1 may be directly regulating many aspects of hair cell function. Finally, we show that the supporting cell death that accompanies loss of Atoh1 in hair cells is likely caused by the abortive trans-differentiation of supporting cells into hair cells. Together our data suggest that Atoh1 regulates multiple aspects of hair cell development and function.

Contributors: Cai, Tiantian; Seymour, Michelle; Zhang, Hongyuan; Pereira, Fred; Groves, Andrew
Cancer is a very complex disease that adapts during progression and therapeutic treatment. Numerous studies have highlighted the need for analyzing various biomarkers to aid clinicians with patient stratification and therapeutic choices. An additional hurdle, which is much less studied, resides in intratumoral variation at the cellular level. This phenomenon can be described by both genetic and phenotypic heterogeneity. Genetic heterogeneity occurs because of chromosomal instability that gives rise to clonal subpopulations in the same tumor, while phenotypic heterogeneity proceeds from epigenetic and/or “stochastic” mechanisms. How phenotypic heterogeneity impacts patient stratification, prognosis and therapeutic choices remains unclear. Moreover the concept of heterogeneity has been largely ignored in terms of assay development and drug screening, although it remains possible that it is an essential component of success in screening and/or therapeutic efficacy.

In order to begin understanding and quantifying phenotypic heterogeneity in cancer cell biology, we have begun analyzing the levels and subcellular localization of three hormone nuclear receptors (HNRs; estrogen receptor, androgen receptor, and glucocorticoid receptor) by simultaneous multiplexed immunofluorescence and single cell analysis. These receptors were selected as they are members of a class of transcription factors that play pivotal roles in the pathophysiology of breast cancer. To optimize the analysis of phenotypic heterogeneity, we began studying well established breast cancer models and found that the levels of these three HNRs vary greatly across the genetically homogeneous MCF7 cell line. We queried if and how their relative nuclear protein levels change after different agonist/antagonist combination treatments across dose and time. By employing various multi-dimensional statistical methods, we have found a way to describe and quantify phenotypic differences between HNRs levels over time, dose and agonist/antagonist treatments. Our future directions will focus on expanding our analysis of the heterogeneity of several cancer hallmark biomarkers in various tumor cell lines. This analysis will lead towards multiplexed screening for drug and/or drug combinations that affect the heterogeneity of multiple cancer biomarkers simultaneously.

Contributors: Callison, Austin; Stossi, Fabio; Dandikar, Radhika
Saturated fatty acids increase inflammatory gene expression in macrophages (CCL2, TNFα, IL-6), while unsaturated fatty acids increase anti-inflammatory M2 macrophage markers (CD206, Arg1, MGL1). In the fasted condition, macrophages are required for the removal of excess free fatty acids that have been mobilized from adipocytes. TLR4 is required for recruitment of pro-inflammatory M1 macrophages during long term high fat feeding. We have analyzed the polarization of macrophages recruited following fasting and tested whether TLR4 is required for fasting-induced macrophage recruitment. In wildtype mice, high-fat feeding is required for distinct macrophage polarization in the fed or fasted state. Some adipose tissue macrophages are a unique population of mixed macrophages expressing both CD11c and MGL1. These markers are increased on macrophages cultured in vitro with both palmitic and oleic acid. TLR4 is not required for macrophage recruitment, but it appears to inhibit macrophage polarization in the fasted state, as TLR4−/− mice had increased F4/80+CD206+CD11c− and F4/80+MGL1+CD11c+ macrophages. Taken together, these data identify a new role for mixed macrophages in the clearance of fatty acids following fasting and show that both fatty acids and TLR4 play a role in their polarization.

Contributors: Camell, Christina; Smith, Wayne
HUMAN ENDOGENOUS RETROVIRAL ELEMENTS (HERVS) THROUGHOUT THE GENOME MEDIATE RECURRENT DELETIONS AND RECIPROCAL DUPLICATIONS VIA NAHR.

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The vast majority of recurrent deletions and duplications are mediated by nonallelic homologous recombination (NAHR) between low-copy repeats (LCRs) distributed throughout the human genome. However, recurrent genomic rearrangements are also mediated by non-LCR structures such as AT-rich cruciforms and retrotransposable HERV elements. Recently, we reported nine de novo, identically-sized 3.3 Mb deletions in 3q13.2q13.31, mediated by NAHR between two flanking HERV elements 4,954 and 5,684 bp in length sharing 4,839 bp of 95.71% DNA sequence identity. We hypothesized that other HERV pairs throughout the human genome may serve as substrates for NAHR and lead to genomic instability. Based on the HERV properties at the 3q13.2q13.31 and Yq12 loci, we developed predictive features of HERV elements mediating NAHR. We identified 76 HERV pairs matching such criteria and thus potentially predisposing ~ 9% of the human genome to HERV-mediated recurrent rearrangements. Using a custom-designed comparative genomic hybridization array, we screened eight genomes for these predicted susceptibility regions. Moreover, cross-referencing the predicted regions with CNVs in our clinical chromosomal microarray databases revealed deletions and apparently reciprocal duplications, ranging in size between 189 kb and 1.36 Mb and mapping to LCR-free chromosomal regions 1q41, 2p12 (two loci), and 11q24.3 in nine unrelated patients. DNA sequencing of long-range PCR-amplified junction fragments showed that all CNVs had different breakpoints mapping within HERVs 5,711-6,136 bp in size and sharing stretches of 92.6-95.1% sequence identity with their partner. The presence of HERV-mediated deletions and reciprocal duplications indicates NAHR as a causative mechanism, even though the length and the sequence homology of the HERV elements are less than that currently thought to be required for NAHR. Further supporting the NAHR hypothesis is significant clustering of recurrent HERV-mediated breakpoints near predicted PRDM9 hotspot motifs. Our data demonstrate an underappreciated role of HERV elements in human genome instability. We propose that in addition to HERVs, other repetitive elements such as LINEs may also be responsible for the formation of recurrent CNVs via NAHR.

Contributors: Campbell, Ian; Gambin, Tomek; Beck, Christine; Hixson, Patricia; Cheung, Sau Wai; Shaw, Chad; Rosenfeld, Jill; Stankiewicz, Pawel
Background— Stroke is the fourth leading cause of death in the United States and a major cause of long-term disability. The limitations of therapeutic hypothermia restrict the potential application for neuroprotection against ischemic stroke. Transient receptor potential vanilloid 1 (TRPV1) is a temperature-sensitive ion channel expressed in epidermis and nervous system. Our previous studies show activation of TRPV1 induces reliable and reversible hypothermia. Through activation of TRPV1 by agonist dihydrocapsaicin (DHC), we examined the effectiveness, safety and specificity of DHC induced pharmacological hypothermia (PH) in conscious mice for the treatment of focal cerebral ischemia and reperfusion (I/R).

Methods—Focal cerebral I/R (1 hour ischemia + 24 hours reperfusion) was generated by distal middle cerebral artery (MCA) and common carotid artery (CCA) occlusion. Hypothermia (8 hours) was initiated in conscious mice starting at 90 minutes reperfusion by DHC infusion via osmotic pump. Mice were divided into WT, WT with vehicle, WT with DHC (hypothermia), TRPV1 KO, and TRPV1 KO with DHC groups. Neurofunction (by behavioral testing) and infarct volume (by TTC staining) were measured at 24 hours reperfusion. In addition, heart rate (HR), mean arterial blood pressure (BP) and laser Doppler cerebral cortical perfusion (CCP) were measured and compared within TRPV1 WT and KO mice in response to a single dose of DHC. Lastly, WT and TRPV1 KO mice were evaluated for DHC desensitization at therapeutic dose (1.25, 1.25 mg/kg) and high dose (30, 15 mg/kg). Mice were allowed to recover for one month and were then evaluated for a hypothermic response to DHC (1.25 mg/kg).

Results—1.25 mg/kg/hr DHC-induced hypothermia initiated at 90 minutes post-reperfusion significantly decreased the infarct volume by 84.4% from 2.0±1.3% compared to 12.8±1.9% in I/R alone group and improved behavioral neurofunction median score by 14.0% from 14.25 to 16.25 of a maximum score of 18. There was no significant difference among I/R alone, TRPV1 KO and TRPV1 KO with DHC treatment groups. The effective therapeutic dose of DHC (1.25mg/kg) showed no effect on HR and CCP, but transiently decreased BP by 5.8±1.5mmHg. BP recovered to pre-drug level within 4 minutes and could be prevented by atropine pre-treatment.

Conclusions—TRPV1 agonist induced hypothermia provides an effective and safe method for acute cerebral I/R neuroprotection. The protection is specifically to DHC activation of TRPV1 channels. We propose that this method may provide a more broadly applicable means to produce therapeutic hypothermia in the stroke population.

Contributors: Cao, Zhijuan; Balasubramanian, Adithya; Marrelli, Sean P.
PRDM14 is a putative histone methyltransferase that is critical for embryonic stem cell (ESC) maintenance, primordial germ cell (PGC) specification, and is misexpressed in several human cancers. To study the in vivo consequences of Prdm14 misexpression, we developed a ROSA26-loxP-STOP-loxP-Prdm14-IRES-EGFP (R26PR) mouse line which constitutively expresses Prdm14 and EGFP following Cre recombinase-mediated STOP excision. Mice carrying both R26PR and either Mx1-cre or MMTV-cre developed rapid onset acute lymphoblastic leukemia (ALL), with full penetrance and median survival of 41 and 64 days, respectively. Leukemic cells were predominantly immature single-positive CD8+ T-cells (CD8+CD24+TCR(lo). Analysis of hematopoietic stem and progenitor populations several weeks prior to overt leukemia onset detected both a 100-fold expansion of cells resembling long-term hematopoietic stem (LT-HSC-like) cells and a 25-fold expansion of cells with a common lymphoid progenitor immunophenotype. Furthermore, these expanded cells had restricted developmental potential, as they did not contribute to the myeloid lineage and preferentially contributed to the T-cell rather than B-cell lineages. Thus, Prdm14 misexpression in LT-HSCs leads to the rapid proliferation of this population and biases differentiation towards T-lineage fates. This bias phenocopies models of constitutively active NOTCH1, which is mutated in more than 50% of human T-ALLs; therefore, we examined R26PR;Cre T-ALLs for mutations in Notch1. The endogenous Notch1 promoter was deleted at cryptic recombination signal sequences (RSSs) recognized by RAG recombinases; tumors expressed truncated, ligand-independent NOTCH1 protein from a downstream promoter. PRDM14 may facilitate these deletions through direct and/or epigenetic mechanisms, as PRDM14 binds at the Notch1 locus in mESCs and R26PR;Cre T-ALLs have significantly elevated global and Notch1-specific H3K4me3, which is required at RSSs for RAG-mediated recombination to occur. Establishing R26PR;MMTV-cre on a RAG1-/- genetic background abolishes T-ALL development, though LT-HSC-like cell expansion still occurs. Furthermore, a R26PR;MMTV-cre-derived T-ALL cell line does not grow in the presence of the gamma-secretase (NOTCH1 cleavage) inhibitor DAPT. Therefore, either RAG-mediated Notch1 deletions or unperturbed T-cell developmental pathways are necessary for leukemogenesis. Moving forward, we believe that the short latency, penetrance, and NOTCH1 pathway involvement will make the R26PR model ideal for the future preclinical testing of novel treatment regimens for T-ALL.

Contributors: Carofino, Brandi L.; Ayanga, Bernard; Justice, Monica J.
Rationale: Status epilepticus (SE) is associated with the development of epilepsy and comorbidities, including deficits in cognition. Previous studies have implicated the mammalian target of rapamycin (mTOR) signaling cascade in the pathophysiology of SE and epilepsy using the mTOR complex 1 (mTORC1) inhibitor rapamycin (Rap). Under basal conditions, mTORC1 regulates protein synthesis and is an important mediator of synaptic plasticity. We have recently shown that aberrant mTOR signaling contributes to behavioral deficits that occur 2 weeks following SE. In the studies reported here, we determined whether aberrant mTORC1 signaling plays a role in hippocampal-dependent learning and memory tasks, locomotion, and anxiety in the epileptic animal and whether early Rap rescue is long-lasting.

Methods: SE was induced in rats using pilocarpine while controls (Sham) received saline. Two weeks after SE induction, we administered Rap or vehicle (Veh) every other day for one week. The treatment groups were Sham+Veh, Sham+Rap, SE+Veh, and SE+Rap (n=7-12/group). Animals were tested in the Morris Water maze (MWM), Novel Object Recognition (NOR), and the Open Field (OF) tasks. To determine whether early Rap was long lasting, we tested animals 5 months after treatment (n=6/group). After behavioral testing was completed, we performed western blotting to verify inhibition of mTORC1 (S6 phosphorylated at S240/244).

Results: SE+Veh rats exhibited significantly longer escape latencies during the acquisition phase in MWM test and decreased time spent in the target quadrant searching for a hidden platform compared to Sham+Veh animals (p< 0.05 and p<0.01, respectively). In NOR, SE+Veh animals displayed no preference for the novel object as compared to controls (p<0.001). In the SE+Rap group, the deficits in MWM and NOR were rescued to performance levels of Sham+Veh animals. SE+Veh rats spent more time in the inner portion of the OF arena than Sham + Veh and displayed increased distance traveled and travel velocity (p<0.05). When tested 5 months after SE, early Rap did not attenuate any hyperactive locomotion in OF nor did it rescue any deficits in MWM or NOR (p-values not significant). For all behavioral tasks, Rap had no effect on sham animals.

Conclusions: These data suggest that aberrant mTORC1 signaling contributes to deficits in hippocampal-dependent learning and memory. Future studies are planned to evaluate whether late mTORC1 inhibition may rescue these deficits in animals in which epilepsy has already been established, particularly since the early treatment is not long lasting.

Contributors: Carter, Angela; Brewster, Amy; Lee, Wai-Ling; Anderson, Anne
Our laboratory is interested in understanding the effects of early-life recurring seizures on the developing hippocampus. Magnetic resonance imaging, such as MRI and DTI, within a clinical setting have shown reduced hippocampal white and grey matter volumes while cognitive assessments routinely reveal intellectual disabilities in children diagnosed with epilepsy. Previous studies by our laboratory as well as others have repeatedly shown that recurring seizures in infant rats and mice produce hippocampal dependent learning deficits - particularly in the acquisition of spatial memories. In addition, in vivo and in vitro murine models have shown reduced dendritic branching complexity and dendrite length within CA1 of the hippocampus following brief recurring seizures. We have previously suggested that the decrease in dendritic growth may be explained by homeostatic mechanisms that gradually mitigate the aberrant activity, resulting in the suppression of dendritic growth. Yet, following just 4 hours of epileptiform activity, we have recently found similar alterations in dendritic length and complexity. Clearly, multiple mechanisms may be contributing to the observed reductions in dendrite length and complexity following periods of hyperactivity. Dendrite development is known to be a stochastic process by which dendritic protrusions rapidly extend and retract throughout development. We recently used live-cell multi-photon microscopy, to evaluate seizure-induced alterations in growth dynamics of CA1 neurons. Experiments were performed using slice cultures prepared from infant mice expressing GFP. Following two days of growth in an incubator, slices were placed in an imaging chamber and continuously perfused with aCSF. After an equilibration period of 20-30 minutes, the imaging session commenced by imaging a 3d volume every 15 minutes for a period of 4 hours. Following the first hour of imaging, randomly selected slices were either maintained in aCSF or switched to aCSF containing the potassium channel antagonist 4-aminopyridine (4AP) to induce epileptiform activity. During the one hour baseline period, there were no changes in the dendritic terminal lengths between the two groups. However, after 45 minutes of epileptiform activity, significant differences in the length of the terminal segments were observed between untreated and 4AP treated terminal dendrites. Terminal segments exposed to 4AP retracted in length by ~2µm compared to untreated terminal segments. After 4 hours, 4AP-treated terminal segments retracted by ~3 µm while untreated terminal segments added ~1.25 µm to the growing tips. Taken together these data suggests that an acute seizure insult may have unexpected consequences on the growth of the developing hippocampus.

Contributors: Casanova, Jose; Swann, John
Glial cells are the most abundant cell type of the central nervous system (CNS). Astrocytes in particular are emerging as central players since they are involved in myriad functions (i.e. blood brain barrier formation, synaptogenesis...) and have been linked to several neurological disorders (leukodystrophies, Rett’s syndrome, ALS and tumors). Recent research has been mostly focusing on astrocyte functions in the adult, however astrocyte development remains astonishingly understudied; especially due to the paucity of reliable markers to characterize astrocytes in vivo and the inability to perform specific gene manipulation without affecting neurogenesis. Indeed, neurogenesis precedes gliogenesis and many aspect of neuronal development appear to be re-utilized during gliogenesis including neural tube patterning and the use of step-wise cell lineage progression. While oligodendrocytes follow distinct step-wise development, astrocyte lineage progression is poorly defined.

We designed a screen to better understand the intermediate stages of astrocyte development, using the previously validated reporter mouse line GLAST::DsRed. Glast is a glutamate transporter involved in glutamate uptake by astrocytes and its expression is tightly linked to gliogenesis. Spinal cords were harvested at mE12.5, mE14.5, mE16.5, mE18.5, P4 and P7 to encompass all the stages of astrocyte lineage progression from birth to functioning astrocytes. Dissociated spinal cords were sorted based on their GLAST-DsRed and CD15 (progenitor marker) expression. The GLAST+/CD15- (+/-) population represents putative astrocyte precursors emerging in the mantle zone, while GLAST+/CD15+ (+/+ ) represent progenitor cells. We also sorted GLAST-/CD15- (-/-) as a negative control. A microarray was performed for each of the 3 populations, at every time point. Using a combination of bioinformatics and literature and databases searches, we generated a list of novel genes enriched in the (+/-) population with no previous association to glial cells.

We have validated the temporal and spatial expression of 5 genes (Tsc22d4, Tom111, Errfi1, Mfge8 and F-Spondin) using In Situ Hybridization in the developing mouse spinal cord and brain. These genes are a combination of transcription factors (Tsc22d4), signaling molecules (Tom111, Errfi1 and Mfge8) and extracellular matrix proteins (F-spondin). Next we propose to perform gene manipulation to assess their function during gliogenesis. To do so, we will use in ovo electroporation in the embryonic spinal cord or in utero electroporation in the embryonic mouse cortex. We expect that some of these novel genes will affect certain aspect of astrocytogenesis from specification to migration or differentiation.
THE MECHANISMS OF DNA DAMAGE RESPONSE IN MAMMARY STEM CELLS AND TUMOR INITIATING CELLS FROM A p53-NULL BREAST CANCER MODEL

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The DNA damage response (DDR) includes activation of cell cycle checkpoints, apoptosis, and DNA repair. To repair DNA double strand breaks, cells may undergo homologous recombination (HR) or non-homologous end joining (NHEJ). Adult stem cells tend to have different DDR mechanisms compared to differentiated cells since they reside in the tissues for extended periods and continuously ensure tissue homeostasis. Studies have shown that mouse hair follicle bulge stem cells and hematopoietic stem cells have higher NHEJ activity and lower apoptosis, resulting in their resistance to radiation. However, little is known about the DDR in mammary stem cells (MaSCs). In addition, previous studies from clinics and mouse models have demonstrated that tumor initiating cells (TICs) from mammary tumors have an increased resistance to conventional therapies. However, the mechanisms underlying the DDR to this therapeutic resistance are still mainly undiscovered. Using a syngeneic p53-null tumor model, our lab has identified a Lin-CD29HCD24H subpopulation in these tumors with functional TICs properties, and these TICs are more resistant to radiation. To study the specific mechanisms that contribute to this resistance, we hypothesized that TICs from p53-null tumors exhibit increased DDR and elevated NHEJ activity to repair DNA damage, which might be the similar DDR mechanisms used in MaSCs.

To date, we have shown that MaSCs and basal cells have lower apoptosis as compared to luminal cells in mammary glands. In cell cycle analysis, MaSCs exhibited an increased G2-M phase, indicating stem cells might have increased G2 checkpoint activation. In addition, using a high-throughput NHEJ assay, we showed that basal cells, which contain MaSCs, have higher NHEJ activity as compared to luminal cells. In tumor studies, TICs have more efficient DNA repair according to γH2AX foci staining and neutral Comet assay. We also showed that TICs exhibit decreased apoptosis and higher NHEJ activity. In addition, IF staining showed that TICs have lower activity of Rad51, a key element of HR, indicating that the NHEJ pathway may play a major role to repair DNA damage in TICs. Ongoing studies are designed to identify the “Achilles heel” of these DDR pathways in TICs, which may act as therapeutic targets.

Contributors: Chang, Chi-Hsuan; Zhang, Mei; Rosen, Jeffrey
Along myelinated axons, action potentials are regenerated at gaps between myelin sheaths, called nodes of Ranvier. Immediately flanking the nodes are paranodal junctions. They play a crucial role in the function of myelinated axons as diffusion barriers, which isolate the periaxonal space underneath myelin, separate the molecular domains of nodes and juxtaparanodes and contribute to node formation by restricting the nodal domain. Loss of paranoidal junctions dramatically slows myelinated nerve conduction and mutant mice show severe tremor and ataxia. Despite their importance, the molecular mechanisms underlying the formation and function of paranodal junctions are largely unknown. AnkyrinB (AnkB) was identified as a cytoskeletal scaffold highly enriched at paranodes. Ankyrins have been implicated in formation and stabilization of membrane domains. Therefore, in this study, we elucidate the roles of AnkB in the structure and function of paranoidal junctions. We found paranoidal AnkB is a Schwann cell component, instead of an axonal component. In contrast to the 440-kDa isoform found in unmyelinated axons, paranoidal AnkB is comprised of the 220-kDa isoform. Conditional knockouts of AnkB by CNP-Cre in myelinating glia revealed that paranoidal AnkB is not essential for the integrity and barrier functions of paranoidal junctions. Interestingly, the known nodal cytoskeletal scaffold AnkG is also observed at paranodes, but only in the CNS, where paranoidal AnkB is not frequently seen. During early development, we found paranoidal AnkG staining appeared to be derived from oligodendrocytes. Conditional knockouts of AnkG by CNP-Cre confirmed its glial source. There was no obvious defect in mature paranoidal junctions. However, defective paranoidal junction formation and delayed nerve conduction were observed during early development. Interestingly, AnkB was accumulated at more CNS paranodes in the absence of paranoidal AnkG. Consistent with a compensatory role of AnkB, double conditional knockouts of AnkG and AnkB by CNP-Cre showed more severe defects in paranoidal junctions. It is known that the AnkG isoforms at the axon initial segment and node of Ranvier are 270- and 480-kDa ones; our analysis indicates the paranoidal isoforms are 270- and 190-kDa ones expressed from the promoter at exon 1b, among the five known exons 1 of AnkG. Our study indicates a conservative scenario where myelinating glia in both the PNS and CNS deposit ankyrins at paranodes, and suggests that paranoidal ankyrins play an essential role in rapid formation of functional CNS paranoidal junctions.

Contributors: Chang, Kae-Jiun; Zollinger, Daniel; Susuki, Keiichiro; Sherman, Diane; Brophy, Peter; Cooper, Edward; Bennett, Vann; Mohler, Peter; Rasband, Matthew
ENVIRONMENTAL STRESS INDUCES TRINUCLEOTIDE REPEAT INSTABILITY

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Although expanded trinucleotide repeats (TNRs) are known to be de-stabilized by a host of DNA metabolic and biochemical processes within the cell, that environmental stresses from outside could also influence their stability is undetermined. Here we demonstrate that four different type of environmental stresses increase CAG-repeat instability measured as contraction frequencies in our assay. While this stress induced repeat instability is modulated by stress response factors; DNA repair or transcription is not involved in this pathway. Intriguingly all stresses propels DNA over-replication in cells, increasing >4N DNA content and driving repeat instability at the CAG loci. Removal of an origin licensing factor, CDT1 by siRNA knockdown obliterates stress induced over-replication and the resultant repeat instability. Collectively this study unravels a yet unidentified source ‘environmental stress’ directly capable of triggering mechanisms inside cells which culminate in TNR instability.

Contributors: 1Department of Biochemistry and Molecular Biology, 2Department of Molecular and Human Genetics, 3Center for Cell and Gene Therapy.

CRYO-EM STUDY OF TYPE II CHAPERONIN ASSISTED FOLDING OF H_D-CRYSTALLIN

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Chaperonin is a class of protein that plays an essential role in protein folding for all cells from bacteria, archaea and eukaryotic cells. Recently it is reported that a type II chaperonin from archaea Methanococcus Marapaludis (Mm-Cpn) could assist refolding of human γD-crystallin to its native conformation. Human γD-crystallin is a lens protein which is associated with the onset of cataract when it partially unfolds. We are interested in understanding the structural mechanism of how type II chaperonin recognizes the γD-crystallin.

Here, cryo-electron microscopy (cryo-EM) single particle analysis method was applied to resolve the structure of Mm-Cpn and human γD-crystallin during their initial recognition step. A challenge in studying the chaperonin-substrate structure is the compositional and conformational heterogeneity in the reaction product. We used a multi-model refinement protocol to sort out particle images according to their structural uniformity. Our analysis showed three conformations: 33% of the particles (Subset II) resembled the apo state Mm-Cpn conformation, 39% of particles (Subset I) had one-
ring less open, one-ring open conformation and 28% of the rest particles (subset III) did not yield a reliable map. The control cryo-EM map from the Mn-Cpn showed both ring open which was similar to the map from the subset II of the Mm-Cpn and human γD-crystallin complex.

Based on the results above, we conclude that the subset I corresponds to the Mm-Cpn population with substrate binding. 3D variance analysis also validated that the variance of subset II was mostly caused by flexible N- and C-terminal tails, while subset I showed higher variance level inside the top ring. Subsequent symmetry-free reconstructions of subset I particle images converged to one-ring less open and one-ring open conformation. Furthermore, differential conformations of each of the 8 subunits in the less open cis-rings were observed to form a tetramer of dimers while both rings in subset II appeared to have a good 8-fold symmetry.

In conclusion, our results demonstrate the conformational changes and symmetry broken features of type II chaperonin upon binding to γD-crystallin.

Contributors: Chen, Bo1; Sergeeva, Oksana2; Goulet, Daniel2; Knee, Kelly2; Joanita, Jakana3; King, Johnathan2; Chiu, Wah1,3*
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Using whole genome sequencing, our lab has recently identified compound heterozygous nonsense mutations in a novel gene, TMPRSS9, in a patient with developmental regression at 2.5 years of age, leading to severe intellectual disability and autistic disorder. TMPRSS9 encodes for polyserase-1, a transmembrane serine protease that is poorly studied. To date, the physiological role of TMPRSS9 is unknown and its endogenous substrates or protein interaction partners have not been identified. Our qPCR and Northern blotting results showed TMPRSS9 expresses in various tissues in mice, including brains, with the highest expression in kidney. Further studies by qPCR demonstrated that glomeruli and cerebellum have higher expression in kidneys and brains, respectively. Fluorescence in situ hybridization will be performed to further identify the specific cells types in glomeuli and cerebellum. To investigate the protein interaction partners of polyserase-1, truncation domain of polyserase-1 that was tagged with HA and FLAG was first expressed in 293T. Subsequently, the purified polyserase-1 was incubated with mouse tissue lysate (kidney and brain) and later subject to tandem affinity purification. Silver staining identified at least one putative interaction partner of polyserase-1. The samples were sent to Pathway Discovery Proteomic Core in BCM for mass spectrometry protein identification. To test whether loss of function mutations in TMPRSS9 causes developmental regression leading to intellectual disability and autism spectrum disorders, Tmprss9 fl/+ mice were generated and crossed with Hprt-Cre mice (“Cre-deleter”) in order to constitutively knockout the gene. A battery of behavioral tests will be used to evaluate the social approach activity, learning and memory, anxiety-like responses, and motor activity of homozygous knockout mice, using heterozygous and wild type littermates as control.
ROLE OF PLIN2 IN THE INNATE IMMUNE RESPONSE IN MICE IN VITRO & IN VIVO

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*Advisor: Lawrence Chan, D.Sc.-Department of Medicine*

PLIN2 is the major lipid droplet (LD) protein in macrophages. It plays a pivotal role mediating the innate immune response and in the atherosclerotic process. We have previously shown that Plin2 regulates foam cell formation in apoE-deficient mice, a mouse model of atherosclerosis characterized by severe hyperlipidemia with markedly enriched circulating β-VLDL particles that contain high levels of cholesteryl ester. Deletion of Plin2 by gene targeting increases cholesterol export and limits foam cell formation in macrophages, ameliorating atherosclerotic plaque formation in apoE deficient mice. Studies have also shown that TLR ligands, such as lipopolysaccharide (LPS), regulate LD formation in macrophages. For example, in RAW264.7, a common mouse macrophage cell line, treatment with LPS stimulates Plin2 expression. We hypothesized that Plin2 is also involved in mediating the innate immune response. We used lentiviral shRNA to downregulate Plin2 mRNA expression in RAW264.7 cells and found that Plin2 knockdown led to a reduction in NO production in response to LPS treatment in vitro. Plin2 knockdown also associated with a reduction in the expression of matrix metalloproteinase 9 (MMP9), TNF-alpha and MCP-1. To examine the role of Plin2 in vivo, we treated wild-type and Plin2-knockout mice with LPS injections and found that Plin2-knockout mice displayed a better survival rate (70%) compared to wild-type mice (20%). Taken together, these data indicate that Plin2 modulates the innate immune response in vitro and in vivo.

Contributors: Chen, Elaine; Tsai Luke; Chang, Benny; Chan, Lawrence
Signaling across biological membranes is critical to living organisms and involves membrane-embedded receptors that transduce extracellular stimuli into cytoplasmic responses. G protein-coupled receptors (GPCRs) are one of the largest families of these receptors, and increasing evidence indicates that they signal through long-range conformational changes. However, the atomic-level mechanisms by which such allosteric transitions are propagated and the trade-off between membrane protein flexibility and stability remain poorly understood, thereby hindering the development of more effective therapeutics targeting GPCRs. By combining bioinformatics, computational protein design, and experimental techniques we sought to design mutations that stabilize GPCRs in specific conformations. We used RosettaMembrane to computationally design beta-1-adrenergic receptor (B1AR) variants stabilized in the inactive state, which yielded a combination of five mutations that were experimentally validated to provide more than 10°C increase in apparent melting temperature. Additionally, we integrated homology modeling and multistate design to engineer conformational switches on transmembrane and loop regions of structurally uncharacterized dopamine D2 receptor (DRD2) that shift the receptor towards its active conformation. Models of inactive and active DRD2 conformations were generated via the homology modeling mode of RosettaMembrane and used as inputs for mutagenesis design. A combined design of eleven mutations in the transmembrane region of DRD2 resulted in an eight-fold increase in basal activity over wild type receptor. Starting from homology models of a DRD2/Gi active state complex we redesigned the flexible 140-residue-long native intracellular loop 3 (ICL3) into a five-residue loop stabilizing the active conformation. Predicted conformational shifts toward the active state and increased active state stability of the designed DRD2 variants were confirmed by fluorescence spectroscopy and functional assays. Our successful redesign of residue interactions switching the receptor’s conformation and function indicate that conformational switches play a key role in regulating the signaling properties of GPCRs. This method should prove useful for designing variants of membrane receptors stabilized in specific conformations, potentially facilitating structural determination of uncharacterized receptors in various functional states.

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Contributors: Chen, Kuang-Yui Michael; Barth, Patrick
Friedreich ataxia (FRDA), the most prevalent form of recessive cerebellar ataxia, is characterized by neurodegeneration and cardiomyopathy. FRDA is caused by mutations in Frataxin (FXN), which is highly conserved across evolution, with homologues in vertebrates, invertebrates, bacteria, and plants. It has been shown that FXN is localized to the mitochondria, and its deficiency in yeast leads to impaired iron-sulfur (Fe-S) cluster biosynthesis and iron accumulation in the mitochondria. Fe-S clusters are ubiquitous cofactors, which are required for a variety of Fe-S containing proteins to perform diverse functions. Although the mutation was identified 17 years ago, the pathological mechanism is still unknown. In a forward genetic screen designed to identify mutations in genes that cause neurodegeneration in Drosophila, we isolated the first mutation in frataxin homolog (fh), a Drosophila homolog of mammalian FXN. fh mutants show an aged dependent eye degeneration, suggesting Drosophila Fh possess a conserved function in neuronal maintenance. From TEM analysis and immunohistochemistry, we found a lipid droplet like structure accumulation in both photoreceptors and glia cells. In addition, the photoreceptor nuclei show abnormal morphology. Furthermore, mitochondria display a round and fragmented morphology in the larval brain, muscle, and adult eye, indicating that loss of fh cause dysfunctional mitochondria. Besides the neuronal maintenance function of Fh, ferric iron accumulates in many tissues in fh mutants, including larval brain, muscle, gut, and adult eye, providing evidence that Fh plays an important role in iron homeostasis. We are currently characterizing the function of Fh.
Losartan increases bone mass and accelerates chondrocyte hypertrophy in developing skeleton

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Angiotensin receptor blockers (ARBs) are a group of anti-hypertensive drugs that are widely used to treat pediatric hypertension. Recent application of ARBs to treat diseases such as Marfan syndrome or Alport syndrome has shown positive outcomes in animal and human studies, suggesting a broader therapeutic potential for this class of drugs. Multiple studies have reported a benefit of ARBs on adult bone homeostasis; however, its effect on the growing skeleton in children is unknown. We investigated the effect of Losartan, an ARB, in regulating bone mass and cartilage during development in mice. Wild type mice were treated with Losartan from birth until 6 weeks of age, after which bones were collected for microCT and histomorphometric analyses. Losartan increased trabecular bone volume vs. tissue volume (a 98% increase) and cortical thickness (a 9% increase) in 6-week old wild type mice. The bone changes were attributed to decreased osteoclastogenesis as demonstrated by reduced osteoclast number per bone surface in vivo and suppressed osteoclast differentiation in vitro. At the molecular level, AngiotensinII-induced ERK1/2 phosphorylation in RAW cells was attenuated by Losartan. Similarly, RANKL-induced ERK1/2 phosphorylation was suppressed by Losartan, suggesting a convergence of RANKL and angiotensin signaling at the level of ERK1/2 regulation. To assess the effect of Losartan on cartilage development, we examined the cartilage phenotype of wild type mice treated with Losartan in utero from conception to 1 day of age. Growth plates of these mice showed an elongated hypertrophic chondrocyte zone and increased Col10a1 expression level, with minimal changes in chondrocyte proliferation. Altogether, inhibition of the angiotensin pathway by Losartan increases bone mass and accelerates chondrocyte hypertrophy in growth plate during skeletal development.

Contributors: Chen, Shan *; Grover, Monica *; Sibai, Tarek *; Black, Jennifer *; Rianon, Nahid; Rajagopal, Abhirmi; Yang, Tao; Munivez, Elda; Bertin, Terry; Dawson, Brian; Chen,Yuqing; Jiang, Ming-Ming; Lee, Brendan Lee.
The Role of the Rac GTPase Regulatory Proteins Tiam1, Tiam2 and Bcr in Adult Neurogenesis

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Adult Neurogenesis is the process of generating functional neurons from adult neural precursor cells throughout life. It is thought to happen in two discrete brain regions, the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricle. Defects in adult neurogenesis correlate with impairments in learning, memory and mood regulation, and are often seen in neurological disorders such as Alzheimer’s disease, bipolar disease and schizophrenia. Thus, adult neurogenesis has been considered as a potential therapeutic target for the treatment of mental disorders. Our lab is interested in studying the factors that regulate adult neural precursor cells proliferation and differentiation. Emerging evidence indicates that the Wnt pathway regulates both adult neurogenesis and neuronal development. Our lab has also demonstrated that the Rac guanine nucleotide exchange factor (GEF) Tiam1 and the Rac GTPase-activating protein (GAP) Bcr cooperate to control dendrite and excitatory synapse development in the hippocampus. Interestingly, these Rac GTPase regulatory proteins remain highly expressed in the adult dentate gyrus and appear to function in the Wnt pathway. Preliminary data indicates that Tiam1 and closely related Tiam2 regulate dendrite and spine development in hippocampal dentate granule cells and loss of Tiam1 and/or Tiam2 decrease adult neurogenesis. Our results suggest that these Rac regulatory proteins play important roles in adult neurogenesis. Ongoing studies are focused on determining how Tiam1, Tiam2 and Bcr regulate adult neurogenesis and newborn neuron development in the dentate gyrus in response to Wnt signaling.

Contributors: Cheng, Jinxuan; Niu, Sanyang; Firozi, Karen; Tolias, Kimberley
A fundamental issue that remains actively debated about the pathogenesis of Alzheimer’s disease is the relative contribution made by soluble assemblies of Aβ vs. insoluble amyloid. Mouse models over-expressing mutant forms of amyloid precursor protein (APP) have demonstrated a relationship between overproduction of APP, Aβ, amyloid deposition, and cognitive deficits, but few experimental systems have been capable of dissecting their individual effects. Here we use a controllable APP transgenic model to distinguish the impact of APP and soluble Aβ from that of deposited amyloid on cognitive function and synaptic structure. Doxycycline was used to rapidly suppress both the production of transgenic APP and from it, the release of Aβ. Short-term suppression of APP and soluble Aβ restored cognitive performance to levels equal with controls in multiple tasks, including Morris water maze, radial arm water maze, and fear conditioning. Cognitive improvement coincided with increased expression of pre- and post-synaptic markers, synapsin and PSD95, greater synaptic density surrounding amyloid plaques, and reduced levels of synaptotoxic Aβ oligomers. Investigation of downstream effectors of the synapse-promoting Rac-GTPases revealed that APP/Aβ suppression normalized the phosphorylation of cofilin, which regulates actin depolymerization and synapse stability. These synaptic changes suggest that expression of mutant APP and production of soluble Aβ assemblies actively impairs synaptic structure via the actin cytoskeleton in a way that can be largely reversed once they are removed.

Contributors: Chiang, Angie C. A.; Fowler, Stephanie W.; Savjani, Ricky R.; Larson, Megan E.; Schuler, Dorothy R.; Cirrito, John R.; Lesne, Sylvain E.; and Jankowsky, Joanna L.
Rationale: Previous studies suggest that protein phosphatase 1 (PP1) is dysregulated in a number of diseases including atrial fibrillation (AF). However, these studies mainly examined the global changes of the PP1 catalytic subunit (PP1c) and not the changes of the numerous PP1 regulatory (R) subunits that confer localization, substrate specificity, and activity of the PP1 holoenzyme.

Objective: To develop a novel unbiased method of quantifying all R subunits bound to PP1c in vivo and to investigate the regulatory network of PP1 in AF patients.

Methods and Results: Using co-immunoprecipitation (IP) followed by mass spectrometry (MS) and label-free quantification (LFQ) algorithm, we developed a novel method of interrogating all R subunits and other putative binding partners that are bound to PP1c in human heart tissues. Using 2 mg of protein input, we identified more than 20 binding partners of PP1c including 7 known R subunits. Based on this we quantified the levels of these partners bound to PP1c in atrial samples obtained from AF patients and patients in sinus rhythm (SR). Comparing the two cohorts we discovered an increase in the binding of CSDA (a novel putative binding partner of PP1c), PPP1R7, and PPP1R12C to PP1c in AF patients.

Conclusions: We developed and validated a novel unbiased method for studying PP1 at the level of the R subunits and demonstrated its usefulness in studying the regulatory network of PP1 in atrial fibrillation.

Contributors: Chiang, David; Lebesgue, Nicolas; Beavers, David; Damen, Mirjam; Dobrev, Dobromir; Heck, Albert; Scholten, Arjen; Wehrens, Xander.
The role of SRC-2 in the anti-diabetic and anti-lipogenic effects of LRH-1.

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Type 2 diabetes is a metabolic disease characterized by a high level of blood glucose due to insulin insensitivity. This disorder is tightly correlated with increased fat accumulation in the liver. It has been proposed that a vicious cycle of increased fat accumulation and insulin insensitivity drives the early stages of this disease. In the forward direction of this cycle serum insulin levels rise in response to obesity. The elevated insulin results in increased lipogenesis and lipid deposition in the liver, which drives further whole body insulin resistance. This in turn results in even higher serum insulin levels and further liver fat accumulation.

Several nuclear receptors are able to reverse this cycle by directly repressing lipogenesis in the liver. In this case, nuclear receptor activation suppresses liver fat accumulation and thereby promotes whole body insulin sensitivity. The resulting decrease in serum insulin feeds forward to further decrease liver fat accumulation, with the net result of reversing hepatic steatosis and insulin resistance, even in the absence of effects on overall body weight.

My project focuses on Liver Receptor Homolog-1 (LRH-1), which can be activated by Dilauroyl Phosphatidylcholine (DLPC). The Moore lab has linked the ability of this LRH-1 agonist to reverse insulin resistance in mouse models of type 2 diabetes to decreased expression of the key lipogenic transcription factor SREBP-1. But the specific mechanisms involved remain unknown. My goal is to understand the mechanism of action of the anti-diabetic effects of DLPC.

The transcriptional effects of nuclear receptors are mediated by coactivators and corepressors, and recent results from the O'Malley laboratory have identified the coactivator Steroid Receptor Complex-2 (SRC-2) as a key metabolic regulator (PMIDs 21195347, 19039140). Additional preliminary results show that SRC-2 can coactivate LRH-1 transactivation, and indicate extensive overlap between genome wide LRH-1 and SRC-2 binding sites in the liver. In vivo, there are also phenotypic similarities between SRC-2 binding sites in the liver. In vivo, there are also phenotypic similarities between SRC-2 liver specific knockouts and LRH-1 liver specific knockouts in terms of lipid metabolism and bile acid metabolism. Thus, my specific hypothesis is that SRC-2 is an essential mediator of the anti-diabetic and anti-lipogenic effects of DLPC. I am now trying to determine the impact of both acute and chronic DLPC treatments in SRC-2 liver specific knockout mice and the littermate controls.

Contributors: Choi, Sungwoo; Kim, Mi-Sun; Kim, Seung-Whan; Lee, Jae Man; Moore, David
Digital sorting algorithm identifies novel genes in adult neural progenitor cells

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Adult hippocampal neurogenesis has been associated with spatial learning and mood control. Deciphering the mechanisms that participate in control of adult neurogenesis has a substantial significance for human health. To discover putative new genes important for the function of neural progenitor cells, we applied Digital Sorting Algorithm (DSA), a blind-source deconvolution method, to microarray data obtained from the adult mouse dentate gyrus. DSA identified 602 candidate markers enriched in neural progenitor cells. We validated these potential markers by in situ hybridization and qRT-PCR in neural progenitor cells. We also generated a mouse that uses the promoter of one of these potential markers to drive the expression of eGFP. Our study utilizing DSA in identifying novel genes in adult neural progenitor cells shows high yield, high reproducibility and high sensitivity.

Contributors: Choi, William T.; Deudero, Juan JP; Semerci, Fatih; Thakkar, Aarohi; Liu, Zhandong; Maletić-Savatić, Mirjana
Confined DNA Thermodynamics: Structure, Pressure, Elasticity

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We have shown that excluded volume, electrostatic forces and surface-induced correlations are sufficient to predict most of the major features of the current structural data of DNA packaged within viral capsids without assuming any elastic conformational ordering. Current models assume highly-ordered, even spooled conformations, based on interpretation of cryo-EM density maps. We have shown that surface-induced ordering of unconnected DNA polymer segments is the only necessary ingredient in creating ringed densities consistent with experimental density maps. This implies that the ensemble of possible conformations of polymeric DNA within the capsid consistent with cryo-EM data may be much larger than implied by the traditional interpretation that such rings indicate a highly-ordered spool conformation. This suggests a more disordered, entropically-driven view of phage packaging may be possible.

We have also shown the electrostatics of the DNA contributes a large portion of the internal hydrostatic and osmotic pressures of a phage virion, suggesting that non-linear elastic anomalies might be responsible reduction of overall elastic bending energies, allowing more disordered conformations to be free-energetically favorable. Currently MC path sampling techniques, phage genomic data, and sequence-dependent DNA elasticity predictions are being employed to accurately model the elastic bending contributions. Biophysical prediction of the pressure and structures of DNA confined within phage capsids will aid prediction of DNA expression in vivo, as well as the design of phage sequence-delivery methods.

Contributors: Myers CG1,2, Pettitt BM1,2
Background: We have previously developed a novel model of breast cancer metastasis involving shRNA knockdown of Rho GDI. Although it is known that androgen receptor (AR) is frequently expressed along with estrogen receptor (ER) in breast tumors, AR’s role in resistance or metastasis is unknown. Here we explore the role of AR in these processes in the Rho GDI knockdown model.

Materials and Methods: shRNA knock down was used to block expression of Rho GDI in the ER(+)positive breast cancer cell lines MCF-7 and ZR-75-B. Reverse phase protein arrays (RPPA) were used to analyze global changes in growth factor pathway expression. Metastasis was analyzed by injection into athymic mice and histological analysis of isolated tissues. Western blot analysis was used to look at activation of the AR, ER, and Rho-GTPase pathways. The effects of AR inhibitors, AR agonists, and tamoxifen (Tam) on anchorage independent growth, proliferation, invasion, and motility were measured in soft agar colony formation assays, MTT growth assays, matrigel invasion assays, and scratch plate motility assays respectively. Microarrays on Tam treated cells were used to explore pathways promoting Tam resistance.

Results: MCF-7 Rho GDI knockdown cells exhibited increased lung metastases, and were resistant to the growth inhibitory effects of Tam when grown as xenografts in vivo. We discovered that AR was overexpressed in the knockdown cells as well, as confirmed by both western blot and RPPA. Increased activation of the Rho-GTPases was seen in knock down cells through immunoprecipitaion assays. The synthetic AR agonist R1881 increased invasion of knock down cells but not parental control cells. In MCF-7 and ZR-75-B knockdown cells Increased basal levels of anchorage independent growth, proliferation, and Tam stimulated growth were seen which could be blocked by inhibition of AR function using the antagonist bicalutimide. In ZR-75-B knockdown cells R1881 and Tam stimulated motility. Microarray showed an activation of EGFR and MAPK signaling in Tam treated cells.

Discussion: Since AR was significantly overexpressed with the metastatic and Tam-resistant phenotype, and AR agonists/ inhibitors modulated the phenotype, we hypothesize that AR plays a role in these processes. EGFR signaling may also contribute based on its activation in Tam treated cells. We are currently asking if resistance conferred by AR overexpression is pivotal for the multistep process of metastasis. Our results suggest that AR may represent a new clinical target for hormone therapy resistance of ER(positive breast cancer.

Improper aggregation of tau protein occurs in many neurodegenerative dementias, including Alzheimer’s disease. A major target of degeneration in these tauopathies is the hippocampus, a classical brain region for memory. In the normal hippocampus, spatial memory is encoded by place cells, a class of pyramidal neurons that fire preferentially when the animal is in a specific location in space. Additionally, proper memory encoding is facilitated by stereotyped oscillations in the local field potential (LFP) and entrainment of neuronal firing to those oscillations. However, we do not know which aspects of hippocampal memory processing are altered in tauopathies and when in disease alterations occur. Such knowledge would bridge our understanding of the molecular and behavioral level changes that occur in these disease states. Here, we study this question using the rTg4510 (Tau) mouse line, in which human tau with the P301L mutation found in patients with frontotemporal dementia is expressed in the mature forebrain. In this mouse, tau becomes hyperphosphorylated and begins to aggregate in the hippocampus as young as 2.5 months of age. Neurodegeneration begins soon after, with 60% of the CA1 layer of the hippocampus already degenerated by 5.5 months of age. Previous experiments in our laboratory have shown deficits in place cell stability as well as synchrony deficits and LFP alterations in older Tau mice that already exhibit massive neurodegeneration. However, we do not yet know if such changes depend on abnormal tau or neurodegeneration. We hypothesize that at least some of these functional changes at the network level occur prior to neurodegeneration. To test this we have recorded in vivo from the hippocampus as Tau mice at 2 to 5 months of age run through a linear track, explore an open field, and sleep. The data we show here suggests that while place cells are not in fact altered until later disease points, network synchrony and LFP changes may occur prior to major degeneration. Specifically, there is a decrease in the amplitude of sharp-wave ripples during slow-wave sleep in Tau mice, which are high-frequency LFP oscillations associated with memory consolidation, and a corresponding decrease in the synchronicity of cell firing during ripple events. These results allow us an insight into the network level alterations that lead to the debilitating memory loss symptoms seen in patients with various types of tauopathies, an aspect of these diseases that is poorly understood.

Contributors: Cheng, Jingheng; Ji, Daoyun
Implantation of an embryo in the uterus is a multistep process tightly controlled by a number of ovarian, uterine, and embryonic factors interconnected by an intricate regulatory network. Upon mating, oocytes are fertilized, develop to blastocysts and move to the uterine lumen. If the uterus is receptive, embryos attach to the luminal epithelium and invade into the uterine stroma. Uterine stromal cells respond to the presence of the embryo by undergoing a functional and morphological transformation called decidualization. The focus of my thesis research has been to define the roles of the type 1 BMP receptors, ALK2, ALK3, and ALK6, during pregnancy using knockout mouse models and human endometrial stromal cells.

BMPs, which signal through heterodimers of BMP type 1 and type 2 serine/threonine kinase receptors, can bind different receptors in different contexts: the physiological association with a specific receptor depends on both the binding affinity and the availability of the ligand and the receptor in a specific environment. To study the implantation process, we first investigated the effects of deleting each of the three BMP type 1 receptors in the uterus. Although ALK6 null female mice are sterile, this receptor is not required for uterine decidualization. However, I discovered that ALK2 and ALK3 are essential for mouse fertility and are required at different phases during the peri-implantation period. When ALK3 is deleted in the uterus, the embryo cannot attach to the uterine epithelium; hence, there is no implantation. Our work suggests that ALK3 is required to regulate uterine receptivity. Alternatively, deletion of ALK2 causes a delay in embryo invasion and a defect in stromal cell decidualization. The evolutionary importance of ALK2 during decidualization is confirmed by the observation that human endometrial stromal cells with knockdown of ALK2 fail to decidualize.

Our data show that BMP signaling is required to regulate different steps of pregnancy. Although the existence of compensatory mechanisms among different ligands and receptors makes this regulatory network particularly difficult to detangle, investigating these different models is a valuable tool to dissect the mechanisms that regulate early pregnancy. The importance of these findings can be fully appreciated if we consider that ~6.1 million of women of reproductive age in the United States have difficulty getting pregnant or staying pregnant. In particular, 75% of lost pregnancies are due to failure at the implantation stage. Moreover, considering the broad involvement of the BMP members of the TGFβ superfamily in many processes, it is crucial to further dissect these pathways in uterine biology to identify possible targets for future therapeutic manipulation.

This research is supported by Eunice Kennedy Shriver National Institute of Child Health and Human Development grant HD32067. Contributors: Clementi, Caterina; Hawkins, Shannon; Creighton, Chad; Kaartinen, Vesa; Lydon, John; DeMayo, Francesco; Martin, Matzuk.
The use of dendritic cell vaccines to treat cancer has significant potential, however that potential has yet to be achieved in treating patients. Fortunately, pioneering biotechnologies are paving the way for more effective cancer vaccines. Our lab has developed a drug inducible MyD88/CD40 (iMC) composite adjuvant that promotes robust cytotoxic T cell priming by iMC activated DCs. Despite the iMC innovation, its implementation in patient-tailored ex vivo DC vaccines is impractical for widespread use, due to issues of scalability and cost. We have therefore begun to address the delivery of DC vaccines as “off-the-shelf” therapies using in vivo electroporation (EP) of plasmid DNA (pDNA) encoding both the iMC adjuvant and tumor antigen (eVac). Intradermal EP of pDNA encoding the model antigen LacZ in mice primed LacZ-specific CD8+ T cell responses, as mice receiving LacZ + EP had significantly greater portions of antigen responsive, IFNγ secreting T cells than mice that received that same LacZ vector without EP. Mice vaccinated prophylactically with LacZ + EP were either protected from establishment of B16/LacZ tumors or demonstrated significantly slower tumor growth compared to controls. In mice bearing pre-established B16/LacZ tumors, LacZ-eVac, but not EP with LacZ antigen alone significantly reduced tumor burden and increased overall survival. Furthermore, activation of the eVac via a chemical inducer of dimerization (CID) improved the vaccine efficacy in this model suggesting that iMC adjuvantanation is a required element of the EP mediate therapeutic vaccine. Lastly, eVac encoding the human Prostate cancer antigen, PSMA, was able to stimulate significant antigen-specific CD8+ T cell and serum antibody responses. Taken together, these data indicate that eVac with iMC-antigen is an effective “off-the-shelf” cancer vaccine and potentially a platform for a wide range of disease targets. These findings warrant elucidation of the underlying immunological mechanisms responsible for the anti-tumor responses observed with eVac treatment to guide further refinement of the vaccine in a pre-clinical model.

Contributors: Collinson-Pautz, M; Seethammagari, M; Decker, W; Spencer, D; Levitt, J
New antibiotics are needed because antibiotic resistance has rendered most existing antibiotics ineffective. We discovered that the repurposed antifungal drug, ciclopirox, is effective against problematic multidrug-resistant clinical isolates (Carlson-Banning et al. 2013 PLoS One 8:e69646). Even though ciclopirox has been used for over thirty years in the clinic, how ciclopirox works is poorly understood. Using E. coli, we showed that both sugar metabolism and the availability of free iron in the growth medium affect ciclopirox activity. Additionally, iron-acquiring siderophore production in P. aeruginosa was altered in the presence of ciclopirox, suggesting a connection to iron utilization. Previous microarray data in C. albicans suggested that ciclopirox is an iron chelator. From these fungi and bacteria data, general iron chelation is a proposed mechanism of action for ciclopirox. To examine which iron utilization pathways might be affected by ciclopirox, we used single-gene deletion strains to screen for enhanced sensitivity to ciclopirox. Somewhat surprisingly, given the importance of the fur and fnr gene products in iron regulation in bacteria, strains lacking these genes were inhibited at the same ciclopirox minimum inhibitory concentrations (MICs) as the isogenic parent strain. Similarly, strains lacking genes encoding the ferric citrate (fecA, fecB, fecC, fecD, fecE) or ferrichrome (fhuA, fhuB, fhuC, fhuD, fhuE) uptake systems had unchanged ciclopirox MICs. In contrast, strains with deletions of any gene encoding a function in the biosynthesis (entB, entC, entE, or entF) or uptake (tonB, exbB, exbD, fepA, fepB, fepC, fepD, fepG) of the siderophore enterobactin had at least 2-fold lower ciclopirox MICs compared to the isogenic parent strain. We are currently testing whether this increased susceptibility is specific to ciclopirox by measuring MIC changes of these mutant strains in response to the general iron chelators 2,2'-bipyridyl and 1,10 phenanthroline. Although different siderophores are used in P. aeruginosa, our previous P. aeruginosa data combined with these enterobactin data suggest that siderophore production may be altered when gram-negative bacteria are exposed to ciclopirox. Together, these data show that ciclopirox is not a general iron chelator, but specifically affects siderophore pathways. These data are an important step toward developing ciclopirox or new derivatives of ciclopirox against multidrug-resistant infections by gram-negative pathogens for which few therapeutic options exist currently.
Dictyostelium discoideum is an amoeboid eukaryote that leaves its unicellular vegetative state upon starvation to undergo aggregative development into a mature multicellular fruiting body. Its simplicity and genetically tractable properties make it an excellent model of multicellular development. An important component of all multicellular organisms is the use of a cell adhesion system for organizing and anchoring cells and tissues. Dictyostelium cell adhesion has been studied during the aggregation stage of development: the cadA gene product is a calcium-dependent cell adhesion protein that functions during streaming and aggregation, and gp80 and tgrC1/tgrB1 cell-adhesion proteins function in an EDTA-resistant manner during streaming and the mound stage of development. However, cell adhesion has not been studied after this stage when the expression of these genes falls dramatically, though development continues with apparent increasing complexity. We now have data that suggests that a paralog of tgrC1 and tgrB1, tgrM1, may function as a cell adhesion protein during late development. A strain which overexpresses tgrM1 shows development-independent aggregation in shaking culture, suggesting that tgrM1 is a hemophilic adhesion molecule. tgrM1 mRNA levels rise as gp80 mRNA levels fall during the streaming and mound stages of development. Like gp80, tgrM1 is predicted to be GPI-anchored, and may exist in lipid microdomains such as the triton-insoluble floating fraction. tgrM1 knockout cells exhibit an EDTA-resistant cell adhesion defect during mid- to late development and produce thinner slugs which frequently break apart. When tgrM1 cells are developed under a layer of soil, fewer spores are produced at the surface, resulting in a fitness disadvantage compared to wildtype cells. Additionally, a tgrM1 ortholog exists in another Dictyostelid, D. purpureum, and has a similar transcriptional trajectory through development, suggesting that the role of this gene product has been conserved for 400 million years. Finally, RNAseq data (S. Hirose et al.) reveals that tgrM1 expression is dependent upon proper recognition of identical or closely-related cells via the tgrC1/tgrB1 allorecognition system, suggesting that the tgrM1 cell adhesion system follows the allorecognition checkpoint for proper development. Together these data suggest that tgrM1 is an important component of the cell adhesion machinery. Currently we are attempting to tag this protein to follow it spatially and temporally during development.

Contributors: Cordill, W Justin; Hirose, Shigenori; Shaulsky, Gad; Kuspa, Adam
To allow for high throughput analysis of neuronal circuits in living tissue, we are developing experimental tools that allow spiking activity in individual cells to be precisely controlled and monitored by optical means without the need for whole-cell patching of each cell. This will be achieved by combining optogenetic manipulation of defined cell populations and Ca2+ imaging with random-access multi-photon (RAMP) microscopy. Generating arbitrary illumination patterns with traditional galvanometer-based two-photon microscopes is difficult due to their scanner inertia. By contrast, RAMP microscopes employ acousto-optic deflectors (AODs) for inertia-free steering of a laser beam and thus are well-suited to generating precise custom spatio-temporal patterns of stimulation. These illumination patterns allow us to monitor and control many cells by interleaving excitation and recording locations.

By varying the RAMP scanner generated stimulation pattern, we are able to control the amount of two-photon-activated light-gated ion channels (i.e. channelrhodopsin-2 (ChR2)) in selected neurons. For stimulation, RAMP technology is used to scan a pulsed near-infrared beam across the soma of each neuron. RAMP technology also records any resulting spiking in the optically activated neurons and potentially connected cells. Optical recording of spiking activity is achieved by monitoring changes in Ca2+ indicator fluorescence (e.g. OGB) using the same laser wavelength but a different illumination pattern that does not activate light-gated channels.

Our initial application is to generate neural connectivity maps. Such cellular-level connectomes are typically generated from “dead” tissue using electron microscopy. While this provides an anatomical description, it does not reveal whether “connected” neurons exert a causal effect on others. Our goal is to generate comprehensive maps showing connectivity in cortical sub-networks. Existing techniques used to study such connectivity, such as simultaneously recording from multiple cells, are of low throughput and can only map connections between a few cells. Our long-term goal is automatic high-throughput mapping of functional connectivity in the intact cortex.

Contributors: Cordiner, Keith; Tolias, Andreas, Saggau, Peter.
SINGLE STIMULUS VENTROMEDIAL PREFRONTAL CORTEX RESPONSE TO PICTURES OF FAVORITE FACES DURING FMRI DISTINGUISHES COHORTS OF ASD AND TD CHILDREN

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Dysfunction of the brain’s reward circuitry had been speculated to contribute to the social and cognitive deficits observed in autism spectrum disorders (ASD). Using functional magnetic resonance imaging (fMRI), we tested the hypotheses that (1) previously determined valuation responses in the ventromedial prefrontal cortex (vmPFC) can be elicited using pictures of subjects’ self-selected favorite person and object; and (2) vmPFC responses to a single presentation of one’s favorite face or object can be used to distinguish cohorts of TD (n = 50) and ASD children (n = 24). Pictures of subjects' favorite person and favorite object were shown in a stream of images that also included images from the International Affective Picture System (IAPS) database; images from the IAPS database were selected for positive, neutral, and negative valence (i.e. pleasant to unpleasant). Whole brain and region of interest (ROI) analyses were used to assess blood oxygen level dependent (BOLD) responses to faces and objects. Subjective behavioral assessments of the pleasantness of each image were determined post-scan. In TD children, BOLD responses to favorite images in reward-processing related areas such as the vmPFC and ventral striatum were significantly greater than to responses to IAPS images. The BOLD response in the vmPFC to a single stimulus of the subjects’ favorite person was diminished in the ASD group compared to age- and gender- matched TD controls. Single stimulus responses in the vmPFC to subjects’ favorite person are diminished in children diagnosed with ASD, however the response to favorite objects remains undifferentiated across the two groups. The ability to measure these differences with a single visual stimulus suggests the possibility of rapid fMRI paradigms for assessing biological markers of ASD.
THE ROLE OF DNMT3A IN SOMATIC STEM CELL REGULATION OF SELF-RENEWAL AND DIFFERENTIATION

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Two of the most important biological functions of stem cells are their ability to both self-renew and differentiate into varied cell types. Great amounts of research have been done to elucidate the complex biology underlying the regulation and activity of these two inherently different processes. Among the proteins involved in regulating these processes in stem cells, de novo methyltransferases (Dnmt3s) are important both in embryonic stem cells and adult stem cell populations. Dnmt3a and b, whose primary known function involves methylation of cytosine residues to covalently modify DNA, comprise an epigenetic method of gene regulation essential to normal development.

Research in our lab has indicated a major role of de novo methyltransferases, particularly Dnmt3a, in the regulation of hematopoietic stem cells (HSCs). With the aid of an inducible deletion system and HSC transplantation, it was shown that HSCs lacking de novo DNA methylation both largely lose their capacity for differentiation and increase their self-renewal capacity. As a possible mechanistic explanation, it was also shown that deletion of Dnmt3s leads to a downregulation of differentiation genes and an upregulation of multipotency genes in HSCs.

These data were of particular interest considering the work done by the original investigators to describe Dnmt3a and b. Dnmt3a knockout mice developed by Okano et al. (1999) were shown to develop to term and appear normal at birth, but became runted and died around 4 weeks of age, without a well-elucidated cause of death. The data our lab has previously generated studying Dnmt3a deletion in the hematopoietic system leads to our central hypothesis that Dnmt3a deletion leads to an accumulation of stem-like cells and a block of differentiation in multiple tissues. This disruption may cause negative phenotypic effects, eventually resulting in death. We are utilizing a Dnmt3a-null mouse model to examine multiple tissues and various stem cell populations in Dnmt3a-/- mice. We are also performing conditional Dnmt3a deletion in specific somatic stem cell populations using an inducible Cre recombinase, with expression driven by a Leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5) promoter, which labels intestinal and hair follicle stem cell populations. Transplantation and cell culture assays will study the effects of Dnmt3a deletion on the ability of these stem cell populations to self-renew and differentiate.

Concurrently, we are elucidating the molecular interactions of Dnmt3a in vitro to better understand the protein’s mechanism of action. Using a mouse embryonic stem (mES) cell line capable of doxycycline-induced expression of biotinylated-Dnmt3a, we identified ~760 proteins that interact directly with Dnmt3a through co-immunoprecipitation with magnetic streptavidin beads and subsequent mass spectroscopy. We are now validating direct interaction with proteins of interest. We are also using this mES cell line for blastocyst injection to generate a mouse model capable of examining the similarities and differences of Dnmt3a-interacting proteins in adult stem cells, such as HSCs, and mES cells.

Contributors: Cullen, Sean; Luo, Min; Wang, Fengchao; Challen, Grant; Nguyen, Hoang; Goodell, Margaret
HUMAN NOROVIRUS INFECTION ELICITS CROSS-REACTIVE SURROGATE NEUTRALIZING ANTIBODIES

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Robert Atmar, M.D.-Department of Medicine

Noroviruses (NoVs) are the leading cause of epidemic nonbacterial gastroenteritis. Our understanding of the determinants of protective immunity to NoV is incomplete. Serum antibody that inhibits virus binding in vitro to the histo-blood group (HBGA) family of host glycans, the putative NoV attachment factors, is the only known correlate of protection from NoV gastroenteritis. Antigenic drift in the capsid protein of human NoVs is well-documented, but the genotype-specificity of HBGA-blocking antibody is not known. In this study, we investigated the breadth of the blocking antibody response elicited by NoV infection.

The study population consisted of healthy adults who participated in a previously conducted experimental challenge study with Norwalk virus (genotype GI.1). Serum was collected before (-3 to 0 days) and 7, 14, 28, and 180 days after infection. A modified ELISA to measure blocking antibody in serum was developed and carried out against NoV virus-like particles representing homotypic and heterotypic NoVs. NoV virus-like particles (VLPs) structurally and antigenically recapitulate native antigen and were produced in a baculovirus expression system for the capsid proteins of selected NoVs representing genotypes GI.1, GI.4, GI.7, and GII.4. All persons infected with Norwalk virus developed an increase in blocking antibody titer to Norwalk VLPs (n=18). Furthermore, 22-39% of infected persons had a serological rise (≥4-fold rise between pre-infection and peak blocking titers) to at least one heterotypic NoV VLP. In contrast, no seroresponse to any antigen occurred among uninfected or placebo recipients.

There is no antiviral therapeutic for the human NoVs, underscoring the need for a vaccine. In the absence of cell culture or small animal replication models, blocking antibody is considered a surrogate neutralizing antibody and serves as an endpoint in the evaluation of norovirus vaccine candidates. However, the substantial genetic diversity and co-circulation of distinct NoV genotypes pose challenges to the development of a broadly effective vaccine. Our results suggest that a broadly effective vaccine may be feasible.

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Contributors: Czakó, Rita; Opekun, Antone; Gilger, Mark; Graham, David; Atmar, Robert; Estes, Mary
Sickle cell disease is an autosomal recessive genetic disease caused by a single point mutation in the β-globin subunit of hemoglobin. This point mutation changes the hydrophilic glutamate to a hydrophobic valine, resulting in non-covalent polymerization of the full hemoglobin protein under low-oxygen conditions. Formation and elongation of these fibril bundles leads to red blood cell distortion, with repeated sickling episodes causing damage to the cell membrane, decreasing the cell’s elasticity and its ability to return to a normal biconcave disc shape when normoxia conditions are restored.

Current techniques for imaging red blood cells provide limited and potentially misleading information because they are restricted to two-dimensional data collection. Our goal is to obtain a three-dimensional structure of sickle red blood cells and the hemoglobin aggregates inside in order to gain a better understanding of the sickling process. To do this we use soft X-ray cryo-tomography in conjunction with scanning electron microscopy of plastic-embedded red blood cells.

Upon visualization of sickled red blood cells, it became clear that the route from a biconcave disc to a fully sickled cell involves multiple stages. We have identified and begun characterizing four distinct morphological stages in the severity of a sickled cell. Additionally, we have created a processing protocol to identify and isolate densities that we believe correspond to hemoglobin internal to the cell. Lastly, we have tested four inhibitors of sphingosine-kinase-1 as potential drugs for treatment of sickle cell disease.
THE ROLE OF WACKY IN NEURODEGENERATION AND AUTOPHAGY

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*Program in Developmental Biology*  
*Advisor: Hugo Bellen, Ph.D./D.V.M.-Department of Molecular & Human Genetics*

Our lab has conducted a forward genetic screen on the Drosophila X chromosome to identify essential genes that play a role in the development, function, and maintenance of the nervous system. From this screen, we isolated six mutant alleles of the Drosophila homolog of WW domain-containing adapter with coiled-coil region (WAC), which we have named wacky. Wacky mutant eye clones exhibit morphological degeneration with loss of ommatidia that begins before eclosion and dramatically increases with age. In addition, wacky mutant clones in the eye and larval fat body exhibit increased autophagic vacuoles and mitochondria, expansion of the ER, and destabilization of Rab11. Since Rab11 has been shown to play a role in eye development and autophagy, we believe that the eye development and autophagy defects associated with loss of Wacky can be linked to its interaction with Rab11. We are currently deciphering the role of Wacky in neuronal maintenance and autophagy by pursuing the mechanisms by which Wacky interacts with Rab11 and other proteins.

Contributors: DAVID, GABRIELA; JAISWAL, MANISH; XIONG, BO; ZHANG, KE; BAYAT, VAFA; SANDOVAL, HECTOR; CHARNG, WU-LIN; YAMAMOTO, SHINYA; BELLEN, HUGO
Aggressive behavior is widespread in the animal kingdom. However, its mechanisms are poorly understood, and the degree of molecular conservation between distantly related species is unknown. In mice, loss-of-function of the transcriptional repressor Nr2e1 causes extreme aggression. Neuropeptides released from the hypothalamus and electrical activation of the hypothalamus also cause aggression in mammals. Here, we show that knock-down of tailless (tll), a fly ortholog of Nr2e1, increases aggression in Drosophila melanogaster. Tll localizes to the adult pars intercerebralis (PI), which is composed of neurosecretory cells with similarity to the mammalian hypothalamus. Knock-down of tll in the PI is sufficient to increase aggression and is rescued by co-expressing human NR2E1. Knock-down of atrophin, which encodes a co-repressor of Tll, also increases aggression and both proteins physically interact in the PI. The tll-knock-down induced aggression phenotype is fully suppressed by blocking neuropeptide processing or release from the PI, showing that a neuropeptide-based mechanism is involved. In addition, genetically activating PI neurons, which leads to neuropeptide release in flies, increases aggression and mimics the aggression-inducing effect of hypothalamic stimulation in mammals. Together our results show that Tll regulates aggression in flies through a neuropeptide-based mechanism in the neurosecretory cells of the adult PI. The remarkable similarities of these findings in flies with three mechanisms involved in aggression in mammals suggest that this transcriptional neuropeptide control mechanism in neurosecretory cells in the brain represents an evolutionarily ancient aggression control system.

Contributors: Thomas, Amanda; Nomie, Krystle; Huang, Longwen
**NEGATIVE REGULATION OF AUTOPHAGY BY PSEUDOMONAS AERUGINOSA TYPE 3 SECRETION SYSTEM IN AIRWAY EPITHELIAL CELLS**

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*Integrative Program in Molecular and Biomedical Sciences*

*Advisor: Tony Eissa, M.B.,B.Ch.-Department of Medicine*

Rationale: Airway epithelial cells (AECs) provide initial protection against airborne pathogens by acting as a cellular barrier, but represent a common target for gram-negative bacteria *Pseudomonas aeruginosa* (P. aeruginosa). The type 3 secretion system (T3SS) is a virulence mechanism through which P. aeruginosa injects up to four toxins, ExoS, ExoT, ExoU and ExoY into the cytoplasm of host cells; thereafter affecting processes including phagocytosis, cytokines production and other antimicrobial pathways yet to be identified. Autophagy is a process in which cellular components are engulfed by double membrane organelles (autophagosomes), which then fuse with lysosomes for degradation. Autophagy is also important in pathogen clearance. Studies have shown that P. aeruginosa’s T3SS enables the bacterium to persist inside AECs. However, it is unknown whether or not T3SS promotes bacterial survival by negatively modulating autophagy. We hypothesized that P. aeruginosa’s T3SS inhibits autophagy in host cells.

**Methods:** We established AEC line (A549 cells) deficient in autophagy by knocking down the autophagy gene 7 (Atg7). We infected cells with wild type (WT) P. aeruginosa, P. aeruginosa deficient in injection apparatus (T3SS mutant) or P. aeruginosa mutants deficient in individual toxins. We analyzed P. aeruginosa survival in cells by performing colony forming unit assays. We evaluated autophagy by monitoring the levels of autophagy marker, LC3II, and autophagosome formation by Western Blot and fluorescence microscopy, respectively.

**Results:** Knockdown of the critical autophagy gene Atg7 in A549 cells had no effect on survival of WT P. aeruginosa but greatly enhanced survival of both T3SS mutant and ExoS deficient P. aeruginosa, suggesting that WT P. aeruginosa but not ExoS deficient P. aeruginosa are resistant to autophagic destruction. T3SS mutant and ExoS deficient P. aeruginosa colocalized with autophagosomes more than the WT strain. Inhibition of autophagy, shown by reduction in LC3II and autophagosomes in A549 cells, was observed in cells infected with WT P. aeruginosa but not with ExoS deficient strains, indicating that ExoS is the main toxin required for autophagy inhibition.

Contributors: De La Rosa, Indhira; Xu, Yi; Eissa, N.Tony.
DISSECTING THE ROLE OF THE RNA BINDING PROTEIN RBM17 IN THE PATHOGENESIS OF SPINOCEREBELLAR ATAXIA TYPE 1

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Spinocerebellar ataxia type1 (SCA1) is an inherited neurodegenerative disorder characterized by impairment of coordination mainly due to loss of cerebellar Purkinje cells. SCA1 is caused by expansion of CAG repeats in the gene encoding the ATAXIN1 (ATXN1) protein leading the protein to have an expanded polyglutamine (polyQ) tract. PolyQ-ATXN1 causes diseases via a gain-of-function mechanism, but the expansion by itself is not sufficient to trigger pathogenesis. It has been shown that toxicity of polyQ-ATXN1 requires integrity of additional domains and amino acids residue in the protein, and also depends on alteration of critical ATXN1’s interactions. Investigation of the ATXN1’s interactome unveiled an interesting finding: many ATXN1 protein partners are RNA binding proteins (RBPs). Within this group the splicing factor RBM17 stood out because its binding to ATXN1 is strengthened by the poly-Q expansion and relies on the phosphorylation of S776 (pS776), both aspects proven critical for SCA1 pathogenesis. These data supported the hypothesis of a critical role for RBM17 in SCA1 pathogenesis and led us to focus on studying the physiological function of this RBP. Hence, we generated an Rbm17 knockout mouse and observed that Rbm17 loss of function causes early embryonic lethality. We also found that conditional deletion of Rbm17 from Purkinje cells (Pcp2-Cre) or from the broader domain encompassing developing midbrain and cerebellum (En1-Cre) causes, respectively, progressive Purkinje cells degeneration and perinatal lethality. Our findings pinpointed an essential developmental function of Rbm17 and its requirement in Purkinje cells maintenance. The overarching goal of this project is to provide insights into the molecular mechanism linking Rbm17’s function to SCA1 pathogenesis. To do this, we will take advantage of the iCLIP technique combined with deep-sequencing to unveil Rbm17’s RNA targets and the gene expression changes dependent on this RBP’s activity. We expect to reveal aberrant splicing of specific factors whose altered function could contribute to SCA1 pathogenesis. We believe that this study will shed new light on the subtle link between RNA homeostasis and neurodegeneration, providing potential new therapeutic perspectives for SCA1 and other polyglutamine diseases.

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(p)ppGpp Inhibits Replication Elongation by Direct Targeting of Primase

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Xiangwei He, Ph.D.-Department of Molecular & Human Genetics

DNA replication is regulated in response to environmental constraints such as nutrient availability to ensure faithful duplication of the genetic material. Previously, we have shown that rates of replication elongation are reduced during starvation. This effect is mediated through the starvation signaling molecules guanosine pentaphosphate and guanosine tetrphosphate collectively known as (p)ppGpp. We believe that (p)ppGpp likely regulates DNA replication elongation directly via its action as a competitive inhibitor of the primase, DnaG. To clearly demonstrate that (p)ppGpp reduces replication elongation rates almost exclusively by targeting primase requires the identification of a mutant that is refractory to (p)ppGpp inhibition while retaining priming activity. We have preliminary evidence that a Q230E substitution in Bacillus subtilis DnaG satisfies these criteria. The Q230E variant maintains some primase activity, as it complements a dnaGts allele at the non-permissive temperature. Furthermore, this strain fails to inhibit replication during starvation. Characterization of the mutant in vitro confirms that pppGpp is no longer capable of inhibiting priming activity, although the basal activity of the mutant is compromised. Using this mutant, we have can now begin to probe the physiological importance of (p)ppGpp-mediated inhibition of replication.

Contributors: DeNapoli, Jessica; Mei, Sally; Wang, Jue D.
Neuronal responses to identical visual stimuli are highly variable, but the source of this variability is not known. The most widely accepted hypothesis on its origin is that neurons are inherently noisy. Despite this convergence of opinion on the noisy nature of neurons, there exists an alternative explanation: this variability is not actually noise but reflects, and is due to, computations internal to the brain. Internal signals such as cortical state and attention interact with the processing of sensory information in primary sensory areas. However, little research has directly examined the effect of fluctuations in these internal signals on information processing, leaving a number of uncontrolled parameters in perceptual tasks that may contribute to neuronal variability. One such typically uncontrolled variable is attention, a multifaceted cognitive process with varying effects on the neuronal response. We test the hypothesis that fluctuations in attentional signals contribute to neuronal response variability in early visual cortex, and that controlling for such fluctuations will reduce this variability. Our hypothesis predicts attentional effects on neuronal variability that differ from those predicted by the standard model of attention, in which attention reduces background cortical noise to improve sensory processing. The standard model predicts an inverse relationship between degree of attention and neuronal variability, which has found recent experimental support. In contrast, our model, which considers the effects of fluctuations in the attentional signal on recipient neurons, predicts the greatest degree of neuronal variability at an intermediate level of attention, corresponding to a state where the focus of attention is most variable. To test this hypothesis we will control for fluctuations in the attentional signal in a demanding change-detection task, varying across blocks of trials the degree to which subjects must attend to one of two stimuli presented in the visual field, while recording from neurons in primary visual cortex of the macaque.

