Autophagy and Development

Background and Significance

Drosophila is a holometabolic insect that undergoes metamorphosis during development. Prior to becoming an adult, the fly goes through several stages of development. Prepupa leave the rich medium for a suitable environment for development, and the larval cuticle transforms into a hard pupal casing. Metamorphosis occurs within the pupal casing and the larval organs and tissues break down. Finally, the fly develops from the imaginal discs. During metamorphosis, the developing drosophila must obtain energy from itself in order to sustain itself for four and a half days.

Autophagy can be viewed as the recycling of cellular components. Membranes derived from the ER enclose portions of the cytoplasm or enclose organelles to form the autophagosome. The autophagosome fuses with the lysosome, which degrades the contents for reuse. Autophagy is tightly regulated by a group of approximately twenty genes, termed autophagy-related genes (atg). These gene products activate the autophagy pathway during periods of starvation, such as the pupae stage, and the recycling provides energy for cells. Given that activation of autophagy occurs during the pupae stage, it was proposed that knocking out regulators of the autophagy pathway would result in lethality. Although this is the case for the majority of Atg genes, Atg7 and Atg3 knock-outs escaped a lethality based selection screen. In fact, the Atg7 knockout results in an 80-85% reduction in autophagy. The drastic reduction of autophagy in the Atg7 knockout without killing the developing fly suggests that the autophagy pathway may not be essential for developmental stages, which would be an interesting and novel finding.

Atg1 is a regulatory kinase that, upon knock out, results in lethality in the late pupal stage. Although Atg1 plays a role in the autophagy pathway, its homolog plays non-autophagic roles in other species. For instance, Unc51, the atg1 homolog in C.elegans, plays a role in axon outgrowth by interacting directly with VAB-8, a protein necessary and sufficient for posteriorly directed migrations. Because the function of atg1 is known in other species, this gene can be used to investigate whether autophagy is essential during development. Specifically, it remains to be determined whether atg1 lethality is due to autophagy or due to a non-autophagic process. In addition, the temporal and spatial requirement of atg1 is not known.

By continuing to work with Drosophila, we will take advantage of its well defined pupal stage, in which the fly is under starvation conditions, to study the requirement of autophagy during development.
Hypotheses to be Tested
We hypothesize that autophagy will not be required to meet the energy needs of the fly during metamorphosis. Therefore, we expect Atg1 expression to be required in areas other than the fat body, the major energy store. Knowing that many of the Atg1 homologs are responsible for axon guidance, we expect atg1 expression to be necessary during nervous system development. We also expect the inactivation of the kinase domain in atg1 responsible for regulating autophagy will result in viability.

Specific Aims

Aim 1: Explore functional domains of Atg1.
First, we will analyze the primary amino acid sequence of Atg1 to identify functional domains. Using an evolutionary trace, we will identify amino acids in the protein that are functionally conserved. Next, we will use a knock-in strategy to alter each functional domain while leaving the rest of Atg1 intact structurally. We would screen for viability in offspring and assay for the amount of autophagy and we expect that disruption of domains necessary only for autophagy will not result in lethality.

Aim 2: Determine temporal requirement of Atg1
We will genetically engineer an RNAi specific for Atg1 under the control of UAS. We will use the TARGET system for temporal activation since adjusting the temperature will knockdown Atg1. We will then screen for lethality of the flies when exposed to different temporal conditions. We expect Atg1 to be required during development of the adult nervous system.

Aim 3: Determine spatial requirement of Atg1.
We will use the same UAS-RNAi for Atg1 as the above aim and cross with various Gal4 promoter flies to achieve spatial control of RNAi expression. The expression of RNAi will knock down Atg1 in a cell specific manner, and we will screen for lethality and assay for autophagy. Also, to achieve more specific RNAi knockdown, we will use the split Gal4 system to gain more specific knockdown of Atg1 in subsets of cells. We expect Atg1 to be required in neural precursor cells rather than the fat body assuming autophagy is not required for development.

