UNIVERSITY OF CYPRUS
DEPARTMENT OF BIOLOGICAL SCIENCES

The Department of Biological Sciences cordially invites you to the thesis defense of the PhD candidate

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entitled

"*Drosophila* intestinal homeostasis and tumor growth is sustained by the tissue-intrinsic Eiger/TNF cytokine and through the active remodeling of the adult tracheal network"

Abstract

Chronic inflammation has been identified as an important risk factor for gastrointestinal cancer. Nevertheless, the cellular events and the signaling pathways triggered by chronic inflammation that drive the transformation of normal epithelial cells to cancerous cells require further study. Inflammatory responses, such as reactive oxygen species, pathogenic infections, immune response deregulation, and pro-inflammatory cytokine production might lead to carcinogenesis. In this study we use our established model of bacterial intestinal pathogenesis in *Drosophila melanogaster*, whereby a pathogenic bacterial strain of *Pseudomonas aeruginosa* induces intestinal injury and regeneration mediated by the activation of intestinal stem cell (ISC) proliferation, in order to characterize the signaling pathways and the molecular mechanisms that regulate tissue regeneration.

By studying the role of tissue-intrinsic regenerative inflammatory signaling in stem cell mitosis of the adult *D. melanogaster* midgut at the baseline and the infected state through a quantitative genetic screen we found that stem cell mitosis is positively linked with the expression of *eiger*, *Delta*, *upd3* and *vein* in the midgut, as well as with dysplasia and host defense, but negatively with enterocyte endoreplication. We provide evidence that intertwined trade-offs fine-tune midgut homeostasis, according to which stem cell mitosis through *cyclin E* in stem cells promotes the optimal host defense to infection, unless dysplasia ensues. However, *cyclin E* in enteroblasts promotes enterocyte endoreplication and counterbalances stem cell mitosis and dysplasia, providing an alternative but less efficient mechanism to support host defense. Moreover, we showed that the cytokine Eiger/TNF - independently of its systemic role - acts as a dual tissue-intrinsic switch of the *D. melanogaster* intestinal mitosis. While abundant and inducible in the fat body, Eiger has a basal expression in the midgut progenitors and mature enterocytes that is not inducible by intestinal infection and concomitant regenerative signaling. In the midgut progenitor cells, Eiger accelerates regeneration stem cell intrinsically, via the Wengen-JNK-Upd module. In the mature enterocytes Eiger signals the visceral muscle via the Grindelwald/Wengen-JNK module to inhibit the expression of *wingless*, a niche growth factor that sustains stem cell mitosis. We identify zones of high and low Eiger expression in the

midgut with distinct progenitor and enterocyte expression. We demonstrate that the intensity of Eiger/TNF signal in progenitors vs. enterocytes explains differences in regeneration potential among genetically different individuals, and along the midgut of each individual.

In a second line of research, we