Contributors: Denfield, George; Ecker, Alexander; Tolias, Andreas
Alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV) is a rare neonatally-lethal diffuse developmental disorder of the lungs caused by haploinsufficiency of FOXF1. All affected newborns die in the first month of life due to severe respiratory distress and pulmonary hypertension. Foxf1 null mice die by midgestation as a result of defects in mesodermal differentiation and cell adhesion. Foxf1 heterozygous mice exhibit up to 90% neonatal mortality, depending on genetic background. For the current study, Foxf1 heterozygous mice with a deletion of the forkhead binding domain were generated and are congenic on the C57BL/6J background. In contrast to recent reports that FOXF1 is incompletely paternally imprinted in the human lungs, early postnatal mortality was observed regardless of parental transmission of the deleted allele; no differences were seen in Foxf1 expression in embryonic and postnatal lung tissues from reciprocal crosses. Analysis of RNA from postnatal day 0.5 Foxf1+/- and wildtype lungs using Illumina mouse WG-6 v2.0 expression bead microarray revealed statistically significant deregulation (p<0.05, fdr<0.05) of several genes, including those involved in pulmonary vascular development (Sema3C, Dll4, and EdnrB), lung branching morphogenesis (Fgf10 and Lama1), and the blood pressure regulating renin-angiotensin system (Ren1, Cma1, and Cpa3). To study the effects of Foxf1 overexpression, we knocked a Cre-inducible Foxf1 allele into the ROSA26 locus. These mice have been mated to CMV-cre and Tie2-cre mice to obtain whole body and vascular endothelial cell specific overexpression of Foxf1, respectively. We are currently mating ROSA26Foxf1; Tie2-cre mice to Foxf1+/- mice, which we hypothesize will rescue early postnatal mortality. This could inform future gene therapy studies in patients with ACDMPV.
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*Department of Molecular & Human Genetics*  
*Advisor: Kenneth Scott, Ph.D.*, *Department of Molecular & Human Genetics*

Tumor sequencing projects such as The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) have revealed the high complexity of cancer genomes that are comprised of both pathogenic “driver” aberrations and many neutral “passenger” events. The cancer research community is faced with the daunting challenge of discriminating between drivers and passengers to inform the most promising therapeutic targets and diagnostic markers. Much of these efforts are focused on identifying new oncogenes given that such factors have served as successful therapeutic targets to date.

To expedite oncogene discovery based on large-scale sequencing datasets, we established a target discovery pipeline involving a high-throughput mutagenesis and molecular barcoding (HiTMMoB) platform permitting (1) efficient site-directed DNA mutagenesis to model tumor somatic mutations into our collection of >32,000 human open reading frames (ORFs or “genes”), (2) integration of a 24 nucleotide DNA barcode sequence into ORF clones to facilitate pooled genetic screens and (3) their simultaneous recombination into lentiviral expression backbones enabling their expression in cancer cell models. Cells infected with barcoded ORF libraries can be entered into a variety of individual or pooled in vitro and in vivo genetic screens to identify ORF drivers of cancer-related activities (e.g., proliferation, tumor growth, invasion, metastasis among others).

We have successfully used HiTMMoB to generate numerous wild-type and mutant ORF clones currently being used in multiple screening projects in our laboratory. One such project involves a gene focused screen designed to functionally characterize numerous lower frequency somatic ‘tail’ mutations within the PIK3CA oncogene found mutated in breast and other cancers. Here, we have generated 36 mutated clones for PIK3CA that have been entered into in vitro and in vivo screens for drivers of (1) anchorage-independent growth (2) growth factor-independent growth (3) drug sensitivity/resistance and (4) orthotopic tumor formation in a pooled fashion. The goal of these screens is to functionally annotate these low frequency PIK3CA mutations, which may ultimately lead to tailored patient therapies based on the PIK3CA mutation status of an individual's tumor.

In summary, we have developed a prioritization pipeline being used in a variety of target screens across diverse cancer types, and this platform promises to provide the cancer research community functional annotation on the most promising cancer aberrations for downstream biomarker and drug development.

Contributors: Dogruluk Turgut; Dogruluk Armel; Tsang, Yiu-Huen; Nair, Nikitha; Minelli, Rosalba; Wu, Ping; Scott, Kenneth L.
Targeted therapies are medication that strongly impairs tumor growth by interfering with genetic drivers that propel carcinogenesis, with minimal side effects to the patient. Since their inception, many have been developed for to treat a broad range of malignancies, and have had a significant impact in cancer treatment. However, while our arsenal has been increasing, understanding of the rules that govern how a cancer responds to targeted therapies remains poor. Clinical and experimental data suggest that there is substantial heterogeneity in drug-response between patients and even between cells of the same tumor, so understanding the components and mechanisms of targeted therapy will help towards increasing their effectiveness, and preventing tumors from becoming resistant to it. Our lab is using forward genetic approaches to discover the genetic networks that govern response to the anti-cancer drug lapatinib in Her2+ breast cancers as a model.

We performed a pooled RNA interference-based genetic screen in a panel of Her2+ breast cancer cell lines using a short hairpin RNA (shRNA) library with 9,109 unique hairpins targeting 2,236 genes. We then assessed the effects of these shRNAs in untreated cells and in lapatinib-treated cells. Afterwards, we identified ~300 Lapatinib Sensitivity Regulators (LaSRs); from which we validated ~100 using small interference RNA (siRNA) based assays, and organized in protein networks using public protein-protein interaction (PPI) databases. We focused on 12 LaSRs that show a combinatorial effect with lapatinib in multiple Her2+ models, and we systematically tested whether they shared a PPI and genetic coregulation. We identified WEE1 as a critical hub in regulating sensitivity to lapatinib, and using a chemical inhibitor we determined that double inhibition affects growth, induces mitotic errors, and causes cell death in Her2+ cell lines in vitro. We are testing this combinatorial inhibition to determine if there is an effect in Her2+ xenografts.
We have previously shown that poliovirus infection disrupts cytoplasmic P-bodies in infected mammalian cells. During the infectious cycle, poliovirus causes the directed cleavage of Dcp1a and Pan3, coincident with dispersion of P-bodies. We now show that over-expression of Dcp1a prior to infection surprisingly restricts poliovirus infection. This inhibition of infection was independent of P-body formation, since expression of GFP-Dcp1a mutants that cannot enter P-bodies restricted poliovirus infection similar to wild-type GFP-Dcp1a. Expression of wild-type or mutant GFP-Dcp1a induced phosphorylation of eIF2α through the eIF2α kinase PKR. Activation of PKR requires the amino-terminal EVH1 domain of Dcp1a. This PKR induced translational inhibition appears to be specific to Dcp1a, as the expression of other P-body components Pan2, Pan3, Ccr4, or Caf1 did not result in the inhibition of poliovirus gene expression, or induce eIF2α phosphorylation. The translation blockade induced by Dcp1a expression suggests novel signaling linking RNA degradation/decapping and regulation of translation.

Contributors: Dougherty, Jonathan; Reineke, Lucas; and Richard Lloyd
NR4A1 ESTABLISHES A LYMPHOID-BIASED ENHANCER PROFILE IN ACUTE MYELOID LEUKEMIA

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Mice with null mutation of both Nr4a1 and Nr4a3 develop an acute myeloid leukemia (AML)-like disease characterized by the abnormal proliferative expansion of hematopoietic stem cells. Both NR4A1 and NR4A3 transcripts are severely downregulated in human AML patient samples and AML cell lines regardless of the oncogenic insult or individual patient cytogenetics. Furthermore, rescued expression of any member of the NR4A family in several cytogenetically distinct human AML cell lines inhibits their viability and long term proliferation.

Integration of NR4A1 ChIP-seq data with microarray revealed that NR4A1 directly coordinates activation of lymphoid determining genes and suppression of genes important for myeloid development. Binding sites associated with repressed myeloid genes occur in pre-activated regions occupied by the ETS transcription factors ERG and FLI-1 and are characterized by loss of histone H3 acetylation upon NR4A1 expression. Conversely, binding sites associated with activated lymphoid genes occur in primed regions that are not occupied by ERG or FLI-1 before NR4A1 expression. However, NR4A1-mediated enhancer establishment requires these transcription factors for full gene activation, which is mediated by NR4A1-dependent recruitment of p300.

Interestingly, conditional deletion of Nr4a1 on an Nr4a3-/- background in adult mice also results in AML development with reduced numbers of lymphocytes as well as dysregulated expression of several important lymphoid and myeloid genes. Retroviral rescue of Nr4a3 in these mice restores lymphocyte numbers to wild type levels, which is preceded by restoration of appropriate gene regulation, suggesting that NR4As play an important role in lymphoid cell fate determination.

Contributors: Duren, Ryan; Conneely, Orla
Foxi3 is required for development of branchial arches and anterior arch derivatives.

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The bones of the vertebrate face develop from transient embryonic branchial arches that are populated by cranial neural crest cells. We have characterized a mouse mutant for the Forkhead family transcription factor Foxi3, which is expressed in branchial ectoderm and endoderm. Foxi3 mutant mice are not viable and display severe branchial arch-derived facial skeleton defects, including absence of all but the most distal tip of the mandible and complete absence of the inner, middle and external ear structures. Although cranial neural crest cells of Foxi3 mutants are able to migrate, populate the branchial arches and display some elements of correct proximo-distal patterning, they succumb to apoptosis from embryonic day 9.25 (?) onwards. We show this cell death correlates with a delay in expression of Fgf8 in branchial arch ectoderm and a failure of neural crest cells in the arches to express FGF-responsive genes. Zebrafish foxi1 is also expressed in branchial arch ectoderm and endoderm, and morpholino knockdown of foxi1 also causes apoptosis of neural crest in the branchial arches. We show that heat shock induction of fgf3 in zebrafish arch tissue can rescue cell death in foxi1 morphants. Our results suggest that Foxi3 may play a role in the establishment of signaling centers in the branchial arches that are required for neural crest survival, patterning and the subsequent development of branchial arch derivatives.

Contributors: Edlund, Renee; Ohyama, Takahiro; Kantarci, Husniye; Riley, Bruce B.; Groves, Andrew K.
Shohat Type Spondyloepimetaphyseal Dysplasia (SEMD) is Caused by Mutations in DDRGK1, Encoding an Endoplasmic Reticulum Protein

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Shohat type spondyloepimetaphyseal dysplasia (SEMD) is part of a heterogeneous group of disorders characterized by a combination of vertebral, epiphyseal and metaphyseal anomalies. Patients with Shohat type SEMD present with disproportionate short stature, a short neck, a small chest, abdominal distension with hepatosplenomegaly, lordosis, short limbs, genu varum and joint laxity. Radiographically, these patients also have a delayed bone age, platyspondyly with central notches in the vertebral end-plates, radiolucency of the femoral metaphyses as well as fibular overgrowth. The genetic basis of Shohat type SEMD is unknown. By exome sequencing of three individuals from two families, we identified a homozygous donor splice-site mutation in DDRGK1 in all affected individuals, and confirmed it in a separate family by Sanger sequencing. Patients with this splice-site mutation, a predicted frameshift mutation, have significantly reduced levels of DDRGK1 compared to controls. DDRGK1 is an ubiquitylated ER protein that has a conserved PCI domain, a domain predicted to participate in protein-protein interactions. To understand the role of DDRGK1 in skeletal development we knockdown DDRGK1 in zebrafish using morpholinos. DDRGK1 morphants had abnormal craniofacial features, such as disfigured cartilages and reduced number of branchial arches. Furthermore, there were reduced levels of SOX9, a collagen type II transcription activator, when DDRGK1 was knockdown in differentiated ATDC5, a chondrocyte cell line. Therefore, we hypothesize that DDRGK1 regulates the stability of SOX9 and a deficiency in DDRGK1 decreases the SOX9 levels and thus disrupts the normal secretion of matrix proteins, such as collagen type 2, in cartilage secreting cells. In the future, we will perform Co-IP to determine whether SOX9 and DDRGK1 form and complex. In addition, we will determine whether overexpression of SOX9 rescues the craniofacial phenotype seen in DDRGK1 morphants.

Contributors: Bae, Yangjin; Campeau, Philippe; Lu, James; Cohn, Daniel; Lachman, Ralph; Swindell, Eric; Shohat, Mordechai; Gibbs, Richard; Lee, Brendan
1.1 million patients suffer from mild traumatic brain injury (mTBI) at a cost of $17 billion per year (CDC, 1999). Most mTBI patients recover, but 15% have persistent disabling problems (Alexander et al., 1995; Kushner et al., 1998).

To improve our understanding about mTBI, we examined PubMed (http://www.ncbi.nlm.nih.gov/pubmed) mTBI articles spanning 21 years from 1990 to 2011. The functional MRI (fMRI) meta-analysis used Ginger ALE (Eickhoff et al., 2009; Turkeltaub et al., 2012; Eickhoff et al., 2012). All fMRI coordinates were converted into Talairach space. We used the default Ginger ALE parameters, and additionally added the number of subjects per experiment. We chose a false discovery rate threshold level of 0.05. mTBI publications mostly focused at working memory tasks, but the tasks also included resting state fMRI, an auditory odd-ball task, and a spatial navigation task (Krivitzky et al., 2011; Mayer et al., 2011; McAllister et al., 2001; McAllister et al., 2011; Slobounov et al., 2010; Witt et al., 2010; Chen et al., 2007; Mayer et al., 2009).

Importantly, the map significantly ($p < 0.05$, corrected) depicted dampened regions, in mTBI, at: right middle frontal gyrus, right anterior cingulate cortex, right precentral gyrus, bilateral dorsolateral prefrontal cortex and right middle temporal gyrus. The ALE map also detected significantly higher mTBI signal in: cerebellum, right inferior frontal gyrus, right insula and right supramarginal gyrus. Spatially, these regions suggest an anterior-to-posterior pattern in which activity is reduced in anterior regions and increased in posterior regions. The mean Talairach anterior-to-posterior coordinate for regions with reduced activity was $Y = 15$ mm, compared to $Y = -23$ for increased mTBI activity, and a two-sample t-test of $Y$ between the decreased and increased regions was significant ($p = 0.05$, two tailed).

Conclusion is that the fMRI signal, in mTBI, may be significantly different to the control group and that mTBI may affect the frontal lobe by decreasing the fMRI signal locally.

Contributors: Eierud Cyrus; Craddock, Cameron; Fletcher, Sean; Aulakh, Manek; LaConte, Stephen M
The NAD-dependent ligase, LigB, functions in the base excision repair pathway in E. coli

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NAD-dependent ligases are a family of proteins found only in bacteria that use nicotinamide adenine dinucleotide (NAD) as a substrate instead of ATP, which is used by most bacteria and all eukaryotic ligases. Escherichia coli and the members of the family of Enterobacteriaceae encode two NAD-dependent ligases denoted as LigA and LigB. LigA, an essential protein, has been well-studied; it is highly expressed and is responsible for ligating Okazaki fragments during replication. LigB, however, is not essential and has less than 1% the ligase activity of LigA in vitro. No role for LigB has been found despite its strikingly high amino acid, chromosomal location, and sequence conservation across the entire family of Enterobacteriaceae. Because of this conservation and our recent discovery of a SNP in the ligB gene associated with fluoroquinolone-resistance (Swick, Evangelista et al. 2013 PLoS One 8:e65961), we hypothesized that LigB has a role in DNA repair that affects fluoroquinolone resistance. Indeed, comparing a ligB deletion to the parent strain, we uncovered that the deletion strain was 50-fold more susceptible to hydrogen peroxide than the isogenic parent strain. Comparing these strains after treatment with UV irradiation, cumene hydroperoxide, mitomycin C, and bleocin, we found that the ligB deletion strain was only more susceptible to treatments that resulted in the oxidation or alkylation of DNA (hydrogen peroxide, cumene hydroperoxide, and mitomycin C). Because oxidized and alkylated bases are removed and repaired through the base excision repair (BER) pathway, we propose a model whereby LigB is the terminal ligase for the repair of these types of DNA damage. LigB is, thus, not essential under ideal laboratory growth conditions but becomes essential in the presence of oxidating and alkylating agents, which includes the fluoroquinolones and many other antibiotics.

Contributors: Evangelista, Michael; Chang, Huan Ting; Zechiedrich, Lynn
POST-TRANSCRIPTIONAL REGULATION OF THE KRAS PROTO-ONCOGENE

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The post-transcriptional regulation of messenger RNAs by small RNA molecules and RNA binding proteins (RBPs) influence gene expression via modulation of the stability, subcellular localization and efficiency of translation. Our studies aim to identify novel post-transcriptional regulators of the KRAS oncogene. KRAS is a member of the RAS family of oncogenes, which are known to be mutated and overexpressed in approximately 30% of all human tumors. This family of oncogenes functions as intermediate signaling proteins from upstream cell surface receptors in the activation of a number of signaling pathways controlling cellular proliferation, differentiation and apoptosis. We have observed a significant upregulation of K-Ras protein expression, independent of mRNA levels, upon stimulation of both human T cells and the Jurkat human T cell leukemia cell line. We have ruled out regulation of KRAS at the protein level by treating resting and stimulated human T cells with the proteosome inhibitor MG132. By employing polyribosome fractionation assays, we have also shown that KRAS is not actively translated in resting Jurkat cells, whereas it is actively translated and polyribosome bound in stimulated Jurkat cells. We have systematically investigated putative regulatory elements and mechanisms of KRAS by the use of luciferase-based KRAS 3' and 5' UTR reporter assays and poly(A) tail-length assays. Through these approaches, we have now shown that the induction of K-Ras protein expression upon T cell stimulation is largely 3' UTR mediated. While several microRNAs have been implicated in the post-transcriptional regulation of KRAS, the effect we observe seems to be independent of let-7 microRNAs and miR-143. We now aim to map the exact location of and identify the cis-element(s) in the KRAS 3' UTR by which it is regulated and will subsequently use RNA affinity pull-down assays followed by mass spectrometry to identify novel KRAS 3' UTR binding proteins. Regulators identified by this approach will be characterized via gain- and loss-of-function assays. Cumulatively, these experiments have the potential to further our knowledge of the mechanisms by which KRAS may be dysregulated and overexpressed in human cancers.

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SHIVERING AND TACHYCARDIC RESPONSES TO EXTERNAL COOLING IN MICE ARE SUPPRESSED BY TRPV1 ACTIVATION BUT NOT BY TRPM8 INHIBITION.

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Background: Mild decrease of core temperature (32-34°C), also known as therapeutic hypothermia, is a highly effective strategy of neuroprotection from ischemia and holds significant promise in the treatment of stroke. However, induction of hypothermia in conscious stroke patients by traditional physical cooling methods is complicated by cold-defensive responses, such as shivering and tachycardia, which increase metabolic rate and counteract neuroprotection. Thus, there is need for safe and effective methods of inducing hypothermia in conscious subjects. TRPM8 is a cold-sensitive ion channel of the primary nerve fibers in the skin, which is involved in the activation of a number of cold-defensive responses, such as non-shivering thermogenesis and peripheral vasoconstriction. However, its role in shivering and tachycardia is unknown. Therefore, we tested the hypothesis that TRPM8 is required for shivering and tachycardic responses to external cooling and that pharmacological inhibition of TRPM8 facilitates hypothermia by suppressing cold-defensive responses. TRPV1 is a warm-sensitive ion channel expressed within the afferent neural pathways for skin warming. Activation of TRPV1 by agonists is also known to suppress heat generating responses, presumably by mimicking skin counter-warming, but its effect on shivering and tachycardia is similarly unknown. Thus, we tested an additional hypothesis that TRPV1 activation suppresses shivering and tachycardic responses to external cooling and facilitates the induction of hypothermia.

Methods: Conscious mice were treated with TRPM8 inhibitor “compound 5” or TRPV1 agonist dihydrocapsaicin and exposed to cooling at 10°C. Shivering was measured as a total electromyographic muscle activity recorded using implanted electrodes in back muscles, tachycardic response by electrocardiography and core temperature by wireless transmitters in the abdominal cavity. The role of TRPM8 was further determined using TRPM8 KO mice.

Results: TRPM8 ablation had no effect on total electromyographic muscle activity (vehicle: 24.0±1.8; “compound 5”: 23.8±2.0; TRPM8 KO: 19.7±1.9 V*s/min), tachycardia (ΔHR=124±31; 121±13; 121±31 beats/min) and drop in core temperature (-3.6±0.1; -3.4±0.4; -3.6±0.5°C) during cold exposure. TRPV1 activation substantially suppressed muscle activity (vehicle: 25.6±3.0 vs. DHC: 5.1±2.0 V*s/min), tachycardia (ΔHR=204±25 vs. 3±35 beats/min) and produced a profound drop in core temperature (-2.2±0.6 vs. -8.9±0.6°C).

Conclusions: External cooling-induced shivering and tachycardia are suppressed by TRPV1 activation, but not by TRPM8 inhibition. This suggests that TRPV1 agonists may be combined with external physical cooling to achieve more rapid and effective hypothermia.

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A SIMPLIFIED SEQUENCE/STRUCTURE ALPHABET FOR MULTIPLE TRANSMEMBRANE HELICES ASSEMBLIES

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TransMembrane Helical (TMH) domains of integral membrane proteins, like G-protein coupled receptors and ion channels, play critical roles in diverse physiological functions such as the signaling transduction, bioenergetics and ion transportation. Our understanding on their detailed mechanism, however, is limited by lacking structure information due to technique difficulty in conventional experimental methods. Such situations underscore the importance of the knowledge provided through bioinformatics analysis on known structures of TMH domains.

We conducted an exclusive bioinformatics analysis on the sequence and structure motifs defining the packing of multiple TM helices. We generated a library of more than 800 closely packed TMH trimer structure elements by dissecting available crystallographic structure of TMH domains. Based on structure similarity, we found that more than half of the structure elements can be classified into 6 major clusters with distinct geometric feature, which shows the limited conformational space TMH trimers search. The two most abundant clusters contain TMH trimers that have all left-handed pairs and all right-handed pairs, respectively. With a sequence pattern search method (TMSTAT), we also found statistically enriched sequence motifs that correlate with the geometric feature of major classes, suggesting that the identification or the engineering of specific sequence motifs may simplify the prediction or design of TMH trimer structures. Further, we demonstrate that sequence motifs that are enriched on trimer assembly interface also correlate with interaction patterns, indicating the convergent rule of multiple TMH packing.

It is, to our knowledge, the first time that research shows the limited conformational space that the TMH trimer can search. The results will lead to important novel insights in convergent multiple-pass TMH domain folding, structure and will provide information on structure prediction and redesign of complex TMH domains.

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MICROVILLI ARE DISASSEMBLED TO FUEL CLEAVAGE FURROW INGRESSION

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Cell shape change requires cell surface growth, but the source of new membrane fueling this growth is in many cases unknown. A decades-old hypothesis proposes that unfolding microvilli and other cell surface specializations can provide new membrane for cell surface growth during cytokinesis, phagocytosis, wound healing, and cell spreading, although this has never been definitively demonstrated. Here we show that microvilli serve as a membrane source for cleavage furrow growth during cellularization, the first complete cytokinetic event in the Drosophila embryo. Using scanning electron microscopy, we have calculated that microvilli at the start of cellularization contain approximately half the membrane required for furrow ingestion. Accordingly, we see many microvilli before cellularization, but almost none after. Using 3D imaging of live cells over time, we find that microvillar membrane is depleted slowly at the start of cellularization and rapidly later in the process. Remarkably, these microvillar depletion kinetics follow the biphasic kinetics of furrow ingestion. Microvillar membrane is depleted linearly with increasing furrow length, suggesting a mechanistic link between depletion of the microvilli and growth of the furrows. We find that experimental manipulations preventing full microvillar disassembly disrupt furrow ingestion, and conversely, disrupting furrow ingestion kinetics has a similar effect on microvillar depletion kinetics. Together, these results indicate that microvilli provide membrane for furrow ingestion and that furrow ingestion itself drives disassembly of the microvilli. Furthermore, we find that pulse-labeled apical microvillar membrane moves along the plane of the cell surface into the furrows during furrow ingestion, suggesting that microvillar membrane is translocated predominantly by a pulling mechanism, rather than by endocytosis. This work shows that microvilli are disassembled to provide membrane for furrow ingestion and suggests that their disassembly is fueled by a pulling force originating at the ingressing furrows.

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In the neocortex, individual cells of like type can undergo heterogeneous plasticity responses from depression (LTD) to potentiation (LTP), or no change (NC) in response to a common fixed time delay synaptic conditioning protocol. However it is not known whether all synapses onto a common cell have the same plasticity outcome. Nor is it known what role visual experience plays in shaping the distribution of differential plasticity outcomes. Thus, we evaluated the synaptic plasticity responses of separable sets of synaptic inputs onto common postsynaptic neurons in primary visual cortex in response to simultaneous stimulation of distinct sets of afferents in acute brain slices from visually intact and in binocularly deprived mice. Animals were binocularly deprived from before the natural time of eye-opening. The two stimulation sites were isolated by occlusion testing followed by alternative activation of each pathway to evoke a postsynaptic potential (PSP) every 10 seconds in an interleaved fashion. After a stable ten minute baseline period, the activation of both pathways was simultaneously paired with direct postsynaptic activation that preceded the synaptic stimulation by 10 milliseconds resulting in 4-7 postsynaptic spikes at 0.1 Hz over a 10 min period followed by reversion to the interleaved stimulation protocol for an additional 30 minutes. The ratio of the average amplitude of the evoked PSP post/pre conditioning was calculated for each pathway taking the 5 minutes average peak amplitude over 25-30 minutes post-conditioning compared to 5 minutes just before or pre-conditioning. Our results from 57 pathways inputs validate in the mouse cortex our previous findings from other species demonstrating heterogeneous plasticity outcomes ranging from LTD to LTP for individual cells with a mean post/pre ratio of 0.87 +/- 0.32sd for the grouped results. Preliminary data comparing 30 pathways in the binocularly deprived animals also resulted in a similar range of individual plasticity outcomes with an average post/pre ratio of 0.88 +/- 0.40sd for the grouped results that was not significantly different from visually intact animals (p=0.95 t-test). When the plasticity outcomes of the separate pathways were compared to determine whether each set of synapses onto a common cell had similar plasticity behavior, we found that they were independent both in the visually intact animals with a $\chi^2$ of 1.6, 5 df, p=0.9 and in binocularly deprived animals $\chi^2$ of 5.6, 5 df, p=0.35. Thus, synaptic plasticity outcome variability is a local synaptic phenomenon vs. a whole cell property and appears to be intrinsic vs. modifiable by visual experience.

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Our laboratory has defined Steroid Receptor Coactivator 2 (SRC-2) as a “master” regulator of systems wide energy homeostasis. Ablation of SRC-2 results in fatty liver, resistance to diet-induced obesity, and other metabolic disturbances in murine models, therefore, SRC-2 activity may be an important factor in metabolic syndrome associated diseases. Recently, our group published that the interaction between SRC-2 and adenosine monophosphate kinase (AMPK) regulates energy homeostasis. AMPK phosphorylates and activates SRC-2, which in turn increases transcription of the bile salt export pump (BSEP), and therefore fat accretion and whole body energy. Preliminary data suggests that SRC-2 increases AMPK mediated histone phosphorylation, defining a novel mechanism by which these two energy regulating molecules cooperate to identify, interact, and activate target gene transcription. Therefore, we hypothesize that SRC-2 functions as a “master” regulator of energy homeostasis via its interaction with AMPK by altering the post-translational modification code of SRC-2 and histones, which leads to alterations in expression of metabolically relevant genes. To understand the cistromic regulation of metabolic genes by SRC-2 and AMPK, we have overlapped ChIP-seq peaks of these respective proteins to determine common biology. We will be able to confirm the interaction of SRC-2 and AMPK on their target promoters using a novel DNA pull-down technique. By using these two techniques in tandem, we will be able to fully characterize the interaction of SRC-2 and AMPK and determine how these two key players in energy homeostasis can regulate genes with related metabolic functions.

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Lysosomal lipolysis promotes longevity through a lipid-responsive nuclear hormone receptor signaling pathway

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Fat storage and metabolism have long been associated with metabolic health and aging, but the role that lipid signaling plays in the regulation of longevity has not been elucidated. Lysosomes are cellular organelles well known for their function in the digestion and recycling of intra- and extra-cellular macromolecules, and lysosomal dysfunction is associated with lipid storage diseases. Despite the importance of these organelles, a signaling role for the lysosome has not been previously identified. To explore the role of the lysosome in metabolism and aging, we focused on lipl-4, a homolog of human lysosomal acid lipase, which had been identified as a novel regulator of longevity in C. elegans. We hypothesized that lysosomal lipolysis may generate lipid messengers to modulate lifespan-regulating signaling processes. Through metabolomic profiling, we found lipl-4 over-expression increases abundance of oleoylethanolamide, an N-acyl ethanolamide known to function as an endogenous agonist of PPAR( in vertebrates. Both lipl-4 over-expression and feeding of oleoylethanolamide are sufficient to induce the expression of the fatty acid binding protein lbp-8 and the acyl-CoA synthetase acs-2. This transcriptional induction is dependent on the nuclear hormone receptors NHR-49 and NHR-80 and the mediator subunit MDT-15. Interestingly, lbp-8 is not only required for the lifespan extension, but is itself sufficient to promote both longevity and the transcriptional induction of acs-2. We found that LBP-8 is localized to the lysosome under normal conditions, but translocates to the nucleus when lipl-4 is over-expressed supporting a role for LBP-8 in transducing a lipid signal from the lysosome to the nucleus. Additionally, nhr-49, nhr-80 and mdt-15 are also required for the lifespan extension phenotype. These results suggest that lipl-4-mediated lysosomal lipolysis extends lifespan by activating an oleoylethanolamide-NHR-49/NHR-80 transcriptional signaling pathway. In summary, we have identified a signaling role for the lysosome in the regulation of longevity and metabolism, and identified a potential longevity-promoting lipid metabolite.

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Defining the Roles of Cellular Senescence in Ovarian Tumorigenesis

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Ovarian cancer is the most lethal gynecologic malignancy and the fifth most common cause of cancer death in women. Based on previous studies, defects in cellular senescence have been implicated as critical to understanding the pathogenesis of cancer. The ability of senescent cells to cease division is an important response to block the proliferation of cancer cells. Moreover, dysfunction in senescence pathways can lead to a decreased ability for cells to be removed and/or allow for aberrant proliferation. Our lab has been studying ovarian cancer in a mouse model lacking inhibin (Inha). These Inha null mice exhibit dysregulation in important cell-cycle complexes and cellular senescence regulators, and develop fatal ovarian granulosa cell tumors. S-phase kinase protein 2 (SKP2), an E3-ubiquitin ligase, marks p27 and p21 for degradation. Degradation of these tumor suppressors allow for the up-regulation of Cdk2 and the subsequent G1 to S-phase transition, generating proliferation and cell growth. Our microarray data showed that multiple genes encoding cell senescence regulators are upregulated in Inha null granulosa cells prior to cancer formation. This information lends the hypothesis that cellular senescence pathways regulated specifically by Skp2, and proteins upstream (MYC family members) and downstream (cyclin/CDK complexes), control the development of granulosa cell cancers. The central hypotheses are that: 1) Cellular senescence in the ovary controls the balance of apoptosis, proliferation, and differentiation of ovarian cancer; and 2) Alterations in key components of senescence (i.e., Skp2, cyclinA2/E1/E2/Cdk2) can influence ovarian tumorigenesis. Inha/Skp2 and Inha/Cdk2 double knockout (DKO) female mice have been created to study cellular senescence and cell-cycle regulation in granulosa cell proliferation and differentiation. Inha/Cdkn2a DKO mice have also been created to elucidate the involvement of alternate cellular senescence pathways in sex-cord stromal tumorigenesis. Initial survival analysis shows a delayed onset of tumor formation in the Inha/Cdk2 double knockout mouse, with tumors being evident at approximately 30 weeks of age in contrast to the Inha single knockout mice, which develop tumors at 4 weeks of age. Further, Cdk2 single knockout mice do not develop tumors but express a reduction in overall body weight, highlighting the proliferative role of Cdk2 in cellular development and senescence. In stark contrast, tumor formation in the Inha/Cdkn2a double knockout mice mirrors the Inha single knockout mice, as opposed to Cdkn2a single knockout mice which show tumor formation at 34 weeks of age, typically as sarcomas and lymphomas. Further, the Inha/Cdkn2a DKO mice show comparable tumor histology to Inha single knockout, indicating no difference in type of ovarian tumors. This research is supported by NIH grant HD32067 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Cancer Institute CA138628.

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NR4A1-3 REGULATES THE PROLIFERATION OF ADULT HEMATOPOIETIC STEM AND PROGENITOR CELLS

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The transcription factors NR4A1, NR4A2 and NR4A3 are orphan nuclear receptors that regulate the transcription of genes involved in different biological processes such as energy metabolism, DNA damage and differentiation. Our group has previously shown that germline deletion of both Nr4a1 and Nr4a3 causes acute myeloid leukemia in mouse models within 2-4 weeks after birth. In adult mice, global depletion of Nr4a1 and Nr4a3 causes acute myeloid leukemia in 12 to 15 weeks after Nr4a1/3 ablation. Moreover, studies in AML cell lines have shown that forced expression of NR4A1/3 represses a MYC-regulated gene signature characteristic of leukemia-initiating cells. Here, we investigate the biological mechanisms by which NR4A1/3 control the cell cycle and differentiation in hematopoietic stem and progenitor cells in adult mice. The cell cycle effects of acute Nr4a1/3 ablation in hematopoietic cells were analyzed using a tamoxifen inducible Rosa26-Cre-ERT2; Nr4a1f1/fl; Nr4a3-/- mouse model. The acute deletion of Nr4a1/3 caused a significant accumulation of long-term hematopoietic stem cell (lin- c-Kit+ Sca1+ CD150+ CD48-) 4 days after Cre induction. Cell cycle analysis using the Ki67 marker revealed that the hematopoietic stem cells and progenitors cells of both myeloid and lymphoid lineages have a significant increase in the proportion of proliferative cells. Analysis of MYC expression by flow cytometry indicates that the expression of MYC is increased upon Nr4a1/3 ablation in hematopoietic stem and progenitor cells. To observe the long-term effects of Nr4a1/3 ablation, the same analyses were repeated 4 weeks after Cre induction. At this time point, hematopoietic stem and progenitor cells were less frequent in the absence of Nr4a1/3 when compared to control mice, although they maintained a higher proportion of Ki67-positive cells and higher MYC expression. These results suggest that stem cells are being exhausted due to uncontrolled proliferation. We are currently performing a system-wide analysis of NR4A1/3 binding and their effect on gene expression by combining ChIP-Seq and RNA-Seq analyses in the rare long-term hematopoietic stem cell population. The study of the mechanism by which NR4A1/3 control the cell cycle and differentiation of hematopoietic stem cells might reveal new pathways controlling normal adult hematopoiesis and neoplasia.

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The visual system has evolved to process ecologically relevant information in the organism’s natural environment, and thus it is believed to have adapted to its statistical properties. Based on theoretical models, neural populations in primary visual cortex have been suggested to encode natural scenes with few active neurons (“sparsely”), driven by the higher-order correlations in such images. Experimental tests of this hypothesis have been limited to recordings of few neurons at a time, leaving the structure of the population response and the functional benefits of this optimized representation an open question. We recorded simultaneously from up to 510 neurons in the primary visual cortex of anesthetized and awake mice using two-photon imaging, recording from most of the neurons in a small region of cortex. We show that natural movie scenes were encoded more reliably and sparsely in mouse V1 than movie scenes from which the critical higher-order structure had been removed. In addition, we found that the increase in population sparseness and reliability strongly correlated with an improvement in discriminating natural scenes. This link argues for a functional benefit of sparse coding facilitating read-out of natural scenes by neural populations in higher visual areas. Thus, our results provide new insights into sparse coding as a principle for explaining the response properties of neurons in primary visual cortex.

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Metastasis-Associated Alteration of Gata6 and TG2 Expression in Osteosarcoma

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Osteosarcoma (OS) is the most common form of primary bone cancer. Age of OS incidence follows a bimodal distribution with a higher incidence in pediatric and adolescent patients. It is found primarily in long bones such as the femur and humerus, with metastases most often arising in the lung or other bony sites. Metastatic spread of OS from the primary tumor to distant metastatic sites drastically decreases survival rate. Current treatment of metastatic OS still largely parallels that of the non-metastatic disease, usually consisting of surgical resection of the primary tumor and adjuvant chemotherapy. Treatment of metastatic OS would be facilitated by a better understanding of factors driving osteosarcoma metastasis. Metastatic progression is often enabled through altered expression of genes affecting motility, invasive potential, immune evasion, and other significant functions. We hypothesize that specific genetic and molecular changes are required for OS cells to metastasize from the primary tumor and colonize a distant, physiologically distinct site.

Because chemotherapy is begun immediately following diagnosis of OS, there is limited access to samples of lung metastases which have not already been exposed to chemotherapeutic regimen and, thus, some selection. In order to investigate untreated lung metastasis, our lab has developed genetically engineered mouse models (GEMMs) which phenocopy either metastatic or non-metastatic osteosarcoma. Samples of metastatic osteosarcoma were isolated from several animals within our GEMM. Using high-throughput genome-wide microarray, we screened RNA isolated from these metastatic samples for genes showing higher levels of expression in the metastatic lung lesion than in the primary tumor. These microarrays were subsequently repeated with cell lines derived from corresponding primary and metastatic lesions. Several factors exhibited higher mRNA levels in lung metastases than their corresponding primary tumor, both in tissue and cell line mRNA. Statistical analysis and stringent filtering yielded high-probability candidates which were subsequently corroborated through multiple-sample quantitative PCR (qPCR). Some cell line protein levels were also checked through Western Blot to confirm upregulation. Corresponding data in human-derived samples were analyzed and confirmed upregulation of Gata6 and TG2. Analysis via Ingenuity Pathway Analysis (IPA) showed important connections to immune evasion, cell motility, and other pro-metastatic survival functions for two of the consistently-upregulated factors: Gata6 and Transglutaminase 2 (TG2). These pro-metastatic mechanisms may be required for OS dissemination, colonization and metastatic tumor growth. Functional studies indicate that alterations in Gata6 and TG2 affect cellular phenotypes which might facilitate metastatic disease through multiple functions. In vivo experiments investigating metastasis enhancement are ongoing.

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THE ROLE OF ONCOGENIC PHOSPHATASE WIP1 IN CELL CYCLE INHIBITION BY PPM1B AND CDKN1B

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A mammalian cell undergoes the DNA Damage Response (DDR) after encountering various types of genotoxic stresses, such as ultraviolet and ionizing radiation, which results in single or double-stranded breaks in the DNA. The sensor kinases ATM and ATR detect this damage and activate downstream effectors through phosphorylation cascades to promote various cellular responses, including cell cycle arrest. Once DNA repair is complete, the expression of Wild-type p53-induced phosphatase 1 (Wip1) is induced to return the cell to a homeostatic state. Wip1 has been shown to dephosphorylate and downregulate various DDR mediators and effectors, including p53, Chk1/2, ATM/ATR, Mdm2, and H2AX.

Wip1 is amplified and overexpressed in numerous human cancers. Wip1 overexpression in cancer cells expressing wild-type p53 leads to suppression of p53 activity. Wip1 inhibition, either chemically or through the overexpression of an inhibitory miRNA, has been shown to suppress tumor growth via induction of cellular senescence or apoptosis. Thus, understanding the influence of Wip1 on various cellular pathways involved in cancer is critical, since Wip1 has been suggested to be a potential therapeutic target.

Currently, our knowledge of the function of Wip1 is mainly limited to its role in the DDR pathway. Preliminary data from our lab suggests that Wip1 may play a role in cell cycle regulation. We found that Wip1 can target a possible inhibitor of the cell cycle, PPM1B. PPM1B, a protein phosphatase that is closely related to Wip1, has been implicated in the direct dephosphorylation of CDK1 and CDK2. Our findings also suggest that Wip1 can target CDKN1B (also known as p27Kip1), another known inhibitor of CDK2 and associated cyclins that also directly regulate the cell cycle. Thus, we believe that Wip1 may play a more direct role in controlling the cell cycle than has previously been suggested.

We hypothesize that Wip1 dephosphorylates PPM1B and CDKN1B after completion of DNA Damage Response to promote cell cycle progression. To investigate the role of PPM1B, we are examining the ability of Wip1 to target PPM1B T166, and testing the ability of Wip1 to activate CDK1 and CDK2 through suppression of PPM1B. We are also examining the ability of ATM to reverse this process by negatively regulating PPM1B at this putative ATM/ATR target site. To investigate the role of CDKN1B, we are examining the ability of Wip1 to target CDKN1B S140, a site implicated in DDR signaling by ATM, and testing the ability of Wip1 to promote cell cycle progression by deactivating CDKN1B, a known cell cycle inhibitor. The significance of these findings is the potential for Wip1 to significantly affect tumor progression in novel pathways involving CDKs, PPM1B, and CDKN1B, leading to additional targets for cancer treatments that involve Wip1 modulation.

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SIGNALING THROUGH SMAD4 IN PANCREATIC CANCER

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Pancreatic cancer is fourth-leading cause of cancer death in the US with approximately 43,000 cases diagnosed each year. The disease is very severe with a 5-year survival rate of 5% and no effective treatment options for the 80% of patients who present with advanced disease. SMAD4 is mutated or lost in around 55% of pancreatic cancers, and mutation/loss correlates with both metastatic disease and poor prognosis. SMAD4 is a downstream signaling protein in the TGF-β family pathways, and SMAD4 loss could act as a switch that changes responses to TGF-β signaling from anti to pro-tumor. However, the role SMAD4 loss plays in this transition and more broadly in promoting disease progression remains poorly understood in pancreatic cancer. Understanding the contribution of SMAD4 loss to aberrant TGF-β family signaling responses in pancreatic cancer will not only enhance our understanding of this deadly disease, but also boost efforts to develop treatments. Our previous studies have found that lentiviral restoration of SMAD4 expression in the SMAD4-null human pancreatic cancer cell line BxPC3 has powerful anti-proliferative effects, but the mechanism underlying this effect is unknown. Using an inducible system for SMAD4 restoration in SMAD4-null human pancreatic cancer cell lines, BxPC3, CFPAC1, and Hs766t, we will interrogate the effects of SMAD4 restoration on global gene expression in response to TGF-β. By comparing the expression profiles of these cell lines with and without SMAD4, we hope to elucidate the direct and downstream regulatory targets of SMAD4 in pancreatic cancer relevant to the roles of SMAD4 and TGF-β signaling in promoting pancreatic cancer disease progression.

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Microbial derived histamine: effects on TNF production by intestinal macrophages

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Chronic intestinal inflammation reduces quality of life and is deleterious to intestinal tissues. Due to the intimate relationship between the intestinal microbiota and host intestinal tissues, probiotic therapies are an attractive approach for treating chronic colitis, yet no such therapy has been proven effective in inducing remission and maintenance of chronic intestinal inflammation. Currently, therapies with the TNF-α directed monoclonal antibodies are indicated and effective for IBD refractory to corticosteroids, but are prohibitively expensive for some patients and require long-term administration [1]. In vitro, administration of the versatile, biogenic amine, histamine reduces expression and production of TNF-α in human monocyte derived macrophages [2]. The probiotic, lactic acid bacterium, Lactobacillus reuteri is a native inhabitant of the mammalian gut and produces and secretes histamine. Administration of L. reuteri ATCC 6475 to mice with TNBS-induced colitis ameliorates local and systemic markers of inflammation in a histamine-dependent manner [†]. Production and secretion of histamine by L. reuteri ATCC 6475 in the gut is, therefore, hypothesized to reduce colitis by inhibiting TNF production from intestinal macrophages; however the mechanism by which this occurs in not fully elucidated. The mechanism by which histamine signaling reduces TNF transcription in intestinal macrophages will be determined by assaying phosphorylation states of MAP kinase pathway proteins in macrophages exposed to histamine in vitro, or L. reuteri ATCC 6475 in vivo. Elucidation of this mechanism is essential for understanding the probiotic functions of microbial-derived histamine and its clinical applications and limitations.

†. Unpublished data.

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Autophagy is the only known biological process in which entire defective organelles within a cell can be degraded in addition to damaged proteins. Autophagy is characterized by the formation of a double membrane structure called autophagosome that sequesters intracellular cargo and delivers it to the lysosomes for proteolytic degradation. Such a quality control mechanism has been proposed to be important in long-lived post-mitotic neurons and defects in autophagy have been implicated in several neurodegenerative diseases. We are interested in identifying novel genes involved in autophagy that affect neuronal function. We generated a collection of X chromosome mutants to identify essential genes in Drosophila involved in neurodegeneration and synaptic transmission using electroretinogram as an initial read out. One of the genes we isolated from this screen was cacophony (cac) and the ERG traces show a loss of on transient but not a significant decrease in depolarization amplitude even in aged mutants. Cac encodes the pore-forming α1 subunit of voltage gated calcium channel (VGCC). Transmission electron microscopy (TEM) images at the retina of mutant flies show no major morphological defects even in aged animals. However, the mutant lamina are affected even in young animals. As the mutant flies get older, the photoreceptor terminals start losing their capitate projections, active zones and their basic cartridge structure. In addition, they also accumulate mitochondria and autophagic vacuoles (AVs). Upon quantification, we observed that the number of autophagosomes and lysosomes is unchanged in the mutant terminals, but there is a massive increase in the intermediate AVs and especially fusion-defective AVs. This suggests that Cac is required for autophagosomal maturation. Cac, its accessory subunits and the SNARE complex are involved in neurotransmitter release through synaptic vesicle fusion in a calcium dependent manner. Surprised to find that Cac affected autophagy, we also tested eyes mutant for the α2δ subunit of the VGCC, straightjacket (stj), and observed a similar accumulation of late stage AVs in photoreceptor terminals. Interestingly, the autophagy defects rescued if the flies are raised in complete darkness, suggesting that autophagy defects are not only synapse specific, but also activity dependent. Mutations in these highly conserved VGCC subunits in mammals cause severe neurological diseases such as spinocerebellar ataxia 6, episodic ataxia type 2 and migraines, but the molecular mechanisms for these diseases are still undefined. Although calcium and certain SNAREs have been shown to be required for autophagy, a VGCC has not previously been directly implicated to play a role in the autophagy pathway. We propose that Cac plays a role in autophagy by regulating the fusion of the autophagic vacuoles with the lysosome, similar to that in synaptic transmission. Defects in Cac-mediated autophagy may also be responsible for the aforementioned diseases.
In structural biology traditional single particle analysis (SPA) can produce structures of macromolecular assemblies in solution at resolutions now approaching x-ray crystallography. However, there remain a number of situations where it cannot be readily applied. When macromolecules in solution undergo substantial motion or there is a need to study assemblies in-situ, single particle cryo-electron tomography (SPT, aka subtomogram averaging) offers a powerful alternative approach. By providing a 3-D view of individual macromolecular complexes, it minimizes the problem that arises in SPA of distinguishing between different conformations and different orientations of the complex in solution. As such, SPT can address the study of macromolecular structures that are not amenable to other techniques.

Although SPT has gained much popularity over the last few years, more reliable and efficient methodologies are still under active development. We present EMAN2’s (1) complete suite of tools for SPT, including a graphical 3-D particle picker, a complete pipeline of alignment and averaging applications, and post-averaging analysis tools. This development leverages our previously published methods (2), introducing an alternate missing wedge compensation metric based on FSC thresholding. The alignment algorithms include a CUDA implementation for computation using GPUs, providing significant speedup. Additionally, angular sampling has been optimized, among other implementations to further improve processing speed and accuracy, such as parallelization to run alignments on workstations and clusters. Examples using two chaperonins and the epsilon-15 phage demonstrate some of the software’s capabilities.

Beta adrenergic receptors are important for cardiovascular regulation and for physiological responses to the hormones/neurotransmitters adrenaline and noradrenaline in the nervous system and throughout the body and they are the targets of numerous widely used drugs. The downstream signaling of these receptors was long been thought to depend on cAMP-dependent protein kinase (PKA) and to be distinct from those downstream of G-protein coupled receptors which activate phospholipase C (PLC) and elevate intracellular Ca2+. By monitoring of intracellular Ca2+ levels in real time using a fluorescent indicator dye we found that an endogenous receptor in HEK-293 cells responds to the adrenergic agonist norepinephrine, by a delayed rise in intracellular [Ca2+]. The response is blocked by ICI 118,551, a selective antagonist for beta-2 adrenergic receptor (β2-AR), and the relative potency of agonists is isoproterenol > epinephrine > norepinephrine, consistent with the pharmacological profile of β2-AR.Treatment with cholera toxin indicated that activation of Gαs is sufficient for Ca2+ release. Increasing intracellular cyclic AMP levels by treatment with phosphodiesterase inhibitors IBMX and rolipram, potentiates the response. However, treatment with PKA inhibitors H-89 and KT5720 had no effect on the Ca2+ signal, nor did treatment with the cAMP analogue 8-bromo-cAMP which strongly suggests that the cAMP effector PKA is not involved in this signaling pathway. Treatment with thapsigargin (an inhibitor of the SERCA Ca2+ pump of the endoplasmic reticulum) and chelation of extracellular Ca2+ revealed that the Ca2+ is released from intracellular stores. The release is sensitive to inhibition of PLC with U73122 and of InsP3 receptors with 2-APB. These results reveal a novel pathway by which β2-AR can trigger the release of Ca2+ from intracellular stores in a PKA independent manner. Preliminary evidence suggests that the cAMP-activated guanine nucleotide exchange factor (GEF), EPAC couples cAMP production to PLC activation and Ca2+ release. These results point to previously unrecognized modes of action of beta agonists and blockers.
Supplementation with certain probiotic Lactobacillus reuteri strains that naturally colonize the gut of mammals has been found effective at ameliorating intestinal inflammation in rodent colitis models, but the underlying mechanisms are unknown. Pangenomic studies showed that L. reuteri strains with anti-inflammatory properties contain a complete hdc gene cluster which is responsible for synthesis and secretion of histamine. These findings indicate a potential role for histamine in alleviation of inflammation. L. reuteri 6475 which contains an intact hdc gene cluster was found to suppress TNF production in activated THP-1 cells through the production of histamine and activation of histamine receptor 2 (H2R). Targeted mutagenesis of the hdc genes demonstrated diminished anti-TNF activity and loss of histamine production, indicating the anti-TNF activity of histamine in vitro. Using a trinitrobenzene sulfonic acid-induced mouse model of colitis, L. reuteri 6475 administration was found to protect eight-week female BALB/c mice against colitis, as indicated by significantly decreased weight loss, colonic damage graded by the Wallace score and serum amyloid A protein concentrations compared to media control. The hdcA mutant of L. reuteri 6475 which failed to produce histamine showed diminished ability to attenuate colitis. Moreover, H2R was detected in the mouse colon by immunohistochemistry and blocking H2R with its specific antagonist ranitidine diminished the anti-inflammatory ability of L. reuteri 6475. In addition, feeding mice with a histidine free diet diminished L. reuteri’s ability to attenuate colitis. These combined investigations indicate that L. reuteri 6475 attenuates experimental colitis via histamine production, and provides important insights into understanding the molecular mechanisms underlying probiotic immunomodulation.
Local neuropeptide signaling promotes synaptogenesis, plasticity, and neuronal circuit integration in adult brain tissue

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Neuronal circuit plasticity and integration underlie brain development, physiological homeostasis, memory, and behavior. Diverse forms of neural activity either enhance or decrease synapse and circuit plasticity, but how activity is mechanistically relayed to lead to circuit integration is largely unknown. With the goal of identifying signaling mechanisms linking activity to circuit integration in the adult brain, we sought to identify cell types that provide presynaptic inputs onto new neurons that are continuously generated in the adult rodent olfactory bulb. Using viral transsynaptic tracing, optical imaging, behavioral assays, electrophysiology, and optogenetic stimulation, we have uncovered a novel neuropeptidergic signaling mechanism whereby the secreted neuropeptide Corticotropin-releasing hormone (CRH) acts locally to promote and/or stabilize chemical synapses in adult brain tissue. These data reveal a novel mechanism linking in vivo neuronal activity to a downstream signaling pathway for continued circuit plasticity, synapse formation, and integration of new neurons in the adult brain.

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Melanoma-associated chondroitin sulphate proteoglycan (CSPG4) is a well characterized antigen first identified in melanoma and then reported in other solid tumors such as head and neck carcinoma, mesothelioma and basal breast carcinoma. CSPG4 participates in tumor migration, angiogenesis and metastasis and is frequently over-expressed in tumors. Taking into consideration its broad expression in cancer cells, its limited expression in normal tissues and its pivotal role in tumor survival, targeting this antigen with CAR-redirected T cells may offer a valid therapeutic approach for several solid tumors. We constructed a CAR (encoding the CD28 costimulatory endodomain) targeting CSPG4 (CAR.CSPG4) into a retroviral vector. After transduction, T lymphocytes stably and efficiently expressed the CAR (65%-88%) and lysed the melanoma cells SENMA significantly better (59%±6%) than control T cells (11%±8%). Furthermore, in long-term coculture assays, CAR.CSPG4+ T cells efficiently and consistently eliminated several CSPG4+ targets including melanoma (SENMA and CLB, residual tumors: 0.1%±0.06% and 0.1%±0.1, respectively), mesothelioma (PH1 and MILL: 3.8%±3.1%; 3.2%±5%), head and neck carcinoma (PCI-30, 0.5%±0.5%), and basal breast carcinoma (UACC-812 and MDA-MB-231: 5.7% ±6.1%; 3.1% ±2.5%, respectively) while having no effect on a CSPG4– targets (38% ±10%). As expected all tumor cells expanded in coculture with control T cells. The antitumor activity of CAR.CSPG4+ T cells was paralleled by release of Th1 cytokines, such as IL2 (from 6±10 pg/µL to 190±98 pg/µL) and IFN (from 105±48 pg/µL to 3710±975 pg/µL) upon coculture with different CSPG4+ tumors. Both CAR.CSPG4 transgenic CD4+ and CD8+ cells proliferated in response to SENMA tumor cells as compared to control T cells, as assessed by CFSE dilution assays. In vivo experiments were conducted using NSG mice (n=10/group) either melanoma (SENMA) or head and neck carcinoma (PCI-30) or basal breast carcinoma (UACC-812) cells were engrafted s.c. Across all tumor models, mice treated with CAR.CSPG4+ T lymphocytes consistently showed tumor control as compared to mice receiving control T lymphocytes by days 40-50 post tumor engraftment. In summary, CAR.CSPG4-redirected T cells can be used for the treatment of a variety of solid tumors.

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LOSS OF MIR-148 PROMOTES METASTASIS OF OVARIAN CANCER BY TARGETING MTMR9 AND PHOSPHATIDYLINOSITOL PHOSPHATE METABOLISM

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RATIONALE: Epithelial ovarian cancer (EOC) is the 5th leading cause of cancer death for women and results in >15,000 deaths annually because of recurrence of chemoresistant cancer. Treatment of recurrence is especially difficult and alternatives must be developed to increase overall patient survival. Utilizing data from the Cancer Genome Atlas Consortium (TCGA), we recently found that levels of two microRNAs (miR-148a and miR-148) independently correlate with progression free (PFS) and overall (OS) ovarian cancer survival (TCGA dataset, n = 581 patients; HR for miR-148a= 0.84 [0.75, 0.94], p=0.003; HR for miR-148b: 0.71 [0.58, 0.87], p=0.001). This translates to over a year of extra life for women whose cancers express high levels of miR-148a/b. Collectively, our data clearly establishes that both miR-148a/b play a critical role in determining ovarian cancer outcome. However, the mechanisms by which these miRNAs impact ovarian cancer are not clear. Our preliminary data linking levels of miR-148a/b with survival have led us to hypothesize that these miRNAs directly impact ovarian cancer growth and metastasis.

RESULTS: Endogenous levels of miR-148a/b are differentially expressed in the cell lines and in the primary samples. Over 50% of ovarian cancer samples tested had significantly lower expression of both miR-148a/b. We have uncovered that overexpressing miR-148a/b in OVCAR8 (p53 mutant) and SkOViP31 (p53 null) cells leads to a significant decrease in cell proliferation and an increase in apoptosis, but this trend is inverted in HeyA8 cells which are p53 wildtype suggesting that miR-148a/b may be working in a p53 dependent manner. Overexpression of miR-148a/b leads to significantly lower rates of migration and invasion in both HeyA8 and OVCAR8 cells suggesting that miR-148 plays a role in metastasis. Using a new method called Lasso, we have identified and validated a novel gene target, MTMR9, which has never been classified in a cancer model. Our data shows that miR-148a/a*b directly targets MTMR9, an inactive myotubularin, in EOC. In HeLa cells, MTMR9 dimerizes with other MTMRs, such as MTMR7 and 13, to increase their catalytic activity. By immunoprecipitation (IP) of MTMR9 from EOC cell lines, we have shown that MTMR9 dimerizes with MTMR6 and MTMR8 to prevent apoptosis and autophagy, respectively, which has never been shown in cancerous cells. The mechanism of this binding will be investigated by enzymatic assays of these complexes toward PtdIns(3)P and specific autophagy assays. MTMR9’s novel biological significance in EOC was proven by knocking down MTMR9 with siRNA showing that this decreases proliferation and induces apoptosis in HeyA8 and OVCAR8 cells.

CONCLUSION: Overall, we have shown that miR-148a and miR-148b play a critical role in EOC biology and may be working as a tumor suppressor by targeting MTMR9, a novel target that hasn’t been classified in any cancers. MTMR9 may be contributing to ovarian cancer progression by dimerizing with MTMR6 and MTMR8 to prevent autophagy and apoptosis. Using the mechanism of miR-148a/b, our ultimate goal will be to determine whether synthetic mimics for miR-148a/b can be used in conjunction with resection and chemotherapies to directly improve outcomes for women diagnosed with ovarian cancer.

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LETHAL LOVE: BIOENGINEERING A BACTERIUM WITH ANTIMICROBIAL ACTIVITY BY EXPLOITING BACTERIAL CONJUGATION TO DELIVER TOXIC ELEMENTS

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Bacterial infections resistant to antibiotics are an alarming threat as the incidence numbers continue to rise, leaving us in need of new antimicrobial methods. We propose to engineer an antimicrobial bacterium, or rather, a bacterium able to kill other bacteria. With this novel antimicrobial approach, we highjack bacterial conjugation as a platform to deliver toxic elements to bacteria. Conjugation is the horizontal transfer of genes between bacteria via a conjugative plasmid through a sex pilus from the donor to the recipient bacterium. This process is a very efficient mode of gene transfer and is quite promiscuous, theoretically allowing us to target a variety of bacteria. Additionally, with the current methodologies of bacterial genetic engineering, we are able to construct a variety of conjugative plasmids encoding toxic genes of choice with ease. For the toxic component of our antimicrobial platform, we envision utilizing either toxins from bacterial toxin-antitoxin systems or the Cas endonuclease from bacterial CRISPR/Cas systems. Both options are highly advantageous, untapped resources for potential antimicrobials. The TA system toxins are known to be quite toxic to their bacterial hosts in absence of the cognate antitoxin, and have thus often been suggested as putative alternatives to antibiotics. Here, the engineered bacterium carrying the toxin-encoding conjugative plasmid mates with a recipient, transferring and expressing the toxin, killing the recipient bacterium. For our other choice toxic element, the CRISPR/Cas systems are also known to be lethal to the host bacterium if targeted to cleave bacterial host DNA. The CRISPR system is very exciting as a putative antimicrobial as the Cas nuclease can be targeted to cleave very specific SNPs of DNA, and this specificity allows us to target its lethal activity to bacterial pathogen SNPs. In this case, the engineered bacterium carries a conjugative plasmid encoding the Cas nuclease targeted to a desired pathogen SNP. Upon mating, the Cas nuclease specifically cleaves the pathogen DNA, thereby only killing the desired pathogen.

Taken together, this antimicrobial platform combines the toxic elements of TA systems and CRISPR/Cas systems with conjugation as a delivery mechanism, transferred by a harmless bacterial carrier. We are initially testing this antimicrobial strategy by performing mating assays between our engineered strains against non-pathogenic lab strains of E. coli, scoring for recipient cell viability. Preliminary data shows this method is effective against E. coli, and next, we will be performing mating and viability assays against a variety of bacterial pathogens. In the future, this work may lead to the development of this system for application in biotherapeutics or biocontainment.

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BLADDER BIOPSY AT 3 MONTHS POST- BACILLUS CALMETTE-GUERIN (BCG) INDUCTION FOR HIGH-RISK NON-MUSCLE INVASIVE BLADDER CANCER (NMIBC) IS NOT CORRELATED WITH ONCOLOGICAL OUTCOMES

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Introduction and Objective: The clinical response of high-grade (HG) NMIBC to BCG induction is traditionally assessed by biopsy 3 months after treatment. Few studies examine the contribution of the biopsy to outcomes, given the high sensitivity of cystoscopy and cytology. Therefore the objective of this study is to examine the association of the 3 and 6 month cystoscopy, cytology and biopsy with oncological outcomes of HG NMIBC receiving BCG.

Methods: We queried our IRB-approved database (CAISIS) to identify cases of NMIBC that received BCG between Jan 2005 and May 2012. A total of 98 unique patients were identified after excluding 3 for inability to complete at least 5 instillations of induction BCG. Variables included initial T-stage, BCG therapy, cystoscopy, cytology and pathology results at 3 and 6 months post-BCG induction, and oncological outcomes including recurrence, progression and need for radical cystectomy (RC).

Results: The majority of patients were males (85.7%) with median age of 68 (IQR, 61 – 76) years. Forty-nine (50%), 26 (26.5%) and 23 (23.5%) had T1HG, TaHG, and Tis, respectively. CIS associated with papillary disease was observed in 33 (33.7%) patients. Median follow-up in those free from events was 38 months. Overall, 20 patients recurred with a median time to recurrence of 21.4 months. Disease progression occurred in 7 patients and limited the ability of the regression models to identify significant associations with this endpoint. Twenty-seven patients required RC. Cystoscopy, cytology and biopsy results at 3 months were inter-correlated (Pearson’s correlation 0.564, 0.902 and 0.562, all p<0.001). However, the 3 month cystoscopy, cytology and biopsy evaluations were not significantly associated with recurrence, progression or need for RC in this cohort of patients. Cystoscopy and biopsy at 6 months were significantly associated with recurrence and need for RC, and the 6 month cytology was only correlated with the need for RC.

Conclusions: This data suggests that bladder biopsy at 3 months after BCG induction in HG NMIBC may not provide additional prognostic information. Because of significant correlation between the 3 months variables, a standard office evaluation with cystoscopy and cytology only may suffice. Additionally, cystoscopic assessment at 6 months after BCG induction remains a strong prognostic factor associated with recurrence and need for RC.

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Background: Telomerase maintains telomeric DNA at chromosome ends. Constitutional telomerase mutations are associated with short telomeres and a spectrum of disorders including pulmonary fibrosis and liver disease, myelodysplastic syndrome (MDS), and dyskeratosis congenita (DC). DC confers a 90% lifetime risk for marrow failure, a 200-fold risk for AML, and a 2500-fold risk for MDS. Chemotherapy may accelerate telomere shortening and promote phenotypic manifestations in those with underlying defects in telomerase. We investigated the incidence of constitutional telomerase variants in pediatric AML in relation to therapy-related adverse events (AE’s). We hypothesized that these variants would (1) be more frequent in AML cases than controls, and (2) be associated with a telomerase defect phenotype.

Methods: We sequenced the exons/flanking introns of four telomerase-related genes in a local pediatric AML/MDS cohort (n=104), a national AML cohort (n=115), and controls (n=254). Local cohort medical records were reviewed for suggestions of a telomerase defect, including family history of cancer, liver, or pulmonary disease, chemotherapy delays due to cytopenias(s), liver or pulmonary disease, persistent cytopenias after therapy, second cancers, and skin, nail, and mucosal abnormalities. For the COG cohort, we compared the number of variants and telomere length (TL) in subjects with ANC recovery time >1 SD above the mean for >2 chemotherapy courses (n=53) to those with ANC recovery time <1 SD above the mean for 5 courses (n=62).

Results: In the local cohort, 13 variants resulting in missense changes or deletions were found in 21/101 subjects (20.8%). Of note, a large proportion of variants were novel (8/13) and significantly more common than in controls (p<0.0001). Analyzed by logistic regression, the total number of DC-like clinical features was associated with the presence of a variant (p=0.052). Within the COG AML cohort, no difference was noted in the number of variants with respect to ANC recovery, nor was the presence of a variant or TL predictive of specific AE’s. In the fourth chemotherapy cycle we observed a significant association between the shortest RTL quartile and delays in ANC recovery (p=0.03), with a trend to this effect in the fifth cycle (p=0.08).

Conclusions: Novel constitutional variants in telomerase-related genes are enriched in pediatric AML, and variants are associated with characteristics of defects in telomere biology. Shorter telomeres are associated with delays in ANC recovery in later cycles of chemotherapy, presumably reflecting a stress in capacity for hematopoietic reconstitution. Prospective evaluation of these questions in conjunction with correlative functional analyses may provide valuable insight to therapy-related AE’s in pediatric AML.
REGULATION OF E2F1 TRANSCRIPTIONAL ACTIVITY BY SUMOYLATION

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E2F1 is a crucial and dynamic transcriptional modulator that is frequently deregulated in a myriad of cancer types. It is involved in cell cycle progression, the DNA damage response, and apoptosis. The mechanisms by which E2F1 is able to switch between such contrasting roles has been attributed to changes in post-translational modifications (PTMs) and interacting partners, but the paradigm is by no means fully understood. One such modification that has been shown to further regulate E2F1 activity is sumoylation. The small ubiquitin-related modifier (SUMO) family of proteins acts as an essential PTM for many key cellular processes ranging from response to oxidative stress to serum stimulated growth. Recently, Pc2 mediated SUMO-1 modification of E2F1 was found to be a critical modification for serum stimulated cell proliferation. We have further demonstrated that E2F1 can also be modified by SUMO-2. Additionally, SUMO-2 conjugation to E2F1 is dramatically increased in HEK293T cells upon overexpression of Ubc9, the sole E2 conjugating enzyme in the sumoylation enzymatic cascade. To elucidate the function of this modification, E2F1 transcriptional activity was investigated using a luciferase reporter assay with an E2F1 specific promoter in H1299 cells. Upon expression of E2F1, a drastic increase in activity was observed, and this increase was greatly inhibited with co-overexpression of Ubc9. Moreover, this reduced transcriptional activity was found to be recapitulated in endogenous expression of E2F1 target genes when Ubc9 was overexpressed. These observations lead us to hypothesize that the SUMO-2 modification on E2F1 regulates its transcriptional activity, consequently altering the cell cycle, apoptotic propensity, or the DNA damage response. By further elucidating the manner in which E2F1 activity is regulated, we could potentially harness its pro-apoptotic or curtail its oncogenic abilities as a novel and potent cancer therapeutic target. Furthermore, we are currently investigating if the status and role of SUMO-2 conjugation to E2F1 is altered in the presence of DNA damage induced by conventional chemotherapeutic agents. Should this be the case, the findings of our studies could offer significant insights into the transcriptional regulation that contributes to patient chemo-sensitivity/resistance.

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Non-alcoholic fatty liver disease (NAFLD) is a range of diseases encompassing hepatosteatosis (fat in the liver), non-alcoholic steatohepatitis (NASH-inflamed liver), and cirrhosis. It is considered the hepatic manifestation of the metabolic syndrome and is associated with cardiovascular disease, type 2 diabetes and obesity. In Western countries, NAFLD has a prevalence of 91% in the obese population, while NASH is estimated to affect 37% of this population. Patients with advanced disease face liver failure and possibly death without a liver transplant. NAFLD development and progression is explained as a "two-hit hypothesis", which states that hepatosteatosis is the first hit and serves to sensitize the liver to a second insult such as physical injury or oxidative stress. The involvement of oxidative stress in the disease pathology of NAFLD is well studied; however, current antioxidant treatments have produced mixed results. As the prevalence of NAFLD in the western population continues to increase, it is imperative to investigate all possible treatments for NAFLD. Our lab has access to polyethylene glycol-hydrophilic carbon clusters (PEG-HCCs), a very effective bioengineered nanoantioxidant. Our hypothesis is that PEG-HCCs can lower oxidative stress and slow disease progression in NAFLD. The overall goal is to evaluate the therapeutic effectiveness of these PEG-HCCs at the immunological level of disease, at a gross level using the methionine choline-deficient diet (MCDD) mouse model, and in the event of a lowered endogenous antioxidant defense using the nuclear factor (erythroid-derived 2)-like 2 knockout mouse (Nrf2-/-).

1. To determine the effectiveness of PEG-HCCs in preventing oxidative stress induced T regulatory (Treg) cell death in NAFLD. Within the liver there is a population of Tregs, which prevents the infiltration and proliferation of pro-inflammatory cells such as Th 17 (Th17) cells. In NAFLD these Tregs are depleted, allowing pro-inflammatory cells to infiltrate and damage the liver leading to NASH. The goal of this aim is to determine, by use of flow cytometry, if treatment with PEG-HCCs can rescue intrahepatic Tregs and prevent pro-inflammatory cell infiltration in the liver.

2. To determine if PEG-HCCs can prevent the onset of disease in a diet-induced model of NAFLD. MCDD is commonly used to induce NAFLD in mouse models as it results in rapid development of each stage of disease. Using this mouse model, we will evaluate PEG-HCCs effectiveness at preventing the onset of NASH and fibrosis.

3. To determine if PEG-HCCs can prevent the development of NAFLD in the Nrf2-/- mouse. Nrf2 is a transcription factor that regulates the antioxidant response element, thus controlling the levels of several endogenous antioxidants. Nrf2-/- mice suffer increased levels of oxidative stress and are especially susceptible to the development of NAFLD. Nrf2 activators have been considered as a therapeutic for diseases such as NAFLD. However, Nrf2 activators may prove ineffective in older patients as Nrf2 expression and activity decreases with age. We will determine if PEG-HCCs can lower levels of oxidative stress in the livers of Nrf2-/- mice and if this slows disease progression.

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SYSTEMATIC IDENTIFICATION OF EMS-INDUCED MUTATIONS ON THE DROSOPHILA X-CHROMOSOME: INTERSECTING WHOLE GENOME SEQUENCING AND ROUGH MAPPING.

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Forward genetic screens have proven to be very successful in defining the function of hundreds of genes in Drosophila. The main drawback, however, is the tedious and time-consuming identification of the molecular lesion that is causative of the phenotype. With the recent technical advances and cost reductions in Whole Genome Sequencing (WGS), it is now possible to sequence an entire population of mutants. Although the latter can be done in a fairly short time span, the challenge now lies in weeding out the causative mutation from the numerous variants that are identified when sequencing an entire genome. To facilitate this process, we have developed algorithms and strategies that effectively reduce the number of candidate mutations from an average of 400 to 35 per mutant chromosome. We applied our strategy on ~330 mutant lines that were generated in a forward genetic screen for essential genes on the X-chromosome. Here, we show that by combining WGS with a rough mapping strategy (to ~1.4 Mb), we are able to map about 70% of the mutations.

Contributors: Haelterman, Nele; Jian, Lichun; Bayat, Vafa; Ugur, Berrak; Tan, Kai Li; Zhang, Ke; Bei, Danqing; Li, Yumei; Xiong, Bo; Charng, Wu-Lin; Sandoval, Hector; Yamamoto, Shinya; Jaiswal, Manish; Bellen, Hugo.
2013 GRADUATE STUDENT SYMPOSIUM

IDENTIFYING CANDIDATE GENES IN SYNDROMIC GENOMIC COPY VARIANT REGIONS ASSOCIATED WITH GENITOURINARY BIRTH DEFECTS

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Genitourinary birth defects comprise some of the most common yet least studied congenital malformations and range in severity from conditions such as undescended testes (cryptorchidism) and ventrally misplaced urethral meatus (hypospadias) to highly complex malformations such as dorsally misplaced urethral meatus coupled with exposed bladder (bladder exstrophy epispadias complex, BEEC), cloacal exstrophy, micropenis, and ambiguous genitalia. Congenital anomalies of the upper kidney & urinary tract (CAKUT) are also common and include phenotypes such as duplicated tract components, kidney agenesis, congenital hydronephrosis, horseshoe and cystic kidneys. These birth defects are heavily associated with genomic aberrations such as copy gains and losses. We performed a combination of extensive literature review, genome wide array comparative genomic hybridization for GU abnormal patients, and copy number variation (CNV) qPCR to delineate CNV regions of minimal overlap and to determine a list of candidate genes potentially contributing to the range of GU defects associated with duplications or deletions in the syndromic genomic regions 16p11.2, 9p24.3, and 22q11.2 (DiGeorge Syndrome). We narrowed down each syndromic region to a single candidate gene for further study: 16p11.2 (MAZ), 9p24.3 (KANK1), 22q11.2 (CRKL). MAZ is a transcription factor implicated in wnt signaling with a similar consensus sequence to that of WT1. KANK1 is a maternally imprinted gene with roles in actin polymerization and focal adhesions. CRKL is an adaptor protein known to be involved in FGF8 signaling. In situ hybridization on mouse embryos and isolated mouse GU tracts was performed to determine expression profiles of each candidate gene. In situ experiments showed robust, and for some genes, specific staining central to the GU tract and specifically the genital tubercle. The lab’s full cohort of genomic DNA from GU-abnormal patients and GU-normal fertile controls was screened by CNV qPCR. At least one additional GU abnormal patient screened abnormal for each of the candidate genes. In the instance of MAZ, 9% of our GU abnormal cohort indicated CNV of this gene, as compared to 1.8% in nonsyndromic fertile controls. Knockdown of MAZ in human embryonic kidney (HEK293) cells results in differential expression of several wnt pathway genes, including DKK2 (most heavily upregulated) and WNT11 (most heavily downregulated). Future directions involve further characterization of our in vitro model of MAZ deficiency, as well as creation and characterization of MAZ null mice, and GU characterization of CRKL conditional knockout mice.

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Engineered proteins detect spontaneous regressed replication forks in single living cells

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Regressed replication forks are formed by the base-pairing of the new DNA strands at stalled forks. Regressed forks (called “chicken-foot” structures) both cause DNA breakage, which induces genomic instability, cancer and evolution, and may allow repair of some DNA lesions. Though fork regression is documented in cells with artificially increased replication stalling, spontaneous fork regression and its possible occurrence normally has not been studied. We created a synthetic biological reagent for trapping and quantifying regressed forks in single living cells, mapping their locations and identifying proteins that promote or prevent fork regression, the homologues of which are likely to be important in human disease. We engineered a catalytically inactive fluorescent fusion derivative of the highly four-way junction-specific RuvC protein, RuvCdef-GFP. RuvCdef-GFP binds but does not cleave four-way junctions including both regressed forks and homologous recombination (HR)-promoted (RecA-dependent) Holliday junctions (HJs). RuvCdef-GFP forms visible foci in 0.93% ± 0.09% of undamaged recA cells, indicating that this is the steady-state frequency of cells with regressed forks in growing E. coli. Whereas most spontaneous foci occur singly in cells, some cells display multiple spontaneous foci, implying that some cells stochastically experience cell-wide replication slow-down causing regression of multiple forks. These data, first, imply that foci are not somehow limited to one per cell and so report on numbers of regressed forks present. Second, these are the first data to show single-cell occurrence of regressed forks, including multi-chicken-foot catastrophes, and the first to document fork regression spontaneously. We confirmed previous authors' conclusions that Rep and RecB DNA helicases, and the catalytic subunit of the major replicative DNA polymerase, Pol III, each prevent fork regression. We additionally discovered that DNA helicases UvrD and HelD also prevent fork regression, and we find that two DNA helicases previously thought to promote fork regression, RecG and RuvAB, do not do so usually in growing cells. Direct visualization of regressed forks in single living cells is allowing the first looks at spontaneous fork regression in any organism and will allow the deepest dissection to date of molecular mechanisms, cellular circumstances and consequences of spontaneous fork regression in E. coli, and in future, in other organisms including humans.

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Identification of a Pan-Cancer Oncogenic MicroRNA Superfamily Anchored by a Core Seed Motif

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In order to identify pan-tumor microRNA drivers of cancer we integrated The Cancer Genome Atlas (TCGA) Pan-Cancer dataset with a novel microRNA target atlas composed of all publicly available Argonaute Crosslinking Immunoprecipitation (AGO-CLIP) datasets. This analysis identified a Pan-Cancer, co-regulated, oncogenic microRNA “super-family” consisting of the miR-17, miR-19, miR-130, miR-93, miR-18, miR-455 and miR-210 seed families, that co-targets critical tumor-suppressors via a central GUGC core motif. We subsequently defined mutations in microRNA target sites using the AGO-CLIP microRNA target atlas and TCGA exome sequencing data, expanding the search for tumor mutations to active cis-regulatory sites in the 3’UTR. These combined analyses identified Pan-Cancer oncogenic co-targeting of the PI3-Kinase, TGFβ, and P53 pathways by the miR-17-19-130 super-family members, and a mutation in an active miR-17 seed-family binding site on the SKIL oncogene that relieves auto-regulation of super-family-mediated TGFβ pathway repression.

Contributors: Hamilton, Mark; Rajapakshe, Kimal; Hartig, Sean; Reva, Boris; McLellan, Michael; Kandoth, Cyriac; Ding, Li; Zack, Travis; Gunaratne, Preethi; Wheeler, David; Coarfa, Cristian; McGuire, Sean
CHARACTERIZATION OF A NOVEL HISTONE MODIFICATION

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Nucleosomes are the most basic packaging unit for DNA. They are composed of two of each of the four core histones, H2A, H2B, H3, and H4 along with approximately 146 base pairs of DNA. The core histones are heavily post-translationally modified on their N-terminal tails, but can also be modified within their globular core. Histone modifications include phosphorylation, acetylation, and methylation. These modifications appear to form a decipherable code, known as the histone code, which is written by histone modifying enzymes known as writers and read by effector proteins known as readers. Translation of this code plays an essential role in multiple cellular processes including transcriptional regulation, gene silencing, DNA repair, and cell cycle progression.