Literature Cited


Metastasis, EMT, and Hypoxia

Background and Significance

Metastasis, a process by which primary cancer cells enter the circulatory system or the lymphatic system, travel to distant sites in the body and establish secondary tumors, accounts for approximately 90 percent of all cancer-related deaths. The steps involved in metastasis include tumor proliferation, neoangiogenesis or lymphangiogenesis, invasion of stroma, entry into the circulation (intravasation), entry into the distal organ (extravasation), dormancy, growth and tumor formation. Metastasis is a major contributor to the poor prognosis of cancer patients since these migrated cells can remain dormant for ten years or more. If a therapy can be developed to disrupt the metastatic potential of cancer cells prior to intravasation, this could reduce the number of cancer-related deaths.

Prior to intravasation is the invasion of stroma. A major contributor to this step is the epithelial-mesenchymal transition (EMT), a developmental process normally important for gastrulation and organogenesis. Epithelial cells are held together by tight junctions, adherens junctions, desmosomes, and gap junctions. These, in turn, are organized into well defined cell layers. In contrast, mesenchymal cells interact at focal points with their neighbors but do not form organized cell layers, allowing the cells to become motile. EMT is utilized by cancer cells for migration since these cells lose cell to cell adhesion by a decrease in E-cadherin. Therefore, by disrupting the EMT, and thus migration, this may inhibit the metastatic potential of cancer cells.

Recently, the hypoxia induced response pathway was recognized as an important factor in determining the aggressiveness and metastatic potential of cancer cells. There are a few regulators of EMT, including SNAI1, ZEB2, and TCF3, which are known to be upregulated by hypoxia inducible factor (HIF). We will investigate which cancer-types can be targeted possibly by use of nanotechnology. Since EMT is a reversible process, we also want to explore the time points in which disruption of the hypoxia induced EMT pathway would be successful. Finally, knowing that the ability to successfully metastasize to other areas of the body depends on the tumor cell's ability to adapt to its new environment, we will explore whether cancer stem cells utilize the hypoxia-induced EMT pathway for metastasis.

The mouse is molecularly, histologically and biologically similar to humans. In order to have a translationally relevant model of cancer metastasis, the mouse would be the most effective model for testing the effect of the hypoxia-induced EMT in a variety of tissue types, the time frame for therapeutic effectiveness, and the identification of different therapeutic targets.
Hypotheses to be Tested
Knowing that hypoxia-induced EMT occurs in different carcinoma cell types, we hypothesize that it occurs in all cancer cell types. We also hypothesize that disrupting EMT prior to intravasation would be effective in blocking metastasis since EMT is a reversible process. In addition, cells must be competent to adapt to a new environment. Therefore, we hypothesize that EMT is important for successful metastasis of cancer stem cells.

Specific Aims

**Aim 1:** Test hypothesis that hypoxia-induced EMT occurs in different cancer types.
Obtain different cancer cell types, induce hypoxia conditions and screen for EMT by expression of GFP tagged EMT proteins and stain for the loss of E-cadherin, an epithelial cell marker. This would allow correlation of protein markers with a functional readout by performing cell migration assays. In addition, obtaining mRNA expression profiles of hypoxia-induced metastatic tumors regardless of tumor-type, we can screen for the upregulation of EMT regulators, an EMT signature, and correlate with cell migration assays.

**Aim 2:** Test hypothesis that disrupting EMT prior to intravasation would be effective in halting metastasis. Using an inducible promotor system, induce HIFα subunit with addition of doxycyclin followed by turning off by withdrawing doxycyclin to HNSCC cells and screen for MET and reduced migration. In addition, temporally knock out HIFα in the progression of cancer cells, and perform orthotopic transplants and screen for metastasis in the mouse. Compare knocking out HIFα to knocking out of EMT-specific genes followed by orthotopic transplants to analyze whether HIFα deletion has the same effect as knocking out the EMT pathway.

**Aim 3:** Test Hypothesis that hypoxia-induced EMT is important for cancer stem cells.
Currently, some cancer stems cells can be separated by FACS using CD133, CD44, and Sca-1, positive cell surface markers normally expressed in hematopoietic stem cells and progenitor cells. Upon separation of the cell populations, perform orthotopic transplants and screen for metastasis in the mouse and pair with mRNA expression profiles to observe a correlation between metastatic potential of cell types and a hypoxia-induced EMT signature.

Literature Cited