Of the four core histones, H3 is the most heavily modified. Included in H3’s modifiable residues are threonine 3, serine 10, threonine 11, and serine 28 which are all phosphorylated during mitosis. Notably, all of these sites are located on the N-terminal tail of H3. Phosphorylation sites within the globular domain of H3 are poorly characterized. Included among these sites is H3 threonine 80. While phosphorylation of T80 (T80p) has been shown in three independent mass spectrometry studies, the significance of T80p has not been described. I have demonstrated that H3 T80p is enriched at mitosis and predict that, similar to other H3 phosphorylation sites, it is central for proper spindle attachment, chromatin condensation, and/or segregation of chromosomes during mitosis.

Contributors: Byrum, Stephanie; Graves, Hillary; Tackett, Alan; Tyler, Jessica
SV40 induces tumors, including lymphomas, in the Syrian golden hamster model and has been found in peripheral blood mononuclear cells (PBMCs) of immunocompromised monkeys. SV40 has been detected in human PBMCs, in human tonsils, and in non-Hodgkin lymphoma (NHL), suggesting that SV40 may be lymphotropic in humans. Other polyomaviruses have also been detected in lymphoid tissues and cells, including JCV, BKV, and MCV. We hypothesize that SV40 establishes a persistent infection in B cells that is rarely productive. Preliminary results using SV40-infected DG75 cells (human B cell line) suggest that viral genomes are maintained at low copy numbers and that SV40 early protein T-antigen (T-ag) is expressed. One goal is to determine the mechanism by which the SV40 genome is maintained in human B cells. We speculate cellular mitotic protein(s) could function in tethering T-ag-bound viral DNA to the chromosomes during cell division, allowing for proper segregation to each daughter cell. Genome tethering or a “piggyback” mechanism has been observed in other virus systems that establish persistent infections, including human papillomavirus (HPV) and Epstein–Barr virus (EBV).

Currently, a mechanism for SV40 genome maintenance in human cell lines is unknown. T-ag binds to the origin of replication in the SV40 genome and is predicted to function in a tethering mechanism, allowing segregation of the viral genome during cell division. Transient DNA transfections were used to examine SV40 genome tethering in human B cell lines. The pEGFP-N1 vector was used to represent the SV40 genome in transient transfections with the human B cell line DG75. Cells were transfected with pEGFP-N1 and pCMV-CPC-T (encoding SV40 T-ag). The collected cells were analyzed by flow cytometry for EGFP expression. Through 72 hours post transfection, there were greater numbers of EGFP+ cells in the co-transfected (EGFP+CPC-T) DG75 cells compared to the vector control (EGFP only). The presence of SV40 T-ag led to an increase in EGFP expression detected by flow cytometry, which suggests T-ag promoted the retention of SV40-like DNA during B cell division; the observed “T-ag effect” was significant. To further investigate the genome tethering ability of T-ag, site-directed mutagenesis was used to disrupt the binding site on T-ag for the SV40 origin of replication or binding sites on T-ag for cellular proteins that might act as linkers. These could theoretically block the tethering of SV40 DNA to the chromosome. Transient transfections will be performed with the SV40 T-ag mutants to evaluate changes in the “T-ag effect” on human B cells.

Contributors: Harrigal, Lindsay J.; McNees, Adrienne L.; Butel, Janet S.
Rare Variant Extension of the Transmission Disequilibrium Test Detects Associations with Autism Exome Sequence Data

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Many population-based rare variant (RV) association tests, that aggregate variants across a region, have been developed to analyze sequence data. A drawback of analyzing population-based data is that it is difficult to adequately control for population substructure/admixture and spurious associations can occur. For RV this problem can be substantial, because the spectrum of rare variation can differ greatly between populations. A solution is to analyze trio data, parents and a proband, using the transmission disequilibrium test (TDT), which is robust to population substructure/admixture. We extended the TDT to test for RV associations using four commonly used methods. We demonstrate that for all RV-TDT tests using proper analysis strategies, type I error is well controlled even when there are high levels of population substructure or admixture. The power of the RV-TDT tests were evaluated and compared to the analysis of case-control data using a number of different genetic and disease models. The RV-TDT was also used to analyze exome data from 199 Simons Simplex Collection autism trios and an association was observed with the ABCA7 gene. Given the problem of adequately controlling for population substructure/admixture in RV association studies and the growing number of sequenced based trio studies the RV-TDT is extremely beneficial to elucidate the involvement of RVs in the etiology of complex traits.

Contributors: He, Zongxiao; Wang, Gao; Hooker, Stanley; Leal, Suzanne
The X-linked Methyl-CpG-binding protein 2 (MeCP2) was first identified twenty years ago as a transcriptional repressor that binds to methylated CpG dinucleotides (methyl-CpGs); it became a focus of intense scrutiny after the discovery that mutations in MECP2 underlie Rett syndrome (RTT), a postnatal developmental disorder that causes girls to abruptly lose acquired language skills, dexterity, sociability, and develop respiratory dysrhythmias, seizures, and autistic-like stereotypies after an initial period of normal development. We hypothesize that studying specific RTT-causing point mutations will provide insight into the key functions of MeCP2.

To determine the role of the methyl-CpG binding domain (MBD) of MeCP2, we generated mice carrying the R111G mutation, which completely abolishes methyl-CpG binding in vitro. We confirmed these results in vivo and found that these mice recapitulated the lifespan of the null animals, indicating the importance of the MBD. However, other point mutations in the transcriptional repression domain (TRD) also cause classic RTT by an unknown mechanism. To learn more about the normal function of MeCP2 and in an effort to elucidate the mechanism by which these other mutations cause RTT, we generated a mouse model carrying MeCP2 with the second most common RTT-causing missense mutation: R306C, located in the TRD.

These mice recapitulate many of the phenotypes seen in MeCP2 null animals including obesity, tremors, anxiety, learning deficits, motor dysfunction, and decreased lifespan. Given this, we sought to determine the mechanism by which R306C causes disease. We found that binding to methyl-CpGs, a necessary function of MeCP2, remained intact. However, we have demonstrated that the mutation affects the binding capabilities of the C-terminus of MeCP2 to DNA in vitro, pointing to a mechanism whereby R306C causes disease by decreasing the ability of the C-terminus of MeCP2 to bind DNA.

Contributors: Heckman, Laura D.; Zoghbi, Huda Y.
Latent Herpes Simplex Virus type 1 (HSV-1) infections are a special public health concern as asymptomatic viral shedding is believed to be responsible for over 70% of primary infections and has been shown to increase the rate of HIV transmission in patients with concurrent infections. HSV shares a common evolutionary origin with tailed bacteriophages, which contain a DNA packaging motor, or portal, at one vertex of the icosahedral particle. The bacteriophage tail is a complex structure that assembles at the portal vertex and provides the mechanism for delivering the viral genome into the infected cell. Using electron cryo-tomography (cryo-ET), we have identified a previously unsuspected tail-like structure at the portal vertex (termed PVAT) in the HSV-1 virion. The location of this tail-like structure suggests that it is important for virion architecture and may play a role in herpesvirus infection.

The HSV-1 capsid is released into a host cell following a fusion event between the viral and cellular membranes. The HSV-1 capsid is then transported along microtubules towards the nucleus. Once at the nucleus the viral capsid binds to the nuclear pore complex, allowing viral genome release into the nucleus where viral replication occurs. It remains unclear whether (1) the HSV-1 PVAT structure remains attached to the capsid after entry into the cell and (2) if this structure plays a role in binding of the capsid to the nuclear pore complex.

Until recently cryo-electron microscopy was limited to studying purified macromolecular complexes, which are no longer in their native environment; the cell. Recently our lab has made advances in the field of cryo electron microscopy, which allows the direct visualization of protein complexes in intact mammalian cells. By combining the knowledge that we have obtained from the HSV-1 structures we have solved in vitro, with our recent ability to image whole cells, we will be able to perform structural studies of virus host interactions that have never been possible before.

Contributors: Hecksel, Corey; Schmid, Michael; Rochat, Ryan, Bhella, David; Rixon, Frazer; Chiu, Wah
ONCOGENE ACTIVATION CAUSES MAMMARY LUMINAL CELLS TO EXPAND INTO BOTH LUMINAL AND BASSAL LINEAGES

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Advisor: Yi Li, Ph.D.-Department of Molecular & Cellular Biology

Background: Previous studies have demonstrated that in early mammary development both the luminal and basal epithelial compartments are generated by basal bipotential progenitors. However, after birth each compartment discretely maintains its own lineage-restricted unipotent progenitors. Upon stress associated with isolation and transplantation into epithelium-free fat pads, basal cells, but not luminal cells, can give rise to both basal and luminal cells to reconstitute a complete mammary duct. This phenomenon may be derived from the severe cellular stress received during transplantation. Unclear is whether cellular stresses undergone during oncogenic transformation and tumor evolution may play a similar role. It is known that BRCA1-derived basal tumors likely come from luminal cells, but these cells develop abnormally and express a basal profile prior to tumor initiation. At this time, it is unclear whether sporadically initiated breast cancers may express a basal lineage when derived from committed luminal mammary cells.

Experimental design and methods: Tumorigenesis was induced using the previously described RCAS-TVA viral vector system to induce discrete lesions specifically in committed luminal cells expressing the WAP promoter. Mice were intraductally injected via the nipple with RCAS virus carrying either a control gene (B-Actin) or one of two oncogenes (PyMT or ErbB2). Early precancerous lesions were evaluated either 7 days (PyMT) or 14 days (ErbB2) after injection. Tumors were collected at 2 cm growth.

Results: Viral integration was confirmed to be restricted to the luminal cell population. However, we find that PyMT or ErbB2 driven tumorigenesis driven by either PyMT or ErbB2 and give rise to both luminal and basal lineages from committed luminal cells.

Conclusion: Using in-vivo virus-mediated lineage tracing, we find that upon oncogenic stress, committed luminal cells in the developed mammary gland can give rise to both luminal and basal lineages. These findings suggest that oncogenic stress leads to the loss of a luminal epithelial phenotype and is associated with the gain of a basal-like phenotype, and may provide an explanation for cellular heterogeneity within cancer.

Contributors: Haricharan, Svasti; Johnston, Alyssa; Toneff, Mike; Reddy, Jay; Li, Yi
The number one cause of cancer death for men and women in the US is lung cancer. The poor 15.9% 5-year relative survival rate is attributed to the late-stage diagnosis of lung cancer; 56% of lung cancer patients are initially diagnosed with metastatic disease. The MAGE-A protein family of cancer/testis antigens is expressed in 59-69% of non-small cell lung cancers and is associated with decreased overall survival. In addition, expression increases proportionally as the disease progresses, interfering with the p53 tumor suppression transcriptional program.

Advances in bicistronic adenoviral (Adv) vector-mediated activation of in vivo dendritic cells (DCs) provide a promising platform for the development of a novel adjuvant therapy against metastatic lung cancer. This system is a departure from recent clinical trials involving expensive and lengthy ex vivo DC-maturation protocols that have produced suboptimal clinical outcomes; in these trials, DCs express low levels of costimulatory molecules and TH1-polarizing cytokines, show poor migration to lymphoid tissues, insufficient longevity, and potentially tolerogenic outcomes. In contrast, in vivo bicistronic delivery of our drug-inducible composite activation receptor with a tumor-specific antigen activates DCs and has been shown to successfully enhance potent CD8+ T-cell responses against antigen-specific tumor growth.

Coupled with the widely expressed lung tumor antigen MAGE-A, we hypothesize that our bicistronic Adv will target DCs for potent activation of specific anti-tumor T cell responses against the novel Ptend/d LKB1+/− spontaneous lung tumor model in vivo.

Mage-a3 and Mage-a4 murine mRNA have been found to be expressed in more than one lung tumor model. We have designed and produced the bicistronic adenoviral vector iMC-Mage-a, combining the drug-inducible composite activation receptor MyD88 and CD40, “iMC,” with the tumor-specific antigen Mage-a. The murine Mage-a3 and -a4 proteins are fused using a glycine flexible linker.

Future work will evaluate murine in vitro and in vivo T cell tumoricidal function following activation of iMC-Magea bicistronic Adv-transduced DCs. Then we will evaluate in vivo anti-tumor function and efficacy of the iMC-Magea bicistronic Adv using murine lung tumor models expressing murine Mage-a.

We are also investigating the mechanism by which murine Mage-a proteins participate in tumorigenesis and metastasis to verify whether these proteins also interrupt p53 signaling as shown with the human MAGE-A proteins.

Contributors: Hendrix, AY; Spencer, D; DeMayo, F; Levitt, J; Kheradmand, F in collaboration with the Helis Foundation.
Adapted over millennia, the drive to consume food is an evolutionarily conserved behavior that promotes survival in times of nutrient scarcity. Underlying this drive are numerous mechanisms that coordinate complex signaling pathways with diverse feeding behaviors in order to regulate the metabolic homeostasis of an organism. Recent insights into these processes have highlighted a central role of the brain in coordinating the intricate mechanisms governing food intake and metabolism. To date, studies investigating the neural components that regulate body weight homeostasis have focused largely on signaling mechanisms in the hypothalamus, a key brain region involved in feeding behavior. However, extrahypothalamic brain regions and signaling pathways have also been shown to play important roles in the complex physiology underlying appetite, satiety, and nutrient metabolism. Recently, cholinergic signaling has been implicated in aspects of body weight homeostasis, though the details underlying this process are unknown. To determine a role for cholinergic signaling in regulating body weight homeostasis, we genetically targeted the diagonal band complex (DBB) of the basal forebrain cholinergic system for cholinergic-specific cell ablation. Strikingly, cholinergic cell ablation results in severe, rapid-onset obesity, which has never before been reported. Resultant obesity in cholinergic neuron-ablated mice appears to be driven by significantly increased food intake leading to increased body fat deposition. Furthermore, cholinergic-specific DBB afferents densely project to major feeding centers of the brain. These discoveries provide strong evidence that cholinergic signaling from the basal forebrain plays a critical role in maintaining body weight homeostasis.
Eighteen percent of individuals with Rett Syndrome (RTT) have a prolonged QT (LQT) interval; a heart rhythm disorder that can lead to lethal cardiac arrhythmias. Mice deficient of Methyl CpG binding protein (MeCP2), Mecp2Null/Y, recapitulate the cardiac phenotype seen in patients with RTT. The current standard therapy for patients with RTT and LQT are β adrenergic antagonist used to decrease sympathetic activity. We tested for the incidence of inducible arrhythmias with acute administration of propranolol, a β adrenergic antagonist, and did not prevent arrhythmias. Meanwhile, acute administration of phenytoin in Mecp2Null/Y mice prevented induction of arrhythmias. We have designed a pre-clinical trial to investigate the physiologic and behavioral effects of chronic treatment with phenytoin or propranolol in Mecp2Null/Y mice.

Mecp2Null/Y and wildtype mice were randomly assigned to be treated twice a day for 28 days with either 30 mg/kg of phenytoin or vehicle. In a separate cohort, Mecp2Null/Y and wildtype mice were treated 10 mg/kg of propranolol via osmotic pump or vehicle. The experimenter was blinded for genotype and treatment. Mice were implanted with telemeters at 4 weeks and treatment was initiated soon after implantation. Programmed electrical stimulation (PES) was used to determine their susceptibility to inducible arrhythmias and to measure the electrocardiogram (ECG) for interval analysis. ECG analysis shows that Mecp2Null/Y vehicle treated mice have a prolonged QRS interval and QTc compared to the Mecp2Null/Y phenytoin treated mice. Treatment with propranolol did not correct the ECG abnormalities in Mecp2Null/Y mice. Propranolol treatment did not prevent arrhythmias in Mecp2Null/Y whereas phenytoin completely ablated arrhythmias in Mecp2Null/Y mice. Phenytoin treatment had no adverse behavior effects, and rescued the overweight phenotype in Mecp2Null/Y mice which was identified to be due to decreased abdominal fat accumulation. Mecp2Null/Y mice have a decreased basal heart rate compared to wildtype mice which was not affected by phenytoin treatment. However, treatment with phenytoin was identified to worsen the breathing phenotype seen in Mecp2Null/Y mice. Since phenytoin worsened the breathing phenotype, a more target therapy such as ranolazine should be tested to determine if it is effective in ablating arrhythmias with minimal side effects. Ranolazine has been identified to selectively block the late persistent sodium channel current which is seen in cardiomyocytes isolated from Mecp2Null/Y mice.
ESTROGEN-RESPONSIVE NEURONS IN THE MEDIAL AMYGDALA PREVENT STRESS-INDUCED HYPERTENSION

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Background: Hypertension is the leading cause of cardiovascular disease worldwide. The etiology of essential hypertension is unknown. Psychological stress contributes to development of hypertension in humans. Estrogen has been shown to prevent stress-induced hypertension in rodents with unknown mechanisms. Amygdala neurons have been implicated in controlling blood pressure. Abundant estrogen receptors, including estrogen receptor-α (ERα), are expressed in the medial amygdala (MeA). Thus, we hypothesize that ERα expressed by MeA neurons mediate the anti-hypertensive effects of estrogen during stress. Methods: Experiment 1: We used the Cre-LoxP system to remove ERα from specific MeA neurons in mice (ERαlox/lox/SIM1-Cre). Female ERαlox/lox/SIM1-Cre mice and controls (ERαlox/lox) were subjected to bilateral ovariectomy (OVX), given a vehicle (V) or 17-β-Estradiol pellet (E), and inserted with telemetry probes on day one. After seven days recovery, blood pressure was recorded for 3 hours to establish baseline and then mice were subjected to 1 hour stress restraint. Experiment 2: C57BL/6 female mice were subjected to intracerebroventricular surgery (ICV) and bilateral ovariectomy (OVX). Accurate ICV cannulations were validated via expected rearing and/or drinking behaviors induced by ICV injection of angiotensin II (10 ng in 1 µl saline). On day nine, mice received ICV injection of 10% dimethyl sulfoxide (DMSO) or 500 pmol of 1, 3, 5-tris (4-hydroxyphenyl)-4-propyl-1Hpyrazole (PPT, a selective ERα agonist). Thirty minutes after injections, mice were euthanized and amygdala tissues and plasma were harvested for analyses by western blot, CoIP, and nitrate/nitrite assay. Results: ERαlox/lox OVX + V mice showed increased mean arterial blood pressure (MAP) and systolic arterial blood pressure (SAP) in response to stress, whereas this stress-induced hypertension was blunted in ERαlox/lox OVX+ E mice. Interestingly, ERαlox/lox/SIM1-Cre OVX + E and OVX + V group both showed increased MAP and SAP in response to stress. ICV experimentation revealed that PPT dramatically increased pAKT/AKT ratio, the interaction between ERα-p85α and ERα-nNOS. Also, ICV injection of PPT significantly increased plasma nitric oxide levels, demonstrated by elevated nitrate/nitrite levels. Conclusions: We demonstrated that estrogens protect against increased neural activity and increase ERα under stress. Also, ERα in the MeA is required to mediate estrogenic actions to prevent stress-induced hypertension. Our results also indicate that stimulation of central ERα activates PI3K signaling, increases the interaction of ERα and nNOS in the amygdala, and stimulates nitric oxide production in the circulation, which may mediate the anti-hypertensive effects of estrogen during stress.

Contributors: Hinton,Jr., Antentor; Xia, Yan; Xu, Pingwen; Saito, Kenji; Yan, Xiaofeng; Zhu, Liangru; Cao, Xuehong; Reynolds, Corey; Xu, Yong
The evolution of sociality and altruism is enigmatic because cooperators are constantly threatened by cheaters who benefit from cooperation without incurring its full cost. Kin recognition is the ability to recognize and cooperate with genetically close relatives. It has also been proposed as a potential mechanism that limits cheating, but there has been no direct experimental support for that possibility. Here we show that kin recognition protects cooperators against cheaters. The social amoebae Dictyostelium discoideum cooperate by forming multicellular aggregates that develop into fruiting bodies of viable spores and dead stalk cells. Cheaters preferentially differentiate into spores while their victims die as stalk cells in chimeric aggregates. We engineered syngeneic cheaters and victims that differed only in their kin-recognition genes, tgrB1 and tgrC1, and in a single cheater allele, and found that the victims escaped exploitation by different types of non-kin cheaters. This protection depends on kin-recognition-mediated segregation, because it is compromised when we disrupt strain segregation. These findings provide direct evidence for the role of kin recognition in cheater control, and suggest a mechanism for the maintenance of stable cooperative systems.

Contributors: Ho, Hsing-I; Hirose, Shigenori; Kuspa, Adam; Shaulsky, Gad
THE ROLES OF ANKYRIN-G IN NODE OF RANVIER FORMATION IN VIVO

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The axon initial segment (AIS) and nodes of Ranvier, the subcellular polarized domains along myelinated axon, are essential for neuronal electrogensis. Voltage-gated sodium (Nav) channels specifically cluster at the AIS and nodes of Ranvier to fire action potentials. Disruption of action potential propagation is associated with human diseases. Therefore, one important question is how Nav channels target precisely to these regions. Ankyrin-G (AnkG) is the master scaffold at the AIS and is essential for recruiting Nav channels to the AIS; however, how Nav channels cluster at nodes of Ranvier is unknown.

We hypothesize that AnkG is required for Nav channel targeting to nodes of Ranvier. To study the role of ankG in node of Ranvier formation in vivo, we generated AnkG conditional knockout mice by crossing AnkGFlox mice with different Cre driver lines. Surprisingly, in contrast to the current accepted model that AnkG directs Nav channels to nodes of Ranvier, we found that Nav channels can still target to nodes independent of AnkG. The results suggest there are previously unidentified factors that direct Nav channels to nodes in the absence of AnkG. Thus, we further investigate the unknown mechanism for Nav channel targeting. Unexpectedly and importantly, we found ankyrin-R (AnkR), an erythrocyte ankyrin, is upregulated and clearly clusters at nodes of Ranvier in AnkG knockout neurons to compensate for the loss of AnkG. We showed for the first time that AnkR can be a nodal protein and AnkR can bind Nav channels. Interestingly, the binding partner of AnkR in erythrocytes, spectrin, can also accumulate at nodes and compensates for the loss of nodal spectrin, spectrin, in AnkG-knockout neurons. This indicates that there is not just single molecule replacement but the whole ankyrin-spectrin complex can be switched at nodes. We further generated AnkG and AnkR double knockout in sensory neurons to study the effect on node formation. Nav channels fail to cluster properly when both AnkG and AnkR are removed. Therefore, ankyrins are necessary for Nav channel clustering at nodes of Ranvier.

Contributors: HO, Tammy Szu-Yu; Zollinger, Daniel R.; Xu, Mingxuan; Cooper, Edward C.; Stankewich, Michael C.; Bennett, Vann; Rasband, Matthew N.
Cryo-EM Reveals The Portal Machinery of Membrane-containing Bacteriophage PRD1

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Nearly 100 years ago it was proposed that bacteriophage could be used as antibacterial agents to mitigate bacterial infections. Recently, interest in phage therapy has seen resurgence as an increasing number of pathogens are becoming multi-drug resistant. PRD1 phage, which is known to infect Salmonella typhimurium, is a good candidate for a possible antibacterial agent. PRD1 is an icosahedral dsDNA bacterial virus with an inner membrane (Tectiviridae family). Based on previous X-ray crystallographic results of PRD1, its major capsid protein has similar fold as those of several other viruses such as adenovirus, PBCV-1 and STIV. However, the structure of the non-icosahedrally arranged portal complex anchored in the inner membrane remains elusive. Biochemical and immuno-electron microscopic studies have identified four proteins in the portal complex: the packaging ATPase P9, the packaging efficiency factor P6, and the integral membrane proteins P20 and P22. The goal of our study is to reveal the structures of the portal machinery of PRD1.

We used single-particle electron cryo-microscopy (cryo-EM) to study the mature virion and three procapsid mutants of PRD1 using symmetry-free reconstructions. Their density maps allow us to conclude the locations and features of the four portal proteins at a unique vertex. The P20 and P22 form a hexamer of dimers embedded in the viral membrane and function as a conduit for the DNA packaging. The P20 or P22 cannot exist alone without the other. The P6 and P9 form a 12-mer of a portal complex with ATPase activity similar to other phage portal protein complex. This is the first structural evidence of the PRD1 packaging complex operating at a specific vertex, and shows the connection between the membrane and the capsid shell providing a conduit for DNA translocation in an ATPase-driven reaction.

Contributors: Hong, Chuan; Liu, Xiangan; Jakana, Joanita; Oksanen, Hanna; Bamford, Dennis; Chiu, Wah
Turner Syndrome (TS), the partial or entire loss of an X chromosome, occurs in ~1 in 2,500 female births. Of the many phenotypes associated with TS structural cardiac abnormalities arise in ~30% of individuals and lead to complications that can contribute to early mortality in those who survive beyond birth. Bicuspid aortic valve (BAV) disease is the most common heart defect associated with TS and is the result of the fusion of two of the leaflets of the aortic valve. While BAV is one of the most prevalent congenital heart defects, much is still unknown about the genes that directly contribute to its development. Here we conducted a genome wide association study to identify potential loci that may be associated with the development of BAV in females with TS. 162 individuals, 55 BAV cases and 107 controls, with partial and complete loss of one X chromosome were genotyped for 733,202 variants using the Illumina OmniExpress array. Logistic regression analysis was performed under an additive allelic effect model to detect associations. No markers in this study were significantly associated with BAV after Bonferroni correction for multiple testing. The limited sample size of this study may have contributed to the inability to detect loci associated with the BAV phenotype.
Use of nonviral modification in hematopoietic stem cells to produce HIV-resistant T-cells

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Hematopoietic stem cells (HSCs) are progenitors for all blood cell types, including most immune cells. If HSCs could be modified by gene therapy, and that modification maintained through differentiation, all of the subsequent lineages would keep that modification. This goal can be used to treat any disease that affects immune cells. Our laboratory has developed minivectors, minimized non-viral vectors, which can be smaller than 400 base pairs. Published and extensive preliminary data have shown that minivectors transflect hard-to-transfect cell types, such as HSCs, and have significantly less toxicity than conventional plasmids. RNA interference (RNAi) can be utilized in gene therapy to knock down a gene, but in order to maintain knockdown, interfering RNA must be continually expressed from a gene and the vector maintained in HSCs and subsequent lineages following differentiation. By encoding a scaffolding/matrix attachment region (S/MAR), plasmids have been shown to replicate episomally in mammalian cells, providing a stable modification of dividing cells without disrupting host chromosomes. We plan to test the ability of S/MAR to replicate minivectors in cells while continuing express a RNAi gene.

Our model to test minivector gene therapy in HSCs is Human Immunodeficiency Virus 1 (HIV-1), a pandemic lentivirus with ~34 million people currently infected worldwide. HIV-1 primarily infects and destroys CD4+ T-cells, which are a necessary part of the adaptive immune system, thus rendering its victims susceptible to a multitude of opportunistic infections. Previous studies have shown that individuals with non-functioning CCR5, one of the co-receptors HIV-1 uses for T-cell entry, are resistant to HIV infection, and such resistance can be conferred by transplanting hematopoietic stem cells to a non-resistant individual. HSC transplants have a high mortality rate, but targeting HSCs using gene therapy would provide a safer alternative. We hypothesize that transfecting HSCs with minivectors encoding shRNA to knock down CCR5 will produce HIV-resistant CD4+ T-cells. Most HIV-1 research so far has created drugs that reduce viral load, but cannot eliminate the virus completely, thus turning a deadly disease into a chronic condition. Results from our work may provide the basis for a therapeutic that can serve as a functional cure to those whose immune systems are compromised by HIV-1.

Contributors: Arevalo-Soliz, Lirio; Fogg, Jonathan; Catanese, Daniel J.; Gilbert, Brian; Zechiedrich, Lynn
Background: Tumor necrosis factor antagonists (anti-TNFs) are effective in treating inflammatory bowel disease (IBD) but may cause reactivation of latent tuberculosis (TB) and hepatitis B virus (HBV). Practice guidelines and IBD performance measures recommend screening for latent TB and HBV prior to anti-TNF initiation. However, the performance rates of TB and HBV screening prior to anti-TNF initiation are unknown. The aim of this study is to evaluate the prevalence and determinants of TB and HBV screening prior to anti-TNF initiation in a national cohort of veterans with IBD.

Methods: We identified a cohort of Veterans Affairs (VA) users with IBD diagnoses during fiscal years 2003-2011 using the national VA administrative datasets. Patients with IBD were identified by a previously validated algorithm using International Classification of Diseases, 9th Revision, diagnosis codes for Crohn’s disease (CD) or ulcerative colitis (UC). Among those patients, those with filled prescriptions for infliximab, adalimumab, and certolizumab pegol were evaluated. The anti-TNF start date was defined as the date of the first filled anti-TNF prescription. TB screening was defined as the performance of a purified protein derivative test or QuantiFERON-TB gold test within 6 months prior to anti-TNF start date. HBV screening was defined as testing for HBV surface antibody, core antibody, or e antigen within 1 year prior to anti-TNF start date. Determinants of screening were identified by univariate and multivariate analyses. Results: A total of 3,357 IBD patients were identified with filled anti-TNF prescriptions. Approximately 72% of subjects received TB screening and only 24% of subjects received HBV screening prior to their anti-TNF start date. In multivariate analyses, patients who live in rural areas were significantly less likely to be screened compared to those in urban areas for TB (OR 0.72, 95% CI 0.54-0.95) and HBV (OR 0.72, 95% CI 0.52-0.98). Patients who received care at facilities with an academic affiliation were more likely to have received screening for TB (OR 1.49, 95% CI 1.31-1.95) and HBV (OR 1.97, 95% CI 1.33-2.92). Patients who received care at a facility with a high volume of IBD patients on anti-TNFs (top quintile) were more likely to have received screening for HBV compared to patients who received care at a low volume facility (OR 2.16, 95% CI 1.59-2.93).

Conclusions: Screening prior to anti-TNF initiation for TB and especially for HBV is low in a national cohort of veterans with IBD. Receipt of care at urban, academic-affiliated, high volume IBD facilities is associated with higher rates of screening. Identification of TB and HBV screening processes at these high performing facilities may serve as models for other facilities.
The Lef/Tcf transcription factor Tcf3 (Tcf7l1) has important roles in embryonic development and embryonic stem cell homeostasis, as well as an emerging role in cancer pathogenesis. Previous studies have also demonstrated a role for Tcf3 in development and self-renewal of the mammalian epidermis. Despite its importance in stem cell biology, however, very little is known about the role of Tcf3 in homeostasis of adult tissues.

Here, we examine the fate of Tcf3-expressing cells in adult epithelia in vivo using a novel Tcf3 knock-in mouse, Tcf3-2A-eGFP-2A-CreERT2. Performing Cre/loxP-based lineage tracing with these mice, we show that Tcf3-expressing stem cells in the hair follicle bulge persist for extended periods of time and incorporate into all the differentiated cell types of the hair shaft. During normal homeostasis of the skin, Tcf3-lineage cells are strictly confined to the hair follicle. Following epidermal wounding, however, the normal fate-specification process is disrupted and Tcf3-lineage cells are transiently seen in the stratified interfollicular epithelium as well.

We then turn our attention to other epithelial tissues and show, for the first time, the presence of Tcf3-expressing stem and progenitor cells in the paw skin, tongue, and esophageal epithelia. Unlike the back skin, a substantial proportion of Tcf3-lineage clones are lost over time in these tissues, implying that either (a) Tcf3 is expressed in stem and progenitor cells in a stem-progenitor hierarchy, or (b) Tcf3-expressing cells possess stem potential, but some are lost over time under a “neutral competition” model of homeostasis.

Our findings represent an advance in the understanding of Tcf3 in epithelial homeostasis and raise interesting questions about the exact role of Tcf3 in adult stem cell homeostasis.

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Validated near-atomic resolution structure of bacteriophage epsilon15 derived from cryo-EM and modeling

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Bacteriophages infect nearly all bacteria, making them excellent potential alternatives to antibiotics. In this work, we solved the structure of bacteriophage epsilon15 using cryo-EM and computational modeling to a resolution at which an all-atomic model could be constructed of the intact mature viral capsid. This structure allowed us to segment and model the two primary structural proteins (GP7 and GP10), and describe the intricate interactions required for capsid stability. Beyond the relevance to its own structure, the use of a secondary staple protein in epsilon15 further illustrates the diverse mechanisms of capsid assembly and stability found in tailed dsDNA bacteriophages.

Additionally, the technology development in this work represents the first time that a complete validation of map and model has been used to assess the cryo-EM reconstruction and associated models. Even at near-atomic resolution, the models in this work were fully validated using best-practices from X-ray crystallography. Obtaining near-atomic resolution allowed for a more extensive model building procedure to be used. Previously our C-α models were focused on fit to density and proper bond lengths. Developing an extensive protocol and modeling GP10 separately from GP7 allowed for individual aspects of the model to be analyzed. The modeling procedure for the all-atom structures now accounts for various rotamers, side chain clashes, geometry and side chain fit to density. With this work we hope to have established the standards for which all cryo-EM maps and models are to be evaluated.

Beyond the importance of establishing community wide standards, this work also reveals for the first time that a virus, bacteriophage epsilon15, containing two canonical viral structural proteins folds: the major capsid protein fold from HK97 bacteriophage fold and the jellyroll fold found in a wide range of eukaryotic viruses. We propose that the presence of such folds in epsilon15, in which a vertebrate host acted as a reservoir for both bacteriophages and other viruses, evolved through genetic transfer and recombination. When compared to other dsDNA viruses, a clear pattern has begun to emerge, in which these viruses can adapt to different environments while maintaining the same overall structural architecture.

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A significant hurdle in tissue engineering is adequate perfusion of donor tissue. Success in engineering tissues has mainly been restricted to thin or avascular tissues such as skin, cartilage, bladder etc. A related problem plagues therapeutic angiogenesis approaches to treat ischemic disease. Despite the discovery of numerous pro-angiogenic factors, clinical therapeutic strategies based on single factors have been wholly unsuccessful in repairing damaged tissue; however, recently, combinations of growth factors are beginning to show great promise in promoting stable angiogenesis and improving damaged circulation. These successes are inspiring new designs of pro-angiogenic tissue scaffolds. It has been demonstrated that the combination of PDGFBB and FGF2 has a synergistic effect on angiogenesis to induce stable vessel structures in vivo. However, the cellular mechanism responsible for this effect remains undefined. Several studies indicate that macrophages exhibit a trophic effect on angiogenesis. To more fully define the mechanism regulating the vessel stability, I will carry out experiments to test the role of macrophages in angiogenesis. My goal is to investigate whether macrophages are responsible for the formation of stable neo-vessels. I hypothesize that the recruitment of pro-angiogenic macrophages is required for the induction of stable neo-vessels. A fluorescent reporters mouse model, which labels macrophages (c-fms-GFP) and endothelial cells (Flk1-myr::mCherry), was used to study the role of macrophages in vessel stability. Growth factors delivered by poly(ethylene glycol) diacrylate (PEGDA) hydrogels were used to evaluate the angiogenic potential and host responses in the cornea micropocket assay. Live, confocal microscopy was also performed to examine the dynamics of macrophages in response to different growth factors. Using PEGDA hydrogels, we have been able to confirm the synergistic angiogenic effect of PDGFBB and FGF2. Interestingly, our finding also indicates that the combination of PDGFBB and FGF2 not only induces more stable vessels; it also increases the density of macrophages before the induced vessels reach the hydrogel. This phenomenon suggests that instead of causing regression of VEGF-induced vessel, macrophages may contribute in stabilizing PDGFBB/FGF2-induced vessels. Vital imaging reveals that PDGFBB/FGF2 indeed results in the infiltration of circulating macrophages, which migrate toward the implantation site. In summary, these data indicate that macrophages play a role in stabilizing the neo-angiogenic response. Future studies, using vital imaging to study the macrophage dynamics in macrophage-deficient mice, will be used to further test whether macrophages are necessary and sufficient for the formation of stable vessels. We anticipate the second-generation bio-scaffold design will incorporate activated macrophages or factors to directly activate these cells to optimize the angiogenesis response.

Contributors: Hsu, Chih-Wei; Poché, Ross; Saik, Jennifer; Ali, Saniya; Vadakkan, Tegy; West, Jennifer; Dickinson, Mary
Using Live-cell Imaging to Study the Correlation between NK-cell Lysosomal Maturation and Degranulation Process

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Natural killer cells are critical in innate immune system for their ability to survey and kill the viral-infected, transformed and stressed cells in the human body. NK cell activation triggers a stepwise degranulation process to perform cytotoxicity. During degranulation, preformed lytic granules rapidly converge to the microtubule organizing center (MTOC), and polarize to the immunological synapse (IS) before release onto the target cell. In previous studies, lytic granules are loaded with lysotracker red so that the convergence and polarization process can be visualized by live-cell imaging in real-time. However, this method cannot clearly define the process of granule release. The fusion of acidic granules to the plasma membrane exposes the lysosome-associated membrane protein (LAMP) to the neutral environment. We took advantage of this phenomenon by utilizing NK92 cells expressing pHluorin fused with LAMP-1. PHluorin is a GFP mutant protein that remains non-fluorescent in acidic pH but becomes fluorescent in neutral pH. Thus, we can precisely track the release of granule contents to the IS. Using this system, we found that the granules converge within ten minutes and degranulation event occurs 25 minutes after the start of imaging. However, one limitation of the system is the broad specificity of lysotracker red to all acidic organelles, which potentially interferes with the analysis of granule kinetics. In order to overcome this limitation, we adopted another GFP mutant, the ratiometric pHluorin, which has two pH-dependent excitation wavelengths at 410 and 470nm. The pH of any intracellular compartments containing ratiometric pHluorin can be characterized by distinct 410/470-nm excitation ratio. Therefore, we will generate NK92 cells expressing the ratiometric-pHluorin-LAMP-1 to specifically label lytic granules without lysotracker red. Furthermore, the pH indicative feature of ratiometric pHluorin can provide us unprecedented insights into how the lysosomal acidification/ maturation process may be related to the activation status and the degranulation process of NK cells. Studies showed that the structure and function of lytic granules depends on their acidified condition. In addition, the activity of perforin and granzyme is regulated by pH-dependent proteases within acidified endosomes. Based on these observations, we want to explore the following questions: 1) Does the activation of NK cells regulate the acidification process of lytic granules? 2) Does the pH of lytic granules have a role in granule convergence, polarization and/or degranulation? 3) Does the change of pH affect loading of perforin and granzyme into lytic granules?

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EVALUATING THE IMPACT OF CANCER MUTATIONS

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Whole genome sequencing of cancer cells has uncovered a large number of genetic alterations and estimating the functional impact and clinical consequences of these alterations has become a major challenge. Toward this goal, we derived an “Evolutionary Action” method, based on protein evolution information, to evaluate the impact of any point mutation, such that a greater Evolutionary Action score corresponds to a greater impact on protein function.

In cancer genes, high Action mutations disrupt or alter protein function, and therefore they are positively selected during tumor development since they confer a growth advantage to the cancer cells. In contrast, high Action mutations are not selected for in the genes that control other cellular functions or are non-essential. As a result, the distribution of Action scores for the somatic mutations of each gene is able to identify the genes that are associated with cancer. Identifying cancer genes is crucial for understanding the cancer mechanism, finding potential therapeutic targets and developing novel strategies to treat cancer. The survival of patients can also be predicted from the impact of genetic alterations in key cancer genes, such as TP53. Cancer patients with high impact genetic alterations in cancer genes may have a lower survival rate than patients with no or with low impact mutations. The integration of mutations occurring in various genes is a major challenge that can improve the associations of mutations to disease severity. Using protein networks can help in this integration, and lead to a better understanding of the functional importance of genetic alterations.

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TARGETING SPLICING-INDUCED STRESS IN MYC-DRIVEN BREAST CANCER

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Amplification and/or hyperactivation of c-Myc (Myc) occurs in 20-40% of human malignancies and confers poor prognoses in many breast cancer subtypes. While direct targeting of Myc has been unsuccessful to date, targeting the stress support pathways required to tolerate aberrant Myc activation represent ideal therapies since cancer cells become hyper-dependent on these pathways. Using a genome-wide forward genetic screen, we searched for genes required to tolerate aberrant MYC activation (Kessler et al, Science 2012). We discovered that cells are strongly dependent on the spliceosomal protein BUD31 to tolerate MYC hyper-activation, and BUD31 inactivation impairs MYC-dependent breast cancers in vivo. Although BUD31 is poorly understood in humans, the Bud31 yeast homologue is a member of the core spliceosome, which removes pre-mRNA introns, and BUD31 is required for spliceosomal activation. Our mass spectrometry studies reveal that human BUD31 also interacts with the core spliceosome and regulates splicing in human cells. Many of these Bud31-interacting spliceosomal components also essential for Myc-hyper-activated cells. Putative pharmacologic inhibitors of BUD31-associated spliceosomal complexes also impair the survival of Myc-driven breast cancer cells in vitro. By understanding how BUD31 and its associated complex play a role in coping with Myc stresses, we will identify potential targets for therapeutics that can be specifically applied to aggressive Myc-hyperactive breast cancers. Furthermore, insight into stress support networks may be applied to other Myc-driven malignancies, which will make a substantial impact on the treatment of cancer patients.

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CRYSTAL STRUCTURES OF A SINGLE DOMAIN IN PKGI REVEAL THE MOLECULAR MECHANISM OF CGMP SELECTIVITY

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Cyclic guanosine monophosphate (cGMP) is a key secondary messenger that is produced in response to nitric oxide. One of the key mediators of cGMP signaling, cGMP-dependent protein kinase (PKG), is activated upon binding to cGMP and phosphorylates downstream substrates in a process required for important physiological processes such as vasodilation, nociception, and memory formation. PKGs are also known to mediate most effect of drugs that increase cellular cGMP levels, including nitric oxide-releasing agents and phosphodiesterase inhibitors, which are used for the treatment of angina pectoris and erectile dysfunction, respectively. We have investigated the mechanism of cyclic nucleotide selectivity by PKG by determining crystal structures of the cGMP-selective carboxyl-terminal cyclic nucleotide-binding domain (CNBD-B) of human PKG I bound to cGMP and in the apo form. Our crystal structure of CNBD-B with bound cGMP reveals that cGMP adopts the syn configuration in the binding pocket and is coordinated by a previously unidentified arginine residue. Furthermore, comparison of the cGMP-bound crystal structure of the apo structure suggests a role for a C-terminal tyrosine residue in capping the nucleotide into the binding pocket. The interaction of this tyrosine residue with cGMP appears to result in conformational rearrangement of the C-terminal helix, suggesting a mechanism for kinase activation by cGMP.

Contributors: Huang, Gilbert; Kim, Jeong Joo; Reger, Albert; Casteel, Darren; Bertinetti, Daniela; Lorenz, Robin; Zhao, Chi; Moon, Eui-Whan; Melacini, Giuseppe; Herberg, Friedrich; Kim, Choel
In the nervous system, inhibitory circuitry plays significant roles in sculpting principle neuron output and maintaining proper brain functions. In prominent models of the olfactory bulb, the principle neurons, mitral cells, receive inhibitory input from both periglomerular cells and granule cells, which are involved in gain control and lateral inhibition of mitral cells. However, it remains unclear how other interneurons are connected in the circuitry and involved in odor processing. Here we report a previously uncharacterized population of corticotropin releasing hormone (CRH) – expressing GABAergic interneurons that reside in the external plexiform layer (EPL), and make reciprocal connectivity with mitral cells. Using genetic manipulation, imaging, optogenetics, electrophysiology, and fast scanning microscopy, we have revealed that EPL interneurons make strong, fast inhibitory connectivity onto mitral cells, and are reciprocally excited by direct mitral cell input. Further functional analysis using optogenetics and in vivo electrophysiology shows that EPL interneurons also influence olfactory processing. These findings functionally identify a novel inhibitory circuitry within the olfactory bulb, and uncover a potentially critical player in olfactory information processing.

Contributors: Huang, Longwen; Garcia, Isabella; Cordiner, Keith; Saggau, Peter; Arenkiel Benjamin
ROLES OF MECP2 IN THE AUTONOMIC NERVOUS SYSTEM

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder associated with loss of communication and purposeful hand skills as well as several autonomic deficits. These autonomic problems may contribute to the sudden death observed in a fraction of people with RTT. Mutations in the gene encoding a transcriptional regulator, Methyl-CpG-binding protein 2 (MECP2), cause 95% of RTT cases, and mice lacking MeCP2 function exhibit the pathological features similar to RTT patients, including the autonomic deficits.

Our lab found that removing MeCP2 from brainstem and spinal cord in mice causes early lethality and autonomic phenotypes including decreased heart rate and abnormal respiratory response to hypoxia. In addition, re-expressing MeCP2 within the region is sufficient to rescue these phenotypes. The brainstem is known to contain neural circuits critical for the regulation of autonomic function. To determine which neuronal circuits require MeCP2 for normal respiratory response to hypoxia and survival, I used the Cre/LoxP system to remove or re-express MeCP2 in specific regions that are known to be important for the control of breathing. Using transgenic mice I developed which express Cre recombinase in the HoxA4 domain, I removed MeCP2 from the caudal medulla, spinal cord, and peripheral nervous system (PNS). MeCP2 expression in pre-Bötzinger complex, the pacemaker of breathing, and nucleus tractus solitarii is removed, but pontine respiratory group, retrotrapezoid nucleus, and Bötzinger complex are not affected. The conditional knockout (CKO) mice showed abnormal motor functions and early lethality, but had normal heart rate and ventilation response to hypoxia. In addition, preliminary results suggest that expression of MeCP2 solely within the HoxA4 domain is sufficient for the survival of rescued animals. The possibility that restored MeCP2 expression in PNS is responsible for the survival is ruled out by generating animals expressing MeCP2 function in the Sox10 domain (including PNS and oligodendrocytes); these rescued animals continued to have poor motor coordination, abnormal breathing, and early lethality. The result indicates that MeCP2 expression in caudal medulla and spinal cord is critical for the motor coordination and survival but MeCP2 function within the PNS or oligodendrocytes is not. The following study would help to define the critical cardiorespiratory regulatory components that required MeCP2 function for the survival.

Contributors: Huang, Teng-Wei; Neul, Jeffrey Lorenz
The novel complex mTORC2 controls actin polymerization required for consolidation of long-term memory

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A major goal of biomedical research is the identification of molecular mechanisms that underlie memory storage. Here we report a previously unknown signaling pathway that is necessary for the conversion from short- to long-term memory. The mammalian target of rapamycin (mTOR) complex 2 (mTORC2), which contains the regulatory protein Rictor (rapamycin-insensitive companion of mTOR), was discovered only recently and little is known about its function. We found that conditional deletion of Rictor in the postnatal murine forebrain greatly reduced mTORC2 activity and selectively impaired both long-term memory (LTM) and the late phase of hippocampal long-term potentiation (LTP). We also found a comparable impairment of LTM in dTORC2-deficient flies, highlighting the evolutionary conservation of this pathway. Actin polymerization was reduced in the hippocampus of mTORC2-deficient mice and its restoration rescued both L-LTP and LTM. Moreover, a compound that promoted mTORC2 activity converted early-LTP into late LTP and enhanced LTM. Thus, mTORC2 could be a novel therapeutic target for the treatment of cognitive dysfunction.

Contributors: Huang, Wei; Zhu, Ping Jun; Zhang, Shixing; Stoica, Loredana; Krnjevic, Kresimir; Roman, Gregg; Costa-Mattioli, Mauro
LOSS OF βII-SPECTRIN IN THE CENTRAL NERVOUS SYSTEM CAUSES PROFOUND PHENOTYPES AND AFFECTS VIABILITY

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Spectrins are a family of cytoskeletal proteins classified into α and β subunits. It has been reported that spectrins are required for structural support of cells and they serve as platforms for cell signaling. Spectrins are also implicated in nervous system disorders. For instance, mutations in spectrin can cause cerebral hypomyelination, epilepsy and spinocerebellar ataxia (SCA) in humans. Furthermore, the importance of spectrins is emphasized by embryonic lethality of constitutive knockout mice of αII-spectrin and βII-spectrin. I used Nestin-Cre;SPNB2fl/fl mice to bypass embryonic lethality and study the role of βII-spectrin in the central nervous system. We found that conditional knockout mice were smaller and weaker than control animals; 50% of the animals lacking βII-spectrin in the CNS died by 9 weeks. Behavioral tests showed that they were ataxic. Seizure phenotypes were detected by electroencephalogram abnormalities in cKO mice. Hippocampal CA3 region was found to be disrupted while expression of calbindin and neuropeptide Y (NPY) was increased in this region. Besides, striatum was more susceptible to neurodegeneration labeled by βAPP staining. Ankyrin G and βIV-spectrin staining suggested that loss of βII-spectrin in the central nervous system caused fragmentation and disorganization of axon initial segments. In the cerebellum, Purkinje cells were lost, molecular layers were thinner and potassium channel clustering was aberrant in Nestin-Cre;SPNB2fl/fl mice. Ablation of βII-spectrin also altered expression of other related proteins. For instance, the βII-spectrin interacting partners αII-spectrin and ankyrin B were reduced while other members of β-spectrin family βI-spectrin and βIV-spectrin were up-regulated. These findings suggest that βII-spectrin plays important roles in a variety of CNS functions and can be associated with the mechanisms of human diseases.

Contributors: Huang, Yu-Mei; Zhang, Chuansheng; Rasband, Matthew
Antioxidant nanomaterials as immunomodulatory therapy for rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic, idiopathic autoimmune disease that affects 1 in 100 people worldwide. RA involves the destruction of cartilage and bone in diarthrodial joints, arbitrated by autoreactive CCR7- effector memory T (TEM) cells that promote inflammation and the production of deleterious reactive oxygen species. Current treatments induce generalized immunosuppression and have life-threatening side effects. Interestingly, superoxide radical (SO), the precursor of reactive oxygen species is produced by mitochondria upon T cell stimulation and is crucial for T cell proliferation. Therefore, a selective inhibitor of mitochondrial SO could function as an innovative avenue of immunomodulatory therapy for RA. Here we report that the nontoxic nanomaterials, PEGylated hydrophilic carbon clusters (PEG-HCCs), which are potent antioxidants that specifically and efficiently scavenge SO, may serve as a novel therapeutic for RA by suppressing the TEM cell-mediated pathology. PEG-HCCs were shown to rapidly enter T cells indicating that they will be in contact with intracellular SO. Remarkably, PEG-HCCs preferentially reduced the ex vivo proliferation of rat TEM cells without inducing cell death, while not reducing the proliferation of CCR7+ naïve and central memory T cells essential for combating acute infections. This finding, along with PEG-HCCs having no effect on antigen-presentation or phagocytosis by macrophages, indicated that PEG-HCCs do not behave as generalized immunosuppressants. Furthermore, PEG-HCCs decreased inflammation specifically mediated by TEM cells in vivo and reduced intracellular cytokine production in rat TEM cells. Data from our pharmacokinetic studies in rats administered with a subcutaneous injection of PEG-HCCs demonstrated a gradual release into the bloodstream and a circulating half-life of 25 h. A pilot study on rats with pristane-induced arthritis, an animal model of RA, suggested that PEG-HCC therapy will lower disease severity. We plan on performing a large-scale animal study to confirm efficacy. This work provides critical evidence that PEG-HCCs will be a promising treatment for RA that circumvents generalized immunosuppression, setting the stage for future clinical trials and use in other TEM cell-mediated autoimmune and inflammatory diseases.

Contributors: Lee, Thomas; Tanner, Mark; Khan, Fatima; Samuel, Loic; Tour, James; Beeton, Christine
Identification and Characterization of Novel Peptide Reagents for the Detection of Noroviruses

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Background: Norovirus (NoV) infections are the most common cause of non-bacterial gastroenteritis and lead to about 21 million new cases and $2 billion of expenses per year in the United States alone. Antigen immunoassays currently available for NoV diagnosis detect only certain strains and are only approved in the United States for use during epidemics where NoV is suspected. There is a clear need for more reliable, rapid, and simple-to-use diagnostic tools for the detection of NoV.

Methods: In this study, phage display technology was used to screen libraries of phage displaying random 12-mer peptides for those that bind to NoV virus-like particles (VLPs) composed of the VP1 capsid protein. Since NoV strains classified in genogroups GI and GII cause the majority of human outbreaks, we focused initially on representative strains in genotypes GI.1 and GII.4 to identify binding reagents.

Results: After screening the random 12-mer peptide phage library against G1.1 VLPs, our group recently identified a peptide named NV-N-R5-1 that binds to the P-domain of VP1 (Rogers et al. J Clin Micro. 2013). Enzyme immunoassay experiments revealed that phages displaying the NV-N-R5-1 peptide are efficient detection reagents for GI.1 VLPs or NoV from stool samples. To optimize the binding affinity and solubility of NV-N-R5-1, we are using random mutagenesis to identify mutants with strong binding to GI.1 and improved solubility in order to develop a diagnostic reagent. Additional peptide displaying phages have been identified with binding specificity for GII.4 VLPs, and these phages are currently undergoing investigations for binding specificity and NoV detection sensitivity.

Discussion: The use of phages displaying binding reagents directly as a detection reagent has several advantages including the ease and low cost of phage propagation and purification. Additionally, the clones obtained by phage display can be further developed by directed evolution strategies to increase binding affinity and modify specificity to new pathogens. Therefore, the phage clones recently described by our group represent first generation peptides whose properties can be improved by random or site-directed mutagenesis followed by phage display selections. Ultimately, this work will lead to the establishment of novel tools for the rapid and reliable diagnosis of NoV infection with sensitivity to a broad range of clinically relevant NoV strains.

Contributors: Hurwitz, Amy; Huang, Wanzhi; Rogers, Jennifer; Estes, Mary; Atmar, Robert; Palzkill, Timothy
Metagenomic Analysis of Nitrate Reducing Bacteria in the Oral Cavity:
Implications for Nitric Oxide Homeostasis

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Nitric oxide (NO), which is generated in an oxygen-dependent manner by mammalian NO synthases, plays a critical role in maintaining cardiovascular health. The nitrate-nitrite-NO pathway is an oxygen-independent pathway to NO production. The first step of this pathway is the bacterial reduction of nitrate by oral bacteria, which human cells cannot do, establishing a symbiosis between humans and the oral microbiota. The aim of this study was to dissect nitrate reduction by oral bacterial communities through biochemical and metagenomic analyses, with the goal of creating communities optimized for nitrate reduction to increase host NO availability. Tongue scrapings from six healthy volunteers were inoculated onto poly-methyl-methacrylate (PMMA) biofilm discs in biofilm medium and incubated anaerobically. Medium was collected every 24 hours for 96 hours and nitrate levels measured via HPLC; one PMMA disc was also collected at each time point for bacterial DNA extraction. Based on HPLC results, samples were divided into three groups: best, intermediate, and worst nitrate reducers. 16S pyrosequencing identified several genera that were significantly more abundant in the best nitrate reducing samples compared to the worst samples and that could discriminate between these groups. Whole genome shotgun (WGS) sequencing on a subset of samples identified many known oral nitrate reducers, but also identified species whose genomes do not encode nitrate reductase genes. These results suggest that the community dynamics leading to efficient nitrate reduction are complex, with non-nitrate reducers possibly acting as “helper” species to enhance nitrate reduction by other bacteria. Supporting this hypothesis, four species were grown individually and as a consortium and quantification of nitrate reduction revealed that although the consortium reduced nitrate well, two species were efficient nitrate reducers and two were poor nitrate reducers. Biochemical studies on more candidate species have begun to further elucidate the role of these bacteria in nitrate reduction. Together, our studies have enabled us to begin to dissect the complex oral bacterial interactions leading to nitrate reduction and suggest that bacterial consortiums optimized for nitrate reduction with in vivo probiotic potential can be generated.

Contributors: Andrade, Fernando; Tribble, Gena; Kaplan, Heidi B.; Bryan, Nathan S.; Petrosino, Joseph F.
Oxidative stress plays a key role in the pathogenesis and progression in multiple sclerosis (MS), a progressive and relapsing disease of the central nervous system (CNS). Previous studies have shown the cascade of events that unfold during the initiation of MS, involve oxidative stress events. As a result, many antioxidant-based treatments have been tested unsuccessfully due to low efficacy, efficiency and poor delivery to needed regions. Recently, we have developed a novel antioxidant, PEGylated-hydrophilic carbon clusters (PEG-HCC) in collaboration with the Tour Laboratory at Rice University that has high efficacy, is targetable, can be utilized as a vector and has been shown to be non-toxic. Modifications have been performed to nanoantioxidants to make them blood-barrier permeable with the addition of adamantane (ADA-PEG-HCC), as well as detectable via MRI with the addition of gadolinium (GDAP). As a result, we hypothesize that our novel nanoantioxidant will be able to address previously unmet needs by earlier antioxidants in the treatment of neurodegenerative disease, namely being able to cross into the central nervous system (CNS), as well as be highly efficacious in scavenging radicals. Modifications have been performed to these agents to make detectable via MRI with addition of gadolinium (Gd). The T1-signal intensity and T1-times of Gd-DTPA-PEG-HCCs, ADM-PEG-HCCs, PEG-HCCs were assessed in vitro utilizing MRI. We found that addition of Gd resulted in higher T1-signal intensity and lower T1-time of these agents allowing potential tracking of agents in vivo. Currently, we are carrying out studies to visualize their localization in vitro, ex vivo and in vivo and have seen their uptake into lymph nodes, potentially in the T-Cell zone of these tissues. Ultimately, we believe that these nanoantioxidant agents will address previously unmet needs by earlier antioxidants, namely by being highly efficacious in scavenging radicals, targetable and also, be non-invasively, MR detectable. As a result, they can potentially be utilized as a therapeutic for treating Multiple Sclerosis.
Electron cryo-microscopy (cryo-EM) is a powerful structural technique that can now routinely reach subnanometer resolution (3.5-5 Å) for large, homogeneous specimens, such as protein machines and viruses. The advantage of cryo-EM is that prior to imaging, specimens are frozen instantaneously in liquid ethane, which allows them to retain a near-native hydrated state. Here we use single particle cryo-EM to visualize the dimerization initiation site (DIS) of HIV-1 RNA. At merely 88 nucleotides (~30 kDa), this is the smallest sample to date studied by cryo-EM.

One of the biggest challenges in working with specimens under 100 kDa is the low signal to noise ratio of the raw data. Sufficient signal is crucial for particle identification and orientation determination. One way to obtain higher contrast is to increase the number of electrons hitting the specimen. However, the drawback of this approach is that a high electron dose can destroy the higher resolution features of the sample. In order to increase the contrast, while preserving high-resolution information, we employed a new direct electron detector camera system, which has provided us with the ability to acquire a series of two-dimensional images of the same area and within a very short time period (2.5 seconds). The entire stack of images averaged, yields high contrast, improving particle identification. Furthermore, during the three-dimensional reconstruction, we can focus on a particular subset of the image stack and remove data where the higher resolution features are compromised. Our current results of the DIS RNA show a three-dimensional structure that is consistent in size and shape with the results obtained by NMR. We are able to independently resolve features such as the bend of the molecule and detect the pitch of the RNA helix. By employing this new state of the art imaging we hope to resolve the structure of the DIS region to 10-15 Å resolution, where the grooves of the helix will be well-defined.

Recent technology advances, such as those used in our study, have made it possible to push the limits of cryo-EM, opening the door to opportunities to study specimens much smaller than those traditionally considered. Cryo-EM is a powerful tool and combined with other structural techniques can help unravel the mystery around the structural behavior of critical regions such as the DIS. In the future, we plan to use this technique to also study RNA-protein interactions, providing further insight into the mechanics behind the processes of HIV genome dimerization and packaging.

Contributors: Irobalieva Rossi; Heng, Xiao; Schmid, Michael F; Summers, Michael; Chiu Wah;
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The modern clinical implementation of efficient strategies for selectively sequencing complete coding regions (i.e., “whole exome”) has the potential to contribute to the diagnoses of human diseases. However, such genetic approaches are often challenged by the large number of variants observed in individual patients. We hypothesize that efficient use of available clinical information can improve the prioritization of variants. Unfortunately, phenotypic inputs rarely adhere to a common vocabulary. Natural language processing can be used to parse and standardize Electronic Medical Record text. This generates clarified queries from which diagnostic estimates are calculated. We used these techniques, in conjunction with hierarchical semantic relationships of The Human Phenotype Ontology, to help simplify the problem of abundant variants by improving the matching of phenotypic inputs to known diseases (e.g.: those described in Online Mendelian Inheritance in Man – OMIM). Improved similarity metrics were developed and systematically evaluated via their performance in Monte Carlo simulations of possible feature combinations that may occur in patients being sent for exome diagnostics. These methods can be applied to analyze the correspondence between the requisition content and the ultimate diagnosis.

Contributors: James, Regis; Shaw, Chad
Background Familial exudative vitreoretinopathy (FEVR) is a developmental disease that can cause visual impairment and retinal detachment at a young age. Four genes involved in the Wnt signaling pathway were previously linked to this disease: NDP, FDZ4, LRP5, and TSPAN12. Identification of novel disease causing variants of this disease allows for a deeper understanding of the disease, better molecular diagnosis, and improved treatment.

Methods 86 patients of families with hereditary FEVR were examined in this study. We used a proprietary capture panel to enrich for 294 known or suspected retinal disease causing genes in humans. Samples were processed using next generation sequencing (NGS) techniques followed by data analysis to identify and classify single nucleotide variants and indels. Sanger validation and segregation testing were used to verify suspected variants. This is the largest study of a FEVR cohort utilizing NGS that we are aware of.

Results Of the cohort of 86, 47 patients were probably solved (55%). Of these samples, 34 were due to rare variants in known FEVR causing genes, 25 of which were novel. The remaining 13 patients were found to have possible disease causing variants in other retinal disease causing genes, including four variants with a high probability of causation (i.e. stop-gain, frameshift, or splicing site mutations).

Conclusions We were able to determine probable disease causing variants in a large number of FEVR patients, the majority of which were novel. Knowledge of these variants will help to further characterize and diagnose FEVR.

Contributors: Salvo, Jason; Wang, Wang, Hui; Wang, Keqing; Nguyen, Duy; Zhang, Kang; Chen, Rui;
Improvement of modern medicine has increased human lifespan. However, many old people are suffering from hearing loss. To increase the quality of life, hearing restoration is important. Age-dependent hearing loss is mainly caused by loss of hair cell in the cochlea. Unlike human and other mammals, adult birds can regenerate their hair cells from surrounding supporting cells, thus restoring hearing. Inducing trans-differentiation of supporting cells into hair cells in the adult mammalian cochlea may be a pathway to hearing restoration.

Atoh1 is a bHLH transcriptional factor that is necessary and sufficient for hair cell formation. Ectopic overexpression of Atoh1 can trans-differentiate supporting cells into hair cells in embryonic and neonatal mice, but not in adults. To successfully induce trans-differentiation in the adult cochlea, it is important to decipher the mechanisms that prevent Atoh1-induced hair cell formation.

One of the mechanisms that prevents Atoh1 induced trans-differentiation in adults may be the inability to activate Atoh1’s target genes. To address this problem, we have begun to identify Atoh1 targets. We compared and analyzed hair cell RNA-seq data from the cochlea and Atoh1 ChIP-seq from cerebellum to find possible candidates. We will use in situ hybridization and ChIP-PCR to validate their expression in hair cells. We will also detect their mRNA in conditional Atoh1 knockout to show they are indeed targets of Atoh1. After finding several targets, we will determine if they can be induced by Atoh1 over-expression in neonatal and adult supporting cells.

If there is no expression of Atoh1 target genes in when Atoh1 is over-expressed in adults, it is possible that adult supporting cells lack Atoh1 co-activators. To elucidate this problem, we will use in situ hybridization to probe one identified Atoh1 co-activator, Tcf3, to see if there is expression in the adult. If there is no expression of Tcf3, then we will co-overexpress Atoh1 and Tcf3 in adult supporting cells, to see if this can induces hair cell formation.

In addition, epigenetic changes in enhancers of Atoh1 target genes may also prevent their expression in the adult. We will use ChIP-PCR and compare the epigenetic state of target genes in embryonic and adult supporting cells. If there are epigenetic state changes, we can modulate specific histone modification enzymes in Atoh1 overexpressing cells to see if ectopic hair cells can be induced in adult mice. Our goal is to successfully induce hair cell formation in the adult cochlea, and eventually restore hearing.

Contributors:
Attention-Deficit Hyperactive Disorder (ADHD) is the most commonly studied and diagnosed psychiatric disorder in children. Methylphenidate (MPH, e.g., Ritalin) is the most commonly prescribed treatment for ADHD, and it has been used for decades to treat ADHD, but little is known about its therapeutic mechanisms.

The hippocampus is a center for learning and memory, and synaptic plasticity is a measurable underlying mechanism for memory. This project examines the effects of MPH on synaptic plasticity in the hippocampus that correlates with and may contribute to learning and memory. Our hypothesis is that MPH will affect synaptic plasticity in the hippocampus that underlies learning, and the influence of MPH will arise from its ability to boost catecholamine signaling. The object of this proposal is to determine the effects of MPH on synaptic plasticity in the hippocampus of awake animals, and to more clearly elucidate its mechanism of action in the hippocampus.

We measure synaptic plasticity in response to MPH in the perforant path to dentate gyrus pathway in the hippocampus by recording field potentials with permanently implanted electrodes in freely moving mice. When weak theta burst stimulation was applied along the perforant pathway, it did not produce synaptic change. However, when the same stimulation was applied along with MPH (5 mg/kg), then the stimulation produced long-term potentiation (LTP) of 64 ± 18 % (n=6, p<0.01). These in vivo recordings offer a highly precise measurement of synaptic transmission, while preserving the overall circuitry and neurotransmitter systems that we ultimately wish to understand.

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TORC2 REGULATION OF AGING AND AGE-RELATED MEMORY IMPAIRMENT

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As our population ages, cognitive decline and dementia are becoming more prevalent. The fact that memory declines as a function of age indicates that there must be crosstalk between components of these two processes. Yet, the mechanisms underlying these processes are not fully understood and shared components have not been identified. This information is critical since different mechanisms of longevity extension may be beneficial to certain functions and detrimental to others such as cognition. The Target of Rapamycin complex 2 (TORC2) has been shown to play a role in both long-term memory (LTM) formation and aging. Given the evolutionary conservation of TORC2 between flies and humans and the relatively short lifespan of Drosophila melanogaster, this study investigates the role of dTORC2 in both aging and age-related memory impairment in the fly. We show that compared to young flies, aged flies show a significant impairment in LTM when tested in an aversive olfactory conditioning paradigm. Remarkably, LTM can be significantly enhanced in aged flies treated prior to training with Compound A, a specific activator of TORC2. Ongoing work will determine if dTORC2 regulates lifespan in the fly and if so, define the population of neurons required for this effect. Taken together, these results suggest that dTORC2 plays a role in age-associated cognitive decline in the fly and may provide the first evidence that dTORC2 regulates the processes of memory and aging in distinct neural populations.

Contributors: Johnson, Jennifer; Huang, Wei; Roman, Gregg; Costa-Mattioli, Mauro
From tumorigenesis to memory to viral infection, many fundamental physiological processes are linked to the regulation of translation. A messenger RNA is simultaneously translated by many ribosomes while interacting with many other regulatory and functional cofactors, and these components fold into an ordered supramolecular assembly called the polysome. The structure of the polysome will determine allowed or disallowed mechanisms of regulatory interaction. For instance, it was previously demonstrated that proteins binding specific 3’ UTR sequences can form complexes with 5’-associated proteins to suppress translation in a regulated fashion. Poly(A) binding protein (PABP) is a critical translation factor and regulator which is found both oligomerized on the poly(A) tail and free in solution, and has separable cis and trans activities. Its mechanisms of action are insufficiently understood. One of its many roles is to assist terminating ribosomes to re-initiate on the same mRNA instead of exchanging with the free pool of ribosomes in solution. PABP can bind the eRF3 stop codon release factor, the eIF4F 5’ initiation complex, and other molecules of PABP. Perhaps one or many molecules of PABP fold the mRNA into a stable cloverleaf to bring stop codon and 5’ end together, or maybe PABP dynamically shuttles back and forth between the stop codon and the 5’ end carrying a ribosome with it. As the polysome assembles, the rate of translation increases, but the cause is unknown. The increase is seemingly independent of de novo ribosome initiation and the 5’ cap; it is thought that interactions among the ribosomes themselves may boost the translation rate.

With the recent revelation that both linear and circular polysomes can occur in physiologically relevant systems, I hypothesize that the choice of topology is a regulated event with effects on translation. It remains to be seen what causes a given translating mRNA to adopt one or the other conformation. To that end, we are using or developing several techniques to assay polysome structure at the single-molecule level. Thus far we have purified human polysomes and used cryotomography to determine the orientation of individual ribosomes to extrapolate the trace of the mRNA chain. We have purified bacteriophage MS2 protein to specifically bind a hairpin cloned into a defined mRNA for detection of the UTR and are optimizing immunogold labeling for single-molecule imaging. Finally, we are attempting to locate the nascent peptide chains of translating ribosomes to determine how far a ribosome has advanced on the RNA. Our goal is to expand studies of ribosome topology to compare and contrast different mRNAs and translation conditions, disrupting known players such as the poly(A) tail, 5’ cap, and initiation factors.

Contributors: Kaelber, Jason; Fu, Caroline; Reineke, Lucas; Lloyd, Richard; Chiu, Wah
Metabotropic glutamate receptors (mGlus) are important for modulating signaling by glutamate, the main excitatory neurotransmitter in the central nervous system. The mGlus have been implicated in protection from neuronal excito-toxicity and in learning and memory. The structural basis of ligand specificity differences among Group1 and Group2 (mGluR1, 2, 3, 5) and Group3 (mGluR4, 6, 7, 8) has not been examined in an evolutionary context. Group 3 mGluR have diverged from Group1 and Group2 in having much higher binding affinities for the endogenous ligand L-Serine-O-Phosphate (L-SOP). To confirm the selectivity of Group 3 mGluR to L-SOP, several amino acids known to exist at rat brain were tested against mGluR1, mGluR2, mGluR4, mGluR6 and mGluR7 in HEK-293 cells expressing endogenous G\(_\alpha_q\) or transiently co-expressing G\(_\alpha_{15}\), both of which yield Ca\(^{2+}\) mobilization detectable by high-throughput fluorescence assays. For the Group1 and Group 2 mGluR, only glutamate showed robust response. Even though L-SOP did not activate these mGluRs, we found that L-SOP behaves as an antagonist to mGluR1. For Group 3 receptors, mGluR4, mGluR6 and mGluR7, only L-SOP but not other amino acids activated these receptors in addition to glutamate. In short, L-SOP seems to be the only endogenous ligand specific to Group 3 mGluR and antagonist to mGluR1. To determine important residues for L-SOP binding, the Lichtarge group and ours have used the Evolutionary Trace to analyze sequences from the class C GPCR family, and identified G319, Q170, L342, N415, S344 and S189 as candidates for residues important for ligand binding and/or responses. To test these residues, I have prepared myc-tagged mGluR1 and mGluR4 mutants with reciprocal residue swaps. Among mGluR4 mutants, S319G, P342L showed decreased efficacy (maximal response) and D415N and R344S decreased potency for L-SOP, a ligand of mGluR4. For reciprocal mGluR1 mutants, we tested if these mutants behave as better antagonist to L-SOP than wild type mGluR1. Comparing empirical ki value for L-SOP from this assay with potency (EC50) of L-Glu, we found that mGluR1 single mutant S344R and hextuple mutant G319S+Q170M+L342P+N415D+S344R+S189A showed significantly better selectivity for L-SOP over L-Glu. Therefore, our study demonstrated that Evolutionary Trace has predictive power to uniquely characterize that L-SOP binding for mGluRs.

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Protein Folding and Collapse: Thermodynamics of Aggregation of Gly5

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Intrinsically disordered proteins (IDPs) are proteins that do not fold into a stable, three-dimensional, native structure, except possibly when bound to a ligand, partner protein, etc. Evidence suggests that IDPs function by varying the thermodynamics of recognition and binding by proteins, and thus affect the affinity and specificity with which the proteins bind to their partners. IDPs tend to be rich in amino acids like glycine, which increase their disorderliness. We use oligoglycines as a model to study the behavior of IDPs in solvent in order to determine the thermodynamics of their folding and function.

The solubility of oligoglycine in water decreases as its length increases until, when the peptide contains 5-6 glycines, it aggregates and falls out of solution. However, previous work by our group shows that the solvation free energy of oligoglycine decreases as its length increases from 2 to 5 residues, indicating that solubility should increase with increase in length. We hypothesize that as the peptide length increases, intermolecular interactions between oligoglycines are favored over interactions between oligoglycine and water, leading to their aggregation i.e. local concentration effects play a significant role in driving oligoglycines to aggregate and/or collapse. This aggregation occurs as a liquid-liquid phase separation or a second order phase transition. Here we simulate the behavior of several hundred short (five residues) oligoglycines in water at varying concentrations in order to understand the structural and thermodynamic changes that occur during aggregation of these short peptides. We hypothesize that the thermodynamics of aggregation of short oligoglycines is equivalent to the thermodynamics of folding or collapse of longer oligoglycines in water. We propose to compare the thermodynamics of aggregation of short oligoglycines with the collapse of longer oligoglycines in water in order to understand the thermodynamics of collapse of the longer peptide.

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Breast cancer is a collection of diseases with distinct clinical behaviors and underlying genetic causes. Triple-negative breast cancer (TNBC) is a common subtype of breast cancer that confers a particularly poor prognosis and is refractory to current targeted therapies. Unfortunately, the molecular determinants driving this aggressive malignancy are poorly understood. Using an unbiased genetic screen, we identified an oncogenic network that supports the malignant state of TNBCs in vitro and in vivo. We define a new signaling axis (the STP kinase axis) as a core component in this network. Aberrant activation of the STP axis promotes breast cancer transformation, and STP axis components are frequently amplified and over-expressed in human breast cancer. Mechanistically, the STP axis supports cell transformation by priming ubiquitylation and degradation of the REST tumor suppressor. Notably, REST is frequently lost post-translationally in human TNBCs, and genetic or pharmacologic inhibition of the STP axis restores REST protein levels in TNBC cells. Furthermore, inhibition of the STP axis impairs primary tumor growth and metastatic proclivity in models of TNBC. In breast cancer patients, elevated STP axis expression correlates with decreased REST levels and significantly reduced patient survival. Collectively, these data identify the STP-REST axis as an important oncogenic determinant in TNBC, and provide a tractable entry point for TNBC therapies.
Despite recent developments in treatment strategies, castrate resistant prostate cancer (CRPC) is still the second leading cause of cancer associated mortality among American men, the biological underpinnings of which are not well understood. To this end, we measured levels of 150 metabolites and examined the rate of utilization of 184 metabolites in androgen dependent prostate cancer (AD) and CRPC cell lines using a combination of targeted mass spectrometry and metabolic phenotyping. Metabolic data were used to derive biochemical pathways that were enriched in CRPC, using Oncomine Concept Maps (OCM). The enriched pathways were then examined in-silico for their association with treatment failure (i.e., PSA recurrence or biochemical recurrence) using published clinically annotated gene expression data sets. Our results indicate that a total of 19 metabolites were altered in CRPC compared to AD cell lines. These altered metabolites mapped to a highly interconnected network of biochemical pathways that describe UDP glucuronosyltransferase (UGT) activity. We observed an association with time to treatment failure in an analysis employing genes restricted to this pathway in three independent gene expression data sets. In summary, our studies highlight the value of employing metabolomic strategies in cell lines to derive potentially clinically useful predictive tools.

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Hippocampal Functional Alterations in Mouse Models of Rett and Angelman Syndromes

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Despite the diversity of the underlying genetic defects, there are many clinical features common to most Autism Spectrum Disorders (ASDs). How can such vastly different genetic mutations cause such similar behavioral changes? Considering neural circuits directly mediate behavior, it is likely the overlap is due to common functional abnormalities at the neural circuit level. Probing the neural circuits common to these behaviors is a crucial step to answer this question. Therefore, we propose to examine the neural circuits of two disorders with vastly different genetic and molecular changes, yet common symptoms that are often clinically confused, Rett (RTT) and Angelman (AS) syndromes. RTT is caused by loss of function mutations in the X-linked MECP2 that encodes Methyl-CpG-binding protein 2 (MeCP2). AS is caused by loss of function mutations in the maternal copy of the paternally imprinted gene UBE3A that encodes an E3 ubiquitin ligase called E6-AP. Both syndromes lead to autism, motor dysfunction, and seizures1. The mouse models for RTT (Mecp2+/-) and AS (Ube3amat-/pat+) reliably reproduce many of the behavioral and cognitive deficits seen in the human disorders, specifically, similar memory deficits are found in both models. Since the hippocampus is an area crucial for learning and memory, our hypothesis is that the molecular alterations in RTT and AS lead to common functional changes in the hippocampal circuit during one or more of the stages of memory processing. To test this hypothesis, we plan to examine the hippocampal circuit properties of mouse models of RTT and AS related to memory formation, consolidation and retrieval.

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CIRCADIAN HOMEOSTASIS OF LIVER METABOLISM SUPPRESSES HEPATOCARCINGENESIS

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In the United States, liver cancer shows an average increase of 3.2% annually among men and woman since 1992 and currently ranked second only to pancreatic cancer for cancer-related deaths. More than 80% liver cancers are hepatocellular carcinoma (HCC). Although chronic infections with hepatitis B or C virus (HBV and HCV) are the major risk factors for HCC worldwide, liver metabolic diseases such as alcohol-related cirrhosis and non-alcoholic fatty liver disease associated with obesity account for the majority of HCC cases in the U.S. and other western countries. However, the role of liver metabolic dysfunction in HCC development is understudied. Recent epidemiological studies have revealed that disruption of circadian homeostasis in human night-shift workers, which affects up to 20% of the workforce in industrialized countries, leads to a coupled increase in the risk of obesity, liver metabolic syndromes and cancers. We previously reported that circadian dysfunction disrupts the endogenous circadian clock to promote tumor development, and that liver tumors are the second most common primary lesion induced by circadian disruption in mice. We have further studied the impact of disrupting circadian rhythm in mice by chronic jet-lag following a schedule that mimics the night-shift working schedules in humans. In jet-lagged mice we observed a progressive elevation of serum and hepatic bile acid levels, the induction of liver inflammation and multiple liver metabolic phenotypes including hepatosteatosis, as well as uncontrolled hepatocyte and intra-hepatic bile duct proliferation. Here we report that circadian disruption induces metabolic syndromes in mice prior to the onset of HCC and that deregulation of oncogene MYC and nuclear receptors involved in liver bile acid metabolism and signaling are among the key mechanism synergistically promoting liver metabolic dysfunction and HCC development in vivo.

Contributors: Kettner, Nicole M.; Pan, Michael; Finegold, Milton J.; Moore, David D.; Fu, Loning
TH1 CELLS MEDIATE SKELETAL MUSCLE INFLAMMATION AND INSULIN RESISTANCE IN OBESITY THROUGH JAK/STAT PATHWAY

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Macrophage infiltration in skeletal muscle is associated with obesity-induced insulin resistance. We have shown that in addition to macrophages, skeletal muscle of obese mice has higher total T cell content. Flow cytometry analysis further revealed a 1.4-fold increase in CD4+ and a 2-fold increase in CD8+ T cells in skeletal muscle of C57BL/6 mice fed with high fat diet (HFD) for 3 months. RT-PCR analysis showed significant induction of IL-2 and IL-12, factors important for differentiation of naïve CD4+ T cells into T helper I (TH1), and increase in IFNγ, the main TH1 cytokine, in skeletal muscle of mice on HFD. Consistently, intracellular staining of digested skeletal muscle tissue showed a 4-fold increase in TH1 cells in obese mice compared to lean controls. IFNγ is known to signal through JAK/STAT1 pathway. Immunoblotting skeletal muscle extracts demonstrated 60% increase in phosphorylated STAT1 protein levels in obese mice. We have shown reduced insulin sensitivity and increased inflammatory gene expression in C2C12 myofibers treated with TH1 supernatant. We thus hypothesized that detrimental effects of TH1 on skeletal muscle function can be attributed to activation of STAT1. Treatment of C2C12 myofibers with TH1 supernatant resulted in 15-fold upregulation of STAT1 gene expression, as well as 80% increase in phosphorylated STAT1 protein. To investigate the involvement of STAT1 in TH1-mediated skeletal muscle inflammation and insulin resistance, we used the inhibitor of JAKs. The addition of JAKs inhibitor abolished TH1-induced STAT1 phosphorylation and significantly improved insulin sensitivity in C2C12 cells exposed to TH1 supernatant, evidenced by higher P-Akt (S473)/Akt. The JAKs inhibitor also attenuated TH1-mediated induction of IL-6, TNFα, MCP-1, and RANTES in C2C12 myofibers. In summary, TH1 upregulates and activates STAT1 in skeletal muscle of mice on HFD, which may be important for skeletal muscle metabolism and inflammatory gene expression.

Contributors: Ilvira Khan, Xiaoyuan Perrard, Jerry Perrard, Hua Lui, C Wayne Smith, Huaizhu Wu, Christie Ballantyne
The urea cycle functions to detoxify ammonia derived from the protein breakdown into urea, which is excreted as a component of the urine. Deficiency of any of the six enzymes involved in this cycle will lead to urea cycle disorders (UCDs), the prevalence of which is 1:8200 births in the United States. While the urea cycle occurs primarily in the liver, three of the enzymes (argininosuccinate synthetase [ASS], argininosuccinate lyase [ASL], and arginase [ARG]) also directly regulate the nitric oxide (NO) production in many tissues. In addition to hyperammonemia, the classic manifestation of all UCDs, deficiency of ASS, ASL, or ARG can cause long-term complications. Patients with ASL deficiency, for example, often develop other complex clinical phenotypes, including systemic hypertension and neurocognitive deficiencies, despite early treatment and control of the hyperammonemia. Our recent findings suggested that defects in NO signaling contribute to the complications observed in the hypomorphic mouse model of ASL deficiency.

To develop human cell-based models to further study cell-autonomous NO dysfunction at the molecular level, we generated human induced pluripotent stem cells (iPSC) from patients with ASL and ARG1 deficiency. To derive patient-specific iPSC, we isolated fibroblasts from skin biopsies of the patients and introduced reprogramming genes to these fibroblasts via mRNA transfection or retroviral transduction. Several ASL and ARG1 deficiency clones have been successfully generated by either method and they are characterized to be iPSC on the basis of the morphology, growth characteristics, alkaline-phosphatase staining, expression of pluripotency markers, and capacity to differentiate into all germ layers in vitro through embryoid bodies formation. A normal karyotype was maintained in all of the lines tested. Moreover, we have further differentiated these ASL and ARG1 deficiency iPSC lines, along with iPSC from healthy subjects, into the neural lineage to develop in vitro models to study the molecular neuropathogenesis of the disorders. Our current work specifically focuses on studying the cell-autonomous role of both ASL and ARG1 in regulating neural differentiation and synaptic function through NO signaling.

Contributors: Kho, Jordan; Nagamani, Sandesh C.S.; Erez, Ayelet; Bissig, Karl-Dimiter; Zwaka, Thomas; Lee, Brendan
The adult mammalian brain displays the remarkable feature of continued neurogenesis, whereby neural stem cells divide and integrate into the hippocampus and olfactory bulb to regenerate nervous tissue throughout adulthood. This suggests the existence of mechanisms that coordinate new synapse formation between pre-existing brain tissue and newborn neurons, which could be useful towards development of regenerative brain cell therapies. Using the rodent olfactory system as a model to examine these mechanisms, our lab identified a novel peptidergic signaling pathway between established mouse olfactory bulb circuitry and incoming integrants, which may guide newborn neurons into appropriate circuit positions. This signaling pathway involves release of corticotropin-releasing hormone (CRH) by resident cells of the olfactory bulb onto incoming neurons. Incoming cells upregulate expression of CRH receptor (CRHR) as they migrate into the mature circuitry. Interestingly, we observe high CRHR expression in the most anatomically superficial portions of the granule cell layer of the olfactory bulb, suggesting a spatial pattern to newborn neuron integration. Others reported that this layer is both the longest-surviving and the least remodeled layer within the olfactory bulb. We show here that genetic ablation of the superficial granule cell layer alters the pattern of integration of successive waves of incoming neurons, supporting the hypothesis that the functional state of pre-existing brain circuitry in the adult mammal affects the process of newborn neuron integration. Based on our previous findings that CRH signaling serves as a cue to guide incoming neurons in the unperturbed olfactory bulb, we hypothesize that it may also be involved in directing neural cell maturation and integration in bulbs whose pre-existing resident cells are targeted for death. Future studies are directed toward gain-of-function analyses to ectopically enhance CRH signaling, in order to test this hypothesis directly.
Low rates of early mother-to-child HIV transmission in a routine programmatic setting in Lilongwe, Malawi.

Maria H Kim

Clinical Scientist Training Program
Advisor: Elizabeth Chiao, M.D./M.P.H.-Department of Medicine

Background:
Data on prevention of mother-to-child transmission of HIV (PMTCT) effectiveness within routine healthcare delivery in resource-constrained settings is limited. We sought to evaluate the impact of PMTCT delivery and maternal CD4 count on early HIV transmission within the Tingathe program in Lilongwe, Malawi. Tingathe utilizes community health workers to ensure mother-infant pairs receive all PMTCT services.

Methods:
We reviewed clinical records of all 1088 mother-infant pairs enrolled March 2009-March 2011 who completed follow up to first DNA PCR. The CD4 cutoff for antiretroviral therapy (ART) eligibility changed from 250 to 350 in August 2010. Women on ART at enrollment did not receive CD4 testing. The recommended PMTCT regimen for women ineligible for ART was complete combination prophylaxis- mother: zidovudine (AZT) for at least 6 weeks+ single dose nevirapine (sdNVP)+combivir (AZT+lamivudine) tail, and infant: sdNVP+AZT. Incomplete combination prophylaxis was defined as non-completion of any component of complete combination prophylaxis. Early ART was defined as ART for >14 weeks prior to delivery. We determined transmission rates with confidence intervals and compared these rates using global chi-square tests, followed by post-hoc pairwise testing to evaluate differences between multiple proportions.

Results:
Transmission rate at first PCR was 4.1%. Pairs receiving suboptimal antiretroviral (ARV) prophylaxis were more likely to transmit HIV (10.3%, 95% CI, 5.5–18.1%). ART was associated with reduced transmission (1.4%, 95% CI, 0.6–3.0%), with early ART associated with decreased transmission (no transmission), compared to all other treatment groups (p = 0.001). No association was detected between transmission and CD4+ categories (p = 0.337), trimester of pregnancy at enrollment (p = 0.100), or maternal age (p = 0.164). No transmissions were observed among women on ART for more than 14 weeks prior to delivery.

Conclusion:
Low rates of early MTCT of HIV are possible in resource-constrained settings under routine programmatic conditions. Early ART is more protective than any other regimen. Furthermore, in the context of timely initiation of ART and PMTCT prophylaxis, baseline CD4 does not impact transmission among women not on ART at baseline. Efforts to improve timely initiation of ART and PMTCT prophylaxis are needed.

Reactive stroma in prostate cancer is typified by the co-evolution of myofibroblasts/CAFs. This reactive stroma is associated with most human carcinoma and is predictive of progression. TGF-β1 is a key factor in regulating reactive stroma biology. However, the origin of myofibroblasts and the mechanisms of how TGF-β1 recruits, activates and induces their differentiation are essentially unknown. We have identified mesenchymal stem cells from normal human prostate gland and evaluated their biology in a novel 3D co-culture system. Human prostate-derived mesenchymal stem cells (hpMSCs) were CD44+/CD90+, expressed other mesenchymal stem cell genes, and exhibited multipotent differentiation patterns. When co-cultured with LNCaP cells in Millicell-CM inserts or co-inoculated in nude mice, self-organizing organoids formed with a core of stromal cells and a peripheral mantel of LNCaP cells. To investigate the role of TGF-β1, the hpMSCs were co-cultured with LNCaP cells engineered to overexpress active TGF-β1. Significantly, LNCaP cells engineered to overexpress TGF-β1 induced hpMSC differentiation to prototypical reactive stroma myofibroblasts. Microarray analysis of stroma revealed 1617 gene probes with more than a 2 fold-change in the presence of TGF-β1, showing that expression of TGF-β1 in LNCaP cells drives differential gene expression in hpMSCs. Of those gene expression profiles, RUNX1 was identified as a key transcription factor in hpMSC that mediates TGF-β1-induced myofibroblast differentiation. Knockdown of RUNX1 in hpMSC significantly promoted differentiation to myofibroblasts. Conversely, overexpression of RUNX1 inhibited a myofibroblast gene expression signature. Furthermore, cell cycle analyses following loss of RUNX1 showed a block at the G2/M phase of the cycle in HP-MSCs, consistent with expression changes in genes required for progression through G2 and M phases. These data suggest that RUNX1 plays a permissive role in the regulation of TGF-β1-driven myofibroblast differentiation by mediating G2/M arrest. Together, our data show that reactive stroma in prostate cancer initiates from activation and differentiation of CD44+/CD90+ endogenous hpMSCs and that RUNX1 functions as a major regulator of the stem cell state of those cells and therefore a modulator of the co-evolution of reactive stroma. Understanding these mechanisms is important for developing new strategies to target the microenvironment niche.
Breast cancer is the second leading cause of cancer-related deaths in women. It is a heterogeneous disease divided into multiple subtypes. Claudin-low subtype exhibits mesenchymal properties, is resistant to conventional therapy, and has been shown to be enriched in treatment-resistant Tumor-Initiating-Cells (TICs). TICs exhibit decreased expression of miR-200 family members, critical for Epithelial-Mesenchymal Transition (EMT), a process which renders cells migratory and stem-cell like. We observed that miR-200 expression is down-regulated in our p53-null claudin-low mouse model, and accordingly hypothesize that restoring miR-200 expression in claudin-low tumors will change their TIC profile, therapeutic sensitivity and metastatic potential. To re-express the miR-200 family members in tumors, we created doxycycline-inducible lentiviral constructs and transduced cells from primary claudin-low tumors. Transduced cells were then injected into the cleared fat pad of wild-type mice, and mice were treated with vehicle or doxycycline. Induction of miR-200c led to a decrease in tumor growth, with a significant decrease in proliferation. We observed altered tumor histology, with the spindloid structures changing into more epithelial-like structures post-induction. In vitro migration assay showed a significant difference in migration potential of tumor cells with and without expression of miR-200c. To confirm our findings in vivo, we performed tail-vein injections using transduced tumor cells, and found that the doxycycline treated group had significantly fewer lung metastases. To investigate the potential change in chemoresistance, mice bearing tumors were treated with doxycycline and carboplatin, alone or in combination. Preliminary results suggest that induction of miR-200c in tumors increases their sensitivity to chemotherapy, as combination of doxycycline and carboplatin treatment led to a higher level of apoptosis. Taken together, our data show that miR200c expression alters claudin-low tumor phenotype, affects their growth and metastatic potential, and enhances their sensitivity to chemotherapy. These findings may help in the development of approaches to target TICs in breast cancer. (Supported by grant CA 148761).
Human adenoviruses cause acute illnesses associated with respiratory, gastrointestinal, and ocular infections. In addition to their use as vectors for vaccination and gene and cancer therapy, adenoviruses also serve as tools for revealing mechanisms of cancer due to their tumorigenic potential in experimental animals. The human adenovirus E4-ORF1 gene encodes an oncoprotein that enhances viral replication by activating cellular phosphatidylinositol 3-kinase (PI3K). While the underlying mechanism of activation is not known, this activity depends on a complex formed between E4-ORF1 and the cellular PDZ protein Discs Large 1 (Dlg1).

Mass spectrometry analysis of cellular proteins that associate in vitro with a GST-E4-ORF1 fusion protein identified the PI3K regulatory subunit p85 as a candidate cellular binding partner of E4-ORF1. Confirming and extending this observation, I have shown that in human epithelial cells, E4-ORF1 directly interacts with the p85 regulatory and p110 catalytic subunits of PI3K and elevates their levels, the latter effect of which, like PI3K activation, requires Dlg1. Furthermore, I showed that E4-ORF1, PI3K, and Dlg1 assemble into a ternary complex located at the plasma membrane. At this site, Dlg1 co-localizes with the activated PI3K effector protein Akt, supporting the idea that the ternary complex mediates PI3K signaling. Signifying the functional significance for the ternary complex, the capacity of E4-ORF1 to induce cells to form colonies in soft agar is ablated either by a mutation that prevents E4-ORF1 binding to Dlg1 or by a PI3K inhibitor drug. These findings indicate that adenovirus E4-ORF1 assembles a Dlg1:E4-ORF1:PI3K protein complex that functions to mediate Dlg1-dependent PI3K recruitment to the plasma membrane and dysregulation of PI3K signaling. This novel mechanism is likely to have significance for other pathogenic human viruses, including influenza A virus, human papillomavirus, and human T-lymphotropic virus type I, that likewise encode for proteins that target Dlg1 and activate the PI3K pathway.

Contributors: Kong, Kathleen; Kumar, Manish; Taruishi, Midori; Javier, Ronald T.
BCL2-associated athanogene 6 (BAG6) is a member of the BAG protein family, which is implicated in diverse cellular processes including apoptosis, co-chaperone, and DNA damage response (DDR). Recently, it has been shown that BAG6 forms a stable complex with UBL4A and GET4 and functions in membrane protein targeting and protein quality control. The BAG6 sequence contains a canonical nuclear localization signal and is localized predominantly in the nucleus. However, GET4 and UBL4A are found mainly in the cytoplasm. This raised the question whether GET4 and UBL4A are also involved in DDR in the context of the BAG6 complex. Here, we provide evidence that nuclear BAG6-UBL4A-GET4 complex mediates DDR signaling and damage-induced cell death. BAG6 appears to be the central component for the process, as depletion of BAG6 leads to the loss of both UBL4A and GET4 proteins and resistance to cell killing by DNA-damaging agents. In addition, nuclear localization of BAG6 and phosphorylation of BAG6 by ATM/ATR are also required for cell killing. UBL4A and GET4 translocate to the nucleus upon DNA damage and appear to play redundant roles in cell killing, as depletion of either one has no effect but co-depletion leads to resistance. All three components of the BAG6 complex are required for optimal DDR signaling, as BAG6, and to a lesser extent, GET4 and UBL4A, regulate the recruitment of BRCA1 to sites of DNA damage. Finally, we purified endogenous BAG6, GET4 and UBL4A by affinity purification and analyzed precipitated endogenous protein complexes by mass spectrometry to study the BAG6 complex interactome under DNA damage conditions in HeLa cells. Together our results suggest that the nuclear BAG6 complex is an effector in the DNA damage response pathway and its phosphorylation and nuclear localization are important determinants for its function. In addition, mass spectrometric analysis of the BAG6 complex precipitates revealed new components of the BAG6 complex interaction network in the context of damaged DNA.

Contributors: Liu, Shangfeng; Shi, Yi; Yucer, Nur; Ortiz, Priscilla; Kim, Beom-Jun; Odejimi, Ore Abiola; Qin, Jun; Wang, Yi.
Mammalian epidermis consists of the interfollicular epidermis (IFE) and associated appendages, including hair follicles (HFs) and sebaceous glands (SGs). The differentiation of stem cell progeny into specific epidermal lineage is tightly regulated, as altered growth control results in different types of skin tumors. Reactivation of embryonic signature genes during tumor formation is observed in many types of cancer. In my study, I aim to assess the impact of activating the embryonic gene Sox11 on cutaneous tumor formation.

As a member of SRY-box transcription factor, Sox11 is expressed at early embryonic stage (E12.5 to E17.5) during skin morphogenesis. We have found that Sox11 promotes the expression of a tumor-promoting gene, Tcf7l1 (or Tcf3). Given that Tcf3 overexpression promotes tumorigenesis and Sox11 activates Tcf3 expression, I hypothesize that misexpression of Sox11 plays a role in skin tumorigenesis.

Combining the two-stage chemical carcinogenesis system with the tet-inducible Sox11 mouse model, I ectopically expressed Sox11 in the epidermis and assessed how its overexpression affects tumorigenesis. I found Sox11 induction increased both tumor incidence and multiplicity. These chemical-induced tumors include typical papilloma and atypical sebaceous tumors. Mechanistically, I found that Sox11 increases the transactivating activity of β-catenin and induces the expression of β-catenin's DNA binding partners Lef/TCF in vitro. In summary, epidermal Sox11 induction promotes the formation of skin tumors in the chemically induced skin cancer model and alters Wnt signaling.

Contributors: Ku, Amy T; Miao, Qi; Nguyen, Hoang
DYNAMIN CONTROLS SNARE DENSITY AT THE FUSION SITE

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The convergence of the antagonistic reactions of membrane fusion and fission at the hemifusion/hemifission intermediate has generated a captivating enigma of whether SNAREs and dynamin have unusual counter-functions in fission and fusion respectively. SNARE-mediated fusion and dynamin-driven fission are fundamental membrane remodeling reactions known to occur during ubiquitous cellular communication events such as exocytosis, endocytosis and vesicle transport. Here we demonstrate the influence of the dynamin homolog Vps1 on lipid mixing and content mixing properties of yeast vacuoles, and on the incorporation of SNAREs into fusogenic complexes. We propose a novel concept that Vps1, through its oligomerization and SNARE domain binding, promotes the hemifusion-content mixing transition in yeast vacuole fusion by increasing the number of trans-SNAREs.

Contributors: Kulkarni, Aditya; Alpadi, Kannan; Peters, Christopher
Enhancing chemotherapeutic response by blocking PGE2–induced recruitment of quiescent cancer stem cells from repopulating residual tumors

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Cytotoxic chemotherapy remains a major therapeutic option for a wide spectrum of epithelial cancers including urothelial carcinoma. While chemotherapy is highly effective in debulking the tumor mass and improving survival, certain patients show initial response but their tumors eventually become unresponsive after multiple chemotherapy cycles. Currently the identity of repopulating cancer cells following chemotherapy is unknown, and the underlying molecular mechanisms that initiate tumor repopulation remain poorly understood. Here we hypothesize that cancer stem cells (CSCs) may be recruited to repopulate chemotherapy induced damage within residual tumors, similar to how normal resident tissue stem cells mobilize to wound sites for tissue repair.

To test this hypothesis we utilized our previous data showing that cytokeratin 14 (CK14) marks the most primitive urothelial carcinoma cells and abundance of CK14+ cancer cells in patients correlates with poor survival. We found that exposure to one cycle of gemcitabine and cisplatin (GC) chemotherapy effectively reduced the size and growth rate of tumors in vivo. We then followed the regular clinical regimen with a gap period to allow recovery of normal tissues and found a generalized expansion of CK14+ cancer cells in residual tumors during the time between treatment cycles. Next we investigated whether cancer cells are stimulated to proliferate in between chemotherapy courses to repopulate residual tumors and demonstrated the increase of proliferating CK14+ cells. To further explore the recruitment of quiescent CSCs we utilized a dual-labeling approach with thymidine analogues (IdU to mark slow-cycling or quiescent cells/CldU to define actively proliferating cancer cells) in tumor xenografts and found that there was a pool of label retaining slow-cycling CK14+IdU+ cells recruited to proliferation (CK14+IdU+CldU+) as soon as 12 hours after initial GC treatment. We also found that prostaglandin E2 (PGE2) released by neighboring apoptotic cells induced this CSC repopulation in a paracrine manner, while Celecoxib-mediated attenuation of PGE2 signaling blocked CSC repopulation following the first chemotherapy cycle and consequently improved chemotherapeutic response in a second cycle of chemotherapy.

Here we demonstrated a new mechanism by which CSCs contribute to therapeutic resistance, via repopulating residual tumors between chemotherapy cycles. This repopulation occurs by the recruitment of quiescent label-retaining CSCs to divide and “repair” chemotherapy-induced damage, similar to how normal stem cells are mobilized during wound repair. These findings reveal a new mechanism in the development of clinically relevant chemoresistant cancer, and provide a new paradigm to enhance chemotherapeutic response by abrogating early “wound repair”-like CSC repopulation.

Contributors: Kurtova, Antonina; Pazhanisamy, Senthil; Xiao, Jing; Lay, Erica; Chan, Keith
Metabolic regulation by the MeCP2/HDAC3 transcriptional corepressor complex.

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that affects approximately 1 in 10,000 live female births and presents with severe autistic features. Symptom severity is progressive, and includes loss of speech and motor skills, stereotypic hand movements, difficulty walking, apneas, digestive problems, bone anomalies, and seizures. RTT is primarily caused by mutations in the X-linked gene encoding methyl CpG binding protein 2 (MeCP2). Although RTT has been classically labeled as a neurological disease, recent reports have described an underlying metabolic aspect to the disorder in human patients and in mouse models lacking functional Mecp2. Here we show interaction between MeCP2 and histone deacetylase 3 (HDAC3), a potent regulator of lipogenesis and cholesterol biosynthesis, in the liver and brain. Concurrently, we describe metabolic defects in Mecp2 null mice, including fatty liver disease, an increased rate of lipolysis, and aberrant expression of genes encoding proteins of the lipogenesis pathway. These metabolic symptoms are similar to that in mice with a liver-specific knockout of HDAC3. Our data suggests that subsets of Mecp2 null phenotypes result from the loss of this interaction, resulting in an inability for MeCP2/HDAC3 to remodel chromatin and repress expression of a subset of metabolic genes. Importantly, these findings point to a novel mechanism of action of MeCP2, which may be targeted by pharmaceutical intervention to relieve a subset of symptoms in Rett syndrome patients.

Contributors: Buchovecky, Christie; Justice, Monica
EX VIVO EXPANDED MULTI-SPECIFIC CYTOTOXIC T CELLS DERIVED FROM HIV+ PATIENTS AND HIV NEGATIVE DONORS USING GMP-COMPLIANT METHODOLOGIES RECOGNIZE MULTIPLE HIV ANTIGENS AND SUPPRESS HIV REPLICATION

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Antiretroviral therapy (ART) does not eliminate HIV from latently infected reservoirs, has long-term toxicities and fails to fully prevent immune attenuation. Therefore there is a need for alternative therapies that will decrease dependency on ART. Previous studies have demonstrated the safety and feasibility of infusing single-epitope specific CD8 T cells. However, these T cells were restricted to a single HLA restricted epitope and had limited persistence in vivo. Hence, we hypothesized that broadly HIV-specific T cells could be expanded from patients on ART and HIV negative individuals to effectively target HIV infection using a non-HLA restricted approach. We developed a method by which PBMCs from ART patients were stimulated with gag, pol, and nef peptide libraries (pepmixes). T cells expanded to clinically relevant numbers (Mean=1.62e8 cells, Range (3.72e7, 2.87e8 cells), n=7) in the presence of antiretrovirals. 5 of 7 patient sample lines showed specific activity to all 3 HIV antigens in IFNY ELISPOT assays, with the remaining 2 showing specificity to 1 of 3 antigens. The T cell lines were broadly specific to gag (mean=99.33 SFC/10e5 cells), pol (mean=131.11 SFC/10e5 cells) and nef (mean=337.26 SFC/10e5 cells). Due to the association of gag-specific T cell responses with viral control, we also determined whether gag-specific T cells could be derived from seronegative donors for potential third party use. Gag-specific T cells were expanded from HIV seronegatives using gag-pepmix. These T cells released IFNγ in response to gag (163.79 SFC/1e5 cells, n=9) but not an irrelevant antigen (mean=7.0 SFC/1e5 cells). Importantly, T cells expanded from both ART patients and HIV seronegatives were cytotoxic, as expanded T cells lysed antigen loaded autologous PHA blasts (mean=67.55% specific lysis at 10:1 effector:target ratio) but not PHA blasts alone (mean=0.46% specific lysis at 10:1 effector target ratio). Expanded T cells from ART patients also showed a greater ability to suppress HIV outgrowth in vitro compared to unexpanded CD8 T cells when co-cultured with reactivated resting CD4+ T cells from ART-suppressed HIV+ patients, the authentic latent reservoirs. In 5 patients on ART a lower recovery of virus from resting CD4+ cells was seen in the presence of CTLs as compared to no effectors (p<0.006 by Mann Whitney), while the unexpanded autologous CD8 cells showed only a modest trend towards decreased recovery that was not statistically significant (p>0.9). Similarly, HIV-specific T cells derived from HIV seronegative individuals were able to suppress HIV replication more than unexpanded CD8 T cells when co-cultured with autologous CD4 T cells infected with HIVSF162 (HIV only condition p24=681.95 pg/mL, nonspecific CD8 T cells=448.80 pg/mL, expanded CTL=145.82 pg/mL). We have developed robust GMP-compliant methodologies for expanding functional HIV-specific T cells from both HIV+ patients and HIV negative donors for autologous and third-party use, respectively. We now plan to translate our approach to the clinical setting where we will test HIV-polyspecific T cell products as a part of a strategy to fully eradicate HIV infection.Contributors: Lam, Sharon*; Sung, Julia2*; Cruz, RY Conrad*; Castillo-Caro, Paul4*; Ngo, Minh Tran5*; Garrido, Caroline2*; Kuruc, Joann2*; Rooney, Cliona1*; Margolis, David2* and Bollard, Catherine6
DEMONSTRATING THE CRITICAL ROLE OF UTERINE ERBB SIGNALING IN FERTILITY

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Department of Molecular & Cellular Biology  
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The establishment of the appropriate embryo-uterine interactions necessary for a successful pregnancy requires the coordinate actions of growth factors and their receptors. We identified Egfr (Erbb1) as being induced during the implantation period and hypothesize that EGFR signaling regulates the ability of the endometrium to support embryo implantation. This hypothesis is being tested in vivo using mouse models and in vitro using primary human endometrial stromal (HES) cells.

We generated a mouse model with conditional ablation of Egfr in the uterus by crossing an Egfr floxed (Egfrf/f) mouse with a progesterone receptor cre (PRcre) mouse (PRcre/+Egfrf/f; Egfrd/d). Egfrd/d mice are severely subfertile, exhibiting demise in implanting blastocyst health. Implantation failure is likely due to an abrogated decidual response including defects in stromal cell proliferation, apoptosis and differentiation. Microarray comparison indicates misregulation of 3,195 genes in the absence of Egfr. Analysis of cellular functions affected identified processes such as post translational modification (148 kinases), cell death and survival, cell cycle regulation and cell growth and proliferation. Canonical pathway analysis indicates that the PI3K and WNT signaling pathways are likely key mediators of EGFR signaling. Furthermore, we compared microarrays done with uteri from mice lacking either Wnt4 or Bmp2 and determined that they are downstream mediators of EGFR. Because Erbb receptors act as dimers, we examined the effects of the other receptors in the endometrium. Ablation of either Her2 or Erbb3 results only in a slight reduction in litter size, indicating Egfr plays a predominant role while the other receptors may act as dimerizing partners.

In humans, EGFR is induced during decidualization and its attenuation via siRNA impedes in vitro HES cell decidualization as assessed by cell morphology and marker gene expression. Furthermore, we have used kinase antibody arrays to investigate the phosphorylation level of 44 different kinases in the absence of EGFR and have identified alterations of greater than 1.5–fold change in 34 different phosphorylation events following treatment with hormones, growth factor, or altered in both (11, 12, 11; respectively). Interestingly, we have observed inhibition of progesterone receptor expression and many of the genes identified in the microarray are PR targets. We are currently investigating the role of EGFR in regulating PR expression, phosphorylation and target gene promoter occupancy.

Contributors: Large, Michael; Hartig, Sean; Wetendorf, Margeaux; Threadgill, David; Kovanci, Ertug; Jeong, JaeWook; Lydon, John; DeMayo, Francesco
Oligodendrocytes are responsible for providing the myelin sheath that is essential for the rapid and efficient propagation of an action potential down the axon of a neuron. Understanding the processes that regulate the differentiation of oligodendrocytes and their myelination of neurons is important for determining possible treatments for demyelinating disorders, such as multiple sclerosis and cerebral palsy. Specifically, the progression of chronic multiple sclerosis is partly due to the failure of intrinsic mechanisms for the replacement of lost myelin and the leading cause of cerebral palsy is the loss of myelin during early post-natal due to white matter injury.

Previously our lab demonstrated that NFIA is dynamically expressed in differentiating oligodendrocytes in the embryonic spinal cord, where it is co-expressed with Olig2+, oligodendrocyte precursors within the pMN domain of the embryonic spinal cord and downregulated as these precursor populations differentiate into myelinating oligodendrocytes. These observations suggest that NFIA functions to suppress oligodendrocyte precursor differentiation. Our lab demonstrated that NFIA suppresses oligodendrocyte differentiation during embryogenesis in mouse, chick, and in vitro oligodendrocyte precursor models. Also, our lab found that overexpression of NFIA in an adult mouse model of white matter injury and remyelination, suppressed oligodendrocyte precursor differentiation and remyelination. This, coupled with our observation that NFIA is expressed in oligodendrocyte precursors in human multiple sclerosis lesions and neonatal white matter injury, suggests that it may also play an important role in the suppression of differentiation and the failure of remyelination in these disorders.

Late embryonic lethality of the NFIA knockout mice has limited our basic studies to embryonic stages. However, the clinical application of these findings is that reduced NFIA expression in oligodendrocyte precursors stimulates remyelination in cases of white matter injury and multiple sclerosis in post-natal or adult humans. Therefore, studies in the post-natal and adult mouse are crucial to assess whether the loss of NFIA stimulates remyelination following injury. Recently, we have generated a conditional, floxed NFIA allele that we have crossed with an oligodendrocyte precursor-specific cre to delete NFIA in this population to study its role in adult myelination and remyelination during white matter injury. In order to address the remyelinating capabilities of oligodendrocyte precursors, we will use lysolecithin injection in the spinal cord of oligodendrocyte precursor-specific NFIA knockout mice. Following lysolecithin-induced demyelination, we will analyze for myelin repair through both staining for mature oligodendrocyte markers, such as MAG and PLP, and observe the remyelination by electron microscopy.

Contributors: Laug, Dylan; Glasgow, Stacey; Deneen, Benjamin
The leading cause of death in the United States is heart failure brought on by heart disease, and a loss of functional cardiac muscle. Because heart muscle regenerates poorly loss of cardiomyocytes leads to a weakening of the heart and eventually heart failure. The current understanding of endogenous mechanisms preventing heart regeneration is inadequate. However the Hippo signaling kinase cascade, along with canonical Wnt signaling inhibits developing cardiomyocyte proliferation. Here we examine Hippo and Wnt signaling in adult cardiac regeneration. Hippo deficiency enhances functional recovery and reduces fibrosis in a post-natal and adult mouse model of myocardial infarction. Our findings uncover Hippo signaling as a potential endogenous repressor of adult cardiomyocyte renewal and regeneration.

Contributors: Leach, John; Heallen, Todd; Morikawa, Yuka; Tao, Ge; Greene, Stephanie; Rahmani, Mahdis; Martin, James
CD4+ T helper cells are critical in orchestrating immune responses by ‘helping’ both non-immune and immune cells. The differentiation of T helper cells is driven by a particular cytokine milieu during an immune response and the underlying transcriptional circuitry. Even though many positive regulators have been identified, it is not clear the negative regulation at the transcriptional level. Here we report the inhibitory role of the ETS transcription factor ELF4 in the differentiation of Th17 cells. In vitro culture of naïve Elf4−/− CD4+ T cells in the presence of IL-6 and TGFβ, or IL-6, IL-23, and IL-1β, showed increased numbers of IL-17A positive cells compared to wild-type controls. In contrast, differentiation to Th1, Th2, or Treg was largely unaffected by loss of ELF4. Elf4−/− CD4+ T cells also displayed increased expression of genes involved in Th17 programming, suggesting that ELF4 controls entry to the Th17 differentiation program. Despite a normal proliferation of naïve CD4+ T cells in response to TCR stimulation, loss of ELF4 lowered the requirement of IL-6 and TGF( signaling for IL-17A induction in each cell division. In vivo, Elf4−/− mice showed increased numbers of Th17 cells in the lamina propria at steady state and in lymph nodes following immunization. Most importantly, Elf4−/− mice developed more severe disease than wild-type controls in the EAE model, which correlated with increased lymphocytic infiltration to the CNS. Collectively, our findings suggest that ELF4 restrains Th17 differentiation in dividing CD4+ T cells by regulating commitment to the Th17 differentiation program.

Contributors: Lee, Ping-Hsien; Puppi, Monica; Schluns, Kimberly S.; Dong, Chen; Lacorazza, H. Daniel
Breast cancer is the most common cancer and the second leading cause of cancer death among American women. About 12% women in the US will develop invasive breast cancer during their lifetime. Although the early diagnosis and target therapy have significantly decreased the mortality rates of breast cancer, there is no effective treatment of ER, PR and HER2 triple-negative breast cancer (TNBC) and metastatic breast cancer. To develop a better treatment of breast cancer, we need to understand the molecular mechanisms of the initiation and progression of breast cancer.

RNF197, also known as CGRRF1, is a p53-induced cell growth regulator with RING-finger domain. p53 is a tumor suppressor and approximately 20–35% of breast cancer patients have p53 mutations. Since RNF197 is implicated in the suppression of cell growth and is regulated by p53, it might be related to cancer development. Indeed, over-expression of RNF197 inhibits colony formation of colon carcinoma, ovarian carcinoma and glioblastoma cell lines. However, the role of RNF197 on breast cancer is still uncharacterized. We analyzed the correlation between the level of RNF197 and the survival rates of breast cancer, and found that the low expression of RNF197 is strongly correlated with a shorter survival. Compared with matched normal tissues, the level of RNF197 is lower in several tumor types including breast cancer. The analysis of the level of RNF197 in different subtypes of breast cancer showed a lower level of RNF197 in basal-like, mainly triple-negative, breast cancer. RNF197 contains a C3HC5 RING-finger domain which is a binding motif of ubiquitin-conjugating enzymes. The activity of most E3 ubiquitin ligases is specified by the RING-finger domain. However, studies have not been done to examine the potential E3 ubiquitin ligase activity of RNF197. Based on this information, the central hypothesis of my research is that RNF197 regulates the growth of breast cancer. In particular, I hypothesize that RNF197 inhibits the growth of breast cancer through cell cycle regulation. To understand the mechanism behind RNF197-inhibited cell growth, I hypothesize that RNF197 is an E3 ubiquitin ligase and is able to degrade or regulate the biological function of its substrates associated with breast cancer cell proliferation. Studying the potential E3 ubiquitin ligase activity of RNF197, as well as its role in the development of breast cancer may help us to identify potential therapeutic targets or pathways that would aid in breast cancer treatment.

Contributors: Lee, Yu-Ju; Lin, Weei-Chin
MOLECULAR MECHANISMS BY WHICH RIF2 INHIBITS NON-HOMOLOGOUS END JOINING (NHEJ) AT THE TELOMERE

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Integrative Program in Molecular and Biomedical Sciences
Advisor: Alison Bertuch, M.D./Ph.D.-Department of Pediatrics

Telomeres are nucleoprotein structures at chromosome ends that are important for genome protection. Inhibition of telomere fusions is critical in all eukaryotes as failure of this fundamental safeguard results in chromosomal rearrangements and loss of genetic material, both of which are detrimental to the organism. Telomeres are maintained by telomerase and, in Saccharomyces cerevisiae, consist of TG rich double-stranded regions bound by proteins such as Rap1. Rap1 is essential and contains a C-terminal domain that binds Rif2, an inhibitor of telomere elongation. Rif2 removal in the absence of Tel1, the yeast ATM homolog, results in telomere fusions while tel1Δ alone does not promote fusion formation. Tethering the Rap1 C-terminus to induced double stranded breaks (DSBs) allows for Rif2 binding. In this situation, fusions only occur in the absence of Rif2 indicating that Rif2 inhibits end joining. Additionally, deletion of the Rap1 C-terminus results in the same amount of telomere fusions as Rif2 deletion alone. The mechanism by which Rif2 inhibits telomere fusions remains unknown. Fusions formed due to Rif2 loss require components of the NHEJ machinery including the Mre11/Rad50/Xrs2 (MRX) complex. It has been shown that a recombinant C-terminal fragment of Xrs2 physically interacts with the N-terminus of Rif2 in vitro. We hypothesize that Rif2 inhibits fusions at the telomere by binding and sequestering Xrs2, thus preventing MRX complex formation and assembly at the telomere end. To test this hypothesis, we first set out to recapitulate the Rif2:Xrs2 interaction using co-immunoprecipitation (co-IP) of endogenous proteins. Thus far, an interaction has not been detected in vivo. Although this negative result does not support our hypothesis, it is possible that the Rif2:Xrs2 interaction occurs exclusively at the telomere, making it difficult to detect in whole cell extracts. To determine if this is the case, we will use bimolecular fluorescence complementation analysis (BiFC) to assess Rif2:Xrs2 interaction, as this method would be sensitive to interactions at the telomere. Next, we set out to determine if overexpressing Rif2 would prevent repair of DSBs by sequestering Xrs2 and thus preventing MRX complex assembly. To do so, wild type strains were transformed with Rif2 overexpression plasmids and then plated on various DNA damaging agents. While xrs2Δ strains exhibited growth defects when exposed to the DNA damaging agents, as previously reported, Rif2 overexpression in the wild type strain did not mimic the xrs2Δ phenotype. Thus, overexpressing Rif2 does not prevent Xrs2 from binding its fellow MRX components and repairing sites of damage. Although this negative result does not support our model, we have yet to determine whether the levels of the overexpressed Rif2 exceed endogenous levels of Xrs2 such that Rif2 could sequester Xrs2 in its entirety. Thus additional overexpression studies of Rif2 and the N-terminal region (which binds Xrs2) will be performed to further elucidate the role of Rif2 in prevention of fusions at the telomere. Contributors: Lemon, Laramie D.
The liver is the largest organ in the human body with the remarkable ability to regenerate after injury or surgical resection. The regulation of liver differentiation and proliferation is a quite complicated process which includes a tight co-operation of many signaling pathways. Our lab has shown that CUG triplet repeat binding protein 1 (CUGBP1) is one of the key regulators of liver differentiation and proliferation. Examination of post-natal liver differentiation and proliferation of the liver after partial hepatectomy and after injury showed that CUGBP1 increases translation of C/EBP and a chromatin remodeling protein HDAC1 leading to the accumulation of C/EBP-HDAC1 complex. Our recent data show that the elevation of this complex is involved in the repression of key regulators of liver functions including C/EBP, p53, SIRT1, PGC1, and TERT. Because the translational activity of CUGBP1 is controlled by phosphorylation at Ser-302, we have generated a CUGBP1 S302A knockin mouse which lack translational activity of CUGBP1. In these mice, C/EBP-HDAC1 complexes are significantly reduced resulting in de-repression and activation of down-stream targets. Examination of biological consequences of the lack of translational activity of CUGBP1 showed that CUGBP1-S302A mice develop premature hepatic steatosis at age 2 months and have alteration of metabolic parameters in the blood. Current studies of CUGBP1-S302A mice are focused on liver regeneration. To analyze this, S302A mice have undergone 2/3 partial hepatectomy and cell cycle proteins and cellular proliferation were examined. In addition to this, S302A mice were treated with carbon tetrachloride (CCl4) and liver injury and regeneration were measured. Preliminary data show that liver proliferation is inhibited in S302A mice at early time points after initiation, but it is normalized at late stages of liver regeneration. Taken together, these data show that translational function of CUGBP1 is involved in control of liver steatosis and metabolism and in regulation of early stages of liver proliferation and liver injury.

Contributors: Lewis, Kyle; Wei, Christina; Hong, Il-Hwa; Iakova, Polina; Jin, Jingling; Timchenko, Lubov; Timchenko, Nikolai
The evolution of the roles of cell adhesion molecules TgrB1 and TgrC1 in multicellularity and allorecognition

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Advisor: Gad Shaulsky, Ph.D.-Department of Molecular & Human Genetics

Cellular interactions through heterotypic cell adhesion molecules (CAMs) play essential roles in cell recognition and development. One interesting unresolved question is how CAMs were able to diversify within species given that their essential functions would be expected to impose severe functional constraints. Here we attempt to better understand this puzzle in Dictyostelium discoideum by studying the heterotypic cell adhesion and allorecognition proteins, TgrB1 and TgrC1.

Previous studies have shown that TgrB1/TgrC1 mediates allorecognition and cell differentiation during aggregating development. Firstly, cells lacking tgrB1 or tgrC1 are developmentally arrested at the loose aggregation stage and fail to undergo cell differentiation. Secondly, they function as a receptor pair for allorecognition. Cells with different tgrB1/tgrC1 alleles segregate from one another during streaming. Moreover, tgrB1 and tgrC1 genes evolved rapidly and are highly polymorphic in natural populations. tgrB1 and tgrC1 alleles of one strain are considered as a matching allelic tgrB1/tgrC1 pair and alleles in two divergent strains are considered as mismatching allelic pairs. Cells carrying a mismatched pair of tgrB1/tgrC1 have severe developmental defects, similar to tgrB1-null or tgrC1-null cells. Therefore, tgrB1/tgrC1 genes must co-evolve to maintain optimum heterotypic interactions since mutations in one gene could disrupt these interactions and lead to failure of sporulation.

To elucidate the evolvability of this system, we have to dissect the signal transduction pathways of TgrB1/TgrC1. tgrC1-null cells and single gene replacement strains fail to develop beyond the loose aggregation stage. We have devised screens for genetic suppressors that rescue these developmental defects. Such genetic suppressors allow us to elucidate the underlying signaling pathways. In addition, those molecular mechanisms may play roles in buffering novel tgrB1/tgrC1 recognition mutations and allow the organism to tolerate fitness disadvantages during evolution. Thus, activation of such alternate pathways may promote allelic evolution. In our preliminary results, we discovered several candidate genes, including glycosyltransferases and protein kinases, which may compensate for suboptimal function of TgrB1/TgrC1. We are continuing the genetic screens and analyzing the function of the genetic suppressors in various genetic backgrounds.

This study is using a novel system that could broaden our understanding of signaling components of CAMs and how allelic repertoires expand despite the risk of selective disadvantages. It could also shed light on the allelic evolution of allorecognition systems.

Contributors: Li, Cheng-Lin; Kuspa, Adam; Shaulsky, Gad.
Conditional knockout of Pitx2 leads to cardiac arrhythmia

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Atrial fibrillation (AF) is the most commonly seen atrial arrhythmia. Genome-wide association studies identified a locus on chromosome 4q25, variants of which are associated with increased risk of atrial fibrillation. This locus locates close to the pituitary homeobox2 (Pitx2) gene. Pitx2 is a transcription factor, which plays a critical role in left-right asymmetry establishment and maintenance of the heart. Our previous studies indicate that Pitx2+/− mice show symptoms of atrial fibrillation when given programmed electrical stimulation. However, role of Pitx2 in heart function is still not clear due to the functioning allele in these heterozygous mice. Therefore, we hypothesize that Pitx2 deficiency in heart leads to atrial fibrillation.

We use a conditional knockout mouse model, Pitx2flox/flox MCK-Cre mice, in which Pitx2 expression is specifically disrupted in mice cardiac/skeletal muscle after birth. Surface ECG and ECG telemetry is used to record electric activity of the hearts in Pitx2 knockout mice to determine phenotype in Pitx2flox/flox MCK-Cre mice. In Pitx2flox/flox MCK-Cre mice, there is irregular R-R interval without a second triggering event to induce arrhythmia, which is an indicator of sinus node dysfunction. From this result, we know Pitx2 knockout leads to abnormal heart rhythm, which indicates Pitx2 plays a critical role in heart function.

Since Pitx2 is a transcription factor, we want to investigate its binding region on chromosome. ChIP-sequencing and microarray assay is done to find target genes of Pitx2 and determine changes in genes expression levels. Finally, we find more than 700 genes whose expression is more than two fold in mutant than in wildtype mice, including genes related to ion channels, signaling pathways, transcription factors and so on. Majority of the genes screened have an increase in RNA level in mutant mice, indicating Pitx2 may function mainly as a repressor for the candidate genes.

To confirm the result of microarray, and evaluate molecular mechanisms of the arrhythmia in Pitx2 CKO mice, RNA level of a number of candidate genes, which regulates cardiac function, are analyzed using qRT-PCR. Result indicated upregulation of RNA level of majority of candidate genes and down regulation of a small number of genes, indicating Pitx2 may function mainly as repressor in regulation of genes transcription in cardiac function. Luciferase reporter assay was conducted and confirmed Pitx2 targets, indicating Pitx2’s regulation of candidate genes. TEM showed that Pitx2 CKO mice have damaged mitochondria and disrupted intercalated disc structure, suggesting Pitx2 regulates heart function by stabilize integrity of intercalated disc structure.

Contributors: Tao, Ye; Zhang, Min; Bai, Yan; Zhou, Yuefang; Moon, Anne M; Swinton, Paul; Kaminski Henry; Martin, James
Ubr3, an E3 ubiquitin ligase, regulates the activity of Usher syndrome protein Myosin VIIa through tumor suppressor gene Cullin1 in Drosophila auditory sensory cells

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To identify novel genes that affect hearing, we screened a collection of mutations in essential genes on the X chromosome for morphological defects in the fly auditory organ, the Johnston’s Organ (JO). We used the FLP/FRT system to create mosaic animals to bypass lethality and isolated mutations in a gene that cause apical detachment of the auditory sensory cells. We found that this gene encodes a Drosophila homolog of the mammalian UBR3 proteins, E3 ubiquitin ligases.

Apical detachment of auditory sensory cells is a specific defect that was previously only associated with mutations in the fly homolog of Myosin VIIA (MYO7A) gene. MYO7A encodes an unconventional myosin. Mutations in this gene cause Usher syndrome in human, a disease that causes blindness and deafness and is quite prevalent as it accounts for 50% of the blind-deaf patients. However, the molecular mechanism underlying Usher syndrome is still unclear.

To assess whether Ubr3 and MYO7A function in the same pathway, we performed genetic interaction study. We found that over-expression of Drosophila MYO7A (dMYO7A) in ubr3 mutant cells enhances the phenotype while over-expression of dMYO7A in wild type clones does not cause any defect. This suggests a specific genetic interaction between ubr3 and dMYO7A. Biochemical assays detect an increased level of multi-monoubiquitinated dMYO7A upon loss of ubr3. In addition, loss of ubr3 promotes inter-molecular protein interaction of dMYO7A, probably through dimerization. The fact that Ubr3 negatively regulates the ubiquitination of dMYO7A suggests that Ubr3 indirectly regulates dMYO7A through another E3 ligase.

In our previous studies, we found that Cullin1, an E3 ligase encoded by a tumor suppressor gene, is up-regulated in ubr3 mutant cells. To assess whether Cullin1 is the intermediate E3 ligase, we over-expressed Cullin1 in wild type cells. As expected, we observed similar apical detachment in JO. Intriguingly, knock down of Cullin1 through RNAi results in the same phenotype as over-expression of Cullin1, indicating that Cullin1 levels must fall within a narrow range to function properly.

Based on our data, we propose that Ubr3 negatively regulates the protein level of Cullin1. Cullin1 monoubiquitinates dMYO7A which in turn promotes dimerization of dMYO7A. Since previous data showed that dimerization of MYO7A affects the subcellular localization of MYO7A, the increased levels of MYO7A dimers may be the cause of apical detachment in JO. We will test this hypothesis and examine whether the mechanism is evolutionarily conserved in vertebrates.

Contributors: Li, Tongchao; Groves Andrew; Bellen Hugo
Necrosis is the premature death of cells caused by external factors, such as acute cell injury or trauma. In contrast to apoptosis, the programmed cell death, necrosis is caspase-independent and necrotic cells are morphologically distinct from apoptotic cells. Although these two categories of cell deaths are genetically different, it has been suggested that necrotic cell corpses are actively removed by the same set of genes required in apoptotic cell removal indicating they might share a similar clearance mechanism. In the nematode Caenorhabditis elegans, gain-of-function mutations in certain ion channel subunits result in necrotic-like cell death of six touch neurons. Necrotic touch neurons are subsequently engulfed and degraded inside engulfing cells. However, it is unclear how necrotic cells are recognized by phagocytes. Phosphatidylserine (PS) is an important apoptotic cell surface signal that attracts engulfing cells. Using ectopically expressed MFG-E8, a high-affinity PS-binding protein, we observed that PS was actively present on the surface of necrotic touch neurons. In addition, phagocytic receptor CED-1, whose function is needed for the efficient clearance of apoptotic cells, also acts as a phagocytic receptor for necrotic cells. We demonstrate that necrotic cells, like apoptotic cells, rely on cell-surface PS as an “eat me” signal to attract CED-1. We further found CED-7, the worm homolog of mouse ABC1 transporter, was necessary for PS-exposure on necrotic cell surfaces. Moreover, we discovered ANOH-1, the worm homolog of mammalian scramblase TMEM16 could contribute to the presentation of PS on necrotic cell surfaces as well and act in a parallel pathway to CED-7. Our findings suggest between two distinct cell deaths, a conserved mechanism may exist for the recognition of cell corpse.
Activation of transcriptional factor NFκB induces synaptic over-excitation and reduces neuronal dendritic arborization and total length through astroglia-mediated activation of complement signaling pathway

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Nuclear factor kappa B (NFκB) is a family of transcription factors well known for its master-regulatory role in inflammatory responses. Activation of NFκB is observed in aging and major diseases impairing neuronal function and contributes to neurological disease pathogenesis. However, the cell type-specific target genes and signal transduction cascades initiated by NFκB in CNS are poorly understood. Our study suggests that NFκB in glia presents the most potent cytoplasmic-nuclear translocation and executes the most robust and sensitive gene expression induction. To better understand glia NFκB function in the CNS, we created a mouse model in which the predominant NFκB inhibitor IκBα is specifically knocked out in astroglia (GcKO). When cultured in vitro, IκBα KO astroglia increases neuronal mEPSC amplitude and induces neuronal overexcitation. Coordinate, neuronal dendritic complexity and total dendritic length are reduced. To uncover the molecular mediators, we performed microarray analysis followed by qPCR and ELISA assay. We found that the NFκB target, complement component 3 (C3), the central molecule of complement signaling pathway, is overexpressed in GcKO mice and KO primary astroglia and its overexpression is NFκB-dependent. qPCR of other complement proteins suggested that the classical complement pathway is activated. Adding a receptor antagonist SB290157, which targets C3a receptor (C3aR) downstream of complement activation increases neuronal dendritic arborization and reduced neuronal excitability to normal level. Remarkably, we observed significant reduction of dendritic spine density in GcKO mice which can be rescued by SB290157 treatment. All these data support that the NFκB/Complement signaling pathway regulates neuronal activity and morphology and suggest that targeting astroglia may be therapeutically beneficial in neurodegenerative diseases.

Contributors: Hong Lian, Li Yang, Jennifer Rodriguez-Rivera, Allysia Cole and Hui Zheng
THE ROLE OF MULTI-ENZYME AMINOACYL tRNA SYNTHETASE COMPLEX COMPONENT AIMP1/P43 IN TH1 IMMUNITY

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As a group of professional antigen presenting cells (APC), dendritic cells (DC) are considered as initiator and regulator of adaptive immunity. Previously in an attempt to develop DC-based vaccine against acute myeloid leukemia (AML), we loaded DCs with overlapping class I and II determinants: AML specific mRNA and cell lysate. This event which we termed as homologous loading augmented DC IL-12 production, CD8+ T cell population, and IFN-γ T-cell secretion, which are hallmarks of Th1 immunity. We further identified this Th1 phenotype across a variety of systems in which it is specifically seen in DCs homologous loaded with whole cell lysate/mRNA or single protein antigen or viral peptides, but not in singly loaded DCs or DCs loaded with diverse class I and II determinants, indicating DC intrinsic mechanisms to recognize and compare the antigenic epitopes. In an effort to seek underlying mechanisms, we detected high level of AIMP1/p43 release from DCs loaded with homologous lysate/mRNA, single protein antigen, or overlapping viral peptides.

AIMp1/p43 is a structural component of the multi-enzyme amino-acyl tRNA synthetase complex (mARS) which consists of at least 8 tRNA synthetases and 3 structural proteins. Beyond its potential in translation regulation, studies show that it can be released by both tumor and macrophages under stress or TLR stimuli. Recombinant AIMP1/p43 protein could upregulate pro-inflammatory gene expression in monocytes and macrophages, and it can induce Th1 polarization of bone marrow derived DCs. Encouraged to look into its immunological roles, we found that the level of AIMP1/p43 release is closely related to epitope overlapping. We also detected AIMP1/p43 co-localization with MHC class I and II molecules by coIP and mass spectrometry experiments, suggesting that it plays a role in antigen recognition. Furthermore, the administration of recombinant AIMP1/p43 protein with class II antigen determinants recapitulated the Th1 immune phenotype comparable to homologous loaded DCs. siRNA knockdown of mARS complex components including AIMP1/p43 greatly diminished the Th1 phenotype of homologous loaded DCs both in vivo and in vitro. These data allow us to come up with the hypothesis that p43 released by homologous loaded dendritic cells promotes Th1 immunity.

In this study, we would be revealing how AIMP1/p43 release by dendritic cells is regulated by antigen loading signals and whether tRNA synthetase complex is responsible for this process. Additionally, we are looking for candidate cell surface receptors and downstream signal pathways for extracellular AIMP1/p43 pro-inflammatory functions. Finally, we are seeking to apply AIMP1/p43 as a novel DC vaccination adjuvant and test its potential in anti-tumor immunity and infectious diseases models. More importantly, our study would underscore the importance of homologous antigenic signals in DC polarization.

Contributors: Liang, Dan; Halpert, Matthew; Konduri, Vanaja; Decker, William
Characterization of an Fkbp10-/- mouse model of recessive Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is the most commonly inherited form of brittle bone disease and displays a spectrum of severity from mild phenotypes to severe early lethality. Key clinical features of OI are bone fragility and low bone mass, whereas patients may also display blue sclera, dentinogenesis imperfecta, joint and skin laxity, hearing impairment and/or wormian bones on the skull. Mutations in FK506 Binding Protein 10 (FKBP10) that encode the FKBP65 protein result in recessive OI as well as Bruck Syndrome. FKBP65 is thought to act as a collagen chaperone and possesses PPIase activity for proper trimer formation. Currently, we do not understand the consequences of FKBP10 loss and its role in collagen and ECM formation. Therefore our goal is to elucidate the role of FKBP10 in the skeleton and how null mutations lead to progressively deforming OI and Bruck syndrome. We generated a mouse model using the EUCOMM allele to further elucidate the effects of FKBP65 in bone development. We have utilized the LacZ knockin allele to assess expression during development as well as the knockout first allele to discern the phenotypic outcomes of FKBP65 loss in the mouse. Furthermore, mouse embryonic fibroblasts (MEFs) have been used for comparison to human cells and for collagen studies and analysis. We found that Fkbp10 is expressed at low levels at E13.5 particularly in skeletal tissues and increasing through E17.5 with expression in not only skeletal tissues, but also other mesothelial lined tissues, vessels and villi of intestine. Postnatally, expression is limited to developing bone and ligaments, suggesting a more restricted role at this timepoint. Null mice display neonatal lethality with viable embryos isolated up to E18.5 but not after birth, growth delay and generalized tissue fragility. Fkbp10-/- mouse embryonic fibroblasts show retention of procollagen in the cell layer after ascorbic acid stimulation, similar to what is seen in patient fibroblasts. These data suggest a requirement for Fkbp10 function during embryonic connective tissue development in mice but restricted expression postnatally in bone, ligaments, and tendons correlating with the bone fragility and contracture phenotype in humans.

Contributors: Homan E., Yang, T., Munivez, E., Jiang, M., Bertin, T., Chen, Y., Krakow, D., Lee, B.
Lipid Metabolic Regulation upon Environmental Nutrient Anticipation

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The capabilities of environmental sensation and adaption determine the fitness of organisms in the constant changing world. As food availability plays as one of the most critical environmental variables, we hypothesize that animals can anticipate environmental nutrient status through dietary metabolite signals, and adjust physiological regulation to support the survival and reproduction of the individuals and benefit population sustainability.

We apply the model organism C. elegans to study the gene-environment regulation on nutrient anticipation response. As being soil-dwelling, C. elegans utilize bacteria as both the major food source and the important sensory input. When the environmental nutrient is getting restricted, bacteria will be the first to be affected due to their direct dependency on environmental decaying matters. The physiological responses in bacterial may provide indicators of environmental nutrient status, which are received by their predators like C. elegans. C. elegans then coordinate its metabolic strategies upon nutrient status anticipation.

We have identified C. elegans display fat accumulation phenotype when fed on bacteria cultured from nutrient-limited environment. Both fatty acid de novo synthesis pathway and triacylglyceride synthesis pathway are found upregulated in C. elegans in response to such environmental change. An orphan nuclear hormone receptor, NHR-25, is likely to act as the key upstream regulator of this metabolic adjustment upon nutrient status anticipation.

This integrative gene-environment interaction study will challenge the previous thinking of "starvation response". Organisms may sense future starvation environmental changes from current dietary cues, and remodel their energy metabolic strategy to enable better fitness in the time ahead. My project will dissect the molecular mechanisms by which environmental obesogen signals influence organism lipid metabolism. Moreover, through identifying beneficial probiotic supplements, we can potentially boost metabolic health in human via similar pathways.

Contributors: Lin, chih-chun J.; Wang, Meng C.
FUNCTIONAL ANALYSIS OF CDC14 AND CDH1 IN DNA REPAIR

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DNA damage occurs at a rate of 1,000-1,000,000 molecular lesions within each single cell every day. DNA repair is thus essential for genome integrity and healthy of cells and organisms. This project is to analyze the functions of Cdc14 and Cdh1 in DNA repair. Cdc14 is a dual specificity phosphatase which is known as an essential cell cycle regulator in budding yeast. The mammalian homologs Cdc14A and Cdc14B function not only in cell cycle but also in DNA repair. Previous study in our lab showed impaired DNA repair in Cdc14B knockout (Cdc14B/-) MEFs, and the mice develop early onset aging phenotypes. To further understand the role of Cdc14B in DNA repair, two aims are investigated: redundancy between Cdc14A and Cdc14B, downstream target of Cdc14B in DNA repair.

Preliminary study showed DNA repair defects with Cdc14B deficiency in late passage MEFs rather than in early passage MEFs. Detection of lower Cdc14A mRNA level in late passage MEFs compared to early passage indicates the redundancy between Cdc14A and Cdc14B in DNA repair. LOF study showed Cdc14A knockdown caused defective DNA repair in early passage Cdc14B/- MEFs. GOF study showed Cdc14A overexpression partially rescued the defective DNA repair in late passage Cdc14B/- MEFs.

Cdh1 is a substrate of both Cdc14A and Cdc14B. To assess the effect of Cdh1 in DNA repair, Cdh1 was knocked down in MEFs, which lead to more severe DNA repair defects than Cdc14B deficiency. Cdh1 overexpression partially rescued defective DNA repair in late passage Cdc14B/- MEFs, indicating Cdh1 as a downstream factor mediating Cdc14B’s function in DNA repair.

Besides Cdh1 as potential Cdc14B downstream target in DNA repair, DR-GFP assay was performed to identify which DNA double strand break (DSB) repair pathway is regulated by Cdc14B and Cdh1. The assay indicated that Cdc14B and Cdh1 regulate both NHEJ and HR, the two major pathways of DNA DSB repair. Western blotting showed that Cdh1 might regulate NHEJ and HR by regulating the protein levels of Ku80 and Rad51, respectively.

My study shows that Cdc14A and Cdc14B redundantly regulate DNA repair with Cdh1 as a mediating factor. Cdc14B and Cdh1 regulate NHEJ and HR, with Ku80 and Rad51 as potential targets. These help understand how DNA repair is regulated, and might help develop therapies for DNA repair disorders, such as accelerated aging diseases and cancer.

Contributors: Lin, Han; Zhang, Pumin.
BACKGROUND: Preterm male infants have a higher incidence of bronchopulmonary dysplasia (BPD) compared to females. Hyperoxia contributes to lung injury and inflammation in experimental animals and BPD in preterm infants. The mechanisms responsible for sex differences in the susceptibility towards hyperoxic lung injury (HLI) remain largely unknown.

OBJECTIVE: To characterize sex-specific differences in the transcriptome and inflammatory markers in lung injury due to oxidative stress using microarray and luminex bead arrays.

DESIGN/METHODS: Male and female (8-10 wk) wild type (WT) (C57BL/6J) mice were exposed to hyperoxia (FiO2>0.95). Measurement of cytokine levels in lung homogenates was done using suspension bead array. Gene expression in lung tissue was studied using the Mouse Gene 1.0 ST Array (Affymetrix). Preprocessed gene expression data was analyzed by fitting a linear model, using a moderated t-statistic for pair wise comparison and interaction terms. A combination of fold change ≥ 1.4 and false discovery rate < 5% was used to define differentially expressed genes (DEGs). Overrepresentation of gene ontology terms representing biological processes among the DEGs were tested using a conditional hypergeometric test (p-value < 0.01). Signaling pathway impact analysis (SPIA) was also performed to measure actual perturbation in a given pathway under our experimental conditions. Data was processed and analyzed using Bioconductor software packages in R programming language.

RESULTS: The suspension bead array revealed increases in mouse lung tissue homogenate levels of IL-6 (F>M) and VEGF (M>F) after hyperoxia. A total of 2209 (up-regulated (UR): 1385, down-regulated (DR): 824) and 2467 (UR: 1549, DR: 918) genes were differentially expressed following hyperoxia compared to room-air in females and males respectively. The interaction between these factors did not identify DEGs. Comparison of DEG profiles identified 327 genes unique to females, 585 unique to males and 1882 common genes.

CONCLUSIONS: DEGs involved in lung development, signaling pathways (toll like receptor, Wnt and NF-kappa B) may explain the differences in sex-specific susceptibility to HLI. These findings provide novel insights into the mechanisms involved in HLI and suggest new pathways that need to be investigated as sex-specific biomarkers and possible therapeutic targets for BPD in premature neonates.

Contributors: Lingappan, Krithika; Weiwu, Jiang; Wang, Lihua; Couroucli, Xanthi; Moorthy, Bhagavatula
GTP is an important molecule, as it serves as a building block of genetic materials, provides energy for translation, secretion, cell division and signaling. However, knowledge regarding regulation of GTP levels remains incomplete. Previous work from our laboratory using Bacillus subtilis shows that regulation of GTP levels requires two small molecules, guanosine tetra- and penta-phosphate, collectively named (p)ppGpp. We found that (p)ppGpp directly inhibits guanylate kinase (GMK) and hypoxanthine-guanine phosphoribosyltransferase (HprT), two key enzymes in GTP biosynthesis pathways. One question we were interested in tackling is whether this regulation is conserved in other bacterial species beyond B. subtilis. Using in vitro enzymatic assay, we found that regulation of GMK and HprT by (p)ppGpp is conserved among Firmicutes. Moreover, (p)ppGpp functions as a competitive inhibitor for GMK and an uncompetitive inhibitor for HprT. The structure of GMK co-crystallized with ppGpp has been resolved and agrees with our biochemical results. Since (p)ppGpp is required for bacterial pathogenesis, our findings may contribute to understanding the molecular mechanisms by which pathogens cause infection and diseases.

Contributors: Liu, Kuanqing; Myers, Angela; Krasny, Kathy; Gumuser, Esra; Keck, James; Wang, Jade
Lipid metabolism defects have long been observed in patients with neurodegenerative disorders. However, mechanisms of lipid metabolism defects and the pathogenesis of neurodegeneration have not been established. Importantly, mechanisms controlling lipid metabolism are highly conserved and the transcription factor Sterol Regulatory Element Binding Factor (SREBP) regulates lipid metabolism and energy homeostasis in both Drosophila and vertebrates. Here, we show that that the genes C8ORF38, MARS2 and Mitofusin, which cause the neurodegenerative diseases Leigh syndrome, Charcot-Marie-Tooth type 2A and Autosomal Recessive Spastic Ataxia with Leukoencephalopathy (ARSAL), also cause neurodegeneration in Drosophila. Moreover, we found that the three Drosophila mutant models exhibit a common lipid droplet accumulation phenotype in the glia. We found that the lipid droplet accumulation is caused by an elevated level of reactive oxygen species (ROS), which functions through the proteins c-jun-N-terminal Kinase (JNK) and SREBP to cause these lipid droplet accumulations. Furthermore, we found that the ROS/JNK/SREBP pathway is activated in the neurons, where neuronal defects alone cause glial lipid droplet accumulation. Finally, we show that eliminating the lipid droplet accumulation suppresses neurodegeneration in these three mutants. This study reveals a novel pathway in the pathogenesis of neurodegeneration, where elevated levels of ROS trigger the activation of JNK and SREBP to form lipid droplets in the support cells, and affecting their function, thereby contributing to neurodegeneration.

Contributors: Liu, Lucy; Zhang, Ke; Sandoval, Hector; Yamamoto, Shinya; Jaiswal, Manish; Zhihong Li; Graham, Brett; Bellen, Hugo.
Embryonic stem (ES) cells have the unique properties of self-renewal and pluripotency, which make them promising resource for regenerative medicine. However, the mechanism underlying self-renewal and pluripotency remains elusive. To investigate the mechanism, we carried out bimolecular fluorescence complementation screen to identify interacting proteins for important ES cell factors NANOG, OCT4 and SOX2. We further did a functional screen for the interacting proteins and found DIDO1, when overexpressed, can inhibit differentiation under condition normally don’t promote self-renewal. Dido1 is expressed in ES cells. We are currently investigating the role of DIDO1 in the self-renewal and differentiation.
A STRATEGY TO ARM AN ONCOLYTIC HERPES SIMPLEX VIRUS (HSV) TO SELECTIVELY INDUCE APOPTOSIS IN BYSTANDER TUMOR CELLS

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Advisor: Xiaoliu Zhang, M.D./Ph.D.-Department of Molecular Virology & Microbiology

One method to enhance the therapeutic quality of an oncolytic virus is through the addition of therapeutic genes. Therefore, we have designed a strategy to arm an oncolytic HSV with a unique therapeutic gene (HER2-COL-FasL) that when expressed is targeted to kill surrounding bystander tumor cells through apoptosis, or programmed cell death. The molecule derives its apoptosis activation proficiency from Fas ligand (FasL) which forms a transmembrane trimeric structure that activates apoptosis upon binding its trimeric receptor, Fas, on target cells. Therefore the HER2-COL-FasL C-terminus consists of the FasL topological domain. This domain was then fused to a multimerization domain from collagen (COL) that allows the synthesized chimeric molecule to be trimerized as in its native functional form. A secretion signal and a single chain variable fragment (scFv) against Human Epidermal Growth Factor Receptor 2 (HER2) were fused as the N-terminus of the molecule for secretion and targeting of the molecule to tumor cells overexpressing HER2.

Initially, the expression and function of the chimeric molecule was characterized. Western Blot revealed HER2-COL-FasL is secreted from transfected cells into the cell medium. Under non-reducing conditions HER2-COL-FasL is detected in a multimerized form as either trimers or hexamers. Moreover, transfer of medium containing secreted HER2-COL-FasL to wells of multiple tumor cell lines resulted in significant apoptosis and caspase cleavage. The HER2 scFv domain of HER2-COL-FasL enhances apoptosis in HER2 positive cells when compared to a control molecule lacking the scFv.

Following in vitro studies of HER2-COL-FasL, a cassette encoding the gene has been engineered into an oncolytic HSV. This advanced oncolytic HSV specifically replicates in tumor cells while producing the secreted molecule that will selectively activate apoptosis in surrounding tumor cells. As expected, following infection HER2-COL-FasL is expressed and secreted from infected cells. The addition of HER2-COL-FasL to the oncolytic HSV leads to an enhancement of tumor cell eradication following virus infection in multiple cell lines. An in vivo experiment is under way to determine its efficacy in vivo compared to the parental virus. The implications of these studies will further the development of targeted molecules that can enhance viral vectors and oncolytic viruses by specifically targeting and eradicating uninfected bystander tumor cells surrounding transduced/infected cells while leaving normal cells unharmed.

Contributors: Zhang, Shaun
Metastatic melanoma accounts for 75% of skin cancer-related deaths and carries a survival rate of only 10-15% due to its aggressive behavior. Based on transcription profile analyses of melanomas with differential metastatic potential, a "phenotype switching" model of melanoma progression has been proposed wherein individual tumor cells may alter between proliferative and invasive states through epigenetic regulation. We propose that while proliferative cells would be susceptible to treatment, weakly proliferating, invasive cells might be less sensitive and able to switch to the proliferative state to repopulate tumors once therapy has ceased. Our laboratory has demonstrated that homeobox transcription factor A1 (HOXA1) can promote melanoma cell invasion, and expression of HOXA1 evokes a gene expression pattern reflecting an invasive-switched cell state as described above. Moreover, the HOXA1-induced invasive signature also correlated with melanoma cell line resistance to current melanoma therapeutics targeting the mitogen-activated protein kinase (MAPK) pathway. Indeed, our preliminary data indicate that cells over-expressing HOXA1 are more resistant to the MAPK pathway inhibitors. Thus, we propose a model of melanoma progression whereby HOXA1 controls the phenotype switching program that signals back and forth interchanging between proliferative and invasive cell states leading to tumor heterogeneity, metastasis, and resistance to targeted therapies. Our specific aims are as follows:

Aim 1: To investigate whether HOXA1 drives phenotype switching from proliferative to invasive cell states and promotes metastasis in vivo.

Aim 2: To determine whether HOXA1 modulates resistance to therapeutic inhibitors of the MAPK pathway via transforming growth factor-beta (TGF-β) signaling.

These studies are intended to reveal the molecular mechanism of melanoma phenotype switching in driving metastasis, which accounts for the notoriously lethal nature of this disease. We expect that findings from these studies will reveal the mechanisms by which invasive state cells could resist to the MAPK pathway inhibitors, providing novel insights in therapeutics design targeting invasive cell population.

Contributors:
Functions of ATXN1-ATXN1L and CIC in the brain

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Program in Developmental Biology  
Advisor: Huda Zoghbi, M.D.-Department of Pediatrics

The protein ATAXIN-1 (ATXN1) was discovered twenty years ago for its role in the polyglutamine neurodegenerative disease spinocerebellar ataxia type 1 (SCA1): expansion of glutamine-encoding CAG repeats in ATXN1 causes neurotoxicity. Intense research over the ensuing years, much of it in the Zoghbi lab, has provided much insight into SCA1 pathogenesis. Nevertheless, information about the specific function of ATXN1 has been difficult to come by. Thus, the Zoghbi lab sought out protein interactors of ATXN1 to better understand its function and revealed that the most salient of the interactors that modify the SCA1 phenotype are ATXN1’s functionally redundant paralog Ataxin-1-like (ATXN1L) and the transcriptional repressor Capicua (CIC). The three proteins form a co-repressor complex in vivo, and depend on one another for stability. Atxn1-/-; Atxn1L-/- (double knockout, DKO) mice and Cic-/- mice die perinatally with defects in multiple organs, indicating that these proteins have critical roles during development. ATXN1-ATXN1L and CIC are highly expressed in the brain throughout life, but their neuronal functions remain unclear.

To bypass the lethality caused by peripheral organ defects and to better understand the function of this complex in the brain, we use Cre-Lox technology to generate conditional knockout in the nervous system. When we use Nestin-Cre to conditionally knockout Atxn1-Atxn1l in the nervous system, most of the mice died before weaning. This again confirmed the critical roles of these proteins in the nervous system. We then switched to forebrain specific Emx1-Cre. Interestingly, the Emx1-Cre conditional knockout mice have increased motor activity with impaired learning and memory. Histological studies showed that they have thinner cortex, though the layering architecture is largely preserved. We also generated conditional knockout mice of Cic and confirm similar cortical development defects. We are now analyzing whether the cortical defects come from abnormal proliferation or differentiation of the embryonic neural stem cells, using birth-dating experiments in Emx1-Cre conditional Atxn1-Atxn1l and Cic knockout mice. In addition, we will perform transcriptomic analysis to further dissect the molecular mechanism that leads to these defects.

Contributors: Lu, Hsiang-Chih; Lee, Yoontae; Fryer, John; Liu, Xiuyun; Zoghbi, Huda
MICRORNA-22/HDAC4 AXIS PROMOTES ASTHMA AND COPD BY ENHANCING TH17 RESPONSE

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IL-17A producing Th17 cells are key players in various inflammatory lung diseases, including asthma and chronic obstructive pulmonary disease (COPD). Pathogenic Th17 cell development in lung is initiated by antigen presentation and pro-inflammatory cytokine secretion by fully activated antigen presenting cells (APCs), including dendritic cells and macrophages. Although increasing evidences have linked microRNAs (miRNAs) to APC function, the role of individual miRNA in APC activation during pathogenic environment is still lacking. In this study, we showed that microRNA-22 (Mir-22) had a positive role in mediating Th17-prone lung APC activation in asthma and COPD. Mir-22(-/-) mice were completely resistant to fungal induced allergic asthma and cigarette smoking induced COPD with fewer Th17 cells in the lung. Mir-22 expression level was elevated in pathogenic lung APCs and it was sufficient and necessary for Th17-prone pro-inflammatory cytokines (IL-1β, IL-6, IL-23) secretion and B7 co-stimulatory molecule upregulation. The intrinsic role of Mir-22 in CD11c+ APC compartment was demonstrated by restoration of COPD progress in Mir-22(-/-) mice replete with CD11c+ lung APCs from cigarette smoke exposed wildtype mice. Furthermore, one of Mir-22 downstream targets, histone deacetylase 4 (HDAC4), was significantly upregulated in pathogenic Mir-22(-/-) APCs. HDAC4 is a negative regulator of cytokine transcription through deacetylating histones in gene promoter region. The negative role of HDAC4 in Mir-22 mediating cytokine production in pathogenic APCs was addressed by restoration of cytokine secretion in pathogenic Mir-22(-/-) APCs with HDAC4 knockdown. Finally, we found that anti-Mir-22 treatment reduced disease severity of fungal induced asthma when given after the disease was fully developed in mice. In another pilot human study, we translated what we found in mice to potential human therapies and found that anti-Mir-22 treatment attenuated Th17-prone potential of COPD patient lung CD1a+ APCs. All these findings showed that Mir-22 conferred susceptibility to asthma and COPD by promoting pathogenic Th17-prone lung APC activation through targeting and downregulating HDAC4, identifying Mir-22 as a new target for the therapeutic intervention in asthma and COPD.

Contributors: Lu, Wen; You, Ran; Yuan, Xiaoyi; Kheradmand, Farrah; Corry, David B;
THE POTENTIAL ROLE OF MIR205 IN MAMMARY GLAND DEVELOPMENT AND STEM CELL MAINTENANCE

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Analysis of miRNA expression in the normal adult mammary gland reveals high expression of miR-205 in basal stem cell-enriched populations. Consistent with this, miR205 over-expression in the mouse mammary epithelial cell (MEC) line COMMA-DβGeo leads to an expansion of the Sca-1+ progenitor cell population, decreased cell size and increased cellular proliferation and colony-forming potential. Based on these results, we hypothesize that miR205 is required in mammary gland development and stem cell maintenance. In addition, a recent study by Melino et al. shows that miR205 is a direct downstream target of the tumor suppressor p53 homolog, p63, which is well-known for its role in epithelial tissue development and stem cell maintenance. Therefore we plan to use a conditional p63 knock-out mouse model to further examine the relationship of deltaNp63 (main p63 isoform in mammary gland) and miR205 expression in normal mammary gland development. Using mice with conditional floxed alleles containing a Neo-LacZ cassette, X-gal and H&E staining were performed to determine the expression patterns of miR205 at different stages of mammary gland development. The developmental stages we have studied to date including puberty (5-week-old), mature virgin, early pregnancy (p6), middle pregnancy (p12), late pregnancy (p18), involution (forced involution from D1 to D3). Ad-cre treated mammary epithelial cells (MECs) were used for cell transplantation assay. The localization of miR205 and p63 were analyzed by immunohistochemistry (IHC). Our results show that miR205 is expressed and co-localized with p63 preferentially in the cap cell layer of postnatal terminal end buds (TEBs), in which the mammary stem cell population is enriched. In mature virgin and pregnant mice, miR205 is only expressed in the basal cell layer of both ductal and alveolar structures, and is not detected in the luminal cell layer; The expression of miR205 in the basal cell layer of alveoli is gradually lost from early pregnancy through late pregnancy; miR205 expression is lost during forced involution D1, 2 but is detectable in forced involution D3. Cell transplants from mice homozygous for miR205 alleles show severely impaired ductal outgrowth, which supports our hypothesis that miR205 has a potential role in stem cell maintenance.

Contributors: Lu Yang; Rosen Jeffrey M.

ROLE OF FRAGILE X RELATED PROTEINS IN MAMMALIAN CIRCADIAN BEHAVIORS AND GLUCOSE HOMEOSTASIS

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Fragile X syndrome, the most common form of inherited developmental disability, results from the absence of the fragile X mental retardation 1 (FMR1) gene product FMRP. FMR1 has two paralogs in vertebrates: fragile X related gene 1 and 2 (FXR1 and FXR2). One of the behavioral symptoms observed in fragile X patients is the increased occurrence of sleep disorders. Fmr1 or Fxr2 knockout mice display a shorter free-running period of locomotor activity in total darkness (DD) compared to wild-type mice, while Fmr1/Fxr2 double knockout mice exhibit complete loss of rhythmic activity. DKO mice also display significant alterations in the cyclical patterns of abundance of core clock component messenger RNAs in the liver, but not in the suprachiasmatic nucleus. These findings suggest that Fmrp and Fxr2p are acting downstream of the central clock to control rhythm in mice.

Locomotor assays with restricted feeding demonstrate that the Fmr1/Fxr2 DKO mice were able to adjust their rhythm to food availability, even though they cannot entrain to light. Remarkably, these mice also had a significantly higher mortality rate (~60%) in the first three days of restricted feeding, which suggests a difference in their physiological response to food restriction. Here we present data suggesting the involvement of FXRs in glucose homeostasis. In mice of similar body weight, body fat is significantly lower in the Fmr1/Fxr2 DKO mice compared to WT. They had consistently low levels of glucose, cholesterol, and leptin over a 24hr cycle, while the cycling of the other metabolic markers in the blood is out of phase. The DKO mice also exhibited exaggerated clearance of a bolus of glucose, and hypersensitivity to insulin. Glucose production is also impaired, both via gluconeogenesis and glycogenolysis.

The close association of food input to the circadian system and the timing of sleep and wakefulness, together with the typical disturbances of circadian behavior and sleep in Fragile X syndrome, open up a new perspective for the investigation and treatment of patients suffering from this disorder.

Contributors: Lumaban, Jeannette G.; Nelson, David L.
REGULATION OF NF-κB ACTIVITY THROUGH TYROSINE PHOSPHORYLATION OF IRAK4

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Advisor: Jonathan Levitt, Ph.D.-Department of Pathology & Immunology

Src homology region 2 domain-containing phosphatase-1 (SHP-1) is a tyrosine phosphatase which inhibits NF-κB signaling through interference in MyD88/IRAK4-dependent toll-like receptor (TLR) signaling. However, the specific substrates of SHP-1 in this pathway are unknown. Our lab has previously identified IRAK4 as a binding partner of SHP-1, despite zero reports of tyrosine phosphorylation of IRAK4 in the literature. Further, expression of a dominant negative form of SHP-1 leads to accumulation of tyrosine phosphorylated IRAK4 in dendritic cells. We hypothesized that SHP-1 interacts with IRAK4 to inhibit TLR-dependent NF-κB signaling. We have identified three tyrosine residues in the IRAK4 structure which are consistent with SHP-1 binding motifs: Y48, Y371 and Y413. Since phenylalanine resembles a tyrosine which cannot be phosphorylated we mutated these three tyrosine sites to phenylalanines, singly and in double and triple mutant permutations. HEK293 cells were transfected with an NF-κB-SEAP reporter, an inducible MyD88 and a mutant IRAK4. We induced MyD88/IRAK4 signaling and assayed for NF-κB-SEAP activity. Contrary to our hypothesis, both the Y48/371/413F triple mutant and Y371/413F double mutant show increased NF-κB reporter activity in HEK293 cells, indicating that dephosphorylation of these sites may actually increase IRAK4-induced NF-κB activity. To confirm this phenomenon we generated RAW 264.7 murine macrophage lines which stably express the different IRAK4 mutants. Continuing experiments will explore mutant IRAK4 signaling in endogenous TLR signaling and the involvement of SHP-1 in this pathway.

Contributors: Ramachandran, Indu; Chiou, Shin-heng; Saenz, River; Levitt, Jonathan
Arginase expression can lead to neuroprotection in traumatic brain injury.

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Program in Translational Biology & Molecular Medicine  
Advisor: Brendan Lee, M.D./Ph.D.-Department of Molecular & Human Genetics  
Brett Graham, M.D./Ph.D.-Department of Molecular & Human Genetics

Each year, about 1.7 million people are affected by traumatic brain injuries (TBI) in the U.S. alone (1). If left untreated, TBI can lead to death, coma, stroke or permanent nerve damage resulting in paralysis, motor disabilities, speech impairments, loss of vision, sensory damage, cognitive disabilities and behavioral changes. Hence, understanding the underlying pathophysiology is critical. Significantly higher levels of nitric oxide (NO) have been observed in cerebrospinal fluid of patients with severe TBI (2) and have been shown to cause neuronal damage. Additionally, high NO levels are correlated to severity of brain pathology and increased intra-cranial pressure (ICP) in TBI patients. We hypothesize that reducing NO levels in the brain post-TBI will confer neuroprotection. To test this hypothesis, we developed four mouse lines overexpressing either arginase I (liver isoform) or arginase II (kidney isoform) under a glial or a neuron-specific promoter. Since arginine is the common substrate for arginase and nitric oxide synthase (for NO production), overexpression of arginase divert arginine away from NOS-mediated NO production thereby be protective against NO-mediated brain damage seen in traumatic brain injury. After characterization of these mouse lines, we induced controlled cortical injury at 3 months of age. Post-injury intra-cranial pressure measurements were made and brain sections were analyzed for histopathological changes. We found that intra-cranial pressure was significantly reduced in mice overexpressing arginase in glial cells. Additionally, neuronal arginase overexpression lines had significantly reduced contusion volumes and contusion grading scores. Further studies will include assessment of changes in the levels of nitric oxide, nitrates, nitrites and tissue nitrosylation in the animals post-injury to better understand the mechanistic basis of arginase mediated neuroprotection in these lines.

References:

Contributors: Madan, S; Al-Shamy, G; Keller, B; Campeau, P; Munivez, E; Robertson, C; Goodman, C; Lee, B.
All proliferating cells must coordinate DNA replication with cell division to preserve genomic stability. Consequently, DNA replication factors are among the most highly conserved proteins across all domains of life. Control of replication initiation is fundamentally similar in all organisms: Cell cycle-regulated DNA binding proteins catalyze open complex formation at replication origins, allowing for replisome assembly and chromosomal duplication. Our lab uses E. coli as a model organism to better understand regulation of replication initiation.

Recent findings indicate that localization of oriC within the cell may direct initiation of DNA replication. In order to test if oriC position directs initiation, I have developed a method to disrupt normal oriC positioning in vivo, using a modification of the Fluorescent Reporter Operator System (FROS) technique. Tethering oriC results in a complete and reversible block in replication initiation. In addition, tethering loci even very distant to oriC block initiation, suggesting a surprising complex mechanism of initiation control, potentially regulated at the level of chromosomal structure. Experiments are currently underway to test if oriC position is disrupted when distant sites are tethered, and to test if global nucleoid structure is disrupted by tethering chromosomal loci.

Contributors: Magnan, David; Bates, David
Hydatidiform moles (HM) are abnormal pregnancies with hyperproliferative trophoblast and absent embryo development. While most complete HM are sporadic and androgenetic (containing only paternally inherited DNA), a small subset of HM are highly recurrent with biparentally inherited DNA (BiHM). Molecularly, BiHM show loss of methylation at differentially methylated regions (DMRs) of all maternally imprinted loci. Autosomal recessive maternal effect mutations of NLRP7 (NLR family, pyrin domain containing 7) and more recently, of KHDC3L have been found to cause these recurrent BiHM pregnancies. These observations indicate that NLRP7 and KHDC3L, two genes absent from the genome of most mammals besides primates have a direct role in the process of imprinted acquisition in the oocyte or imprint maintenance in the developing embryo. An unanswered question is the mechanism by which maternal absence of NLRP7 or KHDC3L results in loss-of-methylation at imprinted genes in these pregnancies. To answer this question, which may reveal new important information on mechanisms of imprinting-mark switching in the maternal germline, I am pursuing the following lines of investigation. First, I systematically examined whether proteins with already known roles in imprint acquisition and/or maintenance revealed physical interaction with NLRP7. I determined that CG Binding Protein 1 (CFP1), which is crucial to the process of imprint acquisition in the growing oocyte directly binds to NLRP7, which opens up exciting avenues for studying NLRP7’s functions in the female germ line. I also found that overexpressed KHDC3L, the second protein implicated in BiHM pathology and also enriched in the oocyte, directly interacts with NLRP7. The inability of KHDC3L to rescue loss of NLRP7 and vice versa in human patients suggests functional distinctiveness of these proteins. It is therefore conceivable that NLRP7 and KHDC3L along with factors such as CFP1 form a multimeric maternal protein complex essential for ensuring epigenetic fecundity of the germ cells. Second, I determined that NLRP7-knockdown in human embryonic stem cells causes genome wide differences in DNA methylation and confirmed prior experiments in the lab that indicated that this also alters in vitro trophoblast development. Finally, I am generating a mouse model to study the role of these proteins in vivo. Unlike humans, mice only have Nlrp2, indicating that it assumes the role of both human homologs. Thus, by creating conditional knockouts of Nlrp2 I hope to gain insight into its roles in imprinting in the oocyte and early embryonic development that can be translated to understanding human imprinting and BiHM pathology. I have already generated mice that carry a targeted conditional allele and with a global loss of Nlrp2, which are in the process of being characterized. Finally, I also pursued mutation analysis of NLRP7, NLRP2 and KHDC3L in women with various forms of pregnancy loss and unexplained infertility and found that they are only associated with BiHM, putting to rest debate in the literature on this topic.

Contributors: Mahadevan, Sangeetha K; Wen, Shu; Aghajanova, Lusine; Fisher, Rosemary; Kyba, Michael; Van den Veyver, Ignatia B
E2F1 is a transcription factor that plays a critical role in diverse cellular pathways such as cell cycle progression, apoptosis induction, and the DNA damage response. E2F1 has been shown to be necessary for inducing apoptosis in a chemotherapeutic response by transcriptionally activating target genes such as p73. The mechanism by which E2F1 differentially regulates transcription of proliferative genes and apoptotic genes remains unknown. Our co-IP/MS experiment identifying potential interacting proteins of E2F1 has identified a deubiquitinating enzyme (DUB), UCHL5 as a novel E2F1 interacting protein. Through further investigation, I have shown that E2F1 interacts with ectopically overexpressed UCHL5 in 293T cells. After 293T cells were treated by Adriamycin, a chemotherapeutic drug, the accumulation of ubiquitinated E2F1 (K48 and K63-chain specific) is decreased in the presence of ectopic UCHL5. This suggests that UCHL5 is a potential DUB for E2F1. In addition, a reporter assay has shown that E2F1 transcriptional activity is increased when UCHL5 is present, and QPCR analysis has shown that p73, an E2F1 target gene, is upregulated in the presence of UCHL5. This functional role of UCHL5 has been verified using a stable knockdown cell line with the reporter assay and Q-RT-PCR. Studying UCHL5’s role in apoptosis, I used a Caspase 3/7 reporter assay that showed shUCHL5 cell lines have less apoptosis when compared to a scrambled control. Prior studies have shown UCHL5 is able to interact with the INO80 chromatin-remodeling complex, placing the proteasome and UCHL5 at the site of transcription. I hypothesize that UCHL5 is able to interact with E2F1 on the chromatin, and deubiquitinate E2F1, thus enabling an upregulation of transcriptional activity of genes required for apoptosis. I propose that ubiquitination is an additional regulation of transcriptional activity of E2F1. Discovering a mechanism in which E2F1 transcriptional activity is increased could lead to a possible target therapy that induces apoptosis in cancer cells. In conclusion, I have found that a DUB, UCHL5, interacts with E2F1 and deubiquitinates E2F1 in vivo.
In vivo axonal transport deficits in a mouse model of fronto-temporal dementia

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Rachelle Doody, M.D./Ph.D.-Department of Neurology

Background: Axonal transport is required for the movement of vital organelles and energy in neurons. In vivo deficits in this process have been previously reported in mouse models of Alzheimer’s disease. Specifically, these deficits have been detected prior to the onset of plaque and tangle pathology. However, there are limited in vivo measurements of axonal transport in models of frontotemporal dementia (FTDP-17). In the rTg4510 mouse model, the P301L mutation found in familial forms of FTDP-17 is over expressed primarily in the forebrain and can be conditionally expressed throughout the lifetime of the mouse. Thus, the mouse model has a copy of the tetracycline (tta) gene and a copy of P301L gene (tau) that can be activated separately or together. When both Tta and tau are active (tau+/tta+), this mouse model displays increasing levels of tau filaments and neurodegeneration within the forebrain and hippocampus beginning at 4 months of age.

Methods: The rTg4510 mouse model of FTDP-17 was used for these studies. Mice were given 2 ul of MnCl2 in each nostril at one hour prior to imaging. One hour post nasal lavage, mice were imaged using Manganese Enhanced Magnetic Resonance Imaging (MEMRI) Protocol with 15 cycles, each cycle taking approximately 2 min using Paravision software (Bruker BioSpin, Billerica, MA). Regions of interest (ROI) within the olfactory neuronal layer (ONL) and the water phantom consisting of one pixel (ONL) and 9 pixels (water) were selected and copied across each of the 15 cycles. Signal intensities (SI) of ONL and water phantom ROIs were measured. SI values obtained for ONL were then normalized to water. The correlation between normalized signal intensity in the ONL and time were assessed using Prism.

Results: Using the MEMRI technique on 3.5, and 10 month old rTg4510 mice and wild type littermates, we found significant axonal transport deficits present in the rTg4510 mouse model. Using linear regression analysis, we measured a rate of y=0.01005x ± 0.001510 in WT mice and y= 0.002052x ± 0.001536 in tau+/tta+ mice at 10 months of age. At 5 months of age, we measured axonal transport rates of y=0.01043x ± 0.001074 in WT mice and y = 0.007152 ± 0.0008087 in tau+/tta+ mice. However, we hypothesized that there could be a reduction in transport rates at an earlier age point. We calculated measurements at the 3-month time point of y=0.01665x ± 0.001778 in WT mice and y=0.008626x ± 0.0007962 in tau+/tta+ mice. In order to determine the point at which tau appears in the cortex, we probed for phosphorylated tau levels, and found that pSer262 is present at 3 months of age, but pathological tau is not present until 6 months of age, after the onset of transport deficits. In addition, we see localization of tau in the ONL at 6 months of age.

Discussion: Other studies have indicated that axonal transport deficits are strongly correlated to synaptic dysfunction and cognitive decline as a result of decreased movement of energy-producing organelles such as mitochondria to the synapse. In addition, the olfactory bulb has shown to play a major role early in Alzheimer’s disease patients, and has not been thoroughly investigated in other tauopathies. Further characterization of the rTg4510 mouse model will be conducted at earlier age points in order to determine the earliest age of functional decline in axonal transport to identify possible therapeutic interventions at this time point.

Contributors: Majid, Tabassum; Ali, Yousuf; Venikraman, Deepa; Jang, Ming-Kuei; Lu, Hui-Chen; Pautler, Robia
Responding to environmental stress is essential for an organism to survive. A key signaling molecule that integrates diverse environmental information in bacteria is the nucleotide guanosine tetra(penta)phosphate ((p)ppGpp). A longstanding question has been whether (p)ppGpp regulates stress responses in metazoans.

mesh-1 in C. elegans encodes a protein homologous to SpoT, the bacterial hydrolase that regulates intracellular (p)ppGpp level. I found that a mutant strain with a 500 bp deletion in mesh-1, while showing no other apparent defects, had enhanced tolerance to low temperature (2oC); RNAi inactivation targeting the gene also increased tolerance. I am currently verifying the role of mesh-1 in cold tolerance by genetic complementation. In addition, I observed that mesh-1 transcription increased when C. elegans were relieved from starvation. It is, thus, possible that starvation induces synthesis of MESH-1 substrate(s) in vivo. With chromatographic and biochemical approaches, I will determine the identity of the substrate(s). Altogether the study will test whether (p)ppGpp and its metabolism have a conserved role in stress response regulation in C. elegans.

Contributors: Mak, Keng Hou; Wang, Meng; Wang, Jade
A CONSERVED ROLE OF NUCLEAR RECEPTOR LRH-1/NR5A2 IN ENDOPLASMIC RETICULUM STRESS RESOLUTION

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Endoplasmic reticulum (ER) stress occurs when proteins within the ER can no longer be properly folded, resulting in misfolded protein accumulation. Following synthesis, ~1/3 of proteins fold incorrectly, with increased misfolding observed following a variety of stresses. However, ER stress that is experienced chronically plays a role in development of metabolic and neurodegenerative diseases. For protection, three conserved signaling pathways collectively termed the unfolded protein response (UPR) have evolved to be activated by ER stress. Mice lacking UPR pathways cannot resolve ER stress and exhibit abnormal liver metabolism following treatment with chemical ER stressors. We have discovered an unexpected stress resolution pathway initiated by the nuclear receptor LRH-1/NR5A2 that is independent of known UPR pathways yet similarly required for cell survival and maintenance of metabolism. Similar to mice lacking UPR components, hepatic Lrh-1-null mice cannot resolve ER stress, despite a functional UPR. The role of LRH-1 in ER stress resolution is dependent on induction of the kinase Plk3, which phosphorylates and activates ATF2, a transcription factor with phosphorylation-dependent activity at stress-inducible genes. Plk3-null mice also cannot resolve ER stress, and restoration of Plk3 expression in Lrh-1-null cells rescues ER stress resolution. Treatment of hepatocytes with an LRH-1 agonist increases ER stress resistance, allowing resolution of otherwise toxic ER stressors. Additionally, the role of Lrh-1 in responding to ER stress appears evolutionarily conserved, as its homolog nhr-25 in C. elegans is also required for ER stress resolution. We conclude that LRH-1 initiates a novel, kinase-mediated pathway of ER stress resolution that is independent of the canonical UPR yet equivalently required; importantly, therapeutic targeting of LRH-1 may be employed as a new strategy to treat human disorders associated with chronic or irresolvable ER stress.

Contributors: Mamrosh, Jennifer L.; Lee, Jae Man; Wagner, Martin; Stambrook, Peter J.; Sifers, Richard N.; DeMayo, Francesco J.; Wang, Meng C.; and Moore, David D.
Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease involving both upper and lower motor neurons which leads to muscles weakness and atrophy. Affected individuals will ultimately lose the ability to initiate any voluntary movement. There is no cure for ALS and the pathogenesis is still poorly understood. Although ~85% of the ALS cases are sporadic, 15% are familial. ALS8 is one of the disease causing genes and a point mutation (P56S) in the N-terminal MSP (Major Sperm Protein) domain of ALS8 causes ALS and late onset spinal muscular atrophy. ALS8 encodes the VAPB protein, VAMP-associated protein B. It belongs to a conserved protein family with an N-terminal MSP domain, a coiled-coil motif and a transmembrane domain for anchoring in the ER. We are using Drosophila as a model organism to study the pathogenesis of VapB in ALS.

Previously our lab has shown that the MSP domain of VapB is cleaved and secreted into the extracellular region and that it binds to Eph receptors. Little is known about the mechanism of cleavage or secretion. dVapB localizes to the ER and may function in the ER quality control. The P58S mutation in dVapB (P56S in hVapB) leads to failure to secrete the MSP domain and induces its ubiquitination, and promotes an unfolded protein response. dVapBnull mutants are lethal, and one copy of wild type dVapB can fully rescue the lethality. However, one copy of dVapBP58S partially rescues the lethality and these flies show a reduced life span and an age dependent degeneration of motor neuron activity which mimics the symptom observed in patients. Besides, neuronal specific expression of dVapB in dVapBnull mutants can suppress lethality. We therefore proposed that the MSP domain of VapB is cleaved and secreted from the neuron and function as a ligand to regulate downstream pathways in a cell non-autonomous way. Understanding the secretion or cleavage mechanism of VapB and the function of MSP and its downstream targets might help us to uncover the pathogenesis of VapB mutation in ALS disease.

Contributors: Mao Dongxue; Yoon Wan Hee; Lin Guang; Tsuda Hiroshi; Bellen Hugo
Assessing the effects of amyloid plaques on the structural integrity of the axon initial segment

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Advisor: Matthew Rasband, Ph.D.-Department of Neuroscience

The onset of amyloid plaque formation, the primary pathology of Alzheimer's disease, coincides with the appearance of synaptic abnormalities, aberrant electrical properties, and cognitive decline. The microenvironment surrounding amyloid plaques is synaptotoxic, with axonal varicosities, neurite breakage, and significant decreases in spine density. However, the effects of amyloid plaque formation on the molecular architecture of the axon initial segment (AIS), a subdomain of the axon, have yet to be fully investigated. The AIS, which acts as a diffusion barrier as well as the site of action potential initiation, is a critical neuronal structure. Disruption of the AIS hinders action potential conduction and has been linked to stroke, autism, and autoimmune neuropathies. Using a rodent model of Alzheimer's disease, we have analyzed the effects amyloid plaques have on the architecture of the AIS in the forebrain at various stages of amyloid pathology. We found that the presence of amyloid plaques correlates with the disruption of AIS architecture, an effect that is limited to areas in close proximity to plaques. Our findings suggest that the local environment around amyloid plaques is toxic to the AIS and results in its disruption. Though the effects are local, amyloid plaque formation is cumulative. The AIS loss surrounding amyloid plaques in later stages in the disease could negatively impact neuronal function and be potentially detrimental to the central nervous system.

Contributors: Marin, Miguel; Jankowsky Joanna; Rasband Matthew
Drosophila Deficient for porin Provide a Link Between Fat Metabolism and Neurological Function

Ruchi Masand
Department of Molecular & Human Genetics
Advisor: Brett Graham, M.D./Ph.D.-Department of Molecular & Human Genetics

The voltage-dependent anion channel (VDAC or porin) is an integral membrane protein present in the mitochondrial outer membrane (MOM). VDACs are not only the predominant determinant of MOM permeability but also integrate mitochondrial function and other cellular pathways by interacting with various mitochondrial and cytoplasmic proteins; however, details of these functions as well as the interacting pathways remain poorly understood. VDACs have also been implicated to play a pathogenic role in several human diseases including cancer, diabetes, Alzheimer’s and cardiac ischemia-reperfusion injury. Flies mutant for porin (the predominant VDAC in Drosophila) demonstrate energy metabolism defects, neurologic dysfunction with abnormal mitochondrial distribution in motor neurons and male infertility.

A pilot modifier screen identified multiple deletions that suppress male infertility in porin mutants, including one deletion that also suppressed neuronal dysfunction in porin mutants. Deletion mapping identified Lsp2, a hexamerin expressed in the fat body, as a suppressor of porin mutant CNS phenotype. Expression microarray analysis and qRT-PCR on adult porin deficient flies revealed extremely high levels of Larval Serum Proteins (LSPs) as well as Fat Body Proteins (FBPs), all of which are typically highly expressed in the larval Drosophila fat body, which is analogous to the adipose tissue and liver in mammals as the major site of energy metabolism and fat storage in the fly. In addition, we also observed significantly lower levels of triglycerides (TAG) in flies deficient for porin. Elevated levels of LSPs and FBPs have also been reported in Drosophila mutant for technical knockout (tko), previously reported as showing respiratory chain deficiency, developmental delay, and neurological abnormalities.

A p-element insertion allele of Lsp2 was able to rescue the increased bang sensitivity as well as the secondary complex I deficiency phenotypes seen in porin mutants. Also, both CNS and fat body specific expression of porin in the mutant flies was able to rescue the increased bang sensitivity phenotype.

Since tissue specific expression of porin in the fat body, the key metabolic tissue in the fly, is sufficient to rescue the CNS phenotype in porin mutants and some of the key genes expressed almost exclusively in the fat body are elevated in both porin and tko mutants, our data suggests a possible link between mitochondria, fat body (i.e, adipose tissue) metabolism and neurological function.

Contributors: Masand, Ruchi; Graham, Brett H.
Background: Osteosarcoma (OS) is the most common malignancy of bone. The outcome for patients with metastatic/refractory disease remains poor and new targeted therapies are needed. The use of genetically modified T-cells expressing chimeric antigen receptors (CAR) against the OS tumor antigen HER2 has shown promise as a targeted cancer treatment in murine xenograft models. However, murine xenograft models do not allow for the detailed study of engrafted T-cells in a natural tumor environment since these mice are immunodeficient. OS disease and progression in canines has been shown to closely mimic human pathology in terms of tumor location and metastasis to the lungs. In addition, canines with naturally occurring OS possess an intact immune system, making it an ideal model for the preclinical evaluation and optimization of CAR+ T-cell therapies. The immediate goal of this study is to optimize expansion and transduction of canine T-cells to express CARs against the OS tumor antigen HER2 as a prelude to a future clinical study in canines.

Methods/Results: To activate and expand canine T cells, canine peripheral blood mononuclear cells (PBMCs) were activated with PHA, irradiated artificial antigen presenting cells (K562 cells expressing CD80, CD83, CD86, and 4-1BBL), and IL2. Cells expanded on average 1.5 fold (n=20) within 7 days cells of coculture. The majority of cells were CD3+ (mean: 83%) with a mixture of CD8+ and CD4+ T-cell subsets. Restimulation of T cells in Grex tissue culture devices with the addition of IL21 resulted in 50-fold expansion of cells, generating sufficient number of T cells for future clinical studies in canines. To determine if we can retrovirally transduce canine T-cells, cells were transduced 3 days post-activation with GALV-pseudotyped retroviral particles encoding HER2-specific CAR. Post transduction up to 38% of canine T-cells expressed HER2-CAR as determined by flow cytometry. HER2-CAR T-cells were able to recognize HER2+ cell lines expressing either human or canine HER2 as evidenced by the direct lysis of antigen expressing tumor cells in 51Cr release cytotoxicity assays. In addition, transduced T cells produced canine IFNγ in co-culture assays only in the presence of HER2+ tumor cells.

Conclusions: Our results indicate that it is feasible to activate, expand, and genetically modify canine T-cells. Adapting the canine OS model for T-cell therapies will not only facilitate their translation into the clinic, but will also allow us and other investigators to realistically study how to best combine T-cell therapy with other OS-targeted therapies.
DNMT3A DELETION PREDISPOSES HEMATOPOIETIC STEM CELLS TO MALIGNANT TRANSFORMATION

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Advisor: Margaret Goodell, Ph.D.-Department of Pediatrics

DNA methyltransferase 3A (DNMT3A), a de novo DNA methyltransferase, is mutated in various hematological malignancies affecting both myeloid (20%), mixed (50%), and lymphoid (18%) malignancies and is associated with poor prognosis. The most frequently reported DNMT3A mutation is R882 in acute myeloid leukemia (AML), which results in altered enzyme activity, but various missense and nonsense mutations have also been found throughout the gene, suggesting that loss-of-function mutations in DNMT3A may also contribute to leukemogenesis. Our group recently showed that transplantation of HSCs from Dnmt3a knock-out (KO) mice led to increased hematopoietic stem cell (HSC) self-renewal and inhibition of differentiation, but was insufficient to cause transformation. However, in these experiments, Dnmt3a-KO HSCs were transplanted alongside wild-type whole bone marrow to quantitate HSC function, potentially protecting against malignant transformation.

We hypothesized that if Dnmt3a-KO HSCs were transplanted alone, a predisposition to transformation would be uncovered. We established a large non-competitive transplantation cohort receiving 500 control or Dnmt3a-KO HSCs and monitored the mice closely for disease. Strikingly, mice with Dnmt3a-KO HSCs had significantly shorter survival (246d vs 467d, p<0.0001). As mice succumbed to disease, we analyzed histological changes in hematopoietic organs and performed CBCs and immunophenotyping to diagnose the diseases. We identified multiple disease classes within the Dnmt3a-KO recipients, including T-cell acute lymphoblastic leukemia, myeloproliferative disease (MPD), myelofibrosis (MF), and myelodysplastic syndromes (MDS). The relatively long disease latency suggests that acquisition of secondary hits promotes disease; identification of these secondary mutations is ongoing.

Here, we show that Dnmt3a deletion in noncompetitive transplanted HSCs leads to an array of hematologic disorders that models the spectrum of disorders seen in human malignancies. Since DNMT3A mutations are known early genetic lesions in leukemia development, mutations that cooperate with DNMT3A might influence the type of disease developed. This mouse model serves to validate an important role for Dnmt3a in the development of hematologic malignancies, and is also valuable for the study of future targeted therapies.

Contributors: Mayle, Allison; Yang, Liubin; Challen, Grant; Zhou, Ting; Rebel, Vivienne; Goodell, Margaret
The main source of a spontaneous double stand break (DSB) in replicating cells is a single strand nick which is encountered by a replication fork, creating a single ended DSB break, commonly called a broken fork. Break-induced replication (BIR) is a pathway of homologous recombination known to repair single ended DSBs, and has been hypothesized to be responsible for repairing broken replication forks. However, BIR has been studied at DSBs outside the context of a replication fork, thus it remains unclear whether BIR has similar mechanistic and enzymatic requirements as the repair of broken replication forks. The hallmarks of BIR in yeast are its long duration, taking hours to complete, high mutagenicity, and a requirement of proteins which stimulate a D-loop migration mode of repair synthesis, including Pol32, the non-essential subunit of Polδ, and the helicase Pif1. We used a site-specific nick-induced DSB repair assay to study the process of broken replication fork repair, and compare this to BIR. By studying the genetic requirements and mutagenicity of broken fork repair at two distinct genomic loci, we can determine how closely this pathway resembles traditional BIR. We have placed nick sites between two origins as well as between the first origin and the telomere. The latter situation presents the cell with only one option for repair; synthesis primed from the single end break. In the case of a nick site between two origins, a second fork originating from an adjacent origin could arrive at the break site before synthesis initiates, eliminating the need for BIR. Such a mechanism would have different genetic requirements and mutagenic properties. By comparing the the characteristics of these two repair situations, we will determine the role of BIR in broken fork repair.

Contributors: Mayle, Ryan; Ira, Gregory
DYSREGULATION OF PURINERGIC SIGNALING IN HEPATOCELLULAR CARCINOMA

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Background. Hepatocellular carcinoma (HCC) is the third most lethal cancer worldwide, but molecular mechanisms of its pathogenesis are not well understood. Recent studies suggest that extracellular ATP-mediated activation of P2Y2 purinergic receptor induces hepatocyte proliferation in response to partial hepatectomy and ATP treatment alone was sufficient to induce hepatocyte proliferation in vitro. The purpose of this study was to characterize extracellular nucleotide effects on HCC cell proliferation and to examine the role of P2 purinergic signaling in the pathogenesis of HCC in patients and Mst1/2-/-, a mouse model of HCC. Hypothesis: Dysregulation of purinergic signaling facilitates aberrant cell proliferation underlying hepatocellular carcinogenesis.

Methods. Human HCC-derived Huh7 cells, maintained in serum free media (24h), were treated with ATPγS, or ADP (100µM) for different time intervals. SP600125 pretreatment was used to inhibit c-Jun N-terminal Kinase (JNK) signaling. Western blotting, qRT-PCR and 5-Bromo-2'-deoxy-uridine (BrdU) incorporation analysis were done. WT and Mst1/2-/- mouse livers (1, 3, & 6 months) and HCC patient livers (n=27) were analyzed by qRT-PCR for all 15 P2 purinergic receptor isoforms. Results. Extracellular nucleotide treatment alone was sufficient to induce cell cycle progression in Huh7 cells, evidenced by increased BrdU incorporation and increased cyclin D3, E, and A mRNA and protein expression. We observed downregulation of cyclin D1 mRNA, however, as previously reported in a subset of HCC with high tumor grade. JNK inhibition attenuated nucleotide-induced cyclin D3, E and A protein expression, but enhanced cyclin D1 downregulation. Meanwhile, nucleotide treatment induced de-phosphorylation of AKT (inactivation), and GSK-3β (activation), an upstream regulator of cyclin D1. Mst1/2-/- mouse tumors (at 3-6 months) exhibit dysregulated expression of multiple P2 purinergic receptor isoforms as compared to WT. In HCC patients, multiple P2 purinergic receptor isoforms were elevated ≥2-fold in liver tumors as compared to uninvolved areas in up to 52% of patients. P2 purinergic receptor upregulation was more prevalent among HCC patients infected with hepatitis C virus (75%) as compared to non-viral groups (20%) identifying a unique subset of viral-induced HCC overexpressing P2 receptors.

Conclusions. We show that extracellular nucleotides are potent mitogens in Huh7 cells, inducing downregulation of cyclin D1 and upregulation of cyclin E, which are associated with poor prognosis in HCC patients. Our analysis of HCC patient and Mst1/2-/- mice livers has uncovered a likely role for purinergic signaling in the pathogenesis of HCC, highlighting P2 purinergic receptors as potential biomarkers and novel therapeutic targets for HCC.

Contributors: Maynard, J; Johnson, R; Lopez-Terrada, D; Goss, J; Thevananther, S
A PROSPECTIVE NEWBORN SCREENING AND TREATMENT PROGRAM FOR SICKLE CELL ANEMIA IN LUANDA, ANGOLA

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Over 300,000 infants are born annually with sickle cell anemia (SCA) in sub-Saharan Africa, and 80-90% die young from infection or anemia, usually without diagnosis of SCA. Early identification by newborn screening, followed by simple interventions dramatically reduced the mortality of SCA in the US, but this strategy is not yet established in Africa. We designed and implemented a proof-of-principle newborn screening and treatment program for SCA in Angola, with focus on capacity building and local ownership. Dried bloodspots from newborns were collected from five birthing centers. Hemoglobin identification was performed using isoelectric focusing; samples with abnormal hemoglobin patterns were analyzed by capillary electrophoresis. Infants with abnormal FS or FSC patterns were enrolled in a newborn clinic to initiate penicillin prophylaxis and receive education, pneumococcal immunization, and insecticide-treated bed nets. A total of 36,453 infants were screened with 77.31% FA, 21.03% FAS, 1.51% FS, and 0.019% FSC. A majority (54.3%) of affected infants were successfully contacted and brought to clinical care. Compliance in the newborn clinic was excellent (96.6%). Calculated first-year mortality rate for babies with SCA compares favorably to the national infant mortality rate (6.8% versus 9.8%). The SCA burden is extremely high in Angola, but newborn screening is feasible. Capacity building and training provide local healthcare workers with skills needed for a functional screening program and clinic. Contact and retrieval of all affected SCA infants remains a challenge, but families are compliant with clinic appointments and treatment. Early mortality data suggest screening and early preventive care saves lives.

Contributors: McGann Patrick; Ferris, Margaret; Ramamurthy, Uma; de Oliveira, Vysolela; Santos, Brigida; Bernardino, Luis; Ware, Russell
The Role of α5-containing MHb Cholinergic Neurons in Anxiety

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Nicotine is both anxiogenic and anxiolytic in mice depending upon experimental conditions. This variability is likely due to the specific neural circuits activated, nicotinic receptor (nAChR) subunit composition of those circuits, as well as the desensitization and upregulation of nAChRs occurring in response to chronic nicotine exposure. The α5 and β4 nAChR subunits appear to participate in the regulation of nicotine withdrawal, and specific SNPs of the subunits have been linked to greater pleasure from smoking, earlier onsets of smoking, and increased transitions to nicotine dependence.

α5 subunits are highly expressed within the medial habenula (MHb) and interpeduncular nucleus (IPN), and projections from the MHb to the IPN via the fasciculous retroflexus (FR) form an axis critically involved in somatic signs of withdrawal. α5-null mice exhibit reduced basal anxiety-related behaviors, and fail to exhibit somatic signs of withdrawal following cessation of chronic nicotine administration. Additionally, withdrawal symptoms are reduced when nAChRs in both the MHb and IPN are inhibited. Therefore, we believe that anxiety components of the syndrome observed in mice undergoing withdrawal from nicotine are largely due to the activity of a specific subset of nAChRs present in the MHb and IPN that exert modulatory influence over dopamine (DA) and serotonin (5-HT) neurons in structures such as the ventral tegmental area (VTA), nucleus accumbens (NAc), and raphe nucleus (RN).

Optogenetic, microdialysis, designer receptor, and receptor subunit knock out techniques will be used for targeted circuit analyses in vivo. This project will elucidate the activity of a circuit that contributes significantly to both anxiety and drug addiction, with the potential of providing targets for tailored treatments of both disorders.

Contributors: McLaughlin, Ian; Perez, Erika; De Biasi, Mariella
Background: Adipose tissues contain macrophages and a variety of lymphocyte subsets, including NK cells, αβ T cells and γδ T cells. While macrophages and αβ T cell subsets have been the subject of intense study, the functional significance of γδ T cells is unknown.

Methods: Male C57BL/6J mice were placed on a high milk fat diet (HFD) for 5 weeks. The potential contributions of γδ T cells were investigated in mice deficient in all γδ T cells (TCRδ-/-), deficient in only Vγ4 and Vγ6 subsets (Vγ4/6-/-), or in wildtype (WT) mice treated with anti-TCRδ antibody.

Results: HFD feeding resulted in increases in total and TNFα+ γδ T cells in epididymal adipose tissue (AT) of WT mice that were proportional to the increases in fat mass. Obese TCRδ-/- mice had significantly reduced tissue expression of F4/80, CCL2 and IL-6 compared to littermate WT mice, though food intake and weight gain were not different. WT mice treated with anti-TCRδ antibody during HFD feeding also exhibited significantly reduced F4/80 and CCL2 compared to mice treated with control antibody. Obese TCRδ-/- mice also had significantly reduced TNF-α+ pro-inflammatory macrophage accumulation in AT as determined by flow cytometry of AT stromal vascular fraction, and significantly increased systemic insulin sensitivity compared to WT controls. Three subsets of γδ T cells were found in the AT of both lean and HFD fed mice, Vγ2, Vγ4 and Vγ6, and this differs from the subsets found in liver (Vγ1) and intestines (Vγ7). Obese Vγ4/6-/- had significantly reduced expression of CCL2 and IL-6 compared to obese WT mice, though F4/80 expression was not reduced.

Conclusion: γδ T cells in adipose tissue contribute to the proinflammatory changes that occur within the tissue in the first five weeks of feeding a high milk fat diet to C57BL/6J mice.

Contributors: Mehta, Pooja; Smith, C. Wayne
The β-lactamase enzymes play an important role in bacterial antibiotic resistance by hydrolyzing and thus inactivating the β-lactam antibiotics. The KPC-2 β-lactamase poses a major threat to antibiotic therapy as it can hydrolyze the carbapenem antibiotics which are considered a last line of defense. Despite several biochemical and structural studies, the mechanism of carbapenem hydrolysis by KPC remains elusive. In this study, we have performed alanine scanning mutagenesis of several important active site residues of the KPC-2 enzyme including E166 and N170 that coordinate a catalytic water molecule as well as the R220, T235 and T237 residues that form the binding pocket for the C3/C4 carboxylate common to all β-lactam antibiotics. Each mutant was subjected to detailed kinetic analysis, comparing their ability to hydrolyze carbapenem and cephalosporin substrates with respect to wild-type. The general mechanism of class-A β-lactamase involves the catalytic serine attacking the β-lactam carbonyl in the acylation step forming the acyl-enzyme intermediate. Subsequently, the deacylation step involves an attack on the acyl-enzyme intermediate by the catalytic water resulting in release of the active enzyme. The study reveals that both E166A and N170A mutants are highly deacylation deficient. The crystal structure of KPC-2 shows these residues coordinate a catalytic water that is responsible for the deacylation reaction. Thus, alanine mutants at these positions can no longer hold the catalytic water in place leading to impaired deacylation. The T237A mutant shows greater than 10-fold increase in catalytic efficiency for cephalosporins as compared to wild-type. However, with both the carbapenem substrates viz. imipenem and meropenem, this mutant deviates from Michaelis-Menten kinetics to show a branched kinetic pathway. This indicates that on exposure to carbapenem substrate, the T237A mutant changes conformation and exists as a mixture of kinetically viable and non-viable isoforms. The R220A mutant displayed an 8-fold increase in catalytic efficiency for cephalosporins while its catalytic efficiency was 2-fold lower than wild-type for imipenem hydrolysis. Interestingly, meropenem hydrolysis followed a branched kinetic pathway with R220A. Thus, this preliminary analysis of the KPC-2 active site indentified the importance of T237 for KPC-2 to function as a carbapenemase. The loss of this residue is accompanied by loss in kinetic viability as a carbapenemase. However since the cephalosporinase activity of this mutant seems unaffected, T237 possibly plays a role in substrate class discrimination. Similarly, the R220 residue is essential for the hydrolysis of meropenem.
Nxph4 is Localized in Sub Regions of the Brain and is Functionally Important for the Central Nervous System

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Advisor: Huda Zoghbi, M.D.-Department of Pediatrics

Neurexophilin 4 (Nxph4) belongs to the Neurexophilins family, which forms a conserved family of neuropeptide-like glycoproteins. Nxph1 mutation has been reported in Autism patients and Nxph3 knockout mice reveal functional abnormalities in sensory information processing and motor coordination. Previous biochemical work demonstrated that Nxph1 and Nxph3 interact with an extracellular domain of α-neurexin, a presynaptic protein, and mutations of α-neurexin gene have been reported in Autism Spectrum Disorder patients. However, the expression pattern and function of Nxph4 has not been previously described. We generated lacZ reporter knock-in mice to investigate the distribution of Nxph4 in the central nervous system and to reveal the function of Nxph4 by studying the homozygous (null) mice. In adult mouse brain, Nxph4 expression was restricted mostly to neurons in cortical layer 6b, olfactory bulb, mammillary body, and cerebellum. In situ hybridization experiments showed that the neurons expressing Nxph4 although diverse might belong to circuits critical for learning and navigational memory. Comprehensive behavioral testing of Nxph4 knockout mice uncovered remarkable functional abnormalities including altered anxiety-like behavior, impaired sensory information processing, impaired motor coordination and impaired spatial working memory. Our data indicate that Nxph4 may be critical for the function of specific circuits in the nervous system through binding with α-neurexin or other proteins with similar extracellular domains.

Contributors: McGraw, Christopher Michael
Identification of Two-Dimensional RNA Motifs Using Topological Network Motif Discovery

Kit J Menlove

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Non-coding RNA functions in many processes, including the recruitment of chromatin-modifying machinery. Computational prediction or identification of conserved (and therefore likely functional) structural RNA elements remains a challenge. We aim to predict these conserved elements using available RNA immunoprecipitation data by (1) generating ensembles of predicted RNA secondary structures with pseudoknots, (2) converting these structures into networks or dual-graphs, and (3) locating statistically overrepresented sub-graphs in these networks. In a dual-graph, each node represents an RNA stem and each edge represents a segment of RNA backbone (from its 5' to 3' end) connecting two stems. Using a dataset of Polycomb repressive complex 2-binding lincRNA sequences from Khalil, Guttman et al. 2009, we have identified three motifs that are statistically overrepresented and located in regions of three human IncRNAs (HOTAIR, RepA of Xist, and H19) known to bind PRC2 components.

Contributors: Menlove, Kit; Ma, Jianpeng
Chronic Hepatitis B virus (HBV) affects over 400 million people worldwide and is a major risk factor for the development of hepatocellular carcinoma (HCC). Our laboratory studies the HBV regulatory protein HBx, which is required for virus replication in infection models. We and others have demonstrated that an interaction between HBx and cellular damaged DNA binding protein 1 (DDB1) is critical for virus replication. DDB1 functions as an adapter to the Cullin 4A E3 ubiquitin ligase complex. DDB1 recruits DDB1 Cullin Associated Binding Factors (DCAFs) to the Cullin 4A E3 ubiquitin ligase complex, and the DCAFs in turn recruit substrates to be ubiquitinated and degraded. Each of the ~90 human DCAFs share a 16 amino acid DDB1-binding WD40 motif. The HBx contains a sequence similar to the DDB1-binding WD40 motif and has been shown through crystal structure to interact directly with DDB1, suggesting that it is a viral DCAF. The DDB1 function affected by HBx binding is unknown and there are few biologically relevant systems to study this interaction. We will be using human liver chimeric mice (immunodeficient mice that have been engrafted with human hepatocytes) as an infection model in which to study the HBx-DDB1 interaction.

We hypothesize that HBx binding to DDB1 may alter the DCAF profile, and that this will lead to changes in the array of cellular factors that are ubiquitinated and degraded, thereby creating a cellular environment that benefits virus replication.

Aim 1: The first aim of this project will determine the DCAF profiles of normal, uninfected livers as well as HBV-infected livers and compare the two to determine the impact of HBV replication. This will be done using human liver chimeric mice of the same hepatocyte donor. Immunoprecipitation/mass spectrometry will be used to identify DCAFs that are bound to DDB1 in both infected and uninfected hepatocytes.

Aim 2: The second aim of this project will determine the impact of the HBx-DDB1 interaction on DDB1 functions such as the DNA damage response and innate immunity. We will analyze uninfected and HBV-infected liver tissue for the DNA damage marker γH2AX to determine if the DNA damage response is being activated during HBV infection in vivo. We will also be using a variety of methods to analyze how HBV may interact with DDB1 in order to dampen the immune response.
Hedgehog signaling is a highly conserved pathway important in stem cell function and mammalian organogenesis. Misregulated hedgehog signaling contributes to a variety of cancers, including breast. Breast cancer patients with premalignant and malignant lesions show decreased expression of Patched 1 (Ptch1), an inhibitor of signaling, and increased expression of Smoothened (Smo), the main activator of signaling. Nuclear GLI1, a marker of activated hedgehog signaling, may predict poor patient outcome. Additionally, epithelial expression of hedgehog ligand and stromal hedgehog-dependent transcription correlates with poor patient prognosis.

Previous studies using mice homozygous for a hypomorphic Ptch1 allele showed stunted, dysplastic ducts, while mice heterozygous for a null allele showed hyperproliferative, hyperplastic, filled-in ducts. Other data suggested that Ptch1 heterozygosity prevented quiescence in a mammary stem cell-enriched population. Transplantation experiments using these mutants demonstrated a requirement for epithelial, stromal, and systemic Ptch1; however, the true function of Ptch1 in each compartment has not been dissected.

We hypothesize that Ptch1-mediated inhibition of hedgehog signaling is required in epithelium and stroma for normal mammary development and stem cell function. To test this hypothesis, we used tissue compartment-specific deletion of Ptch1 coupled with phenotypic, molecular, and transplantation analysis.

Outgrowths from transplanted adenovirus-Cre treated, conditional null Ptch1fl/fl mammary epithelial cells yielded hyperbranched, hyperproliferative mammary ducts, indicating a critical role in this tissue compartment. In contrast, stromal loss of Ptch1 via Fsp-Cre produced stunted ductal outgrowths with less proliferation in mature virgin animals. A subset of terminal end buds (TEBs) was dysmorphic, and mutant TEBs had reduced proliferation, which may contribute to the stunted duct phenotype. Fsp-Cre, Ptch1fl/fl animals recapitulated the filled-in duct phenotype seen in the Ptch1 +/−-animals. Further, an increase in the number of estrogen receptor positive cells implicates altered regulation of cell fate. Work is in progress to determine whether phenotypes are due to activation of canonical hedgehog signaling.

Contributors: Monkkonen, Teresa; Landua, John; Lewis, Michael
Nucleoid-Associated Proteins (NAPs) are a super-family of proteins in Escherichia coli required for compaction of the nucleoid. They also regulate, both directly and indirectly, DNA transactions such as DNA damage, repair, and transcription by dynamically controlling access to the genome. When E. coli cells are placed under stress such as starvation, their mutation rate increases transiently until a mutation occurs that allows the cell to escape the stress. In a well characterized assay, E. coli carrying a leaky lac frameshift allele are starved on lactose-only medium. After several days, some cells in a subpopulation acquire compensating mutations that restore the ability to utilize lactose, either compensatory frameshift (“point”) mutations, or amplifications of the leaky allele to 20-50 copies, which confers sufficient enzyme activity for growth. The formation of these stress-induced mutations relies on the creation of a double-stranded DNA break and its subsequent repair, which requires access to the DNA. Since NAPs had been shown to regulate genomic access, we hypothesized that the action of one or more NAPs was required for the phenomenon of stress-induced mutagenesis. We showed that several NAPs are either partially or wholly required for stress-induced mutagenesis, both point mutation and amplification. We have investigated 4 NAPs in depth; H-NS, Fis, Dps, and CbpA. In growth phase, H-NS and Fis are two of the four major NAPs, with Dps acting as the major stationary phase NAP and CbpA a specifically late stationary phase NAP. Deletions of H-NS, Fis, and CbpA caused strong reductions in the frequency of stress-induced mutants, while a deletion of Dps resulted in an increase in mutation frequencies. Currently, we are investigating why H-NS, Fis, and CbpA are required for mutagenesis and how Dps is normally inhibiting the mutagenic pathway.
THERAPEUTIC POTENTIAL OF THE EGFR/HER2 INHIBITOR AZD8931 IN CIRCUMVENTING ENDOCRINE RESISTANCE

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Advisor: Rachel Schiff, Ph.D.-Department of Medicine  
Mothaffar Rimawi, M.D.-Department of Medicine

Moderate EGFR/HER2 levels have been suggested to play a role in endocrine therapy resistance in ER+ breast cancer. Preclinical studies have shown that AZD8931, a dual tyrosine kinase inhibitor of EGFR and HER2, is more effective in blocking ligand-dependent signaling than lapatinib in tumors with low to moderate levels of HER receptors. We found that AZD8931 combined with either Tam or Ful inhibited cell growth more profoundly than lapatinib in the T47D cells resistant to Tam (TamRes), and was also significantly, though modestly, more potent than lapatinib in MCF7 TamRes when combined with Tam. In both TamRes models, AZD8931 significantly inhibited cell proliferation and induced apoptosis. Interestingly, multiple HER ligands are upregulated in both MCF7 and T47D TamRes lines, which could explain the superiority of AZD8931 over lapatinib in these models. Indeed, in EGF and heregulin - stimulated conditions, AZD8931 more potently inhibited HER signaling than lapatinib or gefitinib. Finally, AZD8931 significantly delayed the growth of MCF7 TamRes xenografts in the presence of continued Tam or Ful. The strongest inhibition was achieved with Ful and AZD8931 combination. This study provides evidence that AZD8931 has greater inhibitory efficacy than lapatinib in endocrine resistant models that are dependent on ligand activation of the HER pathway. Although AZD8931 combination with Ful robustly slowed growth of TamRes tumors in vivo, the absence of tumor regression suggests that additional escape pathways contribute to resistant growth and will need to be targeted to fully circumvent Tam resistance.

Contributors: Morrison,Gladys; Fu,Xiaoyong;Klinowska,Teresa;Osborne, C.K;Rimawi,Mothaffar; Schiff,Rachel
Validation of cryo-EM structure of IP3R1 channel

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*Advisor: Steven Ludtke, Ph.D.* - *Department of Biochemistry & Molecular Biology*

Electron cryomicroscopy (cryo-EM) is the imaging of a vitrified specimen with a transmission electron microscope, using the collected two-dimensional images to reconstruct a three-dimensional structure. About a decade ago, three cryo-EM single-particle reconstructions of IP3R1 were reported at low resolution. It was disturbing that these structures bore little similarity to one another, even at the level of quaternary structure. Recently, we published an improved structure of IP3R1 at ~1 nm resolution. However, this structure did not bear any resemblance to any of the three previously published structures, leading to the question of why the structure should be considered more reliable than the original three. Here, we apply several methods, including class-average/map comparisons, tilt-pair validation, and use of multiple refinement software packages, to give strong evidence for the reliability of our recent structure. The map resolution and feature resolvability are assessed with the gold standard criterion. This approach is generally applicable to assessing the validity of cryo-EM maps of other molecular machines.

Contributors: Murray, Stephen; Flanagan, John; Popova, Olga; Chiu, Wah; Ludtke, Steve, Serysheva, Irina
A NEW STRATEGY FOR CO-POSITIONING ANTI-CANCER THERAPIES FOR TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is a collection of diseases with distinct clinical behaviors and heterogeneous molecular features. Such clinical and genetic heterogeneity has called into question whether there are common underlying pathogenic mechanisms (and potential therapeutic targets) driving the TNBC subtype of breast cancer. Our recent studies uncovered that a member of the tyrosine phosphatase family (PTPN12) is a prominent tumor suppressor in TNBC (Sun et al., Cell). PTPN12 is lost in nearly 70% of TNBCs, and we have shown that PTPN12 strongly suppresses the tumorigenic and metastatic proclivity of TNBCs. Reconstituting PTPN12 function in a number of TNBC models is anti-proliferative. In the current study, we have discovered that PTPN12 inhibits a suite of tyrosine kinases (TKs) that are unleashed in PTPN12-deficient TNBCs. Restoring PTPN12 inhibits these TKs and impairs tumor growth and metastasis in TNBC models in vivo. Importantly, pharmacologic inhibition of these PTPN12-regulated TKs in combination (but not individually) suppresses MAPK, PI3K, and other survival signaling and consequently elicits significant tumor cell death. In a pre-clinical trial of 18 patient-derived TNBC xenografts, we demonstrate that combined inhibition of PTPN12-regulated TKs causes dramatic tumor regression in PTPN12-deficient TNBC models. This suggests that TNBCs are dependent on the proto-oncogenic TKs constrained by PTPN12. More broadly, this study predicts that cancers lacking conventional biomarkers for TK dependency (like amplification/mutation in oncogenic TK) may be responsive to combination TKi therapy based on protein tyrosine phosphatase dysfunction.

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Obesity is a growing epidemic worldwide; including the United States, where 35% of adults are classified as obese. Several members of the TGF-beta superfamily play important roles in body composition, adiposity and energy metabolism, including protection from diet-induced obesity. Activins (A and B are TGF-beta family members, encoded by Inhba and Inhbb respectively, that function in a wide variety of biological processes. In vitro and in vivo studies have demonstrated the role of activins in adipocyte differentiation and function. Activin (A inhibits adipocyte differentiation of 3T3-L1 cells and human preadipocytes by decreasing the expression of C/EBP, which results in increased preadipocyte proliferation. Increased expression of activin (B in mice and humans is associated with obesity. Additionally, activin (B decreases lipolysis and down-regulates expression of lipolytic genes. In this study, we seek to explore direct roles of activins in white adipose tissue. We utilize mouse models with an adipose-specific conditional knockout of Inhba (activin (A) and a global Inhbb (activin (B)-null mutation to understand the combinatory effects of activin deficiency in adipose tissue and metabolism. Loss of either Inhba or Inhbb has a modest suppressive effect on diet-induced obesity. A combination of adipose-selective deletion of Inhba on the Inhbb-null background (activin double mutant mice) leads to severe weight loss and reduced adiposity. Combined activin (A and activin (B deficiency up-regulates the gene expression of Cidea, Cpt1b, FoxO1 and Pgc1-( in brown adipose tissue, liver and skeletal muscle in activin double mutant mice when compared to wild type controls. Moreover, we found that activin (A and activin (B deficiency induces ‘britening/beiging’ predominantly in visceral white adipose tissue, where the white adipose tissue adopts a gene expression profile and morphological characteristics of brown adipose tissue. Together our results suggest that disruption of activin signaling reduces body growth and adiposity by up-regulating expression of genes involved in metabolism and mitochondrial function. The ‘britening’ of white adipose tissue suggests that combined loss of activin (A and activin (B probably alters the function of white adipose tissue from storage to energy expenditure in the activin double mutant mice.

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A SYSTEMS APPROACH FOR IDENTIFYING CORRELATES OF PROTECTION FOR NEW VACCINE CANDIDATES

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One significant hurdle in vaccine development for emerging infectious diseases is determining whether or not a particular vaccine formulation will provide protection in the absence of a human challenge study. Diagnostic correlates of protection, such as elevated cytokine levels in response to vaccination, are often used to infer whether a protective immune response has been elicited by the candidate vaccine. However, often these correlates of protection are either poorly defined and/or do not apply to all vaccinated individuals. We are proposing a systems biology platform approach that includes longitudinal analyses of human antibody gene repertoire sequence data, immunoreactive protein response kinetics, and cytokine response profiles to better define correlates of protection for vaccines and adjuvants under development.

We are leveraging our expertise with a Francisella tularensis model as a proof of principle target for this platform. First, based on previously published immunoproteomic studies, we have selected a set of approximately 30 F. tularensis (Ft) proteins that are immunoreactive in humans and mice following Ft exposure. These proteins are being expressed and purified in E. coli and are being used in novel, highly sensitive RT-PCR binding studies (incorporating the LG Tadpole) with serum from vaccinated mice and humans to identify the kinetics of immunoreactivity for each of these proteins. Second, we are sequencing the antibody repertoire from the same vaccinated mice and humans, which will provide information about abundance of antibody isotypes, lineage diversity, and somatic hypermutation over the course of the immune response. Together with cytokine profiles generated in parallel with these longitudinal samples, we will construct a predictive model that will be tested in a murine challenge model where lethal doses of F. tularensis will be administered to vaccinated mice.

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Elucidating the role of TIN2L at the telomere and in dyskeratosis congenita

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TIN2, encoded by TINF2, is a component of the telomeric binding complex shelterin. TIN2 acts as the lynchpin in shelterin, linking the double stranded telomeric DNA binding proteins TRF1 and TRF2 to the single stranded telomeric DNA binding protein POT1 via interaction with TPP1. Both a short (TIN2S) and a long (TIN2L) isoform of TIN2 are expressed in human cells. Little is known of the unique functions of TIN2L beyond its association with the nuclear matrix. Heterozygous TINF2 mutations are found in dyskeratosis congenita (DC), a bone marrow failure predisposition syndrome. Underlying the pathogenesis of DC are very short telomeres, and the nine genes implicated in DC have known telomeric roles. The predominantly de novo DC-associated TINF2 mutations result in earlier age at disease onset and shorter telomere lengths than other DC-associated mutations. The mutations, both missense and truncating, cluster in one region of TIN2, present in both isoforms. Although studies have demonstrated the DC mutations affect some of TIN2S’s functions, whether these account entirely for the severe affects in patients remains unclear. Moreover, no studies have examined the effect of the DC-cluster mutations on TIN2L function. We hypothesize that TIN2L has unique roles at the telomere not shared with TIN2S, and that DC-associated mutations could impact these roles. In support of this, evolutionary trace analysis revealed that portions of TIN2L, including a putative casein kinase 2 (CK2) phosphorylation site, are highly evolutionarily constrained. We found TIN2L was predominantly phosphorylated in asynchronous cells, whereas TIN2S was not. Mutation of the predicted CK2 consensus site eliminated this phosphorylation. Additionally, recombinant TIN2L was phosphorylated by CK2 in vitro. Overexpression of TIN2L with a phosphodead mutation inhibited progressive telomere elongation in HT1080 cells, while overexpression of wild type TIN2L or TIN2L with a phosphomimetic mutation did not. In co-immunoprecipitation assays, TIN2L had increased interaction with TRF2 compared to TIN2S, further supporting the hypothesis that TIN2L and TIN2S can be functionally distinguished. Notably, the enhanced TIN2L-TRF2 interaction was dependent upon an intact DC-cluster and phosphorylation of TIN2L (or expression of a phosphomimetic), whereas TIN2S-TRF2 interaction was not impacted by mutation in the DC-cluster. Further work is underway to delineate the significance of the TIN2L phosphorylation and its interaction with TRF2 and other factors in telomere length maintenance.

Contributors: Nelson, Nya; Bertuch, Alison
Metabolomics, a quickly emerging "omics" field in systems biology, is the global analysis of small-molecules in a biological sample. Metabolomics is becoming increasingly important for the analysis of host-pathogen interactions, particularly in the discovery of host and/or pathogen-specific biomarkers of disease. In particular respect to bacterial infections, the rapid emergence of multi-drug resistant bacteria fuels the need for novel strategies in diagnosis and treatment. Metabolomics provides a unique perspective in bacterial infections as it is able comprehensively characterize all metabolic changes in response to a biological perturbation within the host. The metabolic profiles obtained from this analysis can give insight into the identity and nature of molecules involved in the host immune system, alterations in energy metabolism, and metabolites secreted by the invading bacteria. Bacillus anthracis is a Gram-positive blood-borne bacterium that is associated with high morbidity and mortality resulting from bacteremia. Identification of potential bacterial-specific and host-induced metabolites produced in blood in response to an anthrax infection will be key to understanding the disruption of metabolic pathways by can thus provide insight on the impact of bacterial infection on host metabolic function, mechanisms or biochemical molecules produced by pathogens to cause disease, and mechanism of host immune responses. A metabolomics screen using LC/MS and GC/MS platforms was conducted using whole blood collected from mice subcutaneously infected with B. anthracis. Multivariate analysis involving principal component analysis was used to determine clustering profiles of experimental groups for initial identification of statistically significant metabolites produced in response to infection. Metabolic profiles of early infected mouse blood revealed numerous changes related to the inflammation, apoptosis, oxidative stress, and glucose metabolism before the onsite of noticeable pathological symptoms. Future studies will involve bioinformatics analysis and biochemical characterization of key metabolites and associated metabolic pathways.

Contributors: Maresso, Anthony
EVIDENCE FOR HIPPOCAMPAL MICRO- AND ASTROGLIAL ACTIVATION IN THE NS-PTEN KNOCKOUT MOUSE MODEL OF CORTICAL DYSPLASIA

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Rationale: Cortical dysplasia (CD) is a disorder characterized by malformation of the cortex and is commonly associated with aberrant activation of the mTOR pathway and epilepsy. In addition, recent studies have revealed the induction of inflammatory responses such as increased proliferation and activation of microglia and astrocytes (micro- and astrogliosis) and elevated levels of proinflammatory cytokines in tissue resected from CD patients who underwent epilepsy surgery, suggesting a role for neuroinflammation in the pathophysiology of epilepsy. The mTOR pathway is known to regulate a variety of immune processes; however, the link between mTOR dysregulation, neuroinflammation, and epilepsy remains unclear. In the current study, we investigated whether markers of inflammation were abnormal in the previously described neuronal subset-specific PTEN knockout (NS-PTEN KO) mouse model of CD with hyperactive mTOR signaling and epilepsy.

Methods: Immunohistochemistry was performed in coronal brain sections obtained from 7-9 week-old NS-PTEN KO and WT mice. We used antibodies against IBA1 and CD11b (microglial markers), GFAP (astrocytic marker), NeuN (neuronal marker), and p-S6 (phosphorylated ribosomal S6 protein, a marker for mTOR pathway activation), and evaluated immunostaining of these markers in the hippocampal formation. Results: Staining for IBA1 and CD11b was markedly stronger in the hippocampi of NS-PTEN KO compared WT mice. Many of the IBA1- and CD11b-stained microglia in the KO group were hypertrophied compared to those in the WT group. Staining for p-S6 was notably more intense in the KO group compared to the WT group. This was evident in the granule cell layer dentate gyrus where the majority of cells lacking PTEN reside although scattered p-S6 staining was also observed in CA1 and CA3 regions. p-S6 staining co-localized with NeuN in both the KO and WT groups, and also with CD11b in the KO group. In addition, more intense staining for GFAP was also observed in the hippocampi of KO compared to WT mice.

Conclusion: Our findings reveal evidence of micro- and astrogial activation in the hippocampus of NS-PTEN KO mice. Furthermore, p-S6 staining co-localizes with activated microgla in the NS-PTEN KO mice, suggesting a role for mTOR hyperactivity in this process. Future studies will evaluate the effects of rapamycin, an mTOR inhibitor and immunosuppressant with seizure-attenuating effects, on these findings.

Contributors: Nguyen, Lena; Brewster, Amy; Anderson, Anne
Steroid Receptor Coactivator-3 (SRC-3/AIB1) is a potent transcriptional coregulator for nuclear receptors and other transcription factors and is often amplified or overexpressed in tumors. The growth promoting effects of SRC-3 involve its ability to integrate extracellular signals into discrete patterns of gene expression. SRC-3 is regulated by various post-translational modifications, including multiple phosphorylations that define a combinatorial code that tailors SRC-3 activity in response to specific signaling stimuli. HER2 is a member of the ErbB receptor tyrosine kinase family of growth factor receptors and is often associated with cancer. Clinical studies show that breast cancer patients with tumors expressing high levels of both HER2 and SRC-3 have reduced disease-free survival, and SRC-3 phosphorylation is influenced by HER2. The current study seeks to define the effect of HER2 signaling events on SRC-3 activity in breast cancer cells. HER2 knockdown using siRNA affects multiple kinase pathways, as evidenced by decreased AKT and c-Raf phosphorylation. Loss of HER2 or SRC-3 decreased cell viability and triggered cellular apoptosis in response to treatment with estrogen receptor antagonists. HER2 knockdown causes the isoelectric point of SRC-3 to shift, suggesting that post-translational modification of SRC-3 is actively altered by HER2 signaling. Reduction of SRC-3 phosphorylation in HER2-depleted cells also was evident using phospho-specific antibodies to SRC-3. HER2 effect on SRC-3 is also evidenced by decreased SRC-3 activity in luciferase reporter systems and on endogenous genes. Finally, expression microarray analysis revealed that many genes are altered by HER2 knockdown. Future studies using chromatin immunoprecipitation with deep sequencing (ChIP-seq) aim to identify differential SRC-3 cis regulatory elements upon HER2 signaling in breast cancer cells.

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RE-IMAGE-INING HEME UTILIZATION AS AN IRON-SOURCE DURING INFECTION FOR B. ANTHRACIS

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During infection, B. anthracis, the causative agent of anthrax, must seize sufficient amounts of iron to proliferate. While several iron acquisition systems have been previously investigated, heme has not yet been determined to be utilized as an iron-source during an actual infection. B. anthracis is capable of growth on heme as an iron-source in vitro, employing a network of heme-acquisition proteins which our laboratory has characterized. To address the biology of heme uptake during infection, we aimed to develop a reporter of heme catabolism for in situ use. Since the proposed immediate and terminal heme catabolite in B. anthracis is biliverdin IXα, we hypothesized that the cytosolic expression of infrared fluorescent protein (IFP, which binds biliverdin to fluoresce in the near-infrared region) would allow for direct measurement of the release of biliverdin during heme catabolism. Biochemical characterization of IFP-biliverdin interactions demonstrated a 1:1 binding stoichiometry, with fast binding kinetics and a low micromolar affinity for the ligand. Cytosolic expression of IFP in B. anthracis leads to increased fluorescence when cells are given biliverdin, a result which is paralleled during iron-dependant growth of B. anthracis expressing IFP when supplemented with hemoglobin, a predicted heme-source during infection. Further, infections with IFP expressing B. anthracis demonstrate a near-infrared fluorescent signal localized around the site of infection in DBA/2J mouse infection model shortly after inoculation. This work suggests IFP can be used as a fluorescence reporter of heme breakdown in culture and during infection, a tool that will aid in understanding the kinetics, localization, and regulation of this critical process during real-time, live infections.

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FUNCTIONALLY-IMPORTANT BASES IN NON-CODING RNAS EVOLVE IN HIGHLY COMPACT CLUSTERS: SEQUENCE-STRUCTURE ANALYSIS OF RNA-BASED MACHINES.

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Functional non-coding RNAs (here, used to broadly designate all functional non-translated RNAs) play a vital role in a variety of subcellular processes including RNA and DNA modification, genome stability, and gene regulation. As discovery and characterization of novel ncRNAs continue, the need for new theoretical approaches to guide inquiry into the sequence-structure-function relationship of individual ncRNA molecules will become more pronounced. Here, we employ Evolutionary Trace, a method that relies on multiple sequence alignments and phylogeny, to identify sites of functional and structural importance in the functional RNA molecules represented in the Protein Data Bank. Our analysis shows that, in most cases, bases, that we predict to be evolutionarily important, form highly non-random clusters that often constitute biologically-relevant regions such as metabolite and ion binding sites, protein interfaces, and catalytic pockets. This suggests that evolutionarily-important bases in ncRNAs evolve in a manner that makes them detectable, and that functional regions in ncRNA molecules can be predicted based on sequence and phylogenetic information.

Contributors: Novikov, Ilya; Wilkins, Angela; Lichtarge, Olivier
Alteration in the Lower Limit of Autoregulation with Elevations in Cephalic Venous Pressure.

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BACKGROUND: Recent studies suggest that elevated intracranial pressure (ICP), created by hydrocephalus, can alter the lower limit of autoregulation. However, ICP can become elevated by other clinical scenarios, including those that elevate cephalic venous pressure. Our objective in the present study was to determine if ICP elevation from cerebral venous outflow obstruction would result in comparable alterations in the lower limit of cerebrovascular autoregulation (LLA). METHODS: Anesthetized juvenile pigs were assigned to 1 of 2 groups: naïve ICP (n = 15) or high ICP (>20 mmHg; n = 20). To elevate ICP through venous obstruction, a modified 5F esophageal balloon catheter was inserted via the right external jugular vein into the superior vena cava and inflated to maintain an ICP of >20 mmHg. Gradual hypotension was induced by continuous hemorrhage from a catheter in the femoral vein. The LLA was determined by monitoring cortical laser Doppler flux (LDF). Invasive ICP monitoring and cerebral blood volume monitoring by near-infrared spectroscopy (NIRS) were used to derive the pressure reactivity index (PRx) and hemoglobin volume index (HVx) autoregulation curves, which were then normalized to LLA. Receiver operating characteristic (ROC) curves for PRx and HVx were created using the LLA as the cutoff point. RESULTS: The naïve ICP group had an average LLA of 45 mmHg (95% CI: 41–49 mmHg) by laser Doppler, and the high ICP group had a LLA of 71 mmHg (95% CI: 66–77 mmHg). The LLA was significantly different between the two groups (p < 0.0001), and correlated significantly with ICP (R = 0.7468, p < 0.0001). The area under the curve for the ROC curves were 0.9920 and 1.000 respectively for PRx and HVx. CONCLUSIONS: Elevated ICP from cephalic venous engorgement leads to an increase in the LLA. Autoregulation monitoring using the PRx or HVx can identify autoregulatory impairment during elevation of cephalic venous pressure. These findings suggest that pathologic processes resulting in cephalic venous outflow obstruction and intracranial venous congestion can acutely elevate ICP and may place the brain at risk for impaired cerebrovascular autoregulation.

Contributors: Nusbaum, Derek; Clark, Jonathan; Brady, Kenneth; Kibler, Kathleen; Sutton, Jeffrey; Easley, R. Blaine
Objective: It is understood that light/dark cycles entrain circadian rhythms. How this occurs in the absence of light/dark cues (such as occur in utero during fetal development) is poorly understood. We have previously demonstrated that maternal diet entrains the fetal circadian machinery, and a high fat diet is globally disruptive. Our objective in this study was to identify the role of specific circadian machinery in programming the metabolic response.

Study Design: Npas2 KO (-/-) and control mice (+/+), livers were harvested at day 2 (neonate) and at 25 weeks (adult). Total RNA was extracted, and converted labeled cDNA was loaded onto the GeneChip® Mouse Exon 1.0 ST array. Partek Genomics Suite software and GeneSpring GX 11 were utilized for first pass analysis. Genes meeting statistical and expression thresholds were then compared to genes associated with BMAL1 regulation to identify candidate genes for NPAS2 gene regulation; validation was performed with QPCR.

Results: We identified 2891 genes that were altered in -/- mice compared to +/+ (p<0.05, fold-change >1.5 or <-1.5) and 352 candidate genes for primary transcriptional regulation by NPAS2 (Fig.1B). Consistent with our prior observations, expression of key regulators of the circadian pathway, Bmal1 and Clock, are similarly altered in newborn -/- mice (.73, .43x respectively, p<.05) with expression levels shifting in the adult -/- mice (Bmal1:1.4x, p<0.05; Clock: 1.17x, p=0.45).

Conclusion: We have significantly expanded our prior findings to demonstrate that loss of Npas2 during fetal development alters both circadian and metabolic gene expression. However, gene expression alteration in early development is mitigated in adult life, suggesting a crucial role of Npas2 during early development. Since fetal development is void of light/dark cues, entrainment of Npas2 through other cues (such as maternal diet) is likely key to establishing circadian patterns.

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Deficiency of FKBP12 in the Heart Leads to Impaired Contractility and Pregnancy Induced Cardiomyopathy

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FK506 Binding Proteins (FKBPs) are a family of cis-trans prolyl isomerase that bind rapamycin and FK506. FKBP12 and 12.6 interact with ryanodine receptors (RyR), homotetrameric transmembrane ion channels that regulate Ca2+ release from the sarcoplasmic reticulum (SR). FKBP12 interacts with RyR1 in skeletal muscle and FKBP12.6 interacts with RyR2 in cardiac muscle to regulate the Ca2+ leak properties of these channels. Recently it has been suggested that FKBP12 also plays a role in regulating RyR2 activity. Using mice with a cardiac specific deficiency in FKBP12, we analyzed the role of FKBP12 in cardiac function.

We found that both male and female mice with a α-MyHC Cre/Lox mediated deficiency in FKBP12 in the heart (FKBP12 KD) developed a mild dilated cardiomyopathy, with enlarged left ventricular diameter both during systole and diastole, decreased ejection fraction and decreased fractional shortening. To elucidate the mechanism for these effects we assessed Ca2+ sparks in isolated cardiomyocytes. We found an increase in both Ca2+ spark frequency and spark amplitude in FKBP12 cardiac deficient mice without a change in spark duration. Despite a mild phenotype in adult mice, we found that approximately 25% of all pregnancies (26/106) in the FKBP12 deficient mice resulted in the mothers dying following the birth. Autopsies show that these cardiac specific FKBP12 deficient mice had increased heart weight and significantly dilated ventricles compared to female Cre mice. Our data suggest that a cardiac specific deficiency in FKBP12 leads to the development of pregnancy induced cardiomyopathy. Echocardiography on FKBP12 deficient mice one day after giving birth found that there was no significant difference in ejection fraction or fractional shortening compared to α-MyHC Cre control mice. FKBP12 deficient females, however, had larger hearts and 33% (2/6) displayed heart failure and died. In conclusion, we show that FKBP12 does indeed alter Ca2+ handling in the heart and that a loss of FKBP12 leads to the development of pregnancy induced cardiomyopathies in females.

Contributors: Oakes, Joshua; Hamilton, Susan
The role of Stat5a for progesterone signaling in normal mammary epithelium.

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Progesterone (P4), a proliferative hormone in the mammary gland, is required for ductal side-branching in the adult virgin and epithelial expansion and alveologenesis during pregnancy. Previous microarray analysis of adult virgin mice treated acutely with P4 identified a subset of paracrine growth factors as PR targets and potential mediators of P4 induced cell proliferation. To study mechanisms of PR regulation of these target genes, we have developed a 3D culture system of primary mammary epithelial cells (MECs) embedded in Matrigel that form polarized acini composed of luminal and myoepithelial cells and maintain PR expression similar to the mammary gland in vivo. As assessed by TaqMan Array qPCR, P4 induced expression of many of the target genes identified previously in the mammary gland, including paracrine factors RANKL, Wnt4 and amphiregulin. RANKL has emerged from recent studies to be an essential paracrine effector of P4 proliferation in the mammary gland. In co-transfection experiments, P4 in a PR dependent manner induced activation of 5 different RANKL enhancers linked to a luciferase reporter and PR bound to these same RANKL enhancers in response to P4 detected by ChIP. Stat5a was also recruited in a P4 dependent manner to the same enhancers as PR in the absence of prolactin (PRL), a known Stat5a activator. To determine whether Stat5a has a role in PR-mediated gene regulation, primary MEC 3D cultures derived from Stat5a knockout mice were analyzed. P4 induction of RANKL, Wnt-4, and amphiregulin was substantially reduced in MECs from Stat5a KO mice compared with wildtype. Additionally, PR recruitment was maintained at selective RANKL enhancers in the Stat5a KO MECs, but abrogated at others analyzed by ChIP. Further mechanistic exploration by ChIP with the P4 antagonist, RU486, identified P4 induced Stat5a binding is abolished at selective RANKL enhancers, suggesting the mechanism by which Stat5a regulates P4 signaling is enhancer dependent. These data indicate that RANKL is a direct target of PR and Stat5a is a coregulator of progesterone signaling.

Contributors: Obr, Alison; Grimm, Sandy; Lydon, John; Bishop, Kathleen; Pike, J. Wesley; Edwards, Dean
ABROGATION OF ANDROGEN METABOLIC PATHWAY IMPAIRES FEMALE AND
MALE SEXUAL BEHAVIORS IN CYP7B1 NULL MICE

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Circulating testosterone can be intra-cellularly metabolized in brain tissue to 17-(estradiol by the aromatase enzyme or to DHT by 5( reductase. However, DHT is further metabolized to 3-Diol and to 3β-Diol. The conversion of DHT to 3β-Diol is unidirectional. Moreover, it has been demonstrated that 3β-Diol binds preferentially to the estrogen receptor (ER) to regulate transcription of downstream genes. In contrast, 3-Diol has little affinity for ERβ or ERα. 3β-Diol is further metabolized to inactive 6- or 7-triols by the actions of the enzyme, CYP7B1. Thus, CYP7B1 may be an important regulator of estrogenic functions mediated by 3β-Diol in the brain.

We investigated the role of androgen metabolism on behavior by examining the phenotype of CYP7B1 null mutant mice (CYP7B1-/-). Our results indicate that male sexual behaviors were significantly reduced in CYP7B1-/- compared to their wild type littermates (CYP7B1+/+). This could be due to a defective vomeronasal olfactory system in CYP7B1-/- mutants, since the male CYP7B1-/- mice demonstrated no preference to the female estrous bedding in the olfactory preference test even though testosterone levels were comparable to the CYP7B1+/+ males. A reduction in androgen receptor (AR) and ERβ mRNA levels in the olfactory bulb, but not in the medial amygdala or preoptic area (POA), was also found in the CYP7B1-/- mice. Consistent with this, testosterone administration to gonadectomized mice down-regulated AR mRNA in the olfactory bulb of CYP7B1+/+, but not in the CYP7B1-/- males. No genotype effect was observed in stress-induced anxiety-like behaviors and stress-responsive hormone levels. Female CYP7B1-/- mice, but not their wild type littermates, treated with the ovarian hormones estrogen and progesterone, demonstrated a deficit in female receptive behavior, quantitated by their lordosis response. Despite these changes in behavior in CYP7B1-/- mice, sexual differentiation of the brain appears normal based on the presence of the sexually dimorphic distribution of calbindin-immunoreactivity in the preoptic area. Ongoing studies are aimed at examining the role of 3β-Diol and ERβ on organizational and activational effects of steroid hormones on the brain.

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Neuroendocrine Regulation of Lipid Homeostasis in C. elegans

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Lipid metabolism is under the control of environmental and genetic factors. The nervous system receives environmental sensory inputs and consequently communicates with the adipose tissue. This project aims to dissect the functional roles of insulin/insulin-like growth factor signaling (IIS) in the regulation of lipid metabolism and how it conveys signals from the nervous system to the adipose tissue in Caenorhabditis elegans.

To identify specific neurons and neuropeptides involved in the regulation of fat metabolism, we examined lipid storage in chemosensory mutants that have either defective signal transduction or abnormal sensory cilia morphology. We found that daf-11 mutants that lack a trans-membrane guanylyl cyclase have increased lipid levels in C. elegans main fat storage tissue, the intestine. daf-11 is expressed in 5 pairs of neurons located in the head: AWB, AWC olfactory neurons and ASI, ASJ, ASK gustatory neurons. Increased fat storage of daf-11 mutants requires the forkhead transcription factor DAF-16, which is a major downstream effector of the highly evolutionarily conserved IIS pathway.

In C. elegans, the IIS pathway includes about 40 insulin-like peptides (ILPs), a single insulin receptor homolog, DAF-2 and downstream signaling cascade that ultimately regulates DAF-16. Mutant worms that lack DAF-2 or the downstream effector PI3K catalytic subunit, AGE-1 have increased fat storage, which requires functional DAF-16. daf-2, age-1 and daf-16 are broadly expressed in multiple tissues. We found that reduced IIS specifically in the intestine is sufficient to increase intestinal fat storage suggesting a cell-autonomous regulation. Furthermore, we found that among four different daf-16 isoforms, the daf-16 d/f isoform is specifically required for increased fat storage in daf-2 mutants suggesting its role in the regulation of lipid homeostasis. Last but not least, we compared metabolite profiles of daf-2 mutants and wild type to investigate metabolic changes associated with reduced IIS. Unexpectedly, branched-chain amino acids (BCAA) and related metabolites, but not lipid metabolites, were overrepresented in the daf-2 mutants.

Based on our findings, we hypothesize that activities of sensory neurons modulate the release of ILPs, which bind to the DAF-2 receptor located in the intestine and subsequently transduce signals for the regulation of lipid and amino acid homeostasis that interact bidirectionally. These studies will advance our understanding of the molecular mechanisms by which IIS pathway modulates lipid metabolism, and also shed light on the coordination between BCAA and lipid metabolism.

Contributors: Ozseker, Ayse Sena; Yu, Yong; Wang, Meng
NEONATAL POLYMICROBIAL INFECTIONS INCREASE MORTALITY

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Introduction and Hypothesis: Polymicrobial infections increase mortality in children and adults. Neonatal polymicrobial infections are poorly characterized. We hypothesized that polymicrobial infections comprise more than 5% of neonatal infections, have identifiable risk factors and are associated with greater mortality and morbidity than monomicrobial infections.

Methods: We identified neonates with positive blood and/or CSF cultures at Texas Children’s Hospital, from January 1, 1997 to December 31, 2012. Polymicrobial infection was defined as isolation of more than one organism from blood or CSF during an infectious episode. Clinical data were available from January 2009 to December 2012. For each polymicrobial infection (case), we matched two neonates with monomicrobial infections (control) by gestational age and birth weight.

Results: We identified 2179 episodes of infection during the 15 year study period and 717 (14.96%) of these infectious episodes were polymicrobial. The frequency of polymicrobial infections varied from 11.2% to 21.3% of annual infectious episodes during the study period. Coagulase-negative Staphylococcus, Enterococcus, Klebsiella and Candida were the most common genera isolated from polymicrobial infections. Infants with polymicrobial infections were matched for gestational age and birth weight with infants who had monomicrobial infections. Presence of a central venous catheter was a significant risk factor for polymicrobial infections and mortality was greater in cases compared to controls.

Conclusions: Polymicrobial infections constitute nearly 15% all neonatal infections and are associated with significantly increased mortality compared with monomicrobial infections. Urgent prevention and treatment strategies are needed to counter neonatal polymicrobial infections.

Contributors: Pammi, Mohan, Zhong, Danni, Johnson, Yvette, Revell, Paula, Versalovic, James
Hepatitis B Virus (HBV) infection is one of the leading causes of death worldwide, infecting more than two billion people. Chronic infection affects 350 million people, leading to liver cirrhosis and dramatically increasing the risk of developing hepatocellular carcinomas. HBV has a 3.2 kb partially double-stranded DNA genome, which persists episomally in infected cells as covalently closed, circular DNA (cccDNA). This cccDNA serves as a template for viral mRNA production via four overlapping reading frames, as well as maintaining a source for possible reinfection after acute symptoms subside. Current therapeutics are not very effective, expensive, and often cause several undesirable side effects. Transcription Activator-Like Effector Nucleases (TALENs) are an emerging technology that couples the site-specificity of a naturally occurring DNA-binding protein with a nonspecific endonuclease domain such as FokI, which only functions as a dimer to prevent nonspecific double-stranded cleavage. These TALENs can be modularly designed and targeted to specific sequences of DNA. Therefore, we hypothesize that targeting TALENs to the cccDNA of Hepatitis B Virus will result in erroneous repair of double-stranded breaks and disruption of essential reading frames. By replacing the nuclease domain with various transcriptional repressors, we also intend to demonstrate epigenetic silencing of the HBV genome. We will be using a human liver chimeric mouse model and recently isolated Adeno-Associated Virus (AAV) serotypes to validate TALENs and TALE repressors as novel therapeutic agents against this major human pathogen.
Although slice recordings and model studies provided a lot of information about how neurons integrate their inputs to overall responses, it is still not clear how specific types of neurons, in vivo, in the brain circuits integrate their synaptic inputs to derive their functional properties. Here, I focus on studying pyramidal neurons in layer 2/3 of area V1, which provide the main relay input from V1 to higher visual cortex. Specifically, I propose to study mouse V1 L2/3 neuronal receptive field property before and after selective dendritic ablation. First, I will validate a method for selective deletion of synaptic inputs by laser ablation in vivo in mouse V1 L2/3 pyramidal neurons. As a second step I propose to study how these neurons’ properties are modified after ablating different parts of their dendritic tree input domains (for example apical versus basal dendrites) using genetically expressed calcium indicators. Over time, this approach will provide additional information on understanding how the function of cortical neuronal circuits.

Contributors: Park, Jiyoung; Smirnakis, Stelios Manolis
PRKRIR: A POTENTIAL NOVEL GENOMIC DRIVER OF TAMOXIFEN RESISTANCE

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While 40% of estrogen receptor (ER)-positive breast cancer tumors recur as a result of acquired resistance to endocrine therapies such as Tamoxifen (Tam), the mechanisms underlying resistance remain largely undetermined. We have identified a novel potential driver of Tam resistance (TamR), PRKRIR (protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)). Microarray analysis comparing Tam-sensitive primary breast tumors to Tam-resistant metastatic tumors has shown that PRKRIR is overexpressed in Tam-resistant tumors. The PRKRIR genomic region was also found to be amplified in the Cancer Genome Atlas (TCGA) (Nature 2012). It has been indicated that PRKRIR is regulated by PAK1 (p21 protein (Cdc42/Rac)-activated kinase 1), another protein involved in TamR. PAK1 was also co-amplified and overexpressed with PRKRIR. I hypothesize that PRKRIR cooperates with PAK1 to promote TamR in ER-positive breast cancer.

I have developed MCF-7 ER-positive breast cancer cells lines that overexpress PRKRIR using a lentiviral vector. Preliminary studies demonstrated that cells with PRKRIR overexpression (OE) also express higher levels of proteins known to have important roles in hormone response, including total and phosphorylated PAK1, and ER. Levels of phospho-serine 118 and 305 were enhanced with PRKRIR OE. High levels of PRKRIR were associated with less responsiveness to Tam, as compared to control cells with significant growth inhibition. Preliminary results suggest that PRKRIR OE may activate PAK1. In addition, we discovered that Tam treatment increases levels of PRKRIR and PAK1 protein in parental cells. Inducible PRKRIR experiments are underway to understand the function of PRKRIR as an upstream modulator of PAK1.

We predict that PRKRIR may be a novel biomarker of resistance to Tam, possibly through inappropriate activation of both Pak-1 and ER. By studying the mechanisms of TamR in breast tumors, my project may provide direction for future diagnostic tools and uncover new clinical targets for therapy.

Contributors: Pejerrey, Sasha M.; Gu, Guowei; Rechoum, Yassine; Beyer, Amanda; Wang, Xiasong; Fuqua, Suzanne A.W.
Growth differentiation factor 9: bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions.

**Jia Peng**

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*Advisor: Martin Matzuk, M.D./Ph.D.-Department of Pathology & Immunology*

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), oocyte-secreted paralogs of the transforming growth factor β (TGFβ) superfamily, have been shown genetically to control ovarian physiology. Although previous studies found that GDF9 and BMP15 homodimers can modulate ovarian pathways, the functions of GDF9:BMP15 heterodimers in any species remained largely unknown. Therefore, we produced six ligands (mouse and human GDF9 homodimers, BMP15 homodimers and GDF9:BMP15 heterodimers) and compared their physiological activities in vitro. We discovered that mouse GDF9:BMP15 heterodimer is about 10-30 fold more biopotent than mouse GDF9 homodimer, and human GDF9:BMP15 heterodimer is about 1000-3000 fold more bioactive than human BMP15 homodimer. Moreover, GDF9:BMP15 heterodimers require a unique signaling complex that includes the BMPR2 type 2 receptor, an ALK4/5/7 type 1 kinase receptor, and an ALK6 type 1 co-receptor. GDF9:BMP15 heterodimers bind to this receptor complex to stimulate phosphorylation of SMAD2/3. Our findings of the activity of GDF9:BMP15 heterodimers not only explain many of the puzzling genetic and physiological data that has been generated over the last two decades, but the data also has important implications for improving female fertility in mammals.

Contributors: Peng, Jia; Li, Qinglei; Wigglesworth, Karen; Rangarajan, Adithya; Kattamuri, Chandramohan; Peterson, Randall; Eppig, John; Thompson, Thomas and Matzuk, Martin.
In the developing vasculature of the mouse embryo, one of the earliest events after establishment of the primitive capillary plexus is the specification of arterial and venous vessels. This specification occurs even before the onset of blood flow but is believed to remain plastic even after that point. The key molecular signaling pathways involved in this fate specification are still largely unknown. Recent evidence indicates a critical role for ERK signaling in arterial specification in the zebrafish embryo where it has been shown to augment arterial angiogenesis. The Sprouty2 protein, encoded by the Spry2 gene, has been shown to repress ERK activation in endothelial cells in vitro. However, the role of Sprouty2 in regulating ERK activation during vascular development has yet to be elucidated. Recent data collected by our group suggests a possible role for Sprouty2 in ERK-mediated arteriogenesis.

The precise role ERK signaling plays in arterial specification in the developing mouse embryos has not yet been fully established, in part because analysis of the dynamics of vascular development in this model have until now been limited to conventional confocal microscopy. With the advent of light sheet fluorescence microscopy (LSFM), which supports fluorescence detection deep within large intact tissues, our ability to define such dynamic developmental processes has been expanded upon. LSFM uses illumination of transgenic markers via a vertical light sheet to visualize specifically labeled living tissue components. Our lab possesses mice carrying transgenic markers of vascular membranes (Flk1:myr::mCherry) and endothelial nuclei (Flk1:H2B-eYFP) that can be combined with LSFM to observe the dynamic activity of vessel morphogenesis and remodeling in the mouse extra-embryonic yolk sac vasculature.

We hypothesize that Sprouty2 negatively regulates arteriogenesis by inhibiting ERK signaling. We aim to employ a variety of embryo culture techniques to both over-express and knock down Spry2 expression, and inhibit ERK signaling directly in both wild-type embryos and those displaying altered arterial specification to determine the precise pathway by which Sprouty2 acts in arteriogenesis. Doing so will help provide valuable information on this poorly understood, yet crucial, component of vascular development.

Contributors: Piazza, Victor; Garcia, Monica; Udan, Ryan; Dickinson, Mary E.
Over thirty percent of Rett Syndrome (RTT) cases are due to nonsense mutations in MECP2, where a change in nucleotide sequence leads to a premature stop codon in the mRNA transcript. One strategy to overcome disease-causing stop mutations is treatment with nonsense suppressing read-through compounds, such as gentamicin, which reduce the stringency and fidelity of ribosomes translating mRNA messages to allow expression of full length proteins from a mutated gene. To determine if this strategy may be useful in RTT we characterized a new mouse model of RTT that has a knock-in nonsense mutation (p.R255X) in the Mecp2 locus (Mecp2tm1.1Irsf/J). Mecp2 is a four exon gene that encodes two functional domains: the methyl binding domain from exons 3 and 4 and the transcription repression domain in exon 4. Because the R255X mutation is located in the transcription repression domain of Mecp2, it is possible that a dominant negative DNA binding truncation product could be produced from the disease allele.

To determine if the truncated gene product acts as a dominant negative allele, we genetically introduced an extra copy of MECP2 via a MECP2 transgene. This allows us to determine whether adding a wild-type version of MeCP2 is sufficient to rescue phenotypic abnormalities in Mecp2tm1.1Irsf/J mice, or whether the truncated allele has a dominant negative effect insurmountable by a wild-type copy. Mecp2tm1.1Irsf/J mice have phenotypes nearly identical to complete null animals: decreased weight early in life, decreased heart rate late in life, abnormal breathing phenotypes, poor motor coordination, and decreased survival time. The addition of the MECP2 transgene to Mecp2tm1.1Irsf/J mice abolished the phenotypic abnormalities and resulted in near complete rescue. This provides a proof of concept that this mutation is amenable to read-through therapy. Future studies will include pharmacokinetic and efficacy preclinical trials in the R255X model using read-through compounds that are currently in clinical trials for peripheral indications. We hope to demonstrate that read-through therapy is a viable treatment option for neurological disease caused by nonsense mutations.

Contributors: Pitcher, Meagan R; Herrera, Jose A; Fisher, Amanda R; Schanen, N. Carolyn; Neul, Jeffrey L
S. cerevisiae MTC5 (Maintenance of Telomeric Capping 5) was identified as having a potential role in telomere maintenance, DNA double strand break (DSB) repair and the DNA damage response (DDR) based on a genetic interaction with cdc13-1 and a physical interaction of Mtc5 with Yku80 and Srs2 reported in high throughput screens. Mtc5 is a member of the SEA complex, which localizes to the vacuolar membrane and is thought to be involved in intracellular trafficking and autophagy. Vacuolar proteins have been implicated in telomere length regulation and epistatically linked to the Yku70/Yku80 complex and telomerase, though the nature of these interactions is unknown. Further, vacuolar proteins are hyper-activated during DNA damage and promote cell cycle arrest. Taken together, we hypothesized that Mtc5 impacts the telomere and DSB repair or the DDR via its interactions with proteins such as these. We found an mtc5Δ mutation stochastically affected telomere length as dissection of mtc5Δ/MTC5 heterozygotes revealed two populations of mtc5Δ haploids; one formed wild type (WT) appearing colonies with WT telomere length, whereas the other formed small colonies with shortened telomeres. Notably, haploid null mutants of other SEA complex members did not have short telomeres. Epistasis analysis with yku80Δ revealed mtc5Δ yku80Δ mutant telomeres were stable but had a variable length defect, placing Mtc5 and Yku in different telomere length maintenance pathways. Epistasis analysis with tlc1Δ (TLC1 encodes the RNA subunit of telomerase) showed mtc5Δ tlc1Δ mutants senesced more severely upon successive liquid culturing and did not form telomerase-independent survivors as readily as tlc1Δ mutants, though both formed ‘type II’ survivors. Since type II survivors maintain their telomeres via break-induced replication (BIR) we examined the impact of the absence of Mtc5 in a system in which an induced DSB is repaired largely by BIR. We found mtc5Δ mutants formed colonies more slowly after DSB induction than WT in this system, although the rate of repair and the number of repaired colonies were comparable to WT, indicating that the delay in colony formation was due to effects downstream from repair. The delay in colony formation was unaffected by a yku70Δ mutation, but was completely rescued by exo1Δ mutation. mtc5Δ strains also formed colonies more slowly on bleomycin, though the number of surviving colonies was similar to WT. We, thus, reasoned that as a vacuolar protein, loss of Mtc5 might influence the levels of factors critical for extinguishing the DDR or for survival post-repair. Consistent with these possibilities, the levels of Srs2, which putatively interacts with Mtc5, decreased over time in an mtc5Δ as compared to WT strain when grown in the presence of MMS. As the human homolog of MTC5 was recently found to have direct effects on mTORC1, we recently focused on whether the effects of Mtc5 on the DDR were mediated through TORC signaling and found results that were consistent with the possibility that Mtc5 functions upstream of TORC in response to DNA damage.
Background: The incidence of Diabetes is increasing at an alarming rate with 220 million people worldwide currently suffering from this metabolic disorder. Increased consumption of high fat diet and beverages containing high fructose corn syrup (HFCS) coupled with an increase in sedentary life-style has led to a drastic increase in obesity rates. Obesity increases the likelihood of developing insulin resistance which can lead to impaired glucose tolerance and diabetes in susceptible individuals. Blood glucose is tightly regulated by the glucose sensing and absorptive/excretory tissues in our body. Glucose sensing occurs primarily in the neurons of the hypothalamus, pancreas, liver and secondarily in small intestine and renal tubules. Upon stimulation, these organs secrete hormones to target organs like the liver (glucose production), skeletal muscles and adipose tissues (glucose uptake), and kidneys (glucose resorption) to regulate glucose homeostasis. One such hormone that is secreted by the oxyntic cells of the stomach under low glucose conditions is ghrelin. Ghrelin is the only known orexigenic hormone which increases food intake, and Growth Hormone secretion. Ghrelin also is thought to suppress insulin secretion. Growth Hormone Secretagogue Receptor (GHS-R) is recognized as the physiologically relevant receptor for ghrelin and has been shown to mediate ghrelin’s effects on growth hormone secretion and appetite stimulation. The objective was to study the effects of blocking ghrelin signaling by deletion of GHS-R gene globally and in specific tissues (pancreatic islets and hypothalamic arcuate nucleus) on metabolic parameters regulating glucose homeostasis.

Results: Unlike expected, ablation of GHS-R in leptin deficient mice have lower insulin, higher blood glucose, and worsened glucose tolerance. Histological analysis of islets reveals that Ghsr-/-:ob/ob mice have islets similar in size compared to ob/ob mice, which implies that the β-cell mass of the islets is not affected in the mice; this points toward an effect of β-cell function. Ex vivo studies on the pancreatic islets from global GHS-R KO mice exhibit significantly lower insulin secretion under hyperglycemic conditions (GSIS). This effect might be mediated by activation of AMPK and the uncoupling protein UCP2 in the pancreas. These mice also have reduced oxidative phosphorylation as observed by sea-horse experiments. Surprisingly, GHS-R KO only in AgRP neurons also reduces GSIS similar to global KO mice without any change in glucose tolerance.

Conclusion: The role of ghrelin on regulating plasma glucose might not be mediated directly though it’s signaling via GHS-R. The tissue specific role of GHS-R in brain and in the periphery have not been studied. Further studies need to be done to elucidate the possible involvement of other peptides derived from the ghrelin gene in regulating glucose homeostasis in these mice and assess whether manipulation of ghrelin signaling is a viable approach for treating glucose homeostasis disorders like diabetes.

Contributors: Pradhan, Geetali; Ligen, Lin; Yuxiang, Sun
Loss of argininosuccinate lyase in enterocytes results in increased incidence of necrotizing enterocolitis

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Abstract
Necrotizing enterocolitis (NEC), the most common neonatal gastro-intestinal emergency results in significant mortality and morbidity, yet its pathogenesis remains unclear. Argininosuccinate lyase (ASL) is the only enzyme in mammals that is capable of synthesizing arginine. Arginine has several homeostatic roles in the gut, hence not surprisingly its deficiency has been associated with NEC. Since enterocytes are the primary sites of arginine synthesis in neonatal mammals, we evaluated the consequences of disruption of arginine synthesis in the enterocytes on pathogenesis of NEC.

Methods: We devised a novel approach to study the role of enterocyte-derived ASL in NEC by generating and characterizing a mouse model with enterocyte-specific deletion of Asl (Asl flox/flox; VillinCretg/+ or CKO). We hypothesized that the presence of ASL in a cell-specific manner in the enterocytes has an important role in the pathogenesis of NEC.

Results: Our studies performed on the CKO mice show that the loss of ASL in the enterocytes leads to an increased incidence of NEC which is at least in part due to increased enterocyte apoptosis and an exacerbated inflammatory state. Importantly, IEC-6 cells without ASL were unable to migrate in response to LPS.

Conclusion: The enterocyte-derived ASL controls enterocyte apoptosis, IL6 production, and intestinal neutrophil infiltration in NEC. The protective role of ASL provides new opportunities to develop preventive and therapeutic strategies in NEC.

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Background: Disruptions in cardiac Ca release have been linked to heart failure (HF). Prior studies in the lab have focused on two key proteins of the cardiac Ca release unit—the ryanodine receptor type two (Ryr2) and junctophilin (Jph2). Mass spectometry revealed “striated muscle preferentially expressed gene” (SPEG), a novel kinase, as the only protein that was pulled down by both RyR2 and JPH2 in a co-immunoprecipitation experiment. Thus SPEG may play an important role in regulating Ca release through its interactions with the Ca release unit.

Objective: to define the molecular and physiological role of SPEG in the heart.

Hypothesis: SPEG is cardioprotective by regulating Ca release through its interactions with the Ca release unit.

Methods: Since immunoprecipitation/ mass spectrometry showed that SPEG was pulled down with high specificity for RyR2 in particular, we began by studying the interaction of SPEG with RyR2. To assess co-localization of SPEG and RyR2, WT (C57BL/6J) mouse cardiomyocytes were isolated by Langendorff perfusion and enzymatically and mechanically separated from nonmyocytes. Fresh cardiomyocytes were stained with antibodies against SPEG and RyR2 and were visualized by confocal microscopy, which revealed overlap of RyR2 and SPEG. Next, to test whether SPEG is important in the adult mouse heart, 2 WT (C57BL/6J) mice were subjected to transverse aortic constriction (TAC), creating pressure-overload induced HF and 2 control mice underwent sham surgery. Echocardiography revealed decreased ejection fractions, a key sign of HF, in TAC but not sham mice at 4 weeks post surgery. At this time hearts were harvested and Western blot was performed to assess protein levels of SPEG in TAC versus control mice revealing that SPEG protein levels are downregulated in mice with HF.

Conclusions/ Future Directions: SPEG interacts with RyR2 and appears to support a regulatory role for SPEG in HF. However, to adequately study the role of SPEG in the adult heart and its contribution to HF, better animal models are needed. Therefore, we obtained a SPEG conditional knockout mouse from KOMP (UC Davis) in which the physiological necessity as well as the mechanistic role of cardiac SPEG can be tested in the adult heart. We also developed a Tet-On SPEG overexpressing mouse, which can be used to assess the protective as well as therapeutic value of SPEG in the heart.

Contributors: Quick, Ann; Wang, Guoliang; Reynolds, Julia; Wehrens, Xander H.T
The malaria parasite Plasmodium falciparum causes the most deadly form of malaria in humans. According to the World Health Organization, an estimated 225 million cases of malaria infection were reported in 2009 with 781,000 resulting in death. Before the discovery of artemisinin, widespread use of the antimalarial chloroquine has lead to the emergence of drug resistant P. falciparum strains. In these resistant strains, one up-regulated and important, yet functionally unknown gene is the exported antigen 1 (Exp-1), a human antigen and vaccine candidate that is exported to the parasital vacuole membrane and food vacuole. Exp-1 is essential and may play an important role in malaria and in its human immune response. Here, we functionally predict and biochemically characterize Exp-1 as a glutathione S-transferase using a computational network method. This characterization puts Exp-1 as a direct molecular target of artemisinin as a critical enzyme in parasital response to oxidative stress.
Voltage-dependant Anion Channels (VDACs), also known as mitochondrial porins, are pore-forming ion-channel proteins located in the mitochondrial outer membrane (MOM). In addition to functioning as the main conduit for transporting metabolites between the cytoplasm and mitochondria, VDACs also form complexes with other proteins that localize to the MOM. We are interested in investigating the role of the VDAC isoform VDAC2 in apoptotic cell death, since we have previously shown that VDAC2 directly interacts with and anchors the pro-apoptotic protein BAK to the MOM. Previous reports have also indicated that, due to release of BAK from the MOM in the absence of VDAC2, Vdac2-/- Mouse Embryonic Fibroblasts (MEFs) demonstrate increased sensitivity to mitochondrial-mediated apoptosis. Here, we report that in the absence of VDAC2, in addition to BAK being reduced in mitochondria, BAK levels in the endoplasmic reticulum (ER) are significantly increased. We have further identified that upon prolonged treatment with ER stressors such as Thapsigargin, there is a significantly reduced rate of ER stress-induced apoptosis in Vdac2-/- MEFs. A double knockout of Vdac2/Bak in MEFs restores ER-stress induced rate of cell death to WT levels, demonstrating a dominant negative role for ER-localized BAK in the process. In mice, while Vdac2-/- whole-body knockout mice exhibit embryonic lethality and a heart-specific conditional knock-out develops a dilated cardiomyopathy, a double knockout of Bak and heart-specific Vdac2 rescues the cardiomyopathy phenotype. In order to identify the potential mechanism behind these phenotypes, we performed a microarray expression analysis between Wt and Vdac2-/- MEFs. We observed a consistent downregulation in the transcript levels of many key players in the ER stress response pathway. Follow-up studies with quantitative-PCR and Western blotting have confirmed this pattern. Single knockout of Bak or a double knockout of Vdac2/Bak increases expression of ER stress genes to levels comparable to WT MEFs. We propose that in the absence of VDAC2 there is loss of BAK in the MOM, an accumulation of BAK in the ER, interactions of ER-localized BAK with ER stress response proteins, resulting in blunted transcription of ER stress response genes and a reduced rate of ER-stress induced apoptosis.

Contributors: RAGHAVAN, ADITHYA; SHEIKO, TATIANA, CRAIGEN, J WILLIAM
A FORCE ASSAY TO MONITOR THE MOTILITY OF CANCER CELLS

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Cancer cells acquire motility by forming migratory structures (e.g., filopodia) via actin remodeling. These migratory structures produce forces to help breach the basement membrane and invade surrounding stroma. We are developing a force assay to determine the characteristics of the actin-based force at the leading edge of a filopodium of a cancer cell. Our goal is to elucidate the motility of cancer cells in vitro with this assay and measure the forces and their time course. We perform experiments with a primary/metastasis-derived head and neck squamous cell carcinoma (HNSCC) cell line pair, (HN30/HN31) that exhibits constitutively active Rho-GTPases that can sustain actin remodeling. HNSCC is the 6th most common malignancy with one of the lowest 5-year survival rates among aggressive cancers.

We have built an optical tweezers biosensor with a stiffness of 9.2×10-4 pNnm-1mW-1 and time constant of 23 s-1mW-1 and use it to initiate formation of a filopodium-like structure from a cell and monitor the force. To form the structure a cell is brought into contact with the trapped bead (500 nm/s) to facilitate membrane adhesion between the cell and bead. The cell is then slowly pulled away (50-60 pN/s) from the bead to rupture cytoskeletal-membrane bonds and form a membrane tube of sufficient lifetime to enable F-actin to grow within the tube. We readily form structures (~50%) from HN31 cells and can monitor the magnitude and time course of the force as the cytoskeletal-membrane bonds rupture and tube formation is initiated. We find the mean loading rate during bond rupture is 23 pN/s and the force decreases to 25 pN as the formed structure reached a stationary state. Riding upon the stationary value are force fluctuations which are insensitive to blebbistatin (5 µM) a myosin II inhibitor but are no longer observable after treatment with latrunculin A (1.67 µM) a drug which disrupts the actin cytoskeleton. The frequency of the force fluctuations increases and the lifetime of the stationary state decreases when calcium and magnesium are added to the external solution. We tentatively associate the fluctuations with F-actin dynamics occurring within the cytoplasm at the tip of the membrane tube. We will report the magnitude and time course of the dynamic and stationary forces and confirm whether F-actin has formed within the tube with fluorescence.

Contributors: Rajasekharan, Vivek; Sreenivasan, Varun; Yuan, Tao; Patel, Ameeta; Myers, Jeffrey; Pereira, Fred; Farrell, Brenda
Discerning the relationship between protein structure and function is one of the major goals of modern biology, and many methods have been devised to both predict and observe this relationship. We are using two methods to investigate the structure-function relationship of the E. coli enzyme orotidine-5'-monophosphate decarboxylase (ODCase): a computational method to predict the impact of specific mutations on protein function, and large-scale randomization to experimentally assay the functional effects of mutations.

ODCase is an essential enzyme in the pyrimidine biosynthesis pathway and is found in all domains of life; it catalyzes the conversion of orotidine-5'-monophosphate (OMP) into uridine-5'-monophosphate (UMP). It is one of the most proficient enzymes known, catalyzing the decarboxylation of OMP at a rate 1017-fold faster than the uncatalyzed reaction. This has made ODCase an attractive target for structure-function studies, and yet, despite catalytic residues and overall structure that are virtually invariant among species, its mechanism is still not fully understood.

We have used a computational method of predicting the functional effects of amino acid substitutions in ODCase and are currently undertaking an experimental method of investigating the real outcomes of the same mutations. We are designing mutagenic primers for each of the 245 codons in the pyrF gene (which encodes for E. coli ODCase), which replace the three nucleotides of each codon with NNS – N meaning A,T,G, or C; S meaning C or G. By performing site-directed mutagenesis with each of these NNS primer pairs, we will produce, for each codon, a library containing all possible permutations (all 20 amino acids have at least one possible codon represented by NNS). These libraries will then be transformed into an E. coli strain lacking pyrF, and selected on minimal media, which will only allow the growth of mutants that can synthesize UMP. These selected libraries will then be pooled and submitted for deep sequencing, allowing us to identify all single-residue substitutions that still maintain ODCase function.

For each position in the ODCase protein sequence, we will be able to determine the relative effect on ODCase function of all 20 possible amino acids using their relative frequencies in the pooled sequence data. This information will provide a strong test for the predictions made computationally, and will provide further insight to the mechanistic requirements of this highly proficient enzyme.

Contributors: Atri, Benu; Katsonis, Panagiotis; Palzkill, Timothy; Lichtarge, Olivier
Background: Although 75% of breast tumors are ERα-positive, there are no ER-positive preclinical models which reliably metastasize to study the effects of hormones on invasion and metastasis. We have studied the role of estrogen in metastasis in an ERα-positive MCF-7 sub-line (MCF-7 Met) which has spontaneously acquired the ability to metastasize in vivo.

Experimental design and methods: Microarray analysis was performed comparing MCF-7 Met to parental, non-metastatic MCF-7 cells. The differential gene expression was compared to genes differentially expressed when cells expressed K303R ER alpha. We performed soft agar growth assays, mammosphere assays, signal transduction assays and ERE luciferase assays. We injected MCF-7 Met and MCF-7 cells into athymic nude mice to study in vivo growth properties.

Results: We found that the MCF-7 Met line shared 66% gene expression similarity with cells expressing K303R ER alpha. We also found that upon hormone treatment the MCF-7 Met cells had higher levels of pS118 and pS305 ER alpha, which was observed in cells expressing K303R ER alpha. The K303R ER alpha model also had an increased in anchorage independent growth under estrogen treatment and we observed similar results in the MCF-7 met line. The MCF-7 Met line was able to form more spheres during a mammosphere assay when compared to MCF-7. In preliminary results we also observed estrogen hypersensitivity in the MCF-7 Met line during ERE luciferase assay, which is another phenotype that was observed in cells expressing the K303R ER alpha mutation. 94% of the mice injected with the MCF-7 Met line metastasized to distant sites regardless of hormonal treatment but their was no difference in primary tumor growth when treated with Tam or estrogen withdrawal.

Conclusion: I have determined that many of the genes differentially expressed in MCF-7 Met cells are related to estrogen signaling and tamoxifen resistance using bioinformatic approaches. This suggests that the processes of invasion and metastasis may be influenced by the hormonal milieu. I have also determined that the MCF-7 Met line may share a similar phenotype to cells expressing the K303R ER alpha mutation. Although MCF-7 Met cells remain hormone-dependent in vivo, they have acquired the ability to metastasize, potentially suggesting that hormone resistance is not a prerequisite for ER-positive breast cancer cells to metastasize to distant sites.

Contributors: Corona, Arnoldo; Covington, Kyle; Gu, Guowei
The Degree of Cerebrovascular Dysautoregulation During the 1st 3 Days in Premature Infants.

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Background
The premature infant is vulnerable to neurologic injury due to immature vascular development. Autoregulation is the phenomenon where blood flow to an organ is constant across blood pressure. Vascular reactivity mediates autoregulation. The hemoglobin volume index (HVx) is a near-infrared spectroscopy (NIRS)-based monitor of vascular reactivity. We monitored the HVx in the first 3 days of life in preterm infants and quantified the daily percentages of time with dysautoregulation. We hypothesized there would be greater dysautoregulation on day of life 1 than subsequent days.

Objective
To determine the percent of time premature neonates have cerebrovascular dysautoregulation.

Methods
Premature neonates were prospectively followed at a single, tertiary care Neonatal Intensive Care Unit. The HVx was continuously monitored as a moving, linear correlation coefficient between relative total hemoglobin (from reflectance NIRS) and arterial blood pressure. Dysautoregulation was defined as HVx > 0.3 and the percentage of time with HVx > 0.3 was quantified for each day (% time dysautoregulated).

Results
13 premature neonates (GA 27±1.8 weeks, BW 900±230 grams) were enrolled (mean,±sd). The % time dysautoregulated was 32.6[30.2-39.5] on day 1, 36.9%[19.4-41.7] on day 2 and 32.9%[23.2-42.4] on day 3 (median, IQR; p = 0.79 by Kruskal-Wallis ANOVA).

Conclusions
Preterm infants in this cohort spent roughly 30% of time with cerebrovascular dysautoregulation defined by HVx monitoring in the first 3 days of life. The % time dysautoregulated was not statistically significant across the first 3 days of life. This is the first report of the HVx in preterm population.

Contributors: Rhee, Christopher J.; Kibler, Kathleen K.; Easley, Ronald B.; Andropoulos, Dean B.; Kaiser, Jeffrey R.; Brady, Ken M.
Infertile Men with Non-Obstructive Azoospermia Exhibit Defects in the MSH5 DNA Repair Gene

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The most severe type of male infertility, non-obstructive azoospermia (NOA), results from spermatogenic failure, but the mechanisms involved are incompletely understood. Given that spermatogenesis requires both mitotic and meiotic cellular division, DNA repair systems (i.e. Mismatch Repair; MMR) are needed to maintain fidelity as well as facilitate in homologous recombination. Epigenetic modifications through DNA methylation can cause MMR deficiencies. Given that MSH5 is a key component of the MMR/homologous recombination pathway and that MSH5 deficient mice display sterility, we sought to determine a role for MSH5 in NOA men.

We examined the global DNA methylation status of NOA men (n=21; n=5 controls) using the Infinium HumanMethylation450 BeadChip to identify DNA methylation abnormalities. An additional 10 NOA and 9 controls were screened for aberrant DNA methylation using bisulfite clonal sequencing. We identified significantly aberrant DNA methylation within MSH5 in a cohort (6 out of 31) of NOA men. This cohort also exhibited downregulation of MSH5 gene expression and reduced MSH5 protein when measured by qPCR, Western blot and immunocytochemistry. Removal of the aberrant MSH5 hypermethylation using 5-aza-2-deoxycytidine resulted in an increase in MSH5 expression. In vitro manipulation of MSH5’s endogenous DNA methylation signature via site-specific RNAi directed DNA methylation in gonadal stromal cells resulted in a similar loss of MSH5 gene expression. This data suggests a deleterious role for the aberrant DNA methylation of MSH5 in our NOA patients. Patients with defects in MSH5 may exhibit decreased genetic stability and deficient DNA recombination leading to impaired spermatogenesis that contributes to the NOA phenotype.

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Contributors: Mukherjee, Sarmistha; Lamb, Dolores
THE ROLE OF VITAMIN D METABOLISM IN PROSTATE AND PROSTATE CANCER CELLS

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Epidemiological data suggests vitamin D may play a role in prostate cancer (PCa) prevention and data from our lab and others have demonstrated the active metabolite 1,25-dihydroxyvitamin D3 (1,25D) inhibits the growth of PCa both in vivo and in vitro. The main circulating form of vitamin D is 25-hydroxyvitamin D3 (25-OHD); it is converted to 1,25D, by 1α-hydroxylase (CYP27B1) in the kidney and other tissues like prostate. Normal prostate epithelial cells express CYP27B1 and are capable of converting 25-OHD into active 1,25D allowing pro-differentiating and growth regulatory functions to occur. Conversely, some PCa cell lines exhibit very low CYP27B1 activity resulting in low intracellular 1,25D synthesis insufficient to observe growth inhibitory effects in these cells. Preliminary data from our lab using RWPE-1 cells, an immortal non-transformed prostate cell line, confirms CYP27B1 expression and growth inhibition after 25-OHD treatment. In contrast, the VCaP PCa cell line expresses minimal CYP27B1 and is not growth inhibited after treatment with 25-OHD suggesting CYP27B1 is necessary for 25-OHD mediated growth inhibition. Loss of CYP27B1 expression has been suggested to be an early event in PCa progression that confers a growth advantage by reducing the ability of prostate cells to respond to circulating 25-OHD. This may also cooperate with the early formation of TMPRSS2:ETS fusions in PCa development. The enzyme 24-hydroxylase (CYP24) tightly regulates 1,25D by metabolizing it to a less active form. Our lab has shown ERG and 1,25D can cooperate to hyper-induce CYP24 in VCaP cells. Comparatively, VCaP cells, which contain the TMPRSS2:ERG fusion, express higher CYP24A1 levels than LNCaP PCa cells lacking this fusion. Preliminary data shows a time dependent decrease in 1,25D mediated gene induction in VCaP cells suggesting metabolism of 1,25D by CYP24. Clinical trials using 1,25D (calcitriol) have thus far been unsuccessful. Two reasons for this may be inactivation by CYP24 and hypercalcemia at high doses of calcitriol. We are testing non-secosteroidal VDR agonists, which should be resistant to metabolism by CYP24 and have a larger safety range for hypercalcemia than calcitriol. Early data shows these VDR agonists inhibit the growth of both LNCaP and VCaP PCa cells in vitro and are resistant to metabolism by CYP24. Future studies will include assessing the ability of these VDR agonists to inhibit tumor growth in vivo. The loss of CYP27B1 activity coupled with increased CYP24 expression provides a model of early escape from vitamin D regulation by developing PCa cells. We aim to gain insight into the roles of CYP27B1 and CYP24 in cancer progression and how sensitivity to vitamin D metabolites is altered through deregulation of these enzymes in PCa.

Contributors: Roberts, Justin; Kim, Jung-Sun
Cardiovascular diseases (CVD), mainly strokes and heart attacks, are the major cause of death in the United States. The underlying cause of most CVD is atherosclerosis, the accumulation of fatty deposits in plaques on the luminal side of the arterial wall. CVD events occur when large plaques or thrombi that form on so-called vulnerable plaques block blood flow. It has been hypothesized that plasma high density lipoprotein cholesterol (HDL)—the “good cholesterol,” prevents atherosclerosis. However, several studies have shed doubt on the HDL hypothesis. For example, ablation of the HDL receptor, SRBI, in mice increased HDL-C but produced more atherosclerosis; conversely over-expressing SRBI reduced plasma HDL concentrations and was atheroprotective.

Serum opacity factor (SOF), a protein produced by Streptococcus pyogenes, disrupts HDL structure yielding lipid-free apolipoprotein A-I, a small HDL-like particle called neo HDL, and a cholesteryl ester-rich micro emulsion (CERM). Neo HDL is more atheroprotective than HDL. Neo HDL is more efficient than HDL at removing cholesterol from macrophages, inhibiting macrophage inflammation, and the detoxification of HDL-cholesterol via its esterification. Thus, the chemistry and biology of neo HDL is likely relevant to its potential atheroprotective properties. However, SOF catalyzes the formation of neo HDL in vitro but not in vivo. We hypothesize that the stability of neo HDL vs. plasma factors underlies its rapid turnover in vivo. We have used chaotropic perturbation of NeoHDL with guanidine hydrochloride to quantify its stability vs. HDL and will identify the plasma factors that underlie the differential accumulation of neo HDL in vitro and in vivo.

Contributors: Rodriguez, Perla J.; Gillard, Baiba; Courtney, Harry S.; Rosales, Corina; Pownall, Henry J.
CONTRIBUTION OF EUKARYOTIC CHAPERONIN TRiC/CCT TO BIOGENESIS AND FOLDING OF A LEUKEMIC ONCOGENIC TRANSCRIPTION FACTOR, AML1-ETO.

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Acute Myeloid Leukemia (AML) is the most severe type with 40% frequency in total leukemia and characterized by non-random chromosomal translocation giving rise to oncogenic fusion genes. One of the most frequent oncogenic fusion protein is AML1-ETO (12%) resulting from the translocation at (8;21)(q22;q22). AML1-ETO is hard to be managed by conventional drug development strategies since it does not mediate its function by ligand-binding or enzyme activity, where are eligible drug-targeting sites by small molecules. In order to control this undruggable oncoprotein, a novel approach of altering the oncoprotein proteostasis network through type II eukaryotic chaperonin, TCP-1 Ring Complex (TRiC/CCT), has been suggested. Our study focuses on the functional relationship between TRiC and AML1-ETO and exploring the discrete interaction mechanism between oncoproteins and chaperonin. In this study, Co-IP results showed AML1-ETO is a TRiC client and its folding intermediate directly associates to TRiC through the DNA binding domain (AML_1~175). Chemical cross-linking and cryo-EM study also revealed that multiple TRiC subunits (CCT4, 6) bind cooperatively to AML1-ETO. More interestingly, total expression level of AML1-ETO is dramatically decreased in vitro translational system when endogenous TRiC was immune-depleted, which suggests not only TRiC is required for AML1-ETO’s biogenesis but also translational AML1-ETO-TRiC interaction is a potential drug target to control Acute Myeloid Leukemia.

Contributors: Soung-Hun, Roh; Tweardy, David J; Chiu, Wah
Retinitis pigmentosa (RP) is an inherited degenerative disease of the retina that results in rod and cone photoreceptor death. Initially as rods degenerate, subjects lose peripheral and night vision; this is followed by cone photoreceptor degeneration leaving the patients completely blind in some instances. RP affects 1 in every 4000 individuals worldwide and around 30-40% of these are the result of autosomal-dominant (ADRP) mutations. Rhodopsin (Rho) is a G protein-coupled receptor of the rods and it initiates the phototransduction cascade upon photon capture. Mutations in Rho, which account for about 25% of all ADRP cases, affect many processes, including Rho post-translational modifications, transport, folding and signaling capabilities.

More than 150 mutations that result in RP have been identified within Rho. Seven of these are nonsense mutations of which five cause dominant RP (dRP) and the two cause recessive RP (rRP). One possible explanation for this difference is that rRP mutants undergo nonsense-mediated mRNA decay (NMD) whereas dRP do not, thereby giving life to a toxic Rho truncation. The NMD pathway is the cell’s quality control mechanism for the detection and degradation of aberrant mRNA transcripts. If an mRNA molecule contains a premature termination codon (PTC), defined as a stop codon ~50nt upstream of an exon-exon junction, then degradation will ensue by NMD.

Our lab has found that the transcript of Rho-Q64X, which causes dRP in humans, is present at similar levels to wild type Rho in a mouse model. The same observation was made in a mouse model with a duplicated exon 2 that results in a PTC at the exon-exon junction. One study in cells detected degradation by NMD of the Rho Q249X nonsense mutant that causes rRP, but in vivo studies are absent. These observations raise the question of whether Rho is insensitive to NMD in photoreceptors. I am studying Rho nonsense transcripts and NMD in the context of the retina so as to have a better understanding of RP, its mechanisms and aid in the development of gene therapy strategies based on suppression and replacement of the mutant Rho.

Contributors: Roman-Sanchez, Ramon; Wilson, John H
Runx2 promotes osteosarcoma tumorigenesis and chemotherapeutic resistance

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Advisor: Lawrence Donehower, Ph.D.-Department of Molecular Virology & Microbiology

Osteosarcoma (OS) is the most common bone malignancy in the pediatric population, principally affecting adolescents or young adults. Although the overall survival rate has approached 70% in patients with localized disease, significant advancements in patient prognosis have been minimal over the past two decades due to a lack of understanding disease biology. Runx2, a critical transcription factor in bone maturation and development, is often amplified and overexpressed in osteosarcoma. However, the molecular and biological consequences of Runx2 over-expression remain unclear. Here, we show that compared to normal osteoblasts, Runx2 transcriptional activity is increased in mouse and human osteosarcoma cells, and knockdown of Runx2 results in reduced OS cell proliferation in vitro and tumor growth in vivo. Our functional data is corroborated by gene expression analysis, which shows Runx2 regulates multiple cellular pathways involved in cell cycle regulation, cell death, and proliferation in OS. Moreover, this analysis revealed that Runx2 regulates pathways involved in drug metabolism, and knockdown of Runx2 sensitizes cells to doxorubicin treatment. Finally, we provide evidence demonstrating a critical role for c-Myc in mediating the doxorubicin-induced apoptosis through the regulation of FADD and caspase-3. Thus, we have shown a novel chemoprotective role for Runx2 in OS, and propose inhibitors of Runx2 as therapeutic options for patients with OS.

Contributors: Roos, Alison; Johnson Kirsten; Donehower, Lawrence A; and Yustein, Jason T.
Efficient Apical Transduction of Polarized Human Airway Epithelium by Adenovirus Serotype 3

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Advisor: Philip Ng, Ph.D.-Department of Molecular & Human Genetics

Viral-based Cystic Fibrosis (CF) gene therapy has not, to date, been successful because the receptors for the most widely used vectors are not accessible via the apical surface of the airway epithelium. Indeed, the major receptor for adenovirus serotype 5 (Ad5), CAR (coxackie-adenovirus receptor), is sequestered to the basolateral surface by tight junctions. Ad5 can efficiently transduce the airway epithelium only if the tight junctions are transiently disrupted by agents such as LPC or EGTA. We have found that administration of these agents into nonhuman primate lungs cause temporary but significant increases in respiratory resistance and decreases in compliance measurements. Thus, opening tight junctions may be unsuitable for CF gene therapy considering the compromised pulmonary status of CF patients with advanced disease. Consequently, identification of vectors that transduce via an apical surface receptor is needed to obviate the need for opening tight junctions. Recently, it was reported that adenovirus serotype 3 (Ad3) could mediate apical infection of various polarized epithelial cells using Desmoglein-2 as its receptor. This observation suggested that Ad3 might be useful for apical infection of airway epithelial cells for CF gene therapy. To evaluate this potential, we compared the transduction efficiency of Ad5 versus Ad3 in polarized human bronchiolar epithelium (HBE). We found that Ad3 transduces polarized HBE at least 10-fold more efficiently than Ad5. Indeed, transduction of polarized HBE by Ad3 alone was comparable to transduction by Ad5 only if the Ad5 infection was preceded by opening tight junctions with EGTA. These results suggest that vectors based on Ad3 may be useful for CF gene therapy because high efficient transduction of the airway epithelium may be achieved without the need to open tight junctions. We are now constructing a Helper-Dependent Ad3 vector by creating a novel replication incompetent Ad3 helper virus.

Contributors: Rosewell Shaw, Amanda; Palmer, Donna; Grove, Nathan; Lieber, Andre; Ng, Philip
THE HUMAN VIROME

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There are an estimated 10^31 viral particles on Earth. Human stool contains more than 10^9 viral particles per gram, making them the most abundant and diverse entities found in/on the human body. The field of viral metagenomics has evolved rapidly over the past few years owing mainly to great breakthroughs in next generation sequencing. However, the composition, interindividual variation and temporal variability of viruses in humans have not been well studied. To help fill this dearth of virome data, we generated a dataset consisting of 16 subjects sampled at 3 time points, spanning 1 year.

Approximately 200 giga-bases of total sequence data were generated for these samples, and analysis of the entire dataset is just getting underway. The questions we intend to answer with this dataset include: What viruses are typically present in human feces? Do these change over time? Are there viruses that are always present? Are there viruses that many/most people have in common? How do phages present at a site correlate to the bacterial populations at that site? Can we gain insight into phage population dynamics through a study of CRISPRs? A study of CRISPRs will also shed light on phage/host specificity, as well as present a chronological view of phage/host exposure because phage sequences are added to the CRISPR array sequentially.

Contributors: Ross, Matthew; Wong, Matthew; Holder, Michael; Metcalf, Ginger; Gibbs, Richard; Petrosino, Joseph
MODELLING DUCT GROWTH AND TERMINAL END BUD (TEB) BEHAVIOR IN THE MOUSE MAMMARY GLAND

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The Terminal End Bud (TEB) at the growing tip of a mammary duct is one of the fastest growing structures in mammals. TEBs are transient structures that drive ductal elongation at the onset of puberty and regress once development of the ductal tree is completed. TEBs are highly ordered and are constrained to a characteristic geometric shape and size, implying a set a regulatory parameters within which the cells of the TEB must operate. Successful modeling of the growth of this structure with the ability to manipulate different parameters may yield information about uncontrolled growth. We are compiling measureable biological data (proliferation rate, cell cycle duration, migration, apoptosis, etc.) that will be used to create a mathematical model of TEB growth. Using thymidine analogs, EdU and BrdU, we dual labeled proliferating cells in the TEBs both at time zero (EdU), and at two hour intervals thereafter (BrdU) through 24 hours. Mammary glands from these mice were harvested and analyzed by immunofluorescence. We demonstrate that the TEB consists of populations of rapidly cycling cells (cell cycle ~16 hours). We also found that cells within different regions of the TEB follow different patterns of proliferation, indicating that the outer cap cell and myoepithelial layer growth rate is distinct from, but coordinated with, that of the underlying body cell and luminal layer. Perturbation of the parameters quantified outside their normal range will allow computational modeling of developmental defects and early-stage cancer.

Hypothesis: The parameters which control terminal end bud geometry are the very same as those exploited in tumor progression.

Contributors: Landua, John; Cauviere, Arnaud; Lewis, Michael
COLLAGEN BIOMARKERS IN PULMONARY ARTERIAL HYPERTENSION

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Clinical Scientist Training Program  
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Objective
The goal of this study was to determine if biomarkers of collagen metabolism in idiopathic, hereditary and anorexigen associated pulmonary arterial hypertension (PAH) identify patients with worse disease and higher risk of death.

Background
The relationship between markers of collagen metabolism, degree of disease, and outcome in PAH is unknown.

Methods
Stable idiopathic, anorexigen-associated and hereditary PAH patients were prospectively enrolled. Collagen biomarkers levels were measured: N-terminal propeptide of type III procollagen (PIIINP), C-terminal telopeptide of collagen type I (CITP), matrix metalloproteinsase 9 (MMP-9) and tissue inhibitor of metalloproteinase 1 (TIMP-1). Patients were divided into mild, moderate, and severe PAH groups. Data was compared between tertiles of each biomarker. Pearson correlation and Spearman rank coefficient analyses were performed. Data on time to death or transplantation was examined by Kaplan-Meier survival curves.

Results
Circulating levels of PIIINP, CITP, MMP9 and TIMP1 were higher in the PAH group as compared to age- and gender-matched normal controls (p<0.001 for each). PIIINP levels increased with the severity of disease (p=0.002), it was lowest in patients with mild PAH and highest in patients with severe PAH. PIIINP tertile data indicated that with increasing levels, six-minute walk distance (6MWD) and cardiac index (CI) decreased and WHO FC worsened, and resting heart rate increased. A significant correlation existed between PIIINP with worsening WHO FC (rs=0.319, p=0.008) and a negative correlation with CI and 6MWD (r=-0.304 and -0.361 respectively; p<0.05). PIIINP tertiles showed a trend towards worse outcome in patients with higher tertile (lung transplant or death) (p=0.07, log rank test).

Conclusions
Markers of collagen metabolism were associated with disease severity in PAH patients.

Contributors: Safdar, Zeenat; Frost, Adaani; Deswal, Anita; Bozkurt, Biykem; Entman, Mark
Evaluate the fidelity of piggyBac Transposon based gene delivery system in human cells.

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Cliona Rooney, Ph.D.-Department of Pediatrics

PiggyBac has been successfully used for modification of primary human cells and cell lines with transgene(s) of interest and holds promise as an effective non-viral gene delivery method. Currently, limited information exists about the safety of the piggyBac system for the modification and generation of clinical grade human cells. In this study we began to evaluate the safety of piggyBac for modification of human cells. PiggyBac works through a “cut and paste” mechanism thereby delivering a transgene of interest flanked by inverted repeat sequences into the genome. We found that piggyBac leads to stable transgene integration and transposase expression is undetectable after 7 days in a mixed population of human cells. Although there are no sequences in the human genome with complete similarity to the 17bp terminal repeat sequence (TRS) of the piggyBac transposon inverted repeats (IR), there are sequences with 16, 15 or 14 bp similarities all of which end in the tetranucleotide TTAA required for transposition. We subsequently tested the ability of piggyBac to mobilize these genomic elements. The potential of these sequences mimicking the TRS was assessed by replacing the TRS of the 5’Inverted repeat with these genomic sequences and looking at transposition efficiency using a colony count assay. None of the 14 tested sequences were able to effectively act as a terminal repeat sequence. Nor did they mediate excision of transposons in presence of the transposase. To assess the safety of delivering the transposase and transposon from a single vector, we isolated clones derived from transfections using the transposase and the transposon cassettes in cis (on the same plasmid vector) and found that all clones had residual transposase expression. In contrast, stable clones generated with transposase delivered in trans from a separate non-integrating plasmid showed no residual transposase expression. Studies are ongoing to further evaluate the potential genotoxicity of piggyBac in human cells. Thus, our data suggests that piggyBac transposase expression is short lived (7 days) in a mixed population of human cells, the piggyBac transposase appears incapable of mobilizing TRS-like-sequences in the human genome, and delivering transposase in trans should be safer than delivery in cis when modifying human cells. Human primary cells modified with transposon alone do not undergo clonal expansion and there is no significant increase in DNA double strand breaks in presence of the transposase. The piggyBac system can be optimized to reduce backbone integration in order to improve safety. Thus, piggyBac appears to be a promising and potentially safe non-viral gene delivery system for therapeutic genetic modification of human cells. piggyBac appears to be a promising non-viral gene delivery system for therapeutic genetic modification of human cells.

Contributors: Saha, S; Kaja, A; Rooney, CM; Wilson, MH
Functional Analysis of Genomic Variants Identified through Whole Exome Sequencing of Pediatric Lymphocytic Leukemia Kindreds

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The underlying genetic basis of many childhood cancers remains largely unknown, which places a significant focus on the discovery and understanding of cancer susceptibility genes. Our research focuses on the identification of novel high risk cancer susceptibility genes through the use of next generation sequencing methods, bioinformatics tools, and functional assays. We are analyzing a small cohort of ethnically diverse families with at least two first-degree relatives with childhood ALL or lymphoma transmitting in an autosomal dominant manner. Whole exome sequencing was performed on affected individuals’ normal lymphoblasts and tumor samples when available. In one particular Hispanic kindred, sequencing generated a list of over 1500 heterozygous missense variants shared among three affected individuals. Filtering out common variants found in databases such as dbSNP, reduced this list by 95% and filtering variants seen in a whole exome sequenced intrafamilial control narrowed down the list to 33 potential rare heterozygous cancer susceptibility variants. In order to select which rare variants to analyze by functional methods, various bioinformatics tools were utilized. Variants leading to the change of a conserved amino acid were given a higher priority. In addition, variants had to be predicted to be damaging or deleterious to protein structure or function by 3 out of 4 algorithms. These bioinformatics analyses left us with 8 candidate variant genes (PIK3R4, SUPT3H, NT5C1A, TRPM4, PRKAG3, C6orf136, GLT25D1, and LPIN2). All of these rare variants were retained in tumors from affected family members, but there was no evidence for second hits. RNA-seq data assessing expression in hematopoietic cell populations from murine bone marrow has been a valuable tool to select genes from this variant list for further analysis. Variants that are potential inactivating mutations in genes robustly expressed in murine bone marrow, such as PIK3R4, are prioritized for knock down in murine bone marrow transplantation studies. We are currently developing a novel murine model in which we harvest the bone marrow from 5-FU treated CD45.2 mice. Harvested bone marrow is then transduced with murine stem cell virus (MSCV) retroviral vectors expressing GFP and miRNAi to target the knock down of our gene of interest (GOI). The transduced bone marrow is then transplanted into CD45.1 recipient mice that have had their bone marrow ablated by radiation. These mice are then observed for signs of leukemia and their blood is collected at different time points to detect any alterations in hematopoiesis caused by the knockdown of the GOI. Alternatively, if an activating mutation is predicted of a candidate gene, we can use the MSCV retroviral system to overexpress the mutant cDNA in donor bone marrow transplanted into a recipient mouse. Within our larger cohort of ALL kindreds, none of these 8 variants are present, which demonstrates genetic heterogeneity of familial childhood leukemia.

Contributors: Saliba, Jason; Zabriskie, Ryan; Powell, Bradford; Hicks, Stephanie; Kimmel, Marek; Cheung, Hannah; Ritter, Deborah; Muzny, Donna M; Reid, Jeffrey G; Wheeler, David A; Gibbs, Richard A; Plon, Sharon E
RECRUITMENT OF CD34 POSITIVE FIBROBLASTS IN TUMOR ASSOCIATED REACTIVE STROMA: THE REACTIVE MICROVASCULATURE HYPOTHESIS

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Reactive stroma co-evolves in a focally heterogeneous pattern at sites of cancer initiation and during progression to malignant disease. Although the tumor-promoting role of reactive stroma has been studied in many model systems, its specific nature and cell types of origin are poorly understood. To better understand the tumor microenvironment, several human tumor tissue arrays were evaluated for common markers. We report here a novel CD34 and vimentin dual positive fibroblast-like cell type that was commonly observed in the microenvironment of breast, colon, lung, pancreas, thyroid prostate and astrocytoma. Recruitment of this cell type was observed in non-cancer sites of age-associated disruption of the epithelial layer in a TGF-β transgenic mouse model and in human benign prostatic hyperplasia. Both xenograft tumors and Matrigel plug models in vivo also exhibited early recruitment of this cell type in the provisional reactive stroma. Morphological, cell marker, and in vivo recruitment studies suggest this cell type is derived from the wall of the microvasculature adjacent to regions of epithelial layer disruption or wounding. Moreover, explants of isolated murine microvasculature produced these cells, which rapidly proliferated and migrated from the vessel wall. Accordingly, we propose a “reactive microvasculature” theory for the evolution of early reactive stroma at sites of epithelial disruption common in both benign and malignant disorders.

Contributors: San Martin, Rebeca; Ressler, Steven; Rowley, David
Gene regulation and transcriptional control are essential for cells to perform a variety of functions including cell division, response to signaling pathways, cell differentiation and development. Interactions between transcription factors (TFs) and their DNA binding sites are necessary to achieve transcriptional control. D. discoideum has about 200 predicted TFs, however, their binding site preferences and their downstream target genes, both direct and indirect, are not well understood. D. discoideum cells lacking gtaC, one of 25 belonging to the GATA family of transcription factors, are defective in aggregation and do not form any multicellular structures. Using RNA-seq, we are trying to understand the effects of the mutation (gtaC–) at the mRNA level. In order to understand the genome-wide binding preferences of GtaC and to identify its direct targets, we performed ChIP-seq. We are trying to identify specific DNA motifs that are recognized and bound by GtaC by analyzing the promoter regions that show enriched binding. We are in the process of developing novel data fusion methodologies to integrate gene expression data from RNA-seq experiments, TF binding preferences from ChIP-seq experiments and DNA binding motifs from promoter analyses to identify key components of this transcriptional network in Dictyostelium.

Contributors: Katoh-Kurasawa, Mariko; Cai, Huaquing; Rot, Gregor; Zupan, Blaz; Devreotes, Peter; Kuspa, Adam; Shaulsky Gad
A GFP-BASED ASSAY FOR CAG REPEAT INSTABILITY

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Advisor: John Wilson, Ph.D.-Department of Biochemistry & Molecular Biology

Myotonic dystrophy, Huntington disease, and several spinocerebellar ataxias are members of a group of disorders that correlate with CAG trinucleotide repeats (TNRs) that increase in length (expand) in specific genes. Despite their long-term study and their severe effects on patients, these diseases still lack effective treatment strategies. Our lab is trying to define the mechanisms that underlie CAG repeat instability, with the ultimate therapeutic goal of devising ways to prevent expansion or promote contraction.

In order to efficiently search for modifiers of instability, we have generated a human cell assay carrying an unstable CAG87 repeat within a GFP reporter. A large contraction within the CAG tract will yield a functional GFP transcript, giving rise to a green cell detectable by flow cytometry. Through cell sorting experiments we have shown that the intensity of green fluorescence is dependent on the size of the repeat tract. This allows us to detect a range of contraction sizes and therefore instability events. Furthermore, the ability to sort and subsequently isolate cells enables us to rapidly characterize the entire spectrum of length changes at the TNR locus, including expansions and contractions. Finally the frequency of events is high enough that the system is amenable to large-scale screens.

This system gives us the ability to uncover the major genetic and environmental effectors of TNR instability using small interfering RNAs or drug treatments.

Contributors: Lin, Yunfu; Wilson, John H
Transmission of cellular identity relies on the faithful transfer of information from the mother to the daughter cell. This process includes accurate replication of the DNA, but also the correct propagation of regulatory programs responsible for cellular identity. Errors in DNA replication (mutations) and protein conformation (prions) can trigger stable phenotypic changes and cause human disease, yet the ability of transient transcriptional errors to produce heritable phenotypic change (‘epimutations’) remains an open question. Here, we demonstrate that transcriptional errors made specifically in the mRNA encoding a transcription factor can promote heritable phenotypic change by reprogramming a transcriptional network, without altering DNA. We have harnessed the classical bistable switch in the lac operon, a memory-module, to capture the consequences of transient transcription errors in living Escherichia coli cells. We engineered an error-prone transcription sequence (A9 run) in the gene encoding the lac repressor and show that this ‘slippery’ sequence directly increases epigenetic switching, not mutation in the cell population. Therefore, one altered transcript within a multi-generational series of many error-free transcripts can cause long-term phenotypic consequences. Thus, like DNA mutations, transcriptional epimutations can instigate heritable changes that increase phenotypic diversity, which drives both evolution and disease.

Contributors: Satory, Dominik; Gordon, Alasdair; Halliday, Jennifer; Herman, Christophe
Osteosarcoma (OS) and Ewing’s sarcoma (EWS) are the two most common bone tumors in the adolescent and young adult population. Pediatric sarcomas are extremely morbid diseases with high mortality rates. Improvements in treatment regimens have been stagnant over the past 20-30 years most likely due to our lack of understanding their molecular pathogenesis. Aberrant expression of miRNA and their functional consequences have not been well studied in these very aggressive malignancies.

In order to identify novel alterations in miRNAs, we have utilized our analysis of microRNA expression from localized and metastatic tumors derived from novel genetically engineered mouse models of osteosarcoma. Among the miRNAs most significantly dysregulated, miR-130b was shown to be significantly upregulated in the metastatic lesions. Furthermore, it has been shown that miR-130b is overexpressed in OS and EWS patient samples, and this event is significantly associated with poor patient outcome in the latter sarcoma. Furthermore, we have verified miR-130b overexpression in both mouse and human sarcoma cell lines and in metastatic EWS tumor samples obtained from Texas Children’s Hospital. Functional analysis shows overexpression of miR-130b increases migration and invasion in vitro.

In order to identify novel targets of miR-130b contributing to metastasis, we performed microarray and GeneGo pathway analysis. We identified Cdc42GAP as a novel target through qRT-PCR and 3’UTR targeting. Cdc42GAP is a negative regulator of Cdc42 activity, which induces filopodia formation during cell migration. ELISA analysis of Cdc42 activity reveals that Cdc42 activity is increased with overexpression of miR-130b. Taken together, these findings suggest that overexpression of miR-130b promotes sarcoma cell migration and invasion through targeting of Cdc42GAP and increased Cdc42 activity.

Future experiments include in vivo studies of alterations in mir-130b in OS and EWS as well as targeting the CDC42 pathway. Finally, we intend to expand our analysis of mir-130b targets through comprehensive proteomic investigations.

Contributors: Satterfield, Laura; Kurenbekvoa, Lyazat; Donehower, Lawrence; Yustein, Jason
MULTISENSORY INTEGRATION REDUCES BIASES IN HEADING PERCEPTION CAUSED BY MOVING OBJECTS

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Accurate judgment of self-motion is critical for navigation. Cues from the visual system, such as optic flow, play an important role in making judgments of heading. However, estimates of heading from visual image motion can be confounded by objects moving in the world, since moving objects alter the pattern of optic flow due to self-motion. While previous studies have examined the contributions of visual cues to recovering heading information, the role of the vestibular system in judging heading in the presence of moving objects remains poorly understood. Here we examined whether biases in heading perception induced by moving objects are reduced when self-motion is indicated by combinations of visual and vestibular cues.

Rhesus monkeys were trained to perform a heading discrimination task in which they reported whether their heading was leftward or rightward relative to straight ahead. Animals performed the heading discrimination task while a virtual object moved leftward or rightward across the visual field within the fronto-parallel plane, thus altering the optic flow field. We used both a simple object (a single sphere) and a complex object (9 cylinders in a 3x3 grid) that covered a substantially larger portion of the visual field. The experiment included three self-motion conditions, in which heading was indicated by optic flow alone, whole-body translation by a motion platform (thus providing vestibular cues), or a congruent combination of these visual and vestibular cues.

To quantify the accuracy of heading judgments, we computed the point of subjective equality (or bias) from psychometric functions. Both simple and complex objects significantly biased heading perception. Rightward motion of the simple object caused a significant leftward bias in the visual self-motion condition (6.7 ± 1.1°), as well as a significant bias in the vestibular self-motion condition (6.2 ± 1.5°). However, the bias in the combined condition was significantly reduced relative to the single cue conditions (3.2 ± 1.3°). With leftward object motion we observed similar biases but in the opposite direction. With the complex object, biases were much greater in the visual and vestibular conditions, as expected since the complex object stimulates a much greater portion of the visual field. Importantly, bias was dramatically decreased in the combined condition. Thus, in the presence of moving objects, macaques cannot accurately recover their heading from visual or vestibular cues alone, but combining the two cues markedly reduces the biases induced by object motion.

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THE ROLE OF NOTCH SIGNALING IN THE REGULATION OF NEURAL PROGENITOR CELLS IN THE ADULT HIPPOCAMPUS

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The unique capacity of adult hippocampus to generate new neurons throughout life is of great scientific and medical interest, as adult neurogenesis has been linked to learning and memory and a variety of neuropsychiatric disorders, including depression, epilepsy, and schizophrenia. However, the population of quiescent neural progenitors (QNPs), the main source of the new neurons, is restricted, which ultimately limits the total number of new neurons produced. Thus, investigations of the mechanisms that determine the proliferation capacity of QNPs and differentiation of secondary progenitors, amplifying neuroprogenitors (ANPs) are significant, as increasing their ability to produce newborn cells may have considerable implications for our ability to treat age-related disorders. Interestingly, both proliferation and asymmetric division of QNPs have been independently shown to be regulated by Notch signaling, but the role of Notch in controlling the ultimate fate of QNPs is unknown. We hypothesized that Notch signaling is necessary for the interaction of QNPs and ANPs, ultimately influencing the net contribution of QNPs to neurogenesis. To test this hypothesis, we first developed a reporter mouse line lunatic fringe (Lfng)-eGFP, which showed that Lfng is specifically expressed in QNPs. Lfng is one of the fringe modifiers of Notch and shifts Notch preference from Jag ligand to Dll ligand. Both Jag (on differentiating neuroblasts) and Dll ligands (on granule cells) are expressed in the dentate gyrus in the neurogenic niche. We further characterized the Lfng-eGFP mouse and determined that Lfng-eGFP positive cells gave progeny that followed the established neurogenic cascade, formed expected neural lineages in vitro, and responded to the electrical stimuli known to increase proliferation. In Lfng knockout mice, the number of QNPs and neuroblasts decreased significantly, strongly suggesting the functional role of Lfng in control of QNP behavior. However in Jag1 knockout mice, although the number of proliferating cells in the dentate gyrus and the ratio of proliferating cells increased as much as Lfng knockout mice, QNP numbers didn’t change significantly suggesting a possible interaction between the QNP and its progeny. These results solidified the role of Notch signaling in the control of QNP behavior and also presented Lfng-eGFP mouse as a new reporter mouse model for studies of primary adult stem cells in the hippocampus, QNPs. Further investigations of the Lfng role in adult neurogenesis will lead to better understanding of the regulation of stem cell proliferation by its progeny both in health and disease.

Contributors: Choi, William Tin-Shing; Thakkar, Aarohi A
Alzheimer’s disease (AD) is a neurodegenerative disease caused by accumulation of extracellular Amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles of microtubule associated protein Tau as well as synaptic loss in the brains of patients. Genome-wide-association studies followed by validation experiments in flies indicate that ten AD susceptibility loci modulate Tau toxicity in the fly eye neurons. Three out of the ten loci are known components of the integrin pathway that regulate the subcortical actin cytoskeleton and possibly mitochondrial function. Interestingly, overexpression of Tau and Aβ has been shown to affect F-actin and mitochondrial dynamics. In addition, the actin cytoskeleton is known to function in the local transport, distribution, and function of mitochondria. To assess if overexpression of Tau and/or Aβ causes a demise of neuronal function in Drosophila adult neurons, we overexpressed them in fully differentiated adult photoreceptors and assessed their function using electroretinogram recordings. We found that overexpression of Tau causes a neuronal dysfunction upon aging and that this dysfunction is enhanced by Aβ co-overexpression. In addition, we show that overexpression of Tau or Aβ results in mitochondrial ETC dysfunctions in aged flies. Even though integrins function in neuronal development, their role in the aging nervous system is not defined. Given the link between AD and the integrin pathway, we are testing if integrin signaling is required for the maintenance of adult neurons. Secondly, as integrins and Tau affect F-actin and mitochondria, we are trying to establish how they interact and affect mitochondrial dynamics. Finally, we have identified potential new players in the integrin pathway through a forward genetic screen, and we will explore the function of these new regulators and their potential roles in Tau-induced phenotypes.

Contributors: Shulman, Joshua; Bellen, Hugo
CHOLESTEROL HOMEOSTASIS IS REQUIRED TO MAINTAIN COCHLEAR PERIVASCULAR INTEGRITY AND AUDITORY FUNCTION

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Animal models and human studies support a link between altered cholesterol homeostasis and sensorineural hearing loss, implicating pathologies that affect the cochlea, auditory nerve and/or central auditory pathways. However, knowledge of the relative quantities and species of lipids in the cochlea, as well as how circulating lipids modulate cochlear cellular activities, is currently lacking. The rising prevalence of obesity and hypercholesterolemia demands a better understanding of how systemic cholesterol homeostasis influences cochlear cholesterol homeostasis and hearing function.

We hypothesize that cholesterol homeostasis is essential for hearing function and maintenance, and that changes in cholesterol levels lead to decreased cochlear and hearing function.

We have demonstrated that cochlear levels of total cholesterol and serum lipid panels change significantly during normal maturation of cochlear and auditory function. Interestingly, we have shown that the cochlear sensory epithelium (SE) contains higher levels of cholesterol than the vascular compartment, suggesting the possibility of endogenous synthesis or a mechanism for concentrating cholesterol in the SE. Current studies focus on how systemic hypercholesterolemia affects the hearing system by using the Low-Density Lipoprotein Receptor (LDLR) KO and Apolipoprotein E (Apo-E) KO mouse models. LDLR KO and Apo-E KO mice both display elevated hearing thresholds, indicative of hearing loss, at multiple frequencies. In addition, LDLR KO mice have altered organization of the vasculature within the stria vascularis, the metabolic engine of the cochlea. We are currently exploring how these changes develop with age in wild-type and hypercholesterolemic mice, as well as the contribution of perivascular-resident macrophage-like melanocytes (PVM/Ms) to maintenance of the structural integrity of the blood-labyrinth-barrier (BLB) in the stria vascularis capillaries.

Contributors: Seymour, Michelle L.; Pereira, Fred A.
Androgen ablation therapy is the most common treatment for advanced prostate cancer, but most patients will develop castration-resistant prostate cancer (CRPC), which has no cure. CRPC is androgen-depletion resistant but androgen receptor (AR) dependent. AR is a nuclear receptor whose transcriptional activity is regulated by hormone binding to the ligand-binding domain (LBD). Constitutively active AR splice variants that lack LBDs often are expressed in CRPC. Current therapies involve targeting the hormone-binding domain, which would be ineffective at targeting the variants. The goal of this study was to examine whether AR splice variants require the same components as full-length AR to properly function. The components studied here include chaperone proteins (i.e. Hsp90, Hsp70, and FKBP52) crucial for proper folding. We generated LNCaP cell lines with regulated expression of the AR-V7 variant to characterize splice variant function. Using an Hsp90 inhibitor, Geldanamycin (GA), an Hsp70 inhibitor, YM1, and an AR-Hsp90-FKBP52 specific inhibitor, MJC13, we discovered that AR splice variants are resistant to such treatments. GA and YM1 inhibited AR transcriptional activity and expression but had minimal effect on V7. Furthermore, full-length AR activity was strongly inhibited by MJC13 while V7 was unaffected. Thus, the variants were resistant to inhibitors of the chaperone-AR heterocomplex. Although chaperone inhibitors will continue to inhibit signaling through activated AR in CRPC, signaling through variants will be retained.
C. ELEGANS CLATHRIN AND ITS ADAPTOR EPSIN PROMOTE APOPTOTIC CELL ENGULFMENT THROUGH REGULATING CYTOSKELETON REMODELING

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The engulfment and subsequent degradation of apoptotic cells by phagocytes is an evolutionarily conserved process that efficiently removes dying cells from animal bodies during development. Here we reported that clathrin heavy chain (CHC-1), key component of a vesicle coating protein clathrin, and its adaptor protein epsin (EPN-1), play crucial roles in removing apoptotic cells in C. elegans. Clathrin is a coat protein well known for its function in receptor-mediated endocytosis, but unknown for acting in phagocytosis, neither are epsins or other clathrin adaptors. Our study has identified the novel roles of clathrin and epsin in phagocytosis. Inactivating epn-1 or chc-1 specifically reduces the speed of engulfment through impairing actin polymerization, the driving force for engulfment. Clathrin-actin crosstalk not only induces membrane curvature, but also directs actin polymerization and drives pseudopod extension around apoptotic cells. Epistasis analysis places epn-1 and chc-1 in the same genetic pathway as ced-1, ced-6, ced-7, and dyn-1 for cell-corps engulfment. The CED-1 signaling pathway is necessary for the pseudopod enrichment of EPN-1 and CHC-1. As a result, CED-1, CED-6, and DYN-1, like EPN-1 and CHC-1, are essential for the assembly and stable maintenance of actin fibers along pseudopods, indicating that in addition to driving 'focal exocytosis' for membrane expansion, the CED-1 signaling pathway also regulates the remodeling of the cytoskeleton for cell-corps engulfment. Our work identified a novel mechanism employed by clathrin and its adaptor to promote pseudopod extension and the engulfment of apoptotic cells, and ties the CED-1 signaling pathway to the actin cytoskeleton.

Contributors: Shen, Qian; He, Bin; Lu, Nan; Conradt, Barbara; Grant, Barth D. and Zhou, Zheng
Sensation is a process composed of representation and interpretation. It is supposed that feed-forward projections are involved in the representation of the external stimuli, while the feedback projections may play a role in the selection and interpretation of the sensory information. However, it is largely unknown that within the sensory cortices how the feedback projections affect the information processing of the sensory systems, and how the neurons can integrate the feed-forward and feedback inputs.

Mice are currently more and more popularly used as an animal model to study visual processing, largely because of the diverse and powerful genetic tools. Corticocortical connection between primary visual cortex (V1) and extrastriate areas (simply referred to as V2) is a good model system to study the feed-forward feedback integrations within sensory systems. In order to figure out the role of feedback projections from V2 to V1, we injected AAV ChR2 into lateral V2 areas and record from different cell types in V1 areas with electrophysiology. We found that the PV positive cells receive strong excitatory inputs from lateral V2, while pyramidal cells receive strong inhibitory inputs, but weaker excitatory inputs. Our results indicate that the functional role of feedback is mainly inhibitory and the inhibitory effect may be because of the activation of interneurons like PV positive cells.
Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy in children, among which T-cell ALL (T-ALL) exhibits worst prognosis. Studies on genetic factors supporting T-ALL leukemogenesis will contribute to the development of alternative therapies. Previously our laboratory described that the Krüppel like factor 4 (KLF4) restricts proliferation in normal CD8+ T cells. In addition, we have found that loss of KLF4 increases self-renewal and enhances survival of normal hematopoietic stem cells, which is a feature that cancer cells acquire during transformation. There are emerging evidences showing reduced levels of KLF4 in bone marrow of T-ALL patients compared to healthy individuals and a correlation of KLF4 expression with response of ALL patients to standard treatment. Collectively, these findings suggest a potential role of KLF4 in tumor suppression and drug resistance in hematological malignancies. To study whether KLF4 functions as a tumor suppressor in T-ALL, we used Klf4 conditional knockout mice and NOTCH1-induced T-ALL model. Bone marrow (BM) cells from control (Klf4fl/fl) and Klf4 deleted (Klf4fl/fl; Vav-iCre) mice injected with 5-FU were transduced with retrovirus carrying the NOTCH1-L1601P-P mutant found in human T-ALL patients and then transplanted into irradiated recipient mice. Mice transplanted with transduced control BM cells showed lower incidence of leukemia (21.7%, n=23) with longer median latency (130 days). In contrast, mice transplanted with transduced Klf4-deleted BM cells showed significantly higher penetrance (76.5%, n=34) with shorter median latency (93 days, p<0.01). Moribund mice from both groups showed similar infiltration of leukemic cells in liver, kidney, testis, brain and spleen. Immunoblots of leukemic cells purified from BM of diseased mice showed increased levels of Cyclin D1 and phospho-Rb in Klf4-deleted cells, suggesting enhanced proliferation. In addition to increased proliferative activity, a global gene expression analysis revealed significant differences in metabolic pathways. Concomitantly, Klf4-deleted leukemic cells displayed increased levels of phosphorylated mTOR, an important signaling involved in the metabolic reprogramming of cancer cells. Collectively, our study shows that KLF4 has tumor suppressor function in a mouse model of NOTCH1-induced T-ALL, providing a novel molecular target to prevent expansion of pre-leukemic cells.
INTEGRATED ANALYSIS IDENTIFYING TRANSCRIPTION FACTORS CRITICAL FOR BASAL-LIKE BREAST CANCER

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Intrinsic differences in gene expression between basal-like breast cancer (BLBC) and other breast cancer subtypes imply that transcriptional regulators are differentially activated in breast cancer subtypes and may be promising therapeutic targets. We hypothesized that genomic comparisons between BLBC and non-BLBC will identify transcription factors (TFs) critical for BLBC growth. We identified TFs using an integrative analysis comparing mRNA expression, frequency of TF response elements in differentially expressed genes, and DNA-binding activity of nuclear proteins in BLBC and non-BLBC. We then tested whether inhibition of these specific TFs suppresses the growth of BLBC.

Analysis of mRNA expression identified 132 TFs significantly more highly expressed in TNBC tumors compared to non-TNBC samples across 15 breast tumor datasets. Examining promoter sequences of 117 genes significantly more highly expressed in BLBC tumors compared to non-BLBC samples (p<0.01 in 3 independent datasets), we identified 94 TF motifs over-represented among BLBC promoters versus ~1500 non-BLBC gene promoters (Fisher's exact test p-value <0.05). Binding of DNA motifs by nuclear protein was measured by protein/DNA Arrays (Affymetrix, Santa Clara, CA) using nuclear protein collected from BLBC (BT20, HCC1143, HCC1937, MDA468) and non-BLBC cells (BT474, MCF7, T47D, ZR75-1). This analysis identified 11/365 TF motifs which were more highly bound by BLBC nuclear lysate with a fold change of >1.2 and p <0.05.

To integrate the results of the three individual screens, we are taking the set of TFs identified by at least 2/3 assays. 2-dimensional growth of BLBC and non-BLBC cell lines transfected with siRNA to each candidate TF or non-targeting control siRNA has demonstrated that several of the TFs are critical for BLBC growth. Further investigation of the TFs identified in this study will improve our understanding BLBC and will identify novel targets for the treatment and prevention of BLBC.

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A resource view on visual short-term memory for multi-feature objects

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Studies of visual short-term memory (VSTM) for multi-feature objects have typically asked three questions: 1) Do observers remember integrated objects or individual features? 2) Can observers remember the same number of features regardless of how many objects they belong to? 3) Do salient irrelevant features affect performance? These questions are often studied in the framework that VSTM has a discrete, limited capacity. In an alternative view, VSTM resource is a continuous quantity; the more resource a feature receives, the higher its precision. Within this new framework, we reformulate the above questions as: 1) Is resource shared between features? 2) Are the resources for different features independently allocable to objects? 3) Can salient irrelevant features be ignored during decision-making?

We used the same change localization paradigm to answer all three questions. In Experiment 1, human subjects briefly viewed four ellipses with random orientations and colors. After a 1-second delay, a second display containing four ellipses appeared, exactly one of which had changed. In the one-relevant-feature condition, the change occurred always in the same feature – either orientation or color. In the two-feature condition, the change occurred randomly in either feature. Observers reported the location of the change.

We found that psychometric curves were identical between both conditions, suggesting that VSTM resource is not shared by features. To rule out that resource is shared by features but the irrelevant feature automatically takes up resource, we compared the one-relevant-feature condition with a truly-one-feature condition that used colored discs or grey ellipses. Performance was not affected by the presence of the irrelevant feature.

To address the second question, in Expt. 2, we compared the two-feature condition from Expt. 1 to a condition in which the features of each item are spread out over two objects (producing 8 single-feature objects) and to the two-feature condition with set size 8. We found that performance in the ‘spread-out’ condition lied in between performance in both two-feature conditions, suggesting that some but not all feature resources are allocated together.

Finally, we compared the one-relevant-feature condition from Expt. 1 to a condition in which all objects change in the irrelevant feature and only one changes in the relevant feature. Performance was identical between both conditions, suggesting that observers can ignore changes in the irrelevant feature.

We conclude that there exist independent pools of VSTM resource for orientation and color, but they are distributed over objects in a partially dependent manner.

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ARYL HYDROCARBON RECEPTOR DYSFUNCTIONAL NEWBORN MICE ARE MORE SUSCEPTIBLE TO HYPEROXIA-INDUCED DELAYED ALVEOLARIZATION

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Background: Hyperoxia causes bronchopulmonary dysplasia (BPD) in preterm infants. We showed that adult mice deficient in aryl hydrocarbon receptor (AhR) are more susceptible to hyperoxic lung injury than wild type controls due to marked decreases in the expression of cytochrome P450 (CYP) 1A enzymes that appear to detoxify lipid hydroperoxides generated by reactive oxygen species (ROS). Whether AhR protects newborn mice against hyperoxia-induced developmental lung injury is unknown.

Objective: To test the hypothesis that upon exposure to hyperoxia, aryl hydrocarbon receptor dysfunctional newborn mice will display increased inflammation and decreased alveolarization compared to newborn wild type mice.

Methods: One-day old wild type C57BL/6J (WT) and aryl hydrocarbon receptor dysfunctional (AhRd) mice were exposed to room air or hyperoxia (FiO2 > 95%) for 14 days. Following exposure to room air or hyperoxia, the lungs of the mice were harvested to determine AhR activation, inflammation, and alveolarization. Pulmonary AhR activation was assessed by analyzing the expression of pulmonary CYP 1A1, glutathione S-transferase and NAD(P)H quinone oxidoreductase-1 (NQO1) enzymes. Lung inflammation was determined by immunohistochemistry (neutrophils and macrophages) and real time RT-PCR (monocyte chemoattractant protein-1 expression), and alveolarization was determined by lung morphometry (radial alveolar counts and mean linear intercepts).

Results: In both newborn WT and AhRd mice, exposure to hyperoxia increased lung inflammation. However, hyperoxia-induced lung inflammation was significantly increased in AhRd mice compared to WT mice. Likewise, hyperoxia decreased alveolarization in both newborn WT and AhRd mice with the latter being more significantly affected than the former. Interestingly, activation of the AhR was inversely related to hyperoxia-induced lung inflammation and delayed alveolarization.

Conclusions: Upon exposure to hyperoxia, AhR dysfunctional newborn mice have increased inflammation and decreased alveolarization compared to newborn wild type mice. Our data suggest that strategies directed towards increasing the functional activation of the AhR would be effective in the prevention and treatment of BPD in preterm infants.

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Platelet RNA And eXpression-1 Study Demonstrates RNA Expression Differences that Correlate with Ancestry

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A number of common human phenotypes including cancer, cardiovascular disease and many drug responses show striking epidemiologic differences between groups with different ethnicity or ancestral background. The Platelet RNA And eXpression-1 (PRAX1) study was designed to investigate these differences by measuring mRNAs and microRNAs (miRs) demonstrating inter-individual variation in platelets from individuals of European American (EAs) and African American (AAs) ancestry. Platelets present a unique opportunity for study of ethnic differences for many reasons including the breadth of functional variation and the ease with which samples from normal individuals can be obtained for functional and molecular characterization. We profiled approximately 160 healthy adults using genome-wide RNA expression analysis, and we discovered large-scale differences in platelet gene expression between EAs and AAs. These observations are consistent with prior findings of other studies, including the Thousand Genomes Project as well as prior expression profiling of cell line samples from the HapMap project. These studies have revealed genetic differences and cell line gene expression differences between groups of distinct ancestry. The differences we identified in PRAX1 are observed in purified human platelets both at the mRNA and miR level. At the mRNA level, race (ancestry) is significantly associated with the first principal component of variation in the genome wide expression data. In addition, predicted target binding sites of miRs with expression up-regulated in AAs are enriched among mRNAs down-regulated in AAs. A particular cluster of miRs encoded at the Meg3 locus on human chromosome 14q is strongly up-regulated in EAs compared to AAs. The results of PRAX1 indicate that ancestry is strongly correlated with RNA expression and may have relevance for epidemiologic differences.

Contributors: Simon, Lukas; Chen, Edward; Edelstein, Leonard; Hanchard, Neil; Bray, Paul; Shaw, Chad
ARF MEDIATES AN APOPTOSIS-INDEPENDENT ANTICANCER BARRIER THAT ACTIVATES SENESCENCE AND BLOCKS TUMORIGENESIS

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Background: Oncogene-induced apoptosis is one of the most well-documented mechanisms employed by cells to prevent malignant transformation. More recently, oncogene-induced senescence has also emerged as an important barrier to tumorigenesis. In the mammary gland, epithelial cells experiencing acute oncogene activation undergo apoptosis and senescence. Frank tumors arising from these cells are devoid of apoptosis but not senescence, suggesting that successful formation of tumors required the disabling of the apoptotic, but not senescence, barrier.

Experimental design and methods: To determine the importance of senescence in erecting a barrier to mammary tumorigenesis, we utilized the ARF-null mouse model and intraductally introduced the constitutively active ErbB2 oncogene into the adult virgin mammary gland to generate premalignant lesions and measure tumor incidence.

Results: Premalignant lesions arising from ARF-null mice showed diminished senescence and robust apoptosis. Furthermore, ARF-null mammary glands bear a heavier premalignant lesion load that ultimately leads to significantly shorter tumor latency than wildtype mammary glands.

Conclusion: This study suggests that weakening of the senescence barrier is ultimately associated with more rapid tumor induction. Furthermore, the data we present reveals that the apoptotic barrier alone cannot suppress tumorigenesis.

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IS TRANSCRIPTION FIDELITY A ROADBLOCK FOR DNA DOUBLE-STRAND BREAK REPAIR?

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The regulation of transcription elongation affects DNA repair as both these processes occur on the same DNA template. Lesions or breaks in the DNA serve as obstacles to the elongating RNA polymerase (RNAP) complex and hence transcription has been linked to the repair of DNA. The process of transcription-coupled DNA repair has been studied with respect to nucleotide adducts in the DNA. But, little is known about the fate of a transcribing RNAP molecule at a DNA double-strand break (DSB). Considerable past as well as contemporary research has popularized the idea that cells devote most of their resources to preserve DNA integrity. Transcription errors leading to RNA mutations are classically thought to be transient and therefore unlikely to have significant repercussions. However, recent evidence demonstrating that RNA mutations can lead to heritable phenotypic change has highlighted the importance of maintaining transcription fidelity. Our hypothesis is that the act of maintaining RNA fidelity may at times, become an obstacle to DSB repair.

In Escherichia Coli, transcription factors that interact with the secondary channel of RNAP modulate its processivity. These include the transcription initiation and elongation factor DksA and the transcript cleavage factor GreA. By rescuing stalled or backtracked RNAP complexes, GreA ensures the production of full-length transcripts and hence maintains transcription fidelity. We have found that (greA mutants are resistant to DNA DSB-inducing agents. Using a system that allows us to assay the ability of these mutants to prevent or fix DSBs created by the I-Sce I endonuclease, we have found that loss of GreA improves DSB repair. This ability to repair is through the canonical RecA-dependent homologous recombination pathway. Results from pulsed-field gel electrophoresis, that detects DNA breaks, also indicate better repair of damaged DNA in the absence of GreA. (dksA mutants on the other hand, are highly sensitive to DSBs compared to wild-type. Further, overexpression of DksA in a (greA background increases resistance to DSBs more than in a wild-type background, suggesting that competition between these two factors dictates the DSB repair outcome.

We hypothesize that in presence of GreA, an RNAP that encounters a DSB will undergo a futile cycle of backtracking and restart that may inhibit DSB repair. However, in the absence of GreA or presence of DksA, dissociation of RNAP at the break and release of nascent mRNA is favored, leaving the break free to be accessed by repair proteins. Thus, these factors may have important roles to play in the cellular decision to fix DNA breaks and maintain genome integrity, as opposed to preserving the fidelity of transcription by preventing transcription abortion.

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THE ROLE OF THE INTESTINAL MICROBIOTA IN NORWALK VIRUS INFECTION

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The intestinal microbiome has recently been shown to play a role in the pathogenesis of viral enteric infections by enhancing viral infectivity. Norovirus (NoV) pathogenesis is not fully understood, and the effect of the gut microbiota in the context of NoV infection has not been described thoroughly. To assess the interaction between NoV, the intestinal microbiota, and the human host, we used fecal samples collected from the Norwalk virus (NV) challenge study carried out at BCM. The study population consisted of 55 individuals who participated in an experimental challenge with NV. NV infection was defined as the excretion of virus in stool or a ≥4-fold increase in serum titer of antibody to virus-like particles by total immunoglobulin ELISA. For pilot microbiome analysis, fecal samples were collected from 16 subjects at 6 timepoints to establish a baseline for the microbiome and assess changes in the gut microbiota related to NV infection. Of the 16 subjects, 7 were uninfected (3 non-secretors, 3 secretors, and 1 placebo) while 9 subjects were infected. Bacterial gDNA was extracted, and the V3-V5 hypervariable regions of the 16S rDNA was amplified and pyrosequenced on the Roche 454 platform. During analysis, several parameters were applied to stratify the samples including: secretor status, infection status, symptoms (vomiting and/or diarrhea), antibody response, and shedding duration. In addition, we looked at each timepoint individually due to the inherent similarity of a person's microbiome over time. Quantitative beta-diversity Principal Coordinate Analysis (PCoA) was performed for each stratification. PCoA demonstrated no differences in microbiome composition according to secretor status. Additionally, pre-challenge microbiome composition did not affect susceptibility to NV infection, and infection did not induce changes to the structure of the microbiome. Infected subjects who exhibited vomiting presented a different microbiome composition than those who did not vomit. Subjects who exhibited long viral shedding (>14 days) cluster apart from short shedders (<14 days), indicating differences in microbiome composition. High antibody responders (>100 fold difference in NV specific antibody production) clustered separately from low antibody responders (<100 fold change) prior and post infection (day 2 p.i.). These observed changes in the microbiome of long shedders and high antibody responders were influenced by Bacteroides (days -7 and 2p.i.), Faecalibacterium (day -7), and Coprococcus (day -7). These results suggest that the microbial composition of the gut might have an effect on viral shedding and the immune response to NV infection. To confirm these initial findings, we performed 16S rDNA sequencing of the V4 hypervariable region on the entire sample set using the Illumina MiSeq platform, and the data is currently being analyzed.

Contributors: Smith, DL; Ajami, NJ; Neil, FH; Opekun, AR; Finkbeiner, SR; Graham, DY; Petrosino, JF; Atmar, RL; Estes, MK
Gene-environment Signaling in the Regulation of C. elegans Reproductive Aging

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Reproductive senescence is a hallmark of aging, the onset of which can be modulated by both genetic and environmental factors. It has been shown that reproductive aging, like somatic aging, is a regulated process. However, the molecular mechanisms that integrate environmental and genetic signals to regulate the onset and progression of reproductive aging remain largely unknown. Here we report the first known instance of a gene-environment signaling mechanism functioning to regulate reproductive senescence in Caenorhabditis elegans. We found that C. elegans fed the standard lab diet of OP50 E. coli reproduce significantly longer than C. elegans fed the alternate diet of HB101 E. coli. This effect is mediated by the AWB olfactory neurons, which perceive a volatile odorant signal produced by HB101 E. coli. The presence or absence of this sensory cue specifically affects germline proliferation and maintenance, ultimately contributing to the timing of reproductive senescence. Furthermore, we have identified serotonin signaling as a downstream effector of this pathway. Serotonin signaling via the C. elegans serotonin re-uptake receptor, MOD-5, is necessary for the reproductive span response. Together, these studies describe a previously unknown regulatory mechanism for reproductive senescence, and suggest the significance of gene-environment interactions in the regulation of reproductive aging.

Contributors: Sowa, Jessica; Ozseker, Ayse Sena; Wang, Meng
SRC-2 is a BMAL1:CLOCK coactivator for circadian rhythm and metabolism

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Synchrony of the mammalian circadian clock is achieved by complex transcriptional and translational feedback loops centered on the BMAL1:CLOCK heterodimer. Modulation of the circadian feedback loops is essential to maintain rhythmicity, yet the role of transcriptional coactivators in driving BMAL1:CLOCK transcriptional networks is largely unexplored. Here we show diurnal hepatic recruitment of SRC-2 to the genome and find that SRC-2 extensively overlaps with the BMAL1 cistrome during the light phase targeting genes enriching for circadian and metabolic processes. Notably, SRC-2 ablation impairs wheel running behavior, alters circadian gene expression in several peripheral tissues, alters the rhythmicity of the hepatic metabolome, and deregulates synchronization of cell-autonomous metabolites. We identify SRC-2 as a potent coactivator of BMAL1:CLOCK and find that SRC-2 targets itself with BMAL1:CLOCK in a feed-forward loop. Collectively, our data suggest that SRC-2 is a transcriptional coactivator of the BMAL1:CLOCK oscillators and establishes SRC-2 as a critical positive regulator of the mammalian circadian clock.

Labeling of Macrophages with Novel Gadolinium Oxide Nanoparticles for In vivo Imaging of Inflammation

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Cell labeling with MR contrast agents has been a major focus of in vivo imaging. Labeling of cells with T2 agents has allowed the visualization of cell populations in vivo, but precise concentration and location measurements remains challenging. T1 labeling of cells is preferable to T2 labeling because T1 contrast exudes positive contrast. Quality labeling of cells with T1 agents has been difficult due to difficult synthesis of agents, toxicity, poor stability at physiological pH, and no assay to easily determine novel targeted T1 agent efficacy in vivo. The Colvin group has synthesized gadolinium oxide nanoparticles that are coated with PAMPS-LA. These nanoparticles are stable down to a pH of 3 and are non-toxic to cells at a labeling concentration of up to 250 uM. These nanoparticles also provide about 9000 times more contrast per molecule than Gd-DTPA in water. We have labeled macrophages with these agents and will use them to image atherosclerosis in the ApoE-/- mouse model. This work is significant because it will allow for a translatable strategy of plaque identification in vivo.

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Nonsense mediated decay (NMD) is an evolutionary conserved surveillance mechanism that recognizes and degrades mRNAs containing premature stop codons (PTC) and thus prevents translation of deleterious truncated proteins. Mutations in core components of the NMD pathway, including Upf2 protein, have been described in patients suffering from mental retardation and autism. However, little is known about the mechanism by which NMD mediates these behavior abnormalities.

In an effort to better understand the consequences of dysfunctional NMD in the brain, we generated UPF2 forebrain conditional mice (UPF2cKO) and subjected them to extensive characterization. Similar to patients carrying UPF2 mutations, UPF2-deficient mice display behavioral alterations including impaired social interaction, behavioral inflexibility and learning and memory deficits. Furthermore, these behavioral abnormalities in mice are accompanied by deficits in both synaptic structure and synaptic physiology, including impaired hippocampal long-term potentiation. We are currently exploring the molecular mechanism by which UPF2 regulates these cognitive dysfunctions and autism like behaviors.

In conclusion, we found that dysregulation of Upf2-mediated NMD could contribute to cognitive disorders and propose that UPF2cKO mice can serve as a new model for NMD-related intellectual disability study and drug discovery.

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Serine β-lactamases are bacterial enzymes that hydrolyze β-lactam antibiotics. Mechanistically, serine β-lactamases are very similar to serine proteases such as chymotrypsin. They both utilize acylation and deacylation of an active site serine in their mechanism of catalysis. Also, they have strategically positioned a residue that acts as a general base to activate the catalytic serine. His57 acts as the general base in the hydrolysis reaction of chymotrypsin and is part of the catalytic triad essential for the function of the enzyme. TEM-1, a common plasmid-encoded serine β-lactamase, catalyzes the hydrolysis of early penicillins and cephalosporins. Here we examine a previously identified triple mutant of TEM-1 165-YYG-167 (wild type165-WEP-167) with switched substrate specificity from ampicillin to ceftazidime. When compared to chymotrypsin, the Glu166Tyr substitution in the TEM-1 triple mutant is analogous to a substitution of His57 in chymotrypsin that results in an enzyme that maintains function. Our findings agree with previous observations of altered substrate specificity of the β-lactamase triple mutant, which displays increased hydrolysis of ceftazidime. Additionally, enzyme kinetic analysis of the triple mutant shows that the hydrolysis of ceftazidime follows branched pathway characteristic of substrate-induced reversible inactivation. These results, together with an ongoing crystallography studies, will help elucidate the mechanism of hydrolysis utilized by the triple mutant to switch in the specificity of the enzyme from ampicillin to ceftazidime when the critical Glu166 residue is substituted with tyrosine. This will provide insights into alternate pathways of β-lactam catalysis and, more generally, alternate mechanisms for hydrolysis reactions catalyzed by enzymes.

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Jagged1 Promotes Metastasis of Prostate Cancer Cells

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Prostate cancer remains the second leading cause of cancer-related deaths in American males. Metastasis and related complications are the major causes of mortality and morbidity. Bioinformatics analysis and tissue microarray revealed that Jagged1, one of Notch ligands, was elevated in tumor specimens from patients that developed recurrent metastatic diseases. However, the roles and underlying mechanisms of Jagged1 in cancer metastasis are rarely investigated.

In current study, we firstly examined the effects of Jagged1 alteration on proliferation and migration of the prostate cancer cell lines. Up-regulation of Jagged1 in Du145 or knocking down Jagged1 in PC3 cells did not affect their proliferation. However, Jagged1 positively regulates the migratory capacities of DU145 and PC3 cells in an in vitro wound-healing assay, suggesting that Jagged1 plays an active role in metastasis. To confirm the role of Jag1 in metastasis of prostate cancer in vivo, we injected the control DU145 cells and the DU145 cells overexpressing Jagged1 into NOD/SCID mice via tail veins. Our result showed that Jag1 up-regulation promoted distal colonization of tumor cells in lung and decreased latency of metastasis. Collectively, these studies showed that Jagged1 plays an active role in tumor metastasis. Our future work aims to understand the molecular and cellular mechanisms through which Jagged1 enhances metastatic prostate cancer.

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THE ROLE OF CD1D-RESTRICTED NKT CELLS IN THE IMMUNE RESPONSE TO SALMONELLA-BASED RECOMBINANT CANCER VACCINE

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Our group is developing a novel therapeutic cancer vaccine platform, which exploits the molecular machinery of Salmonella for effective in vivo delivery of tumor-associated antigens to the intact antigen presenting cells in situ. An attenuated strain of S. typhimurium, MvP728 has been engineered to express human survivin and the resulted vaccine (MvP728-survivin) induced potent CD8 T cell-mediated anti-tumor responses in a highly aggressive murine A20 lymphoma model. Moreover, the vaccine therapeutic efficacy was further enhanced in combination with a ligand for NKT cells, αGalactosylceramide as an adjuvant leading to complete regression of 5-day established A20 tumor grafts in mice and tumor-free survival for at least 60 days in 8 of 8 vaccinated animals. The cured mice were able to reject repeated challenge with live A20 cells in both vaccinated and contralateral flanks indicating that the vaccination resulted in the development of a long-lasting protective memory. While these results indicate that NKT cell activation with an exogenous ligand can augment vaccine-induced anti-tumor immune response, the role NKT/CD1d system in the initiation of the immune response to Salmonella-based cancer vaccine remains unknown. To examine the requirement of NKT cells for the vaccine-mediated response we will use a model antigen OVA and compare the immunogenicity of MvP728-OVA vaccine in wild type, Jα18-/- (lack only type-I NKT cells) and CD1d-/- (lack all NKT cells) mice. If results of this experiment show that NKT cells are required for the vaccine immunogenicity, we will use CD1d conditional knockout mice to examine which subpopulation of CD1d-expressing antigen presenting cells is required for NKT cell response to the vaccine. To this end, our lab has generated mice with Cre-mediated CD1d deletion in B cells, macrophages, dendritic cells, or hepatocytes. The results of this study are expected to reveal the mechanism by which Salmonella-based recombinant vaccines generate protective antitumor immune response with the ultimate goal of developing an effective therapeutic cancer vaccine.

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Telomeres, the nucleoprotein structures localized to the natural chromosome termini, consist of tandem repeats of TTAGGG sequence bound by components of the shelterin complex. An important function of the shelterin complex is to protect chromosome ends from fusions. Without such protection, the natural ends of chromosomes appear as DNA double-strand breaks, thereby activating a DNA damage response. This in turn triggers the non homologous end-joining (NHEJ) pathway, which normally repairs chromosome double-strand breaks, but at telomeres can initiate deleterious chromosome fusions and cell growth arrest. TRF2, an integral component of the shelterin complex, is particularly important in inhibiting NHEJ, as mouse and human cells lacking TRF2 at telomeres display multiple joined chromosomes indicative of fusions.

Interestingly, Ku, an upstream factor crucial to the NHEJ pathway, resides at functional telomeres and has been implicated in telomere maintenance. Ku is a heterodimer of Ku70 and Ku80 subunits, which binds to DNA ends with high affinity irrespective of sequence. This end binding property of Ku is critical to initiate NHEJ. Ku’s association with telomeres, however, may pose a danger as the end binding of Ku to the telomeric end could lead to NHEJ mediated end-to-end chromosome fusions. In addition, Ku interacts with shelterin proteins TRF1 and TRF2. However, no studies have demonstrated the mode of Ku association with telomeres. We will investigate if Ku associates with telomeres via shelterin components, TRF1 and TRF2, or end binding, or both. Furthermore, how Ku is prevented from triggering NHEJ at functional telomeres is not clear. Data from our lab suggests that TRF2 binds to a region of Ku, Ku70 α5, which is essential for its NHEJ function. Ku70 α5 is responsible for the dimerization of Ku heterodimers, which in turn contributes to NHEJ via synopsis of the ends to be joined. Based on these results, our lab has proposed a model where TRF2-Ku70 α5 interaction prevents Ku from participating in NHEJ at telomeres. We will test this model by generating TRF2 mutants defective for Ku binding. Additionally, Ku contributes to telomere function by inhibiting homologous recombination at telomeres. We will investigate if TRF2-Ku interaction plays a role in impeding telomeric recombination. To study the dynamics of Ku’s association with telomeres and Ku’s regulation at telomeres, we will use a wide range of telomere-based analyses. Through these experiments we hope to gain a better understanding of Ku’s role at human telomeres.
PPM1G Regulates Cell Cycle Progression as a p27 Phosphatase

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p27kip1 is an important cell cycle regulator, dysfunction of which has been implicated in head and neck, breast, prostate and colon cancers. Mislocalization and accelerated degradation, which are highly regulated by the phosphorylation state of p27 protein, are major mechanisms resulting in p27 dysfunction. Currently only one phosphatase (PPM1H) has been reported to dephosphorylate p27 but the result showed some contradiction with the literatures. Our objective is to identify protein phosphatase(s) that directly dephosphorylates p27 and regulates its activity and further to investigate this dephosphorylation effect during tumorigenesis. By using functional genomic phosphatase screening, we identified PPM1G, a PP2C family phosphatase, could reduce T198 phosphorylation of p27 protein in cells. In further studies, we found PPM1G could interact with p27 in cells and in vitro. Moreover, PPM1G could directly dephosphorylate p27 at T198 in vitro. This suggests that PPM1G is a p27 phosphatase via targeting pT198.

p27 functions as a Cyclin-CDK inhibitor to arrest cell cycle at G1/S transition. Akt mediates T198 phosphorylation, which promotes p27 binding to 14-3-3 and facilitates p27 cytoplasmic localization and degradation (Takashi et al., JBC, 2002). Therefore, we hypothesized that PPM1G could regulate cell cycle progression through dephosphorylation of p27. In the experiments, we demonstrated that PPM1G could remove the phosphorylation of p27 at T198 induced by Akt and reduce its interaction with 14-3-3. Stably knockdown of PPM1G in HeLa cells could increase cytoplasmic p27 and further reduce its stability at G1 phase of the cell cycle. Due to the importance of p27 protein level at G1/S transition, PPM1G knockdown cells showed great resistance to G1 cell cycle arrest conditions, including serum deprivation, TGF- and Vitamin D3 treatment, indicating a key role of PPM1G as a gatekeeper to regulate cell reentry into the cell cycle. Since cancer cells normally regain ability to escape from quiescent state (G0 phase) and have the advantage to survive under nutrient deficient condition, PPM1G could be a potential tumor suppressor specifically functioning at the early stage of tumorigenesis. We will pursue further experiments to study the relationship between PPM1G and p27 and determine the functional impacts of PPM1G in certain types of human cancer cells, where p27 has been shown as a good prognostic marker.

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COMPUTATIONAL DE NOVO DESIGN OF TRANSMEMBRANE INHIBITORS TARGETING CONCOGENIC RECEPTORS
Interaction specificity ensures proper cellular functions mediated by biomolecules and selective action of therapeutics. How this property is achieved in lipid membranes remains poorly understood in absence of structural information on membrane proteins and techniques to probe and recapitulate their interactions. Designing molecules targeting selectively any membrane protein would greatly improve our knowledge of their action and abilities to regulate their disease-associated dysfunctions. However, engineering interaction specificity toward membrane proteins requires atom-level prediction in absence of structural information and selection of physical interactions for many competing targets, which remains a computational and experimental challenge. Here we describe an integrated computational de novo structure modeling, design and experimental method and apply it to design highly-specific transmembrane peptide inhibitors of oncogenic receptor associations from sequence information alone. We have modeled from sequence, designed and characterized peptides strongly inhibiting an oncogenic, constitutively active single-point (non-polar to polar) mutant variant of the Fibroblast Growth Factor Receptor 3 (FGFR3mut) but not the wild type receptor. When compared to peptides selected in vivo for FGFR3mut binding from random mutagenesis libraries, the highest-ranked computationally designed peptides exhibit complex networks of non-polar and polar residue interaction motifs that are close to optimal for binding affinity and specificity to FGFR3mut. Our results indicate that precise atomic-level tertiary interactions can be designed de novo to discriminate between membrane protein targets that differ by a few atoms only. More generally, our approach paves the road for designing molecules that target a large diversity of uncharacterized membrane receptors with unprecedented selectivity.
Analysis of Osteosarcoma DNA Methylation Using the Infinium 450K Array

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Osteosarcoma accounts for approximately 60% of malignant bone tumors diagnosed under the age of 20, making it the most common malignant bone tumor in children and young adults. The tumor primarily manifests in the long bones, such as the distal femur, with metastasis primarily to the lungs. The 5-year event-free survival (EFS) of patients presenting with metastatic disease at diagnosis has remained a dismal 20% over the last two decades, despite the use of current treatment protocols. Little progress has been made in developing customized treatment of osteosarcoma due to the scarcity of reliable prognostic markers available at the time of diagnosis and lack of novel therapeutic targets. Part of the complication also lies in the high level of genomic heterogeneity of osteosarcoma. We therefore propose to perform a comprehensive characterization of these genetic alterations. Identifying genome wide genetic aberrations, which are indicative of tumor biology, will aid in the understanding of osteosarcoma pathogenesis and the differences between tumor subtypes. This knowledge would allow for earlier and more precise classification of patient prognosis and greatly benefit the search for more effective targeted therapies.

A specific focus for my part of the project is to evaluate methods for analyzing DNA methylation data. The new Infinium 450K array is composed of probes profiling over 485,000 CpG sites in the genome. We are currently in the process of developing an integrated and computationally efficient pre-processing method for analysis of 450K data. This includes data processing with built-in QC, reliable methylation status calling and correlation with gene expression while taking into account the statistical and computational challenges present in analysis of such a large data set. In addition to this, we have utilized the methylation to corroborate results from other genomic platforms such as copy number and miRNA.

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SPECIFICITY DETERMINANTS OF DOPAMINE RECEPTOR LIGAND RECOGNITION AND DOWNSTREAM COUPLING

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The D2 dopamine receptors (D2Rs) and 5-HT2A serotonin receptors (5-HT2ARs) both belong to the class A subfamily of G protein-coupled receptors. Both receptors are expressed in the central nervous system and serve as potential targets for antipsychotic drugs. Although their binding sites are predicted to be structurally similar, they are able to discriminate between the neurotransmitters dopamine and serotonin and mediate distinct physiological processes. Previous studies using Evolutionary Trace (ET) identified residues important for functional specificity of D2R and 5-HT2AR. Replacing the ET-residues in D2R with the corresponding ET-residues from 5-HT2AR (ET-residue swapping) in some cases led to a significant enhancement of serotonin-stimulated Gα16 protein activation or reduced dopamine responsiveness. However, some swaps showed no effect at all. One possible explanation for these results is that some ET-residues may work in pairs or larger groups of residues to perform specific functions. Therefore, in this study, we investigated the effects of combined ET-residue swaps, which are predicted to be covariant during evolution to acquire specific functions by ET analysis. The level of Gαi activation induced by agonist-stimulated D2Rs was determined by the membrane potential assay in which activated Gαi leads to opening of the TRPC4β channel and cation influx across the plasma membrane in HEK293 cells. Some combined ET-residue swaps showed more enhanced serotonin or diminished dopamine response compared to the individual swaps, suggesting the functional coupling between the chosen ET-residues in terms of the specificity of G protein activation induced by agonist-stimulated D2Rs. Although most combined ET-residue swaps had effects intermediate between those of the individual swaps for ligand binding affinity, we did observe one combined ET-residue swap showed higher binding affinity for serotonin compared to the individual swaps. This synergistic effect on serotonin binding affinity suggests the functional coupling for specificity of ligand binding. Taken together, these findings demonstrate that combined ET-residue swaps in some cases convert D2R more effectively into 5-HT2AR, implying coevolutionary interactions between the predicted covariant ET-residues during the evolution of GPCR function and signaling.

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Increased Steroid Receptor Coactivator-2 Levels Impair Normal Endometrial Function

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Steroid receptor coactivator-2 (SRC-2) is a member of the p160/SRC family of coregulators, which also includes SRC-1 and SRC-3. Members of this coregulator class exert a wide-spectrum of physiological processes, ranging from mammary morphogenesis to metabolic homeostasis. Importantly, deregulation of SRC expression levels is a causal factor for many tissue pathologies in both human and mouse. In the case of the endometrium, clinical studies reveal that SRC-2 and SRC-3 levels are elevated in endometrial biopsies from patients diagnosed with polycystic ovary syndrome (PCOS). Significantly, the endometrium of PCOS patients displays severe defects in functionality, including increased endometrial cancer susceptibility and miscarriage rate. Elevated expression of both SRC-2 and SRC-3 has also been found in the hyperplastic and neoplastic endometrium. Collectively, these descriptive findings suggest a causal link between elevated expression of one or both coregulators and the emergence of these endometrial disorders. To address this proposal further, we engineered a SRC-2 overexpressor (SRC-2:OE) mouse in which high levels of human SRC-2 expression are specifically targeted to cells that express the progesterone receptor. Long term-breeding studies, a decidual response assay, gonadotropin-induced superovulation, and measurement of serum hormone levels were conducted on SRC-2:OE mice to evaluate fertility status. Although ovulation and serum hormone levels are normal, six month breeding studies show that elevated levels of endometrial SRC-2 result in a severe subfertility defect. Importantly, an artificial decidual response assay revealed that the SRC-2:OE endometrium exhibits an impaired ability to undergo decidualization, an essential cellular process that enables embryo implantation to occur. The inability of the SRC-2:OE endometrium to decidualize is also reflected at the molecular level by a marked decrease in the induction of the decidual biomarkers, Follistatin, Wingless-related MMTV integration site 4, Bone morphogenetic protein 2 and Heart and neural crest derivatives expressed transcript 2. Furthermore, short-and long-term estradiol treatment reveals that perturbation of SRC-2 levels markedly potentiates estradiol-induced uterine epithelial hyperplasia, providing strong support for SRC-2 in the promotion of unopposed estrogen-action. We conclude that tight control of SRC-2— independent of changes in SRC-3 levels—is mandatory not only for normal endometrial functionality but also to prevent unscheduled endometrial hyperplasia which can lead to cancer.

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Background & Aims. Partial hepatectomy (PH) induces hepatocyte proliferation via step-wise induction of immediate early genes, reorganization of extracellular matrix, and cytokine and growth factor-mediated signaling early on during liver regeneration. However, the identity of initial trigger(s) of liver regeneration has remained elusive. ATP is released into the extracellular milieu within minutes of 70% PH. We hypothesized that extracellular ATP, via activation of its cognate cell-surface P2Y2 purinergic receptors, might play a key role in the induction of early events critical for hepatocyte proliferation in regenerating livers.

Methods. Wild type (WT) and P2Y2 purinergic receptor knockout (P2Y2-/-) mice were subjected to 70% PH and liver tissues were analyzed for efficiency of hepatocyte priming and proliferation. Influence of extracellular ATP and P2Y2 purinergic receptor signaling on hepatocyte proliferation was evaluated in vitro.

Results. Our findings suggest that hepatocyte proliferation in response to 70% PH was impaired in P2Y2-/- mice. Early activation of p42/44 MAPK (ERK, 5 min), early growth response-1 (Egr-1) and activator protein-1 (AP-1) DNA-binding activity (30 min) were attenuated in the remnant livers of P2Y2-/-.. Correspondingly, Egr-1 and AP-1 target gene and a key mediator of extracellular matrix remodeling, matrix metalloprotease-9 (MMP-9) protein induction and HGFα/c-Met signaling were attenuated in P2Y2-/-.. Extracellular ATP alone, via the activation of P2Y2 purinergic receptors, was sufficient to activate ERK/Egr-1 and proliferation of primary mouse hepatocytes in vitro.

Conclusions. Extracellular ATP-mediated rapid activation of P2Y2 purinergic receptors plays a key role in the initiation of hepatocyte proliferation in response to PH in mice.

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THE FUNCTIONAL SWITCH IN POTASSIUM CHANNELS IN MYOTONIC DYSTROPHY IMPAIRS PROLIFERATION, MIGRATION AND FUSION DURING MYOGENESIS

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Voltage-gated K+ channels (Kv) are responsible for myoblasts proliferation and differentiation by triggering changes in membrane potential and cell volume. Since individuals with myotonic dystrophy type 1 (DM1) display reduced myogenesis led by prolonged myoblasts proliferation and delayed myotubes fusion, we investigated the roles of K+ channels in primary human myoblasts obtained from DM1 patients and healthy volunteers. DM1 is an autosomal dominant neuromuscular disorder affecting 1 in 8000 people worldwide. It is the most common adult-onset muscular dystrophy and currently has no treatment. DM1 is characterized by muscle wasting and multi-system disorders.

We have identified a switch in functional potassium channel expression from KCa1.1 to Kv1 channels when comparing myoblasts from healthy individuals to myoblasts from patients with DM1. We showed increase in Kv1.2 and Kv1.5 channels, and decrease in KCa1.1 channels in DM1 myoblasts at mRNA level by RT-PCR, at protein level by immunofluorescence, and at channel activity by patch-clamp technique. We hypothesized that this switch in K+ channels plays a role in the reduced myogenesis observed in patients with DM1, and that selective inhibition of Kv1 channels rescues the pathological features of DM1 in skeletal muscle.

We show that pharmacological inhibition of Kv1 channels in DM1 myoblasts normalized proliferation, rescued matrix metalloproteinase-2 (MMP-2, a protease necessary for myotube fusion) production, and partially rescued myotube fusion shown as increase in fusion index. On the contrary, selective inhibition of KCa1.1 in normal myoblasts lowered MMP-2 production, impaired wound healing repair, and decreased myotubes formation. Therefore we conclude that loss of KCa1.1 and up-regulation of Kv1 channels in DM1 impairs early stage of myogenesis and can be partially rescued by modulating such K+ channels.

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Ykt6, a conserved v-SNARE, is required in neuronal function and maintenance

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Membrane fusion is required for vesicular trafficking between various organelles and compartments. Many cell functions rely on membrane fusion, including intracellular transport, hormone or enzyme secretion and maturation of organelles. In particular, neurons utilize membrane fusion for activities that are important for neuronal function and maintenance such as neurotransmission, protein recycling and degradation. One of the key players of membrane fusion is the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein. In a forward genetic screen on the Drosophila X-chromosome, we identified 2 mutations in Ykt6, which encodes the Drosophila homolog of mammalian Ykt6. Ykt6 is a highly conserved gene which encodes a v-SNARE. Most studies about Ykt6 are done on yeast and mammalian cells. It is widely accepted that Ykt6 is involved in ER-Golgi anterograde transport. In yeast, Ykt6 is also known to function in several other types of vesicular transport, especially in vacuole maturation. However, no study has been done on Drosophila Ykt6. Using the Ykt6 mutants, we examined the role of Ykt6 in photoreceptor function and maintenance. ERGs (electroretinogram recordings) of Ykt6 mutant clones show defects in neurotransmission, and a progressive decline of the amplitude of the ERGs as the flies age, a hallmark of neurodegeneration. The functional neurodegenerative phenotype is also confirmed with the demise of the ultrastructural morphology of the mutant photoreceptors. Through immunostaining studies, we found that several photoreceptor-specific transmembrane proteins like Rhodopsin1 and Chaoptin are aberrantly localized to the cell bodies rather than the rhadomeres. Further biochemical studies suggest that these proteins are trapped in the ER. We propose that accumulation of proteins in the ER of the mutant photoreceptors leads to ER stress, and eventually to neurodegeneration.

Contributors: Kai Li Tan, Manish Jaiswal, Shiuan Wang, Bo Xiong, Shinya Yamamoto, Hector Sandoval, Vafa Bayat, Gabriela David, Wu-Lin Charng, Ke Zhang, Hugo Bellen
One-third of all available medications target G-Protein Coupled Receptors (GPCR). Therefore, specificity towards ligands and downstream effectors is important for understanding protein function and the development of selective therapeutic drugs with less side-effects. The bioamine subfamily of GPCR proteins (i.e. D2 dopamine receptor) are known to be involved in nervous system disorders and are often targeted by psychoactive drugs. We hypothesize that by better understanding the molecular basis of the receptors’ function from evolution, we can: (a) identify the functional determinants, rationally target mutations to (b) change binding and (c) activation specificity to different effectors. Difference Evolutionary Trace analysis was previously utilized to identify functional residues in serotonin receptors (5-HT2AR) that could be swapped into the D2 dopamine receptor (D2R) leading to the generation of 15 transmembrane domain D2R mutants. The effect on ligand potency and specificity of those 15 mutants has been previously determined. Now that there's a better understanding of ligand bias, we aim to generate D2R mutants utilizing the Difference Evolutionary Trace method to determine the preferential effector (i.e. G-proteins vs beta-arrestin). D2R specific G-protein, G\textalpha{}i, is a GTPase that leads to membrane depolarization through activation of an ion channel. Beta-arrestin 2 is involved in desensitization through GPCR internalization for receptor recycling or degradation. We plan to elucidate the evolutionary determinants (key residues) in downstream effector bias that will separate D2R function between beta-arrestin 2 and G\textalpha{}i. Furthermore, the Difference Evolutionary Trace methodology can be applied to other proteins to predict functional sites and mutations to change the function of those proteins.
Each year bacterial pathogens kill millions of people worldwide while antibiotic resistant strains threaten our primary means of treatment. Such pressures necessitate a different approach to understanding bacterial pathogenesis for the development of new therapeutics. An obvious factor affecting the outcome of a bacterial infection is the pathogen’s ability to replicate within the host. Bacterial replication is inextricably bound to the acquisition and incorporation of host nutrients to sustain growth. Many pathogens require inorganic molecules and essential amino acids during infection. In this regard, much research has focused on iron acquisition, as iron is necessary for bacterial respiration and DNA replication. However, the details of amino acid acquisition during a bacterial infection remain poorly defined. Blood, the likely source of nutrients during bacteremia, contains free amino acids as well as a multitude of plasma proteins that could fulfill a bacterium’s nutritional needs. Bacillus anthracis, the causative agent of Anthrax disease, is a gram-positive bloodborne pathogen. Highly virulent, B. anthracis secretes toxins and proteases known to degrade host tissues. Here, we employ B. anthracis as a model bacterial pathogen to investigate the hypothesis that it degrades host proteins for amino acid acquisition. To test this hypothesis we develop a new defined media referred to as Blood Serum Mimic (BSM) to increase physiological relevance and analyze the nutritional requirements of B. anthracis. Through growth assays and proteomic analysis, we discover human hemoglobin is proteolyzed by B. anthracis to acquire the essential amino acids valine and methionine. This process appears to occur in an iron-independent and protease-dependent manner. Collectively, these data highlight the importance of amino acid acquisition and suggest host proteins could be targeted for degradation during a bacterial infection. Future endeavors will focus on assessing the specificity of B. anthracis proteolysis and investigating amino acid acquisition in other common bacterial pathogens.

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NICOTINE ACTS THROUGH STRESS HORMONES TO ALTER DOPAMINE RESPONSES TO ALCOHOL AND PROMOTE INTAKE

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Exposure to nicotine and stress correlate positively with alcohol consumption, yet the neural mechanisms contributing to this interaction remain largely undetermined. Nicotine, stress, and alcohol target the dopamine centers in the brain, suggesting a potential locus for mechanistic interactions. The objective of this study was to better understand how nicotine alters subsequent responses to alcohol and to determine the role of stress hormones in this interaction. Using in vivo microdialysis, we show that nicotine pre-exposure decreased the sensitivity of the dopamine system to alcohol. These interactions required the activation of stress hormone receptors. Inhibition of stress hormone receptors within the dopamine system during a nicotine exposure prevented the decreased alcohol-induced dopamine response and the increased alcohol self-administration. These results suggest that nicotine activates stress hormone systems to alter physiological responses to alcohol and promote intake.

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While it is generally accepted that microRNA expression and function play a wide variety of roles throughout an organism, very little is known about the specificity of microRNAs in any specific tissue or cell type. Because of the highly heterogeneous and intermixed neuronal subtypes, cell type specific miRNA analysis is even more pertinent in understanding neuronal function. In microRNA biogenesis, several key proteins play important roles in processing and function of microRNA. One class of proteins, Argonaut (Ago1) proteins, associate with the fully mature, 21-23 nt, microRNA. A technique, called miRAP was originally developed in worms and mice and uses tagged components of the RISC complex to co-immunoprecipitate attached miRs, however no such system exists for cell type specific miRNA profiling in flies.

We have generated a UAS-Ago1::GFP fusion gene which will allow us to examine the microRNA profiles of specific subtypes of neurons when combined with a specific GAL4 driver of choice. Expression of UAS-Ago1::GFP with pan-neuronal driver elav-Gal4 showed expected tissue wide protein localization of our fusion protein, as well as expected subcellular localization of Ago1. Pull down experiments using our fusion tag demonstrated a highly reproducible enrichment of a subset of miRNAs in neurons. We are currently in the process of using this adapted technique to examine miRNA populations in specific neuronal subtypes in Drosophila with the goal of understanding which miRNAs are important for neuronal function in specific neurons and pathways. This newly adapted technology will allow us to identify miRNAs in specific cell type as a first step to functionally characterize their role in these specific neuronal subtypes, but can be used in combination with any Gal4 driver line to examine cell type specific miRNA expression in any cell or tissue type in Drosophila.

Contributors: Amanda Thomas, Pei-Jung Lee, and Herman A. Dierick
The development of HLAnull CD1d-expressing K562 cells as artificial antigen-presenting cells for selective and efficient clinical scale NKT cell expansion

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Advisor: Leonid Metelitsa, M.D./Ph.D.-Department of Pediatrics

CD1d-restricted type-I Natural Killer T cells (NKTs) have been shown to mediate antitumor responses in mouse models and are associated with improved outcome in several types of cancer. However, the therapeutic application of NKTs has been limited by low numbers and functional defects of these cells in patients with cancer. To provide a means for safe and effective ex-vivo NKT-cell expansion for cell and gene therapy applications we explored native and engineered properties of K562 cells to function as artificial antigen-presenting cells (aAPC). Although clinical grade K562-based aAPC products have been shown to be effective for T- and NK-cell expansion for therapeutic use in patients, K562 cells express HLA-Cw3, an NK-cell activating ligand which stimulates expansion of NK and potentially alloreactive T cells that causes competition with NKTs in culture and poses a clinical hazard. Because HLA-C is the only HLA allele in K562, we rendered K562 cells HLAnull by eliminating HLA-C gene from K562 cell genome using a HLA-C-specific zinc finger nuclease. We then transduced parental and HLAnull K562 cells with CD1d cDNA followed by single cell sorting and clonal expansion. The clones were pulsed with αGalactosylceramide and tested as aAPC for NKTs using CFSE proliferation assay. We found that in contrast to K562/CD1d, HLAnullK562/CD1d clones selectively expanded NKTs when added to primary PBMC, and clones with an intermediate level of CD1d expression induced the highest rate of NKT-cell proliferation. Next, a selected HLAnullCD1dmed clone was further modified to express CD86 alone or in combination with 4-1BBL and/or OX40L followed by single cell sorting and clonal expansion. The comparison of the APC function allowed us to select a clone with the phenotype HLAnullCD1dmedCD86highOX40Lmed that consistently induced the highest rate of NKT-cell expansion. Therefore, the engineered aAPC can be used to selectively and efficiently expand primary human NKTs for safe adoptive cell therapy applications both in autologous and allogeneic settings.

Contributors: Gengwen Tian1, Bipulendu Jena2, Daofeng Liu1, Andras Heczey1, Hiroki Torikai2, Dean Lee2, Laurence Cooper2, and Leonid Metelitsa1
The role of adaptive immune system in the escape of breast cancer cells from primary tumors.

Lin Tian  
Department of Biochemistry & Molecular Biology  
Advisor: Xiang Zhang, Ph.D.-Department of Molecular & Cellular Biology

Metastasis is one of the hallmarks of cancer, and is the direct cause of more than 90% of cancer-related deaths. Intravasation into the circulation is a critical step for tumor cells to reach distant organs. We have recently observed a dramatic effect of the adaptive immune system on vascular structures and metastasis. In the project, we hypothesize that adaptive immune cells may prevent the cancer cells from entering into the circulation through normalizing the tumor-associated vasculatures.

We utilized a p53-null murine mammary tumor model to test our hypothesis. To determine the role of adaptive immune cells in intravasation, we transplanted tumor cells into mammary gland of Balb/c mice (immunocompetent) and nude mice (immunodeficient). Through quantifying circulating tumor cells (CTCs) by qPCR, we found that the number of CTCs in Balb/c mice was around 100 times lower than that in nude mice. To further investigate the relation between adaptive immune cells and vascular normalization, we compared pericyte coverage of tumor-associated vasculatures in Balb/c mice and nude mice, and found that pericyte coverage in tumors of Balb/c mice was higher than that of nude mice. To directly test the function of T lymphocytes in vascular normalization, we reconstituted T lymphocytes in tumor bearing nude mice, and found that pericyte coverage of tumor-associated vasculatures increased significantly in nude mice with T cell reconstituted.

This study indicates that adaptive immune cells may decrease vasculature permeability through increasing pericyte coverage. As a consequence, could inhibit intravasation and decrease metastasis frequency.

Contributors: Lin Tian1,3, Thomas Welte1, Xiang Zhang1,2.  
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KCNQ2 and KCNQ3 are homologous voltage-gated potassium channel subunits that form neuronal “M-channels”. M-channels activate slowly in the voltage range between rest and action potential threshold, thereby integrating synaptic inputs and modulating neuronal excitability. Certain KCNQ2 and KCNQ3 mutations cause dominantly inherited forms of epilepsy and myokymia; by contrast, other, de novo, KCNQ2 mutations have been found in a severe syndrome of epileptic encephalopathy. KCNQ2 and KCNQ3 are conspicuously enriched at the axonal initial segment (AIS) of many central neurons, but the time course of their arrival at the AIS has been little studied. We have performed immunofluorescence labeling and immunoblotting for these subunits in tissue samples and hippocampal neuronal cultures during development. Labeling of mouse hippocampal sections shows that in pyramidal cells, KCNQ2 is detectable at P4 and is highly concentrated within the distal portion of AISs by P15. KCNQ3 arrival at the AIS appears slightly delayed, suggesting the existence of two distinct populations of channels rather than only KCNQ2/3 heteromers. Within neocortical pyramidal neurons, KCNQ2 and KCNQ3 are expressed in the distal two-thirds of the AIS, and most concentrated at the distal tip, the location at which action potentials are initiated. Additionally, in KCNQ2 mutant mice which are a model of KCNQ2 encephalopathy (Peters et al., Nat Neurosci. 2005; Millichap and Cooper, Epil. Curr., 2012) KCNQ2 was completely undetectable at the AIS, and was found instead in perinuclear and dendritic aggregates suggestive of ER retention. KCNQ3 was redistributed to the KCNQ2-labelled aggregates, but was detected at low levels within the AIS. These results indicate that certain KCNQ2 mutations may act as dominant-negative trafficking traps, preventing functional surface expression of subunits co-expressed from wild-type alleles and thereby leading to severe, encephalopathic phenotypes.

Contributors: Tran, Baouyen; Cooper, Edward
ROLE OF INFLAMMATION IN SHH SIGNALING DURING PROSTATIC DISEASE PROGRESSION

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*Advisor: David Rowley, Ph.D.-Department of Molecular & Cellular Biology*

Introduction: During normal prostate development, hedgehog signaling is known to regulate cell proliferation, tissue polarity, and cell differentiation. Reactivation of the hedgehog pathway has been implicated in multiple types of cancers. In vivo studies utilizing the human prostate cancer cell line LNCaP indicate SHH signaling promotes xenograft tumor growth via activation of the surrounding stroma. In vitro, it has been shown that activation of SHH signaling in myofibroblasts is sufficient to accelerate tumor cell growth. It appears that the disease-promoting attributes of this pathway are primarily due to paracrine signaling between the stromal and epithelial compartments.

Emerging data indicates an important role of chronic inflammation in prostatic disease. Amongst exposure to infectious agents and other environmental factors, is the possibility of chemical irritation due to urine reflux. A high concentration of uric acid or change in pH can lead to the formation of uric acid crystals (urate). This crystalline structure has been shown to act as a ‘danger signal’ that enables the cleavage and release of pro-IL1( to its active form and the production of inflammatory cytokines.

Therefore we hypothesize that activation of sonic hedgehog signaling facilitates prostatic disease progression via paracrine modulation of prostate specific mesenchymal stem cells (psMSCs) and that inflammation may be a key driver of this pathway.

Methods/Results: Multiple prostate epithelial lines, ranging from normal to disease, were treated with urate and assessed for changes in gene expression associated with SHH signaling and inflammation. While epithelial cells exhibited no change, stromal cells interestingly upregulated inflammatory genes. When exposed to conditioned media from urate treated psMSCs, epithelial cells displayed a modest increase in SHH gene expression. In an indirect co-culture model, SHH pathway members were upregulated in the epithelial compartment. This suggests that an inflammatory event in the stroma may contribute to later paracrine signaling between the compartments.

Contributors: Ressler, Steven; Rowley, David
**2013 GRADUATE STUDENT SYMPOSIUM**

**Arginine methylation is an important signal within the stress response that alters stress granule dynamics**

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*Department of Molecular Virology & Microbiology*

*Advisor: Richard Lloyd, Ph.D.-Department of Molecular Virology & Microbiology*

Stress granules (SGs) are dynamic cytoplasmic structures, which contain aggregates of translationally stalled messenger ribonucleoprotein (mRNP) complexes. SGs are formed in response to environmental stressors such as heat shock, oxidative stress, amino acid deprivation, and viral infection. Protein arginine methyltransferases (PRMTs) are the enzymes responsible for arginine methylation, which provides a means to modulate the charge and localization of target proteins. A central component of SGs is Ras-GTPase activating protein SH3 domain-binding protein 1 (G3BP1), which contains an arginine-rich RGG domain in the C-terminus. It has been shown that PRMT1 can also bind in this region; however, the role of methylation in SGs dynamics is yet to be determined.

The first aim of this study is to investigate whether G3BP1 methylation is required for SG formation. We found three potential methylation sites (R435, R443, R447) in the RGG region of G3BP1. In order to characterize the functional significance of these sites, I will generate methylation mimics (R to F) and deficient mutants (R to K). These mutants will be transfected into U2OS cells and microscopy will be used to study SG behavior under arsenate and thapsigargin stresses. It is reasonable to infer that G3BP1 will switch its binding partners after changing its charge. The second aspect in this part is to verify the components of G3BP1 complex to depict the possible biological function of G3BP1 via pull down assay and ingenuity pathway analysis software.

The second aim of this study will be to investigate the role of methylation in SG dynamics. For this aim, methylation deficient mouse embryonic fibroblasts will be used to ablate specific methylation pathways. Stress granules will be induced in these cells and stress granule assembly and disassembly kinetics will be monitored by immunofluorescence microscopy. Additionally, in vitro and in vivo methyltransferase assays using PRMTs found to be important in the above SG dynamics studies will be employed with G3BP1 as a substrate. These studies will provide insight into whether R435, R443 and R447 are modified by PRMTs.

The last part of this study will focus on the relationship between innate immunity and methylation modification on G3BP1. First, I will overexpress those mutants in either G3BP knockout or PRMT knockout cells. Then infect cells with enterovirus to investigate viral replication rates. If methylation effects viral replication, I will investigate cytokine expression to determine whether SG modulation of various cytokines is partly responsible for the observed changes in virus replication.

Contributors: Wei-Chih Tsai, Lucas C. Reineke, Jon Dougherty, Richard E. Lloyd
The Cell-Adhesion GPCR Brain-Specific Angiogenesis Inhibitor 1 Regulates Dendritic Spine Morphology and Synaptogenesis

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Integrative Program in Molecular and Biomedical Sciences
Advisor: Kimberley Tolias, Ph.D.-Department of Neuroscience

Synapses are specialized sites that mediate communication between neurons. Most excitatory synapses in the brain reside on actin-rich structures called dendritic spines. The formation, regulation and maintenance of excitatory synapses are crucial for normal cognitive function. Here, we identify the adhesion G protein-coupled receptor (GPCR) brain-specific angiogenesis inhibitor 1 (BAI1) as a key regulator of synapse development. We show that BAI1 is highly localized to spines. Furthermore, knockdown of BAI1 results in decreased spine and synapse density in both cultured hippocampal neurons and cortical neurons from intact mouse brains. Synaptic loss caused by BAI1 knockdown can be rescued by full-length BAI1, but not by a BAI1 truncation mutant, which fails to interact with the Par3/Tiam1 polarity complex. Tiam1 is a Rac1-guanine nucleotide exchange factor (GEF) that promotes spine and synapse development by inducing Rac1-dependent actin remodeling. Tiam1 is restricted to spines by the polarity protein Par3, enabling for spatial control of Rac1 activation. We show that BAI1 regulates spine and synapse development by recruiting the Par3/Tiam1 complex to spines, resulting in localized Rac activation and actin polymerization. Although these findings elucidate how BAI1 signals inward to promote post-synaptic development, it is unclear whether BAI1 also signals across the synapse to induce pre-synaptic differentiation. Utilizing a HEK 293T cell-neuron co-culture system, we show that BAI1 increases pre-synaptic termini formation on the axons of cultured hippocampal and cortical neurons that contact BAI1-expressing HEK 293T cells. These results indicate that BAI1 can induce pre-synaptic as well as post-synaptic development. Our future investigations are two-fold: (1) We have also shown that disrupting BAI1’s interactions with synaptic αVβ5 integrins enhances BAI1’s association with the Par3/Tiam1 complex. We aim to identify other synaptic binding partners or ligands for BAI1 and determine how their interactions modulate BAI1’s function at synapses. (2) Given that the two other BAI family members are also highly expressed in the brain, affect neuron morphology, and have been linked to neurological disease, we are investigating their role as regulators of spine and synapse development. Results from our study should help to elucidate the mechanisms that regulate excitatory synapse development, and provide potential therapeutic targets for the treatment of neurological disease.

Contributors: Tu, Yen-Kuei; Duman, Joseph; Tzeng, Christopher; Munjal, Tina; Schwechter, Brandon; Ho, Szu-Yu; Tolias, Kimberley
IDENTIFYING NOVEL GENES REQUIRED FOR NEURONAL MAINTENANCE in D. melanogaster

Ayse Berrak Ugur  
Program in Developmental Biology  
Advisor: Hugo Bellen, Ph.D./D.V.M.-Department of Molecular & Human Genetics

To identify and characterize novel genes that cause neurodegeneration, our lab has performed a large scale, unbiased, clonal, forward genetic screen on the X chromosome of D. melanogaster for essential genes. The screen was based on the ey-FLP system to generate homozygous mutant eye clones in an otherwise heterozygous mutant background and assessing the ability of these mutant photoreceptors to respond to light in aging flies by recording ERGs. I am currently working on the mapping of some neurodegenerative mutants and trying to uncover the molecular mechanism underlying the neurodegenerative phenotype.

Contributors:
Endometriosis is a hormone-dependent gynecological malignancy affecting 2-10% of women in the reproductive age group. This chronic disease is characterized by the growth of endometrial tissue outside the uterine cavity causing inflammation and severe pain. Expression of several nuclear receptors, including Steroidogenic Factor-1, are deregulated in endometriotic lesions. Steroidogenic Factor-1 is an orphan receptor involved in sex determination during development and the transcriptional regulation of steroidogenic enzymes in the ovary. Aberrant epigenomic activation of SF-1 promoter in ectopic endometriotic lesions is hypothesized to cause the up regulation the steroidogenic enzymes and the development of a hyper-estrogenic state that favors growth and inflammation.

However, the role SF-1 in uterine fertility and the pathogenesis of endometriosis has not been evaluated in vivo. We hypothesize high expression of SF-1 in the endometrium results the local production of estrogen that promotes the progression of endometriosis and disrupts hormone signaling, resulting in sterility. To test our hypothesis we developed a mouse model in which SF-1 was inserted downstream of a STOP cassette under the regulation of a cytomegalovirus-beta actin hybrid promoter. The transgene was inserted into the ROSA26 locus and uterine expression of SF-1 was activated after deletion of the STOP cassette by Cre recombinase expressed under the control of progesterone receptor promoter.

SF-1 expression results in cystic endometrial glands in cycling and ovariectomized mice. Mice were completely infertile in a 6-month breeding trial and presented depleted stromal and myometrial compartments as well highly cystic glands. SF-1 expression impaired response to artificial induction of decidualization and results in the up regulation of ERβ and the steroidogenic enzymes StAR, Cyp11a1 and Cyp17a1. The up regulation of steroidogenic enzymes results in the local production of estradiol and the up regulation of several estrogen targets including Ltf, Lif and Igf-1. Finally, we observed a significant increase in the size of ectopic endometrial explants in the endometriosis mouse model.

Stromal cells isolated from human endometriomas exhibited robust expression of SF-1 and dramatically up-regulated the expression of SF-1 targets in the presence of cAMP. Upon knock-down of SF-1 expression of targets was significantly attenuated. Similar to the decidual defect observed in mice, endometriosis stromal cells were unable to differentiate in response to decidualizing agents. Collectively, these results indicate SF-1 plays a pivotal role in the deregulation of steroid signaling in the endometrium.

Contributors: Vasquez, Yasmin; Wu, San-Pin; Hawkins, Shannon; Tsai, Sophia Y.; Tsai, Ming-Jer; Lydon, John P. and DeMayo, Francesco J.
SMOOTHENED FUNCTION AS A G-PROTEIN COUPLED RECEPTOR IN MAMMARY EPITHELIAL CELLS.

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Advisor: Michael Lewis, Ph.D.-Department of Molecular & Cellular Biology

Background: Smoothened (SMO), an Hh signaling effector, is ectopically expressed in breast cancers, and constitutive activation in transgenic mouse mammary glands leads to paracrine stimulation of proliferation and hyperplasia. SMO can function canonically via GLI transcription factor activation, or non-canonically as a G-protein coupled receptor (GPCR). Our goal is to determine whether SMO signals as a GPCR in the mammary gland.

Experimental design and methods: Differential gene expression analysis was performed using RNAseq, with validation by qRT-PCR and/or immunofluorescence microscopy in FACS-enriched SMO-overexpressing or non-expressing cells vs. wild type cells. Gi function was assessed pharmacologically by in vivo treatment with pertussis toxin (PTX), and genetically using Gi-null alleles, in the presence or absence of activated SMO.

Results: Pharmacological disruption of Gi signaling with PTX abolished SMO-induced hyperproliferation in vivo and ex vivo. Gene expression analysis indicated that Gi1 was the only G subunit induced in SMO-overexpressing mammary epithelial cells. In genetic analyses, Gi2 and Gi3 null mice display attenuated proliferation in the presence of activated SMO suggesting Gi2 and Gi3 mediate SMO-induced proliferation.

Conclusion: Our data are consistent with the hypothesis that SMO functions non-canonically as a GPCR via PTX-sensitive Gi G-proteins to regulate mammary gland proliferation.

Contributors: Villanueva, Hugo; Visbal, Adriana, Plummer, Nicholas; Birnbaumer, Lutz; Lewis, Michael
Plk2 regulates mitotic spindle orientation and mammary gland development

Elizabeth Villegas  
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Advisor: Jeffrey Rosen, Ph.D.-Department of Molecular & Cellular Biology

Disruptions in polarity and mitotic spindle orientation contribute to the progression and evolution of tumorigenesis. However, little is known about the molecular mechanisms regulating these processes in vivo. Here we demonstrate that Polo-like kinase 2 (Plk2) regulates mitotic spindle orientation in the mammary gland and is a putative tumor suppressor. Plk2 is highly expressed in the mammary gland and is required for proper mammary gland development. Loss of Plk2 leads to increased mammary epithelial cell proliferation and ductal hyperbranching. Additionally a novel role for Plk2 in regulating the orientation of the mitotic spindle and maintaining proper cell polarity in the ductal epithelium was discovered. Interestingly, Plk2 deletion also results in increased levels of polo-like kinase 1 (Plk1), a key regulator of mitosis and a putative oncogene, suggesting that the Plk2 null mammary phenotype could, in part, be due to the misregulation of Plk1. In support of a tumor suppressor function for Plk2, loss of Plk2 increased the formation of lesions in multiparous glands. Additionally, we find that Plk2 and Plk1 expression are inversely correlated particularly in human breast cancer and this inverse correlation correlates with disease-free survival. Collectively, these results demonstrate a novel role for Plk2 in regulating mammary gland development and as a tumor suppressor in mammary tumorigenesis.

Contributors: Kabotyanski, Elena; Montemayor, Celina; Dominguez-Vidana, Rocio; Creighton, Chad; Shaw, Chad; Westbrook, Thomas F.; Rosen, Jeffrey M.
Uterine adenogenesis is the development of uterine glands and it occurs in all mammalian uteri. Uterine glands are a unique postnatal process that occurs between Postnatal Day [P] 5 and P12 in mice. During P5, the luminal epithelium (LE) of the uterus will invaginate towards the myometrium and form the glandular epithelium (GE) buds. By P12, the endometrial glands will extend from the LE and extend into the nearby, surrounding stroma. Endometrial glands secrete substances (or histotrophs) that are essential for endometrial receptivity to the embryo, conceptus survival, implantation, development and growth. Comparative developmental biology of uterine glands has been reviewed in domestic animals (sheep, cattle and pigs), rodents (rats and mice) and humans. By understanding the fundamental development of gland formation through three-dimensional reconstructions, this information will improve the understanding of the physiology and pathophysiology of the female reproductive tract. Currently, the most common type of gynecologic malignancy is endometrial carcinoma and it also ranks as the second highest cause of gynecologic cancer mortality in the United States. According to the American Cancer Society in 2012, they have predicted that there will be 47,130 new cases and 8,010 deaths this year. The origins of endometrial carcinoma have been linked to uterine glands (adenocarcinomas) and from the supporting stroma (sarcomas). However, the molecular mechanism underlying uterine adenogenesis and homeostasis are currently unknown. By identifying the molecular pathways and factors involved in the initiation of uterine gland development, this vital information will give us insights into understanding the regulatory defects that may give rise to cancer and other disorders involved in gland dysregulations.
NEXT GENERATION SEQUENCING-BASED MOLECULAR DIAGNOSIS OF RETINITIS PIGMENTOSA: IDENTIFICATION OF A NOVEL GENOTYPE-PHENOTYPE CORRELATION AND CLINICAL REFINEMENTS

FENG WANG
Department of Molecular & Human Genetics
Advisor: Rui Chen, Ph.D.-Department of Molecular & Human Genetics

Retinitis pigmentosa (RP) is a devastating form of retinal degeneration, with significant social and professional consequences. Molecular genetic information is invaluable for an accurate clinical diagnosis of RP due to its high genetic and clinical heterogeneity. Using a gene capture panel that covers 163 of the currently known retinal disease genes, including 48 RP genes, we performed a comprehensive molecular screening in a collection of 123 RP unsettled probands from a wide variety of ethnic backgrounds, including 113 unrelated simplex and 10 autosomal recessive RP (arRP) cases. As a result, 64 mutations were identified in 47 probands, including 40 novel pathogenic alleles. Interestingly, we observed that phenotype and genotype were not in full agreement in 21 probands. Among them, eight probands were clinically reassessed, resulting in refinement of clinical diagnoses for six of these patients. Finally, recessive mutations in CLN3 were identified in five retinal degeneration patients, including four RP probands and one cone-rod dystrophy (CRD) patient, suggesting that CLN3 is a novel non-syndromic retinal disease gene. Collectively, our results underscore that, due to the high molecular and clinical heterogeneity of RP, comprehensive screening of all retinal disease genes is effective in identifying novel pathogenic mutations and provides an opportunity to discover new genotype-phenotype correlations. Information gained from this genetic screening will directly aid in patient diagnosis, prognosis, and treatment, as well as allowing appropriate family planning and counseling.

Contributors: Wang, Feng; Wang, Hui; Tuan, Han-Fang; Nguyen, Duy; Sun, Vincent; Keser, Vafa; Bowne, Sara; Sullivan, Lori; Luo, Hongrong; Zhao, Ling; Wang, Xia; Zaneveld, JacquesSalvo, Jason; Siddiqui, Sorath; Mao, Lois; Birch, David; Heckenlively, John; Wen, Cindy; Flagg, Ken; Ferreyra, Henry; Pei, Jacqueline; Khan, Ayesha; Ren, Huanan; Wang, Keqing; LOPEZ, Irma; Qamar, Raheel; Zenteno, Juan Carlos; Ayala-Ramirez, Raul; Buentello-Volante, Beatriz; Fu, Qing; Simpson, David; Li, Yumei; Sui, Ruifang; Silvestri, Giuliana; Daiger, Stephen; Koenekoop, Robert; Zhang, Kang; Chen, Rui
Currently there is great interest in detecting associations of complex human traits with rare SNVs using large scale sequence and exome genotyping array data. Different from association analysis of common SNPs in traditional genome wide association studies, sequence data specific quality control and variant annotations must be performed before association analysis. Statistical tests for rare variants association, which aggregate variants across a region, also differ from those applied to common variants where each variant is analyzed individually. We developed variant association tools (VAT), a tool-set that implements best practices for rare variant association studies. Major features of VAT include variant site/call level quality control, summary statistics, phenotype/genotype based sample selections, variant annotation, selection of variants for analysis and the implementation of rare variant association methods for analysis of qualitative and quantitative traits. We developed the VAT Ensemble algorithm, a regression based association testing framework which readily allows for flexible construction of association models with multiple covariates, weighting (using information from data or external annotations), interactions terms and models for pathway analysis. VAT is capable of rapidly scanning through data using multi-processes computation, adaptive permutation and simultaneously conducting multiple association tests. Results can be view as text or graphically. Additionally a programming interface is provided to readily facilitate user implementation of novel association methods. The VAT pipeline can be applied to sequence, imputed and genotyping array, e.g. exome chip data. VAT can perform association analyses on small to large scale complex trait studies making use of the latest genotyping and sequencing technologies.

Contributors: Wang, Gao; Peng, Bo; Leal, Suzanne M.
Transcription factors (TFs) regulate gene expression by binding in the vicinity of the gene and interacting with the transcriptional machinery of the cell. These interactions occur on a spatial scale (<100 nm) that is too small to be observed using conventional, diffraction-limited fluorescence microscopy. Stochastic Optical Reconstruction Microscopy (STORM) provides an improved resolution of ~10 nm. We will use STORM to quantify transcriptional regulation at a single gene locus. We will measure the dose-dependent binding of TFs to the promoter and how TF binding affects the transcriptional activity of the gene.

Contributors: Wang, Mengyu; Golding, Ido.
In the United States, heart attack and stroke are two major killers, which are the products of arterial thrombosis and thromboembolism. Platelets, blood cellular fragments that are key components in hemostasis, play a critical role in the thrombosis pathophysiology. Platelets adhere to the exposed subendothelium of ruptured atherosclerotic plaques, aggregate and thereby forming a life-threatening occlusive thrombus that blocks blood flow and kills the surrounding cardiac or neural tissue.

Platelet activation, which is obligatory to both hemostasis and thrombosis, catalyzes their granular contents, thereby propagating aggregation. Within this context, it is clear that a better understanding of the structural mechanisms underlying platelet activation will aid in the development of the appropriate interventions for thrombosis and bleeding disorders. However currently, the structural changes associated with platelet activation are poorly understood.

Although conventional electron microscopy reveals structural details of platelet, it fails to uncover platelet structure in its native solution state. Cryo-electron tomography (CET) is a cellular imaging technique that offers some great advantages over other methods, particularly in the study of platelet structure. CET allows direct visualization of cellular structures at molecular resolution. Importantly, CET could deconvolute platelet structure in its native solution state without chemical embedding and fixation. CET and time-dependent rapid vitrification methods also permits kinetic analysis of activation according to changes in morphology. Unlike conventional methods that require chemical pretreatment that distort structure, CET reveals platelet morphology in a native physiological state.

Contributors: Wang, Rui; Chiu, Wah; Michael Schmid; Dong, Jing-Fei; Wensheng Sun; Khant, Htet
The retromer complex is required for rhodopsin recycling and loss of retromer subunits leads to photoreceptor degeneration

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Program in Developmental Biology
Advisor: Hugo Bellen, Ph.D./D.V.M.-Department of Molecular & Human Genetics

Rhodopsins are G protein-coupled receptors that function as light sensors in photoreceptors (PR) and defective trafficking of rhodopsins can cause PR degeneration in humans and flies. Upon light exposure, activated rhodopsin 1 (Rh1, the major rhodopsin) in Drosophila is internalized via endocytosis and degraded in lysosomes. However, whether internalized Rh1 can be recycled and reused remains to be established. Here we report that loss of the subunits (Vps26 or Vps35) of the retromer, a conserved protein complex that retrieves proteins from endosomes, leads to light-induced PR degeneration.

As the retromer is primarily localized to endosomes, we determined if the endolysosomal pathway is affected in retromer mutants with transmission electron microscopy. In the absence of Vps26 or Vps35, the PRs exhibit a vast expansion of endosomes and lysosomes when the flies are kept in a regular dark-light regime, suggesting that the endolysosomal system is stressed. To understand if Rh1 trafficking is affected, we performed pulse-chase assays and Rh1 staining. We found that internalized Rh1 is trapped in late endosomes in Vps26 mutants, suggesting that the retromer is required to recycle Rh1 from the endolysosomal pathway.

Persistent Rh1 accumulation in the endolysosomal compartments can be toxic to PRs. To test whether accumulated Rh1 causes PR degeneration in retromer mutants, we reduced Rh1 endocytosis or Rh1 levels in Vps26 mutant flies. Both approaches significantly suppress defective light response in Vps26 alleles. Moreover, overexpressing Vps35 or Vps26 strongly suppresses PR degeneration caused by other mutations associated with Rh1 accumulation. These data suggest that the retromer is able to shunt Rh1 from the endolysosomal pathway and maintain PR integrity.

Since the visual transduction in vertebrates is conserved, we assessed if the retromer is expressed in mouse retina. X-gal staining on the retina of vps35 lacZ knock-in mice shows that vps35 is expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs). Moreover, expressing the human Vps26 proteins rescues the lethality and PR degeneration of Vps26 mutant flies, indicating a conserved function of Vps26 in the visual system. In summary, the retromer recycles Rh1 and prevents an overload of the endolysosomal pathway. It may also play a similar role in ipRGCs in vertebrates.

A novel disease-gene finder using case control next generation sequencing data

Xia Wang

Department of Molecular & Human Genetics
Advisor: Rui Chen, Ph.D.-Department of Molecular & Human Genetics

Study Objectives: It has been extensively reported that disease-causing genes can be successfully identified in large families. However, to find genetic causes for sporadic patients is challenging.

Methods: 228 sporadic Leber congenital amaurosis (LCA) patients and 997 control individuals whose phenotypes are unrelated with LCA were genotyped by whole-exome sequencing (WES). SNPs and Indels were called in each individual using our own bio-informatics pipeline. A novel method was developed to evaluate the load of pathogenic mutations in each gene, and to test the mutation load differences of each gene between cases and controls.

Results: A list of genes ranked by p-value was provided by our method. Several known LCA genes, such CEP290 and AIPL1, have top ranks in this list. Several novel candidate LCA genes were also identified by this method and will be further studied.

Conclusions: Our method can effectively identify both known and novel disease-causing genes in sporadic patient cohort with high genetic heterogeneity.

Contributors: Wang, Xia; Zhao, Lily; Wang, Fei; Chen, Rui.
Role of TgrB1/TgrC1 in Dictyostelium Development and Self Recognition

Yue Wang
Program in Structural and Computational Biology an Molecular Biophysics
Advisor: Gad Shaulsky, Ph.D.-Department of Molecular & Human Genetics

Dictyostelium, commonly known as social amoeba, live as single cells with nutrients available. Upon starvation, thousand of cells aggregate to form multicellular structures and develop into fruiting bodies. In the final fruiting body, there are two types of cells: 80% are spores in the sorus that can germinate if allowed, and the remaining 20% become vacuolized and dead, making up the supporting stalk. Due to the life-death difference, it is crucial for Dictyostelium cells to be able to distinguish between self and non-self. Our lab has identified TgrB1 and TgrC1 as the recognition molecules. Furthermore, we discovered that both tgrB1 and tgrC1 are required for normal development. Mutating either of the genes result in cells trapped in aggregates in early developmental stage. However, how tgrB1/tgrC1 incorporate the functions of recognition and development is yet unknown. My project is to perform suppressor screen in tgrC1- background to obtain genes that work in the tgrB1/tgrC1 pathway. Then we use mutations of these genes as tools to interrogate tgrB1/tgrC1’s role in development and cell recognition. Briefly, we introduce each of these mutations into different genetic backgrounds and ask the question how it affects the development of the parental strains, and how it affects self-recognition process when the mutated strain is mixed with parental strains or a strain with different tgrB1/C1 allele. Out of the four suppressor genes we have identified, we have observed that all of them are able to suppress tgrC1- developmental defect, but only one of them so far has been shown to be able to suppress tgrB1-developmental defect, suggesting tgrB1 and tgrC1 may have different functions in relaying the developmental signal. The abilities of these mutations in affecting self recognition are still under test.

Contributors: Wang, Yue; Benabentos, Rocio; Hirose, Shigenori; Kuspa, Adam; Shaulsky, Gad
HOXA1 DRIVES MELANOMA TUMOR GROWTH AND METASTASIS AND ELICITS AN INVASION GENE EXPRESSION SIGNATURE THAT PROGNOSTICATES CLINICAL OUTCOME.

Joanna Wardwell-Ozgo

Integrative Program in Molecular and Biomedical Sciences
Advisor: Kenneth Scott, Ph.D.-Department of Molecular & Human Genetics

The majority of melanoma patients present with no evidence of metastasis at diagnosis. For these patients, primary tumor excision generally confers a favorable prognosis. However, approximately 10% of patients with early stage tumors will succumb to metastatic disease. Improving staging criteria to include molecular-based screening is paramount to efficiently identifying patients with risk for metastasis. Currently we possess a limited understanding of the genetic events driving progression of melanoma, particularly those responsible for its metastatic propensity. To address this, we devised a screen of candidate metastasis genes which identified homeobox transcription factor A1 (HOXA1) as the top scoring pro-metastasis gene.

Transcriptome and pathway profiling analyses of HOXA1- and control expressing cells revealed up-regulation of factors involved in diverse cytokine pathways that include TGFβ signaling, which was demonstrated to be active and required for HOXA1-mediated cell invasion. Transcriptome profiling also showed HOXA1’s ability to potently down-regulate expression of microphthalmia-associated transcription factor (MITF) and other genes required for melanocyte differentiation, suggesting a mechanism by which HOXA1 expression de-differentiates cells into a pro-invasive cell state concomitant with TGFβ activation. Analysis of publicly available datasets indicated HOXA1-induced gene signature can stratify patient risk for metastasis based on expression in primary tumors independent of current staging criteria.

To identify HOXA1 effector genes, candidates were generated from examining HOXA1 transcriptome datasets were entered into a screen for functional drivers of in vivo tumorigenesis/metastasis using high-throughput approaches, leveraging our labs collection of ~32,000 sequence verified cDNA clones and molecular barcoding technologies. Top scoring candidates will be validated and their mechanisms-of-action determined.

In summary, the use of a novel screening platform will be employed to discover HOXA1 effector genes that drive melanoma tumorigenesis and metastasis. If successful, these studies may inform new drug targets and/or prognostic biomarkers critically needed for low stage, high-risk melanoma patients.

Contributors: Wardwell-Ozgo, Joanna; Zhang, Yiqun; Heffernan, Timothy P.; van Doorn, Remco; Creighton, Chad J.; Chin, Lynda; Scott, Kenneth L.
STAT3 SIGNALING IS ACTIVATED PREFERENTIALLY IN TUMOR-INITIATING CELLS IN CLAUDIN-LOW MODELS OF HUMAN BREAST CANCER

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Advisor: Michael Lewis, Ph.D.-Department of Molecular & Cellular Biology

Background: STAT3 mediated signaling is a critical regulator of tumor initiation. Aberrant STAT3 activity has been observed in about 50% of human breast tumors, and this activation shows evidence of specificity among different types of tumor cells, including populations proposed to include tumor-initiating cells. We hypothesize that STAT3 signaling is activated in tumor-initiating cells themselves, as well as surrounding niche cells, to support tumor growth.

Experimental design and methods: To purify populations of cells in which STAT3 signaling is activated, we constructed lentiviral fluorescent (EGFP) reporters, which enabled FACS-sorting of cells demonstrating STAT3 activity. We then compared tumor-initiating potential of STAT3 positive and negative cells by mammosphere formation efficiency assays (MSFE) and limiting dilution transplantation assays (LDT) using in vitro and xenograft-derived cells from claudin-low models of human breast tumor. Gene expression analyses (RNAseq) studies are underway to identify differentially expressed genes in the two populations which may be functionally significant.

Results: Using the STAT3-dependent fluorescent signaling reporter, we separated STAT3 positive and negative cells from breast tumor xenografts. We show that the positive population performs better in both MSFE and LDT assays than the negative population. RNAseq analysis is ongoing and may shed light on genes important for stem cell/niche regulation.

Conclusion: Our results suggest that cells with activated STAT3 signaling are enriched with tumor-initiating cells in claudin-low models of human xenograft tumors.

Contributors: Wei, Wei; Tweardy, David; Zhang, Mei; Roarty, Kevin; Rosen, Jeffrey; Lewis, Michael
CD133, also known as Prominin-1, is a pentaspan glycoprotein localized in plasma membrane protrusions. CD133 has not only been established as a stem/progenitor cell marker in many tissues, but also as a marker for cancer stem cells in various malignancies. Interestingly, the expression of CD133 is shown to be correlated with metastasis and poor prognosis in a series of cancer types. However, controversies still exist regarding its expression pattern and validation as a stem cell marker in the prostate. In this study, I will utilize a YFP reporter mouse model, CD133+/CreERT2/Rosa26-EYFP mouse, to clarify these discrepancies and reveal the property of CD133+ cells and the function of CD133 in the prostate.

First, I propose to examine the distribution pattern of CD133+ cells in murine prostate. My hypothesis is that CD133 is expressed in only a fraction of prostate basal and luminal cells. I will utilize a CD133-CreERT2 mouse model in which the tamoxifen-responding CreERT2 transgene is knocked into the endogenous CD133 locus. CD133-CreERT2 mice are bred with the R26-EYFP reporter mice. Then I will treat the bigenic progeny with tamoxifen so that YFP will be turned on only in CD133+ cells. To verify that CD133 and YFP are co-expressed in the same cells, I will conduct RNA in situ hybridization and flow cytometry analysis. After validating this model, I will use it to detect the expression pattern of CD133 in prostate epithelia through immunofluorescence assay and fluorescence-activated cell sorting assay.

Second, I will investigate whether CD133+ and CD133- cells are functionally different in the stem cell capacities. I aim to corroborate previously published data showing that CD133+ cells are enriched in prostate stem cells by using the CD133+/CreERT2/Rosa26-EYFP mouse model. FACS will be performed to sort YFP+/− basal cells, i.e. CD133+/− basal cells. Then individual cell fractions will be tested in the prostate sphere assay and prostate regeneration assay to compare their in vitro proliferative potentials and in vivo regeneration abilities, respectively. I will also use the lineage-tracing approach to determine the capacities of CD133+ and CD133- cells for generating new prostate epithelial cells in vivo.

Finally, I will interrogate the function of CD133 based on the hypothesis that CD133 plays an important role in prostate epithelial development, regeneration, and prostate tumor growth and metastasis. CD133 knockout mice will be used to examine whether the developmental dynamics of prostate epithelia are impaired during early development, and whether their regenerative potential is attenuated, via H&E staining and immunohistochemical analysis. Meanwhile, I will knockdown and overexpress CD133 in representative prostate cancer cell lines to study its effects on cell growth, migration and tumorigenesis in various in vitro and in vivo models. I will also determine whether CD133 single allele or both alleles knockout can affect the disease progression in prostate cancer mouse models such as the prostate-specific Pten null mouse model.

Contributors: Wei, Xing
The spatial and temporal expression of the progesterone receptor defines the window of receptivity in the murine uterus during early pregnancy

Margeaux Wetendorf
Integrative Program in Molecular and Biomedical Sciences
Advisor: Francesco De Mayo, Ph.D. - Department of Molecular & Cellular Biology

During pregnancy, the mammalian embryo attaches and invades into the uterine epithelium at a finite time after ovulation known as the window of receptivity. The timing of the window is controlled by the ovarian steroids, estrogen and progesterone, which signal through their cognate nuclear receptors, the estrogen receptor (Esr1) and progesterone receptor (Pgr). The cell specific expression of Pgr in the uterus varies with the stage of pregnancy. Initially, Pgr is expressed in the epithelial compartment of the uterus prior to the window of receptivity. At the time of implantation, Pgr expression is lost from the epithelial cells and expressed predominantly in the stromal cell compartment. This decrease in Pgr expression in the epithelial compartment is thought to mark the beginning of the window of receptivity and has further been hypothesized to be critical for proper implantation. In order to test this hypothesis, we generated a mouse model in which Pgr is constitutively expressed through the window of receptivity. This model consists of a ubiquitously expressing chicken beta actin cytomegalovirus fusion promoter (CAAG) upstream of a lox-stop-lox cassette and Pgr coding sequence, with the entire construct targeted to the ROSA26 locus. Pgr expression was activated in the whole uterus and uterine epithelium by crossing the mice to the Pgrcre and Wnt7acre mice. Expression of the Pgr in both models rendered the mice sterile due to a failure of the embryo to attach and invade the uterine epithelium. Gene expression analysis of these mice demonstrated a decreased expression of the estrogen target, leukemia inhibitory factor (Lif). Lif is important for the regulation of the window of receptivity. Although Lif is not directly regulated by the Pgr as shown in our laboratory’s chromatin immunoprecipitation sequencing assay, the Lif promoter contains many transcription factor family binding sites including Ets, NF-κB, Nfat and AP-1. Interestingly, many of these transcription factors are direct targets of Pgr, suggesting that increased levels of the Pgr could be acting in an inhibitory manner to modulate the transcription of Lif. Through the use of these novel mouse models, we have concluded that the spatio-temporal expression of the Pgr before the window of receptivity is necessary for proper embryo attachment and that constitutive expression of the Pgr detrimentally affects Lif. Future directions include determining the mechanism behind how Pgr regulates Lif during early implantation.

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THE ROLE OF KU IN TELOMERE LENGTH MAINTENANCE

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Advisor: Alison Bertuch, M.D./Ph.D.-Department of Pediatrics

The budding yeast Ku heterodimer positively regulates telomere length in a telomerase-dependent manner. Ku associates with telomeric DNA and the RNA subunit of telomerase, TLC1, but not simultaneously, and promotes nuclear retention of TLC1 and the recruitment of telomerase to telomeres. Recruitment of telomerase by Ku, however, is insufficient to maintain telomeres, as a pathway dependent on the telomeric proteins Cdc13 and Est1 is necessary. Therefore, the principal role of Ku in telomere length maintenance remains unclear. Here we show forced recruitment of telomerase to telomeres via a fusion between Cdc13 and the telomerase catalytic subunit, Est2, does not promote efficient telomere elongation in the absence of Ku or Ku-TLC1 interaction. Additionally, we show the influence of Ku on TLC1 nuclear localization does not require Ku’s association with telomeric ends. Furthermore restoration of TLC1 nuclear localization, even when combined with Est2 recruitment, does not bypass the role Ku. In contrast, tethering Est1 to telomeres promotes efficient and progressive telomere elongation in the absence of Ku or Ku-TLC1 interaction. We also show Ku enhances Est1 recruitment to telomeres independently of Est2. Together, these results suggest Ku principally impacts telomere elongation via Est1 rather than TLC1 localization or Est2 recruitment.

Contributors: Williams, Jaime; Ouenzar, Faissal; Chartrand, Pascal; Bertuch, Alison
TARGETING SOLID TUMORS AND THEIR VASCULATURE WITH GENETICALLY MODIFIED T CELLS

LaTerrica Chemise Williams
Program in Translational Biology & Molecular Medicine
Advisor: Stephen Gottschalk, M.D.-Department of Pediatrics
Xiao-Tong Song, Ph.D.-Department of Pathology & Immunology

This project’s objective is to improve current T-cell therapy approaches for solid tumors by targeting not only the malignant cells but also the tumor-growth supporting vascular bed. The outcome of patients with advanced, metastatic solid tumors remain poor, and patients who survive suffer from acute and long-term related complications from treatments such as chemotherapy and radiation. Thus, new, targeted approaches are needed to improve patients’ outcome without increasing treatment-related toxicities and morbidities. Since T-cell based immunotherapies are highly tumor-specific and cause minimal bystander cell damage they have the potential to fulfill these needs.

In early clinical studies T cells genetically engineered to express chimeric antigen receptors (CARs) have shown antitumor activity. However their overall antitumor efficacy was limited, especially for solid tumors. This lack of efficacy is most likely due to several factors including a) emergence of immune escape mutants, and b) inability of tumor-specific T cells to recognize and destroy the vascular bed of solid tumors, which is critical for their malignant growth. While we are already pursuing strategies to target multiple tumor antigens expressed in solid tumors, the central hypothesis of this research project is that targeting the tumor vasculature in addition to the tumor cells themselves will enhance the antitumor efficacy of adoptively transferred T cells.

Tumor endothelial markers (TEMs) are overexpressed in the neovasculature of many solid tumors and are ideal targets for T-cell Therapy, since their expression in normal tissue is limited. This project is focused on 2 members of the TEM family, TEM1 and TEM8, which are expressed in a broad range of solid tumors including lung and breast cancer. To test our hypothesis we propose to generate TEM1- and TEM8-specific CARs and compare their function ex vivo (Aim 1) and in vivo (Aim 2). In Aim 3 we will then evaluate if T cells expressing a CAR specific for a tumor antigen (EphA2) and our optimized TEM-specific CAR will have enhanced antitumor activity in comparison to T cells that either target the tumor or the tumor vasculature.

We expect at the conclusion of this proposal to have developed a 2-pronged T-cell Therapy approach that effectively eradicates solid tumors in vivo by targeting tumor cells and endothelial cells of the vascular bed. If successful this strategy could be readily incorporated into our ongoing T-cell therapy clinical trials for solid tumors.

Contributors: Gottschalk, Stephen; Song, Xiao-Tong
The two known functional classes of cancer genes are the “gatekeepers”, which when mutated, cause cells to behave as cancer cells, and the “genomic caretakers”, which are DNA repair genes that when mutated result in high mutation rates, which cause cancer. Virtually all caretaker genes are DNA repair genes and the damage they handle is thought to result from endogenous cellular processes. Yet the origins of endogenous DNA damage remain largely unknown. We hypothesize the existence of a network of genes/proteins that control steady-state levels of endogenous DNA damage in Escherichia coli, and that deregulation of these damage-control genes leads to genome instability, and in humans, to cancer. We developed a high-throughput screen strategy to find these “damage-control” genes in Escherichia coli using specifically engineered cells that fluoresce red when they experience DNA damage. Using an ordered E. coli overexpression library to model many human cancer-causing mutations which are gene amplifications, we have identified an overexpression damage-control network of 214 damage-up and 24-damage-down genes that cause these phenotypes when overexpressed. All have been validated by sensitive flow-cytometric methods and are now bona-fide damage-control overproduction clones. We have shown that these DNA damage-control genes, when overexpressed, overwhelm DNA repair pathways, presumably due to excessive DNA damage.

We will perform powerful secondary screens to provide an initial functional grouping of how the damage-control proteins promote or inhibit endogenous DNA damage, and clarify the relationship between damage-control and genomic-instability phenotypes for the many genes identified by mutation assays to measure base-substitution, indel mutation rates and gross chromosomal rearrangement mutation rate. To conclude, we have already identified the overexpression damage-control gene network and have shown that DNA repair pathways are overwhelmed in many of these overexpression strains. We will further characterize how the damage-control proteins promote or inhibit endogenous DNA damage and determine whether they are regulators of genome instability. We expect that many cancer genes of previously unknown function will be homologues/counterparts of these damage-control genes, and further work will identify these in cancer genome databases.

Contributors: Xia, Jun; Nehring, Ralf; Frisch, Ryan; Gibson, Janet; Rosenberg, Susan
Proper development of the cerebellum is highly dependent on the basic helix loop helix (bHLH) transcription factor Atoh1. Previous studies on Atoh1 have demonstrated its requirement for both proliferation and differentiation of cerebellar granule cell precursors (CGPs), which give rise to all granule cells of the mature cerebellum. In addition, our lab has demonstrated the requirement of Atoh1 for the formation of Sonic hedgehog-driven medulloblastoma, the most common solid pediatric brain tumor. How Atoh1 promotes both cell proliferation and differentiation is not known. A better understanding of this mechanism not only expands our basic understanding of how a transcription factor can regulate seemingly opposing functions, but will also shed light on the mechanism of medulloblastoma formation.

We hypothesized that phosphorylation of Atoh1 regulates its function, ultimately affecting CGP proliferation and differentiation status. Based on evolutionary conservation, serine 193 (S193) emerged as a potential phosphorylation site for regulation of Atoh1 function. Studies in our lab demonstrate that mutating S193 to a non-phosphorylatable alanine decreases Atoh1 transcriptional activity in vitro while mutating S193 to a phosphomimetic aspartic acid residue abolishes transcriptional activity. Further studies show that while the protein stability and E protein dimerization properties of the S193 phosphomutants are unchanged, the DNA binding properties of these phosphomutants are altered. In sum, our results point to a pivotal role of S193 phosphorylation on Atoh1 function. Therefore we are in the process of generating a phosphomutant knock in mouse that allows us to investigate the role of S193 in vivo. In addition, we hypothesized that different binding partners could also regulate Atoh1 function. We identified many putative Atoh1 interactors through an in vivo immunoprecipitation and mass spectrometry screen. After narrowing down the list of potential interactors, we chose to focus on Nfia, a transcriptional factor that has previously been shown to be important for CGP differentiation. To investigate functional interaction between the two proteins, we are generating Atoh1 and Nfia double heterozygous mice.

In the future, we will investigate the effects of S193 phosphorylation in both proliferating and differentiating CGPs. We will also investigate the effect of the interaction between Atoh1 and Nfia on CGP differentiation. In sum, our studies will shed light on the underlying mechanism in which one transcription factor drives the differentiation process from precursor to terminally differentiated neurons.

Contributors: Xie, Wei; Klisch, Tiemo; Zoghbi, Huda
CHARACTERIZING THE ROLE OF THE HER2 L755S MUTATION IN LAPATINIB RESISTANCE OF HER2+ BREAST CANCER

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Advisor: Rachel Schiff, Ph.D.-Department of Medicine

HER2-targeting agents, such as the HER2 monoclonal antibody trastuzumab (T) and the dual HER1/2 tyrosine kinase inhibitor lapatinib (L), have shown great efficacy in HER2+ breast cancer (BC) patients. Yet resistance commonly exists. To investigate resistance mechanisms, our lab has developed a large panel of HER2+ BC cell lines made resistant to L, T, or L+T, and has begun to molecularly profile these cells on a variety of platforms.

We have previously shown that acquired resistance to the HER1/2 tyrosine kinase inhibitor (TKI) lapatinib (LR) appears in two phases in the ER+/HER2+ BT474AZ cell line model. In the early phase of LR (ELR), the HER pathway remains inhibited and the cells activate the ER pathway for survival. In the late phase of LR (LLR), cell growth rate increases and the cells reactivate the HER receptor layer, no longer relying on ER for survival. Interestingly, whole-exome sequencing of our HER2+ cell line panel revealed two HER2 mutations (G572V, L755S) unique to BT474AZ LLR cells. The L755S mutation has been reported to confer LR by a random site-mutagenesis screen in Ba/F3 cells and reported at low frequency (2%) in HER2+ breast cancer patients by the cancer genome atlas (TCGA). Therefore, we hypothesize that in LLR cells, HER2 L755S mutation activates the HER receptor layer and causes LR by inducing an active conformation of HER2 which prevents binding of L. Our hypothesis also includes that this mutation originates by selection from rare clones pre-existing in parental cells by long term L treatment.

Applying a self-developed nested-Q-PCR assay that specifically amplifies mutant DNA, I have detected high HER2 L755S mutation levels in BT474AZ LLR cells, low L755S levels in BT474AZ parental cells, and high L755S levels in LTR cells of BT474ATCC model as well. This finding supports the scenario of two independent clonal selection processes of this mutation in BT474AZ/ATCC models and also excluded the possibility that it was related to the specific AZ subclone of the BT474 cell line. Importantly, we have found that the irreversible inhibitor afatinib (Af), which binds both active and inactive conformations of HER2 kinase domain, showed robust efficacy in inhibiting LLR cell growth (Af IC50 0.02µM vs. L IC50 3.3 µM). The potent inhibitory effect of Af in LLR supports the hypothesis that the HER2 L755S mutation is the driver of resistance in LLR cells. Afatinib might serve as a more effective alternative to Lapatinib in HER2+ BC patients with the HER2 L755S somatic mutation in tumors.

Contributors: Xu, Xiaowei 1,2; Hu, Huizhong 1,2; Nardone, Agostina 1,2; Nanda, Sarmistha 1,2; Osborne, C. Kent 1,2,3,4; Schiff, Rachel 1,2,3,4

1Lester and Sue Smith Breast Center and Dan L Duncan Cancer Center, 2Verna and Marrs McLean Department of Biochemistry and Molecular Biology, and Departments of 3Medicine, and 4Molecular and Cellular Biology Baylor College of Medicine, Houston, TX.
Human effector memory T lymphocytes express pre-existing hypoxia-adaptation genes and are resistant to hypoxia

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Department of Pathology & Immunology
Advisor: Gianpietro Dotti, M.D.-Department of Medicine

Hypoxia that is a hallmark of solid tumors is considered an inhibitory mechanism that hampers the clinical benefits of T-cell-based immunotherapy in cancer patients. Studies have shown indeed that circulating human peripheral blood (PB) T cells activated in hypoxia have reduced proliferation and survival. However, antigen-specific T cells generated ex vivo and adoptively transferred in cancer patients are mostly (>80%) effector memory T cells (TEM) while this subset represents <20% of total circulating PB T cells. How hypoxia selectively influences TEM cells has not been studied. In sharp contrast with T cells from PB, we found that proliferation, survival and cytotoxic functions of ex vivo expanded T cells are augmented in hypoxia. Importantly, we found that TEM cells directly isolated from PB show properties similar to those of expanded T cells when compared to isolated naïve and central memory cells, suggesting that the resistance to hypoxia is an intrinsic property of TEM. Finally, resistance to hypoxia is associated with an increased hypoxia-adaptation genes. Expression of HIF-1a and downstream glycolytic genes are indeed higher in TEM cells, suggesting a genetic "pre-adaptation status" of TEM to hypoxia. Together, we show that primary TEM and ex vivo generated antigen-specific T cells are resistant to hypoxia, and this finding has relevant implication for the clinical translation of adoptive immunotherapy in cancer patients.

Contributors: Savoldo Barbara; Gianpietro Dotti.
While we have a cartoon picture suggesting that non-muscle actin/Myosin-2 (actomyosin) arrays contract like muscle sarcomeres, many molecular details of this contraction remain unclear. For example, we do not know why the contractile rings in cells undergoing cytokinesis do not get thicker or accumulate increasing actin density as they contract. In addition, recent studies suggest that Myosin-2 motor activity is not required for successful cytokinesis, leaving the mechanism of contraction in question.

To better understand the mechanism of actomyosin ring contraction, I am studying Drosophila cellularization, which is a modified cytokinetic event. I find that the process of actomyosin ring contraction during cellularization can be separated into two morphologically and mechanistically distinct phases, called Phase 1 and 2. Using a genetic mutant of the regulatory light chain of Myosin-2 (Spaghetti-squash; Sqh), I find that Phase 1 of actomyosin ring contraction requires Myosin-2 motor activity, but Phase 2 does not. Quantitative live and fixed cell imaging shows that Phase 1 of contraction is accompanied by an increase in actin levels, while Phase 2 is accompanied by a decrease in actin levels. Based on these results, I propose a bi-phasic model for actomyosin ring contraction during Drosophila cellularization. I hypothesize that Phase 1 proceeds like contraction in the muscle sarcomere, but Phase 2 contraction is driven by actin depolymerization.

Contributors: Xue, Zenghui; Sokac, Anna Marie.
APPLICATION OF SPECKLE-TRACKING IN THE EVALUATION OF CAROTID ARTERY FUNCTION IN SUBJECTS WITH HYPERTENSION AND DIABETES

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*Clinical Scientist Training Program*  
*Advisor: Christie Ballantyne, M.D.-Department of Medicine*

**BACKGROUND:** Speckle-tracking enables direct tracking of carotid arterial wall motion. Timing intervals determined with carotid speckle-tracking and slopes calculated from carotid artery area versus cardiac cycle curves may provide further information on arterial function and stiffness. The proposed arterial stiffness parameters were examined in healthy controls (n = 20), non-diabetic patients with hypertension (n = 20), and patients with type 2 diabetes (n = 21).

**METHODS:** Bilateral electrocardiographically gated ultrasonograms of the distal common carotid artery were acquired using a 12-MHz vascular probe. Four timing intervals were derived from speckle-tracked carotid arterial strain curves: (1) carotid predistension period, (2) peak carotid arterial strain time, (3) arterial distension period, and (4) arterial diastolic time. In addition, carotid artery area curves were recorded over the cardiac cycle and subdivided into four segments, S1 to S4, relating to arterial distention and contraction periods.

**RESULTS:** Mean far wall predistension period and peak carotid arterial strain time were more delayed in patients with diabetes and hypertension than in controls. Global mean arterial distension period was prolonged and arterial diastolic time was shorter in patients with hypertension and diabetes than in controls. Slopes of segments S2 and S4 were markedly steeper in the combined group of patients with hypertension and diabetes compared with healthy controls (P = .03 and P = .02, respectively).

**CONCLUSIONS:** Speckle-tracking-based measures of arterial stiffness may provide potential additive value in assessing vascular function in patients at risk for cardiovascular disease.

Contributors: Yang, Eric Y; Brunner, Gerd; Dokainish, Hisham; Hartley, Craig J; Taffet, George; Lakkis, Nasser; Taylor, Addison A; Misra, Arunima; McCulloch, Marti L; Morrisett, Joel D; Virani, Salim S; Ballantyne, Christie M; Nagueh, Sherif F; Nambi, Vijay
Dnmt3a-deletion accelerates FLT3-ITD induced T-ALL in mice by hypomethylation of enhancer sites; implications for therapy

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Advisor: Margaret Goodell, Ph.D.-Department of Pediatrics

The de novo DNA methyltransferase (DNMT) 3A is mutated in 50% of patients with mixed phenotype acute leukemia, 20% with AML and 18% with T-ALL. In mice, deletion of Dnmt3a in hematopoietic stem cells (HSCs) leads to a block in differentiation and abnormal DNA methylation, but alone is insufficient for leukemic transformation. To study the role of Dnmt3a in leukemia, we combined Dnmt3a-null mice with the activated FLT3 proto-oncogene (FLT3-ITD), a frequent co-mutation with DNMT3A in patients, to establish a murine model of Dnmt3a-associated malignancy.

In mice transplanted with Dnmt3a-knockout (KO) or wild-type (WT) bone marrow cells transduced with a retrovirus bearing FLT3-ITD, Dnmt3a-loss dramatically impacted the disease phenotype. Dnmt3aKO/ITD transplanted mice had significantly shortened survival (79 days vs. 116 days) and increased rate of acute leukemia compared to mice with ITD alone (50% vs. 22%). Mice developed CD4+CD8+ T-ALL or a myeloproliferative disease (MPD), or concurrently both in the bone marrow. To determine the leukemia-initiating population, we transplanted sorted HSC, myeloid, and lymphoid progenitors transduced with FLT3-ITD. All mice transplanted with HSC and myeloid progenitors succumbed to malignancies, suggesting that multiple populations are capable of generating leukemia. To determine the mechanisms by which Dnmt3a-deletion accelerates leukemia, we analyzed changes in DNA methylation in T-ALL blasts by whole genome bisulfite sequencing. Compared to Dnmt3aWT/ITD blasts, Dnmt3aKO/ITD blasts exhibited global hypomethylation with the greatest changes at distal enhancer sites associated with binding sites of transcription factors PU.1, Gfi1, and Ikaros1. Gene expression analysis by RNA-seq revealed upregulation of HSC fingerprint genes as well as myeloid genes, reminiscent of early T-cell precursor leukemia. Increasingly, hypomethylating agents are being used to treat myeloid malignancies, including those associated with DNMT3A, so we examined the impact of decitabine treatment on the Dnmt3aKO/ITD mice with T-ALL. Treatment led to reduced presence of ITD-transduced KO cells and increased survival.

In summary, ablation of Dnmt3a in the bone marrow cells accelerated T-ALL and MPN induced by FLT3-ITD overexpression, and is associated with global hypomethylation, specifically at enhancer sites for transcriptional regulators. The malignancies were responsive to decitabine therapy, which has implications for clinical therapeutic interventions. This novel mouse model can be used to study the mechanisms of Dnmt3a-associated hypomethylation and hematologic malignancies.

Contributors: Yang, Liubin; Rodriguez, Benjamin; Luo, Min; Jeong, Mira, Curry, Choladda; Ruau, David; Zhang, Xiaotian; Challen, Grant; Rau, Rachel; Gottgens, Berthold; Li, Wei; Goodell, Margaret
Correlations between the spiking activity of pairs of neurons are among the most familiar descriptive statistics of neural activity. Multineuronal recordings provide richer information than the equivalent number of neuron pairs. For example, the full covariance matrix reveals correlated activity across the entire population as well as partial correlations between pairs. Estimation of covariance matrices can be improved by regularization, i.e. by imposing some kind of structure. Optimal regularization must be determined empirically since its effect depends on how closely the imposed structure matches the underlying regularities. For example, we can use low-rank parameterizations of the covariance matrix to account for common fluctuations across the recorded population. Conversely, if correlations are strongly influenced by a small fraction of pairwise linear associations between the observed neurons, we can impose sparsity on the inverse covariance matrix. We can also use a `sparse+low rank' inverse covariance representation to account for both common fluctuations and pairwise interactions.

To select the optimal structure of neural correlations in a local neural circuit, we compared the performance of several covariance estimators on the activity of 100–300 neurons in mouse visual cortex: sample covariance, covariance shrinkage, factor analysis, sparse inverse covariance, and sparse+low-rank inverse covariance. We inferred instantaneous firing rates in 200 ms bins from the somatic calcium signals acquired with fast 3D random-access two-photon microscopy. Each covariance estimator was optimized and evaluated by cross-validation. As expected, covariance shrinkage reliably outperformed the sample covariance estimate. In turn, factor analysis-based estimates significantly outperformed covariance shrinkage. Yet sparse inverse covariance with or without an additional low-rank component significantly outperformed both factor analysis and shrinkage estimators. The superior performance of the sparse inverse covariance estimator suggests the relative importance of detailed network interactions over common diffuse input in the circuit we studied.

Contributors: Yatsenko, Dimitri; Froudarakis, Emmanouil; Ecker, Alexander; Cotton, R. James; Josic, Kresimir; Tolias, Andreas
Tubulogenesis is a fundamental developmental process for tissue and organ formation. Several tubulogenesis mechanisms have been extensively studied, such as those in neural tube, lung and kidney formation. However, the formation of the Müllerian duct (female reproductive tract primordium) occurs in a distinct manner – its formation depends on an adjacent tube, the Woffian duct. The cellular and molecular mechanisms of this type of tubulogenesis are not fully understood. To determine the mechanisms of Müllerian duct formation, we will characterize cell behaviors by time-lapse imaging of Wnt7a-Cre; R26R-RG mouse embryonic urogenital organs in ex vivo culture. WNT9B produced by the adjacent Wolffian duct is required for Müllerian duct elongation. To determine if WNT9B directs the direction of Müllerian duct elongation, we will ectopically express Wnt9b using a novel photo-activatable Cre system and examine Müllerian duct elongation. These studies should help define the mechanisms of Müllerian tubulogenesis for female reproductive tract development.

Contributors: Yen, Shuo-Ting; Huang, Cheng-Chiu; Behringer, Richard
WILD TYPE PROGENITORS DIVIDE AND DIFFERENTIATE NORMALLY IN AN AMYLOID-RICH ENVIRONMENT

Michael Joseph Yetman
Department of Neuroscience
Advisor: Joanna Jankowsky, Ph.D.-Department of Neuroscience

Adult neurogenesis is modulated by a delicate balance of extrinsic signals and intrinsic responses that maintain lifelong production of new granule cells in the hippocampus. Disorders that disrupt the proliferative niche can impair this process, and substantial alterations in adult neurogenesis have been described in human autopsy tissue and transgenic mouse models of Alzheimer’s disease. Because exogenous application of aggregated Aβ peptide is neurotoxic in vitro and extracellular Aβ deposits are the main pathological feature recapitulated by mouse models, cell-extrinsic effects of Aβ accumulation were thought to underlie the breakdown of hippocampal neurogenesis observed in Alzheimer’s models. We tested this hypothesis using a bigenic mouse in which transgenic expression of amyloid precursor protein (APP) was restricted to mature projection neurons. These mice allowed us to examine how wild-type neural progenitor cells responded to high levels of Aβ released from neighboring granule neurons. We quantified the number of Ki67-positive dividing precursors, doublecortin-positive immature neurons, and BrdU-positive cells at 7 or 30 days after injection in the subgranular zone of bigenic mice with APP overexpression and their single and non-transgenic siblings. We find that hippocampal neurogenesis is unaffected in the APP bigenic mice, despite abundant amyloid pathology and robust neuroinflammation. Our findings suggest that Aβ accumulation is not sufficient to impair adult hippocampal neurogenesis, and that factors other than (or in addition to) amyloid pathology may account for the neurogenic deficits observed in transgenic models with more widespread APP expression.

Contributors: Yetman, Michael; Jankowsky, Joanna
Regressive autism is a group of disorders that are characterized by loss of speech and social skills in a relatively short period in children of 1-2 years old after normal development. Based on current meta-analysis, one-third of children with autism spectrum disorder (ASD) may have experienced significant regression. A study published in International Society of Autism Research in 2012 suggested that autistic regression might have a genetic component. However, mutations in MECP2 are the only established genetic causes of regressive autism.

Recently, Dr. Zoghbi and Dr. Schaaf identified a patient with autistic regression. She developed normally, if not advanced, until two and a half years old, and then experienced profound regression in language, cognition, and social skills within the following two years. She had no mutation in the MECP2 gene. Through whole genome sequencing, we found compound heterozygous nonsense mutations in the TMPRSS9 gene in the patient, and her parents were both heterozygous for TMPRSS9 mutation. Based on this sequencing result, rarity of truncating TMPRSS9 in the control population, and the fact that another gene in the transmembrane serine protease family plays a critical role in neural development, we hypothesized that TMPRSS9 may be important for wiring of the neural circuits and its loss of function may lead to autistic regression.

Based on literature, very little is known about the expression and function of TMPRSS9 and its association with neurological disorders. We started from characterizing its expression pattern. qRT-PCR on multiple tissues from mice after birth showed that TMPRSS9 is expressed in various tissues, with a relatively higher expression in the kidney and low expression in the brain, liver and testis. To examine its expression in earlier stage, I will perform qRT-PCR on the brain and kidney from mice at different embryonic stages.

In order to pinpoint the subcellular localization of TMPRSS9, I transfected EGFP- or 3Flag-tagged TMPRSS9 into multiple cell-lines. Western blot of subcellular protein fractionations and immunofluorescence of the fusion protein co-stained with different cellular markers suggest that TMPRSS9 localizes to the endoplasmic reticulum membrane. Next, I cultured primary mouse hippocampal neurons and transfected with tagged TMPRSS9. Co-staining with synaptic markers is undergoing to specify its localization in neurons.

Meanwhile, we are generating knockout mice to see if they recapitulate the phenotypes seen in the human patient, and transgenic mice that express tagged TMPRSS9 from modified bacterial artificial chromosome (BAC) to further study the function of TMPRSS9. Contributors: Yin, Jiani; Chen, Chun-An; Sabo, Aniko; Gibbs, Richard; Zoghbi, Huda; Schaaf, Christian
Pancreatic cancer (PC) is the 4th leading cause of cancer death in the US with a 5-year survival rate of less than 6%. Due to the asymptomatic nature of PC, most diagnosed cases are advanced cancers with no option of surgical resection. Hence, there is an urgent need for early diagnosis and more effective treatments for PC. A recent report in Nature published by our collaborators has uncovered an increased copy number and/or mutations in axon guidance genes in the pancreatic adenocarcinoma patient tumor genomes, one of which is SEMA3E that codes for the glycoprotein semaphorin-3E (sema3E). Semaphorins are involved in a wide variety of processes including axonal growth, cell migration, immune responses, angiogenesis, and cancer progression. Several recent reports have implicated sema3E in metastasis of cancer, primarily in promoting extracellular matrix invasion, transendothelial migration, and extravasation of cancer cells. Our qRT-PCR analysis of patient samples found that Sema3E expression was elevated in tumors as compared to matched normal tissue. In the spontaneous Pdx-1-Cre; LSL-K-rasG12D/+; LSL-p53R172H/+ (KPC) mouse model, we found that sema3E was overexpressed in ductal epithelial tumor cells via immunohistochemistry staining. We hypothesize that sema3E may facilitate tumor progression and metastasis of PC, and we are currently investigating the effects of sema3E on PC cell proliferation, migration and invasive potential in vitro, and tumorigenic potential in vivo. Several semaphorin molecules have been reported to have functions in immune cell modulation and migration in the context of cancer. Sema3B has been found to facilitate metastasis by inducing tumor production of IL-8, which recruits tumor-associated macrophages into the tumor microenvironment. Sema3A, also highly expressed in some tumors, was found to suppress T-cell activation via binding to neuropilin-1. Sema3E itself is expressed by thymic epithelial cells in the medulla; upon binding to plexin-D1 on double positive thymocytes, it inhibits migration of these cells into the cortex. Moreover, sema3E has recently been found to be expressed by inflammatory macrophages in atherosclerotic plaques and functions to retain these macrophages in plaques. Taken altogether, we hypothesize that sema3E may function to promote tumor progression via modulating the functions and migration of immune cells, particularly M1 and M2 macrophages, in the tumor microenvironment. We are currently investigating the effects of sema3E on the differentiation of RAW 264.7 macrophages to M1/M2 macrophages in vitro, as well as macrophage migration. In all, a better understanding of the roles of Sema3E in progression and metastasis of PC will help to develop novel therapeutic strategies for PC.

Contributors: Yong, Lin-Kin; Fisher, William; Yao, Qizhi
CARBON BLACK-INDUCED LUNG INFLAMMATION IN EMPHYSEMA AND LUNG CANCER

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Advisor: Farrah Kheradmand, M.D.-Department of Medicine

Environmental pollutants and cigarette smoke are major causative factors for lung diseases, such as COPD (chronic obstructive pulmonary diseases) and lung cancer, which are respectively the 3rd leading cause of death and the 1st cancer-related death, however the shared pathogenic substance by all risk factors for these diseases is still unknown. In our study, carbon black (CB), as the component of coal mine dust, cigarette smoke and airborne particles, is found to deposit in the lung antigen-presenting cells (APCs) of emphysema/COPD patients. Therefore, we hypothesized that CB-induced activation of APCs and lung inflammation promote progression of emphysema and lung cancer.

By using mouse model with the intranasal challenge of CB, we found that CB-challenged mice developed similar immunopathological changes in emphysema patients and mice exposed to chronic smoke including enlarged lung volume, infiltration of immune cells into the lungs, upregulated disease-related gene and strong Th17 responses. Furthermore, by comparing the soluble nanoparticles (oxidized-carbon black (OCB), polyethylene glycol (PEG)-CB) (hydrophilic) and elemental CB (hydrophobic), we found that hydrophobic CB induced more severe inflammation and emphysema development indicating that surface features of CB contribute to its pathogenesis. Additionally, CB-treated APCs were potent in promotion of Th17 responses by upregulation of MHC II and IL-6 and IL-1β, two critical cytokines in Th17 differentiation. Inhibition of caspase-1 attenuated the CB-induced APC activation, indicating the importance of inflammasomes and caspase-1-dependent cell death in this process. Together, hydrophobic CB can induce emphysema in mice by the activation of lung APCs.

More interestingly, CB-induced inflammation also promotes lung cancer progression. We used a new mouse model of lung tumor in which two potent tumor suppressive gene, PTEN and SMAD4 are deleted in the airway (Ptend/dSmad4d/d mice). These mice develop invasive adeno-squamous carcinoma at 9 months that can spontaneously metastasize to stomach at one year. Surprisingly, CB-challenged Ptend/dSmad4d/d mice developed both lung tumor and stomach metastasis at the early age of 5 to 6 months. Moreover, in these mice, increased amount of lung APCs corresponds with the elevated Th1 and Th17 responses. In all, CB, as the shared pathogenic substance in environmental pollutants and cigarette smoke, induces severe inflammation and promotes emphysema and early lung cancer progression. Potential mechanisms of CB-induced APC activation revealed in our study provide new insights into the immunopathogenesis of lung diseases caused by environmental risk factors.

Contributors: You,Ran; Shan,Ming; Seryshev,Alexander; Lu,Wen; Cho,Sungnam; Marcano,Daniela; Demayo,Francesco; Tour,James; Corry,David; Kheradmand,Farrah
ALTERATIONS IN THE HUMAN GUT MICROBIOME ASSOCIATED WITH TRAVELERS’ DIARRHEA

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The human body is home to a diverse community of bacteria, a majority of which is found in the gastrointestinal (GI) tract. Some bacterial species within the gut microbiota perform functions that host cells cannot, such as metabolism of complex polysaccharides and biosynthesis of vitamins. Alterations in the gut microbial community have been correlated with diseases such as obesity and inflammatory bowel disease.

Secretory diarrhea is another mechanism by which alterations to the GI community can occur. Up to 60% of individuals traveling from industrialized to developing countries acquire a form of secretory diarrhea known as Travelers’ Diarrhea (TD). Enterotoxigenic Escherichia coli (ETEC), the leading cause of bacterial TD, expresses two toxins, heat labile toxin and heat stable toxin, which ultimately lead to secretory diarrhea. Norovirus, which is the predominant viral etiology of TD, results in diarrhea via an unknown mechanism, which is initiated through interactions with histo-blood group antigens. Presumably, TD causes alterations in the microbial GI community, however the effect of TD on the GI microbiota has not been studied.

Bacterial 16S rDNA 454 sequencing followed by operational taxonomic unit (OTU)-based analysis was used to examine and compare the GI microbiome of individuals traveling to Central America and India. Stool samples were collected from individuals that acquired TD associated with ETEC, NoV, or a mixed infection of NoV plus a pathogenic strain of E. coli (NoV+Ec). Samples from individuals who were TD positive with no pathogen identified and from healthy travelers were included as controls to determine how specific pathogens affect bacterial gut communities. Compared to healthy travelers, gut populations from NoV+Ec subjects showed a significant decrease in bacterial species belonging to the phylum Actinobacteria and a significant increase in the phylum Bacteroidetes. The NoV group also had a significant increase in Bacteroidetes compared to the healthy travelers. The NoV+Ec group had significantly more Bacteroidetes than both the ETEC and no pathogen identified groups. There was no significant difference in alpha diversity, which was measured by the inverse Simpson Diversity Index. There was a significant difference in community structure based on non-metric multidimensional scaling plots of the Theta-YC distance metric when comparing the healthy travelers to the ETEC, NoV, and NoV+Ec groups. These data provide a comprehensive understanding of alterations associated with TD and will aid in future research targeting prevention and treatment of TD.

Contributors: Youmans, Bonnie; Ajami, Nadim; Jiang, Zhi-Dong; Muzny, Donna; Qin, Xiang; Gibbs, Richard; Petrosino, Joseph; DuPont, Herbert; Highlander, Sarah
Ras must act on the plasma membrane (PM) to mediate extracellular signaling and tumorigenesis. To identify key components controlling Ras PM localization, we performed an unbiased screen to seek fission yeast mutants with reduced PM Ras. Only 5 mutants were found, all affecting the same gene, sp-erf2, encoding sp-Erf2, a palmitoyltransferase, with varying activities. Sp-Erf2 localizes to the trans-Golgi, which is mediated by its third transmembrane domain and the Erf4 cofactor. In fission yeast, the human ortholog zDHHC9 rescues the phenotypes of sp-erf2 null cells. In contrast, expressing zDHHC14, another sp-Erf2-like human protein, did not rescue Ras1 mislocalization in these cells. Importantly, ZDHHC9 is widely overexpressed in cancers. Overexpressing ZDHHC9 promotes, while repressing it diminishes, Ras PM localization and transformation of mammalian cells. These data strongly demonstrate that sp-Erf2/zDHHC9 palmitoylates Ras proteins in a highly selective manner in the trans-Golgi to facilitate PM targeting via the trans Golgi network, a role that is most certainly critical for Ras-driven tumorigenesis.

Contributors: Young, Evelin; Zheng, Ze-Yi; Wilkins, Angela D.; Jeong, Hee-Tae; Li, Min; Lichtarge, Olivier; Chang, Eric C.

Delineation of the genomic structure in the human 2q13 region

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Familial juvenile nephronophthisis is an autosomal recessive kidney disorder, which is the most frequent inherited cause of chronic renal failure in children. Loss of function of the gene NPHP1 is responsible for approximately 85% of the nephronophthisis cases. About 80% of these patients carry a large homozygous deletion including NPHP1. NPHP1 maps to 2q13, a region with extremely enriched low copy repeats (LCRs). Previous studies have demonstrated that deletion of the NPHP1 can be mediated by nonallelic homologous recombination (NAHR) between two 45kb flanking repeats. One non-pathologic inversion haplotype involving NPHP1 region flanked by two 358kb large inverted repeats has also been reported. Moreover, extensive evidence suggest that the human 2q13 region is structurally highly polymorphic. Therefore, we hypothesize that different historical genomic rearrangements occurred within the 2q13 region and generated various structural haplotypes observed in the human population today. Individual susceptibility to the
NPHP1 deletion can be affected by the haplotypes they carry in their personal genomes. By exploring the different haplotypes from various individuals, we may glean a more detailed understanding of the correlation between disease susceptibility and structural variation mediated by LCRs.

We aim to find evidence to support the various predicted structural haplotypes. By mining the data from the literature, we observe the polymorphic copy number variation of the 45kb repeats in the 2q13 region in different populations. Optical Mapping analysis of one HapMap individual reveals the inversion haplotype of NPHP1 region as well as the deletion of one 45kb repeat. In this study, we will also use fosmid clones constructed in the Human Genome Structural Variation (HGSV) project to infer potential haplotypes. The discordant fosmid clones whose mapping cannot be explained by the 2q13 genomic structure in the reference genome imply alternative haplotypes. In total, 226 discordant fosmids in the 2q13 region are called from 17 HapMap individuals involved in HGSV project. By examining all the available discordant fosmids, we may be able to identify novel haplotypes. The various predicted haplotypes identified by this study will promote our understanding of the complex structure in the 2q13 region and other regions with similar high-level LCRs.

Contributors: Liu, Pengfei; Beck, Christine; Potamousis, Konstantinos; Schwartz, David; Lupski, James R.
Cigarette smoke-induced sterile inflammation requires C3/C3a receptor to induce Emphysema

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Smoking-induced chronic obstructive pulmonary disease (COPD), which encompasses chronic bronchitis and emphysema, is a progressive inflammatory lung disease with no known effective treatment. Sterile inflammation induced by cigarette smoke can activate lung antigen presenting cells (APCs) expressing CD11b/CD11c markers that can differentiate T helper type 1 (Th1) and Th17 cells. Complement proteins are increased in response to acute infection but their contribution and downstream signaling in response to sterile inflammation induced by cigarette smoke is less clear. We show here that relative to wild type, complement protein 3 (C3) deficient mice showed attenuated emphysema with reduced lung pathology measured by Micro-CT, less inflammatory cells infiltration to the airway and antigen presenting cell (APCs) infiltration to the lung, as well as reduction in inflammatory cytokine production. C3a, an activated fragment of C3 is deposited in the lungs of mice exposed to chronic smoke and human with emphysema. Mice deficient in C3a receptor (C3aR) phenocopied C3-/- mice exposed to chronic smoke which indicated their essential role for recruitment of lung dendritic cells (CD11c+/CD11b+). In the mean while, smokers with emphysema showed higher plasma C3 concentration and its cleaved products were found deposited on the lung tissue. These findings suggest a critical role for C3 and C3aR in the pathogenesis chronic and sterile smoke induced lung inflammation, and could be explored to develop specific new therapeutic targets for treatment emphysema.

Contributors: Yuan, Xiaoyi; Shan, Ming; You, Ran, Hong, Jeong-Soo; Wetsel, Rich; Drouin, Scott, Seryshev, Alexandria; Corry, David and Kheradmand, Farrah
A central question for The Cancer Genome Atlas (TCGA) community is how to use genomic and proteomic data to guide cancer care. We investigated two clinical applications of TCGA data: 1) predicting the prognosis of patients with primary tumors, and 2) identifying clinically actionable alterations that may be informative for patients with metastatic disease. For four TCGA cohorts (glioblastoma, ovarian serous cystadenocarcinoma, renal clear cell carcinoma, and lung squamous cell carcinoma), we systematically evaluated the power of different types of molecular data in predicting patient survival alone or in combination with clinical variables and found that power varies from cancer to cancer. In many cases, incorporating molecular data with clinical variables significantly improves the predictive models. Interestingly, the models built from one cancer type can have substantial predictive power in another cancer. Moreover, from 19 combined cancer types, we identified 13,624 somatic alterations in clinically relevant cancer genes in 3,879 out of 4,429 patients (87.6%), discovering many recurrent targetable alterations across tumor types that would not be revealed from single tumor datasets. Our study provides deep insights into building reliable predictive models for prognosis from diverse molecular data and identifying clinically actionable alterations that may inform clinical trial design and treatment choice.

Contributors: Yuan, Yuan; Van Allen, Eliezer; Omberg, Larsson; Wagle, Nikhil; Sokolov, Artem; Xu, Yanxun; Hess, Kenneth; Diao, Lixia; Han, Leng; Huang, Xuelin; Lawrence, Michael; Stuart Josh; Mills, Gordon; Garraway, Levi; Margolin, Adam; Getz, Gad; Liang, Han
MODULATION OF FXTAS NEURODEGENERATION IN A MOUSE FMR1 PREMUTATION MODEL BY CUGBP1

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FXTAS is hypothesized to arise by an RNA-mediated toxic gain-of-function mechanism, by which the rCGG sequesters RNA binding proteins (RBPs), inhibiting their normal function. Molecular hallmarks of FXTAS include ubiquitin-positive intranuclear inclusion bodies throughout the brain and marked dropout of cerebellar Purkinje neurons. We have previously developed transgenic mouse models to drive premutation-length rCGGs specifically in Purkinje neurons. Purkinje neuron-specific promoter L7/Pcp-2 drives the expression of 90 CGG repeats fused to the cDNA of Fmr1 or EGFP. These mice have been shown to successfully model human phenotypes including inclusion formation (in Purkinje neurons), Purkinje neuron loss, and impaired motor coordination. CUG-Binding Protein Elav-Like Family Member 1 ( CELF1, aka CUGBP1) is an RBP that has previously been demonstrated in a Drosophila model to be a potent modifier of the rCGG premutation. We predict that, in the mouse, CELF1 protein expression is inversely proportional to phenotypic severity – the less CELF1 is being expressed, the more severe the phenotype and vice versa.

We have developed a novel CELF1 knockout mouse model and have utilized existing models to overexpress CELF1 in Purkinje neurons specifically using a Tet-off system, and are assessing their effect on phenotype in the transgenic premutation models by immunohistochemistry, immunofluorescence, and behavioral studies. Thus far, we have demonstrated that haploinsufficiency of CELF1 in the context of the Fmr1 premutation may enhance rCGG toxicity. In littermates aged six to eight months CELF1 depletion in the context of CGG-Fmr1 significantly (p=0.013) increases the percentage of Purkinje neurons containing an inclusion body, relative to CGG-Fmr1 expressing normal levels of CELF1. We are also in the process of gathering iterative behavioral data – primarily rotarod but also additional motor coordination assays – for mice between the ages of 4-8 months. Our current findings support the significance of CUGBP1 and other RBPs in mammalian models of FXTAS, as well as the RNA toxic gain-of-function hypothesis. This serves to underscore the longterm implications of RBP inhibition/sequestration by premutation-length rCGG, whose presence in intranuclear inclusions is coincident with neuronal loss, and highlight the potential future role of RBPs as possible therapeutic targets.

Contributors: Zalewski, Zachary A.; Jin, Peng; Timchenko, Lubov T.; Nelson, David L.
LRP6 causes a novel developmental disorder including microphthalmia and bone defects

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Patients entered into the clinic with a set of ocular and developmental defects including short stature, microphthalmia, brachydactyly type E, choroidal effusions, and glaucoma. We performed a molecular diagnosis via next-generation sequencing and identified a candidate dominant mutation in LRP6. Mouse LRP6 mutants are known to recapitulate all patient phenotypes. The patient mutation was predicted in silico to be damaging, segregated in a small pedigree, and is extremely rare in control populations. In vitro tests demonstrated that the mutation disrupts canonical Wnt signaling. Further, Zebrafish LRP6 knock down and rescue experiments demonstrated that the patient mutation is sufficient to cause phenotypes similar to those experienced by the patients in vivo. These in depth functional studies combined with our in silico sequence analysis provide strong evidence that LRP6 is a novel human disease gene causing a novel developmental syndrome. Our ability to identify molecular cause of a syndrome that, to the best of our knowledge, is experienced by only three individuals in a single family has major implications for the development of personalized medicine. Finally, the similarities and differences between the phenotypes in our patient family and those caused by other Wnt signaling defects may allow future studies to further investigate the etiology of these diseases.

Contributors: Zaneveld, Jacques; Liu, Wei; Xu, Mingchu; Wang, Hui; Wang, Feng; Wang, Xia; Salvo, Jason-scott; Gelowani, Violet; Wang Keqing; Li, Yumei; Zhang, Kang; Chen, Rui;
Haploinsufficiency of RERE Contributes to Cardiovascular Defects Associated with 1p36 Deletions

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Terminal deletions of chromosome 1p36 affect 1 in 5000 newborns and cause a clinically recognizable syndrome characterized by cognitive impairment, eye/vision problems, hearing loss, facial clefting, cardiovascular malformations and cardiomyopathy. Although cardiovascular anomalies and/or cardiomyopathy affect 71% of individuals with 1p36 deletions, and are the most common cause of morbidity and mortality in the newborn period, the genes that contribute to these phenotypes have yet to be identified. Using a clinical and molecular cytogenetic data from individuals with terminal and interstitial deletions, we have created a deletion/phenotype map of the 1p36 region. This map includes five non-overlapping critical regions for cardiovascular defects and two non-overlapping critical regions for cardiomyopathy. The majority of these regions contain positional candidate genes which have been shown to cause cardiovascular malformation and/or cardiomyopathy in mice. These genes include DVL1, SKI, PRDM16, UBE4B, PDPN, SPEN, HSPG2, CDC42, and LUZP1. The arginine-glutamic acid dipeptide repeats gene (RERE) encodes a nuclear receptor coregulator and is located in a critical region for both cardiovascular malformations and cardiomyopathy on 1p36 and is required for normal retinoic acid signaling. Since perturbations in retinoic acid signaling can cause cardiovascular malformations, and retinoic acid signaling suppresses myocardial hypertrophy and cardiac fibrosis, we hypothesized that RERE deficiency contributes to the cardiac phenotypes seen in individuals with terminal and interstitial 1p36 deletions that include RERE. To test this hypothesis, we generated an allelic series of RERE-deficient mice using an Rere null-allele and a hypomorphic Rere allele (eyes3) identified in our laboratory. A portion of Rere+/- mice had aortic arch anomalies. Rere-/eyes3 mice had more serious cardiac defects which included aortic arch, conotruncal, and septal defects. In absence of structural cardiac defects, these mice spontaneously developed cardiac fibrosis and cardiomegaly. Rere null mice (Rere/-) had unlooped hearts and died of cardiac failure at E9.5. Cardiac defects were also documented in mice in which Rere was conditionally ablated in the first and second heart fields. We conclude that RERE functions in a cell autonomous manner to direct cardiac development and that deletion of RERE contributes to the cardiac malformations and cardiomyopathy associated with 1p36 deletions.

Contributors: Kim, Bum-Jun; Hernandez-Garcia, Andres; Beck, Tyler F; Shchelochkov, Oleg.A; Justice, Monica; Lee, Brendan; Lalani, Seema; Scott, Daryl.A.
The important role of miRNAs in bone homeostasis has been reported in several studies. However, there are few reports of microRNA in osteoporosis. Here, we have found the expression of miR-155 is increased in C2C12 osteogenic differentiation model. MiR-155 plays critical roles in hematopoiesis that the miR-155 knockout (KO) mice show several defects in the function of B cell, T cells and dendritic cells. However, little is known about miR-155 function in bone. The gross skeletal analysis of miR-155 KO mice showed an increased trabecular bone mass in the spines of 8-month-old females compared to wild type (WT) littermates. Furthermore, we adopted the ovariectomy (OVX) surgery model to accelerate the progression of osteoporosis in female mice. OVX group of WT mice showed a significant reduction of bone volume compared to sham surgery group after 4 weeks of surgery, while OVX group of miR-155 KO mice showed comparable bone volume to sham surgery group. Indeed, this OVX study suggests that miR-155 is associated with osteoporosis.

To further understand the cellular mechanisms of miR-155 in bone, we performed in vitro osteoblast (Ob) differentiation assay using bone marrow stromal cells (BMSCs) isolated from both genotypes and quantified by Alizarin Red staining. We found increased Ob differentiation and mineralization of BMSCs from miR-155 KO mice compared to WT littermates. To check if osteoclast (Oc) differentiation is affected in miR-155 KO mouse, Ob-Oc co-culture was performed. We found that BMSCs from KO mice have impaired ability to induce Oc differentiation. In addition, expression of several Ob markers, including Col1a1, Runx2 and Ocn, were increased in the calvarial tissue from miR-155 KO mice. These data, taken altogether, suggest that knockout of the miR-155 results in protection of osteoporosis by increasing Ob differentiation and mineralization, and decreasing the ability to induce Oc differentiation.

Contributors: Zeng, Huan-Chang; Bae, Yangjin; Egunsola, Adetutu Taiwo; Dawson, Brian Christopher; Bertin, Terry; Munivez, Elda; Rodriguez, Antony; Lee, Brendan
2013 GRADUATE STUDENT SYMPOSIUM

TRANSCRIPTIONAL RESPONSE TO ACUTE RESPIRATORY VIRAL INFECTION

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BACKGROUND: Acute respiratory infections (ARIs) are responsible for a large number of outpatient visits and hospitalizations in the U.S., and the threat of pandemic influenza will likely add to these numbers. Transcriptional responses to viral respiratory disease have indicated characteristic signatures reflecting an early systemic response involving innate immune mechanisms.

METHODS: 1618 healthy adults, enrolled in fall 2009 and 2010, were followed for acute respiratory disease. Subjects reporting symptomatic acute respiratory illness were monitored for 3 weeks. Peripheral blood samples for RNA extraction were obtained on the first day of illness (day 0) and then on 2, 4, 6, and 21 days after. In addition to an RNA sample obtained at enrollment, a final RNA sample was obtained at the end of the study. RNA samples were analyzed using expression microarrays and the patterns of gene expression were analyzed to identify differentially expressed transcripts. Lineage-specific transcripts were used to quantify changes in blood cell composition during acute infection and recovery. WGNCA, which partitions gene expression into groups of transcripts (modules) with highly correlated behavior, was used to detect host regulatory networks.

RESULTS: By RT-PCR of the virus RNA in nasal washes, we found that 73 subjects were infected with influenza and 36 subjects have noninfluenza viral respiratory infection (e.g. human rhinovirus or respiratory syncytial virus). Gene expression profiles showed highly similar patterns among the major subgroups. There was a dramatic upregulation of interferon pathway and innate immunity on the first day of infection. This persisted for 2-4 days. A convalescent phase was observed on day 4 and 6 after infection. By day 21 and the final visit the gene expression pattern was indistinguishable from baseline. Using lineage and activation state specific transcripts to produce cell composition scores, patterns of depression of the B and T lymphocytes in acute phase and activation of lymphocytes in convalescent phase were observed accompanied by the evidence of dramatic activation of dendritic cells and NK cells. We identified groups of strongly connected transcripts by WGCNA. The severity of illness was correlated with gene network connectivity and the expression level of one group (module).

CONCLUSIONS: Transcriptional profiling gives a genome wide view of a coordinated systemic response to acute respiratory viral infection. The results also demonstrate that serial measurements of gene expression within the context of a prospective clinical trial can be an efficient strategy for identify candidate genetic mechanisms. Contributors: Zhai, Yijie; Belmont, John; Atmar, Robert; Quarles, John; Arden, Nancy; Bucasas, Kristine; Wells, Janet; Niño, Diane; Wang, Xueqing; Zapata, Gladys; Shaw, Chad; Franco, Luis; Couch, Robert
Combined effect of Dnmt3a loss-of-function and Idh2 neomorphic mutation promotes hematopoietic malignancy

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In recent studies on acute myeloid leukemia (AML), genes involving DNA methylation (DNMT3A, IDH1/2, TET2) were identified as frequently mutated. Around 17% of AML patients were found to have a mutation in isocitrate dehydrogenase-1 or -2 (IDH1/2). In these patients, 2-hydroxylglutarate (2-HG), which is generated from a neomorphic activity of mutant IDH1/2, accumulates and inhibits DNA hydroxymethylation mediated by TET family proteins. Interestingly, mutations in the de novo DNA methyltransferase 3A (DNMT3A) and IDH1/2 mutations co-occur in a statistically significant portion of AML patients. In current known mouse models, neither Dnmt3a knockout (KO) and IDH1/2 mutation alone initiates overt hematopoietic malignancy, leading to our hypothesis that Dnmt3a and IDH1/2 mutations may act synergistically to initiate hematopoietic malignancy.

To create a Dnmt3a knockout IDH1/2 mutant double mutant mouse model, we have employed a transplantation approach to create Idh2R140Q (one of the abundant IDH2 mutation in AML patients) overexpressing stem cells on a Dnmt3a KO (Dnmt3a–/–) and wild-type (WT) background by retroviral transduction. Idh2WT overexpression was also used as a control group. A hematopoietic disease with a median survival of 197 days was observed in the Dnmt3a–/––Idh2R140Q group. Anemia, thrombocytopenia and monocytosis were observed in morbid Dnmt3a–/––Idh2R140Q mice. The pathological examination of Dnmt3a–/––Idh2R140Q mice showed myelodysplasia in one or more lineages, an accumulation of less differentiated myeloid progenitors in the bone marrow and pronounced extramedullary hematopoiesis in the spleen. Moreover, approximately 20% of Dnmt3a–/––Idh2R140Q mice developed AML.

Together, these features led to the diagnosis of MDS/MPN (Myelodysplastic Syndrome / Myeloproliferative Neoplasms) with high transformation rate to AML. In comparison, WT–Idh2R140Q mice also developed less severe MDS/MPN characterized by myeloid differentiation bias and extramedullary hematopoiesis without lethality in one year after bone marrow transplantation. The profiling of 2-HG with gas chromatography mass spectrometry (GC-MS) in the serum of morbid mice transplanted with Dnmt3a–/––Idh2R140Q showed an 80-fold increase compared to normal mouse serum, while the 2-HG content in mice transplanted with WT–Idh2R140Q cells was 10-fold higher than that of normal mouse serum. This suggests that Dnmt3a loss-of-function can promote the synthesis of 2-HG by the Idh2R140Q mutation. The metabolomic profiling on cKit+ bone marrow cells identifies glutamine anaplerosis pathway highly upregulated in the Dnmt3a–/––Idh2R140Q group indicated by the increase of α-ketoglutarate and glutamate. In contrast, the WT – Idh2R140Q and Dnmt3a KO groups have no alteration in glutamine anaplerosis pathway metabolites, indicating a synergy on 2-HG synthesis between Dnmt3a knockout and Idh2R140Q. Moreover, an excess of glutamine during in vitro culture significantly promotes the colony forming ability Dnmt3a–/––Idh2R140Q HSPCs, while there’s no effect in Dnmt3 Idh2WT and WT–Idh2R140Q HSPCs.

In summary, our research shows for the first time, Dnmt3a loss-of-function promotes 2-HG synthesis with mutant Idh2 and strongly aggravates the phenotype induced by Idh2 mutation. Our research also shows the synergistic effect on the metabolome of two genetic backgrounds. These data likely explain the high frequency of co-mutations between DNMT3A and IDH1/2 that promotes aggressive AML in patients.

Contributors: Zhang, Xiaotian; Putluri, Nagireddy; Zhou, Ting; Jeong, Mira; Yang, Liubin; George Michailidis; Sreekumar, Arun; Rebel, Vivien; Goodell, Margaret
DksA Guards Elongating RNA Polymerase Against Ribosome-Stalling-Induced Arrest

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DksA, which is known to modulate transcription initiation, also aids DNA replication bypass of arrested RNA polymerase (RNAP) in amino-acid starved cells, suggesting that DksA may affect transcript elongation by RNAP. However, DksA exhibits at most limited effects on elongation in vitro. To investigate this discrepancy, we determined the genome-wide distribution of DksA and its effects on elongating RNAP in vivo. Our results reveal that DksA associates with RNAP throughout transcription units, and promotes RNAP progress specifically at mRNA genes. Upon uncoupling of transcription from translation by depletion of charged tRNA, DksA dramatically increases RNAP progress genome-wide. This effect of DksA is independent of the transcription termination factor Rho but is dependent on ribosome stalling. Our results establish that, in addition to its role in initiation, DksA is a transcription elongation factor in vivo and suggest that, at least in stressed cells, ribosomes may promote rather than inhibit RNAP backtracking.

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Next Generation Sequencing Based Molecular Diagnosis of Retinitis Pigmentosa Patients in Northern Ireland

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Retinitis Pigmentosa (RP) is a heterogeneous group of inherited retinal disorders characterized by progressive photoreceptor apoptosis. RP is the most common of the retinal degeneration with prevalence approximately 1 in 3,000 individuals, affecting approximately 1.5 million people worldwide. Patients with RP may slowly develop night blindness, followed by tunnel vision, and eventually partial or total blindness over the course of several decades. RP is genetically heterogeneous disease. It can be inherited as an autosomal-dominant, autosomal-recessive or X-linked trait. To date, 56 genes have been linked to RP; however, these can only explain less than half of all RP cases. Due to the extensive genetic heterogeneity of RP and complex inheritance patterns, accurate molecular diagnosis of the disease is essential to match patients with appropriate treatments. Furthermore, identification of novel RP genes is likely to provide new insights concerning disease mechanisms of RP and other retinal diseases.

Here we combine retinal capture and whole exome sequencing to study 94 well-characterized RP families from Northern Ireland. There are 68 RP samples found candidate mutations after panel capture analysis, and 48 of them are novel mutations while 20 of them found mutations that have been reported in literatures. For patients had negative results in panel sequencing, we will collect the DNA of additional affected members to perform WES and use integrative analysis to identify novel disease genes.

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Robustness is a property built into biological systems to ensure stereotypical outcomes despite fluctuating inputs from gene dosage, biochemical noise, and the environment. During development, robustness safeguards embryos against structural and functional defects. Yet, our understanding of how robustness is achieved in embryos is limited. While much attention has been paid to the role of gene and signaling networks in promoting robust cell fate determination, little has been done to rigorously assay how mechanical processes like morphogenesis are designed to buffer against variable conditions. Here we show that the cell shape changes that drive morphogenesis can be made robust by mechanisms targeting the actin cytoskeleton. We identified two novel members of the Vinculin/α-Catenin Superfamily that work together to promote robustness during Drosophila cellularization, the dramatic tissue-building event that generates the primary epithelium of the embryo. We find that zygotically-expressed Serendipity-α (Sry-α) and maternally-loaded Spitting Image (Spt) share a redundant, actin-regulating activity during cellularization. Spt alone is sufficient for cellularization at an optimal temperature, but both Spt plus Sry-α are required at high temperature and when actin assembly is compromised by genetic perturbation. Our results offer a clear example of how the maternal and zygotic genomes interact to promote the robustness of early developmental events. Specifically, the Spt and Sry-α collaboration is informative when it comes to genes that show both a maternal and zygotic requirement during a given morphogenetic process. For Drosophilid cellularization, Sry-α and its expression profile may represent a genetic adaptive trait whose sole purpose is to make this extreme event more reliable. Since all morphogenesis depends on cytoskeletal remodeling, both in embryos and adults, we suggest that robustness-promoting mechanisms aimed at actin could be effective at all life stages.

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The role of vitamin D and vitamin D receptor in non-alcoholic liver disease

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Vitamin D is synthesized predominantly in the liver and functions as an important secosteroid hormone with pleiotropic effects. While its key regulatory role in calcium and bone homeostasis is well established, recently there is increasing recognition that vitamin D also regulates cell proliferation and differentiation, and has immunomodulatory, anti-inflammatory and anti-fibrotic properties. Vitamin D plays a function mainly through vitamin D-binding protein and vitamin D receptor (VDR), which belongs to the nuclear receptor family. Vitamin D deficiency is frequently present in chronic liver disease and may predict non-response to antiviral therapy in chronic hepatitis C. Vitamin D deficiency also closely relates to the severity of non-alcoholic fatty liver disease (NAFLD) and is implicated in the pathogenesis of insulin resistance, a key factor in the development of NAFLD. Although there is evidence to support a relationship between vitamin D status and insulin resistance, the underlying mechanism requires further exploration. The endoplasmic reticulum (ER) is the organelle that is responsible for protein folding and assembly, lipid and sterol biosynthesis, and intracellular calcium storage. Recent research demonstrated that ER stress and the UPR signaling are critically involved in the initiation and progression of NAFLD. To address the association between vitamin D-VDR axis and pathology of NAFLD, we used ER stress induced fatty liver mouse model. Tunicamycin is a widely used chemical inducer for ER stress in mouse model, which inhibits the synthesis of all N-linked glycoproteins. The fatty liver can be induced in 24 hours treatment. We did intraperitoneal injection of tunicamycin to both wild type and VDR knock out mice. After 24 hours injection, the wild type mice displayed apparent fat accumulation in the liver tissue, while the phenotype was totally absent in VDR knock out mice. The hepatic triglycerides and cholesterol levels were significantly increased in wild type mice with tunicamycin challenge, but the VDR KO mice showed no change in these indexes. The H&E and Red Oil O staining also proved large lipid droplets accumulating in the wild type group, which was not seen in the VDR KO group. Further experiments in gene expression change and vitamin D diet rescue will be done to test the link between fatty liver disease and vitamin D-VDR axis. Finally, we will consider the therapeutic potential of the vitamin D-VDR axis in non-alcoholic fatty liver diseases.

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Expression and Function of Daam2 in Glioblastoma Tumorigenesis

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Glioblastoma multiforme (GBM) is one of the most common and aggressive malignant high-grade gliomas in human, which only has about half year median survival. Current standard therapies mainly focus on combination of surgery, radio- and chemotherapies. But many challenges remain, including high drug resistance and glioblastoma recurrence.

GBM shares similar characteristics to glia, astrocytes and oligodendrocytes (and their precursors), which are the study direction in our lab. Considering development context is one of the inception of tumorigenesis, understanding how glial development regulation contributes to GBM formation remains a key question. Until now, many genes and signalling pathways have been found to have function in GBM. But there is few evidence to show Wnt signaling functions in GBM, comparing to its function in many other types of tumor, such as colorectal cancer, melanoma, hepatoblastomas, medulloblatoma. It might be some new components in Wnt signalling pathway that links Wnt and GBM. Previously, our lab for the first time described that Daam2 gene can cluster Dvl3/Axin2 complex-mediated Wnt receptor into signalsomes therefore potentiate Wnt signaling. Therefore, my hypothesis is that Daam2 expression is required for GBM tumorigenesis. The mechanism for such a function is due to its function in canonical Wnt signaling pathway.

So far, it showed high Daam2 expression on both xenograft mouse model and human GBM tissue array by in situ hybridization. We also used shRNAi virus to knockdown Daam2 expression in primary GBM cell lines. In vitro test showed that low Daam2 expression has accelerated growth and transforming capability. In vivo test is to inject knockdown cell line inside SCID mouse brain and see whether the tumor growth will change. At the same time, we used in utero electroporation as a method to build a GBM mouse model by overexpressing HRasv12 in PiggyBac system. We plan to generate GBM inside a Daam2 conditional knockout mouse in order to study Daam2 function in GBM. Considering the vital role of Wnt signaling pathway in development, study of Daam2 function in GBM might be helpful to better understand molecular biology of GBM.

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A basic question in neuroscience is to investigate how single neurons perform multiple computations and at what temporal and spatial scale these computations work. We focus on the locust, a model system in which the neural mechanisms underlying visually evoked escape behavior are very well understood and the associated neural computations precisely defined at the single neuron level. In the locust, these neural computations revolve around an identified neuron called the lobula giant movement detector (LGMD) that responds selectively to objects approaching the animal in a collision course. To experimentally test the logarithmic transform between total conductance and membrane potential within the excitatory dendrite of the LGMD in response to looming stimuli, we employ the approach of in vivo two-photon calcium imaging with the calcium indicator Oregon Green 488 BAPTA 1 injected intracellularly into the LGMD. Our goal is to record two-photon calcium fluorescence changes within the excitatory dendrites of the LGMD in response to visual stimuli presented at different locations on the eye.

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Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS) surround axons with myelin to enable rapid and reliable action potential propagation by sequestering sodium channels at nodes of Ranvier. The developmental mechanisms regulating myelin formation and the radial sorting of axons in the PNS are only partially understood. Recently, highly conserved polarity proteins have been implicated in myelination. One polarity protein, Protein Associated with Lin7 (Pals1), which localizes to paranodes, Schmidt-Lanterman incisures, and the adaxonal domain in Schwann cells, has been proposed to regulate myelin thickness, length, and ultrastructure. To determine whether Pals1 is important in myelination, we generated conditional knockout (cKO) mice lacking Pals1 in Schwann cells and oligodendrocytes using the Cre-lox recombinase system under the control of the 2',3'-cyclic nucleotide phosphodiesterase (CNP) promoter. As adults, CNP-Cre;Pals1 cKO mice demonstrate hind limb clasping and a motor coordination deficit. Axons in the CNS and PNS of adult cKO mice are myelinated and subcellular domains are present. However, some axon bundles in the sciatic nerve are aberrantly myelinated indicating radial sorting defects. CNP-Cre;Pals1 cKO mice exhibit a delay in myelination as indicated by fewer myelinated axons and more unsorted axons as compared to littermate controls at postnatal day 7. These data reveal a new role for Pals1 in early Schwann cell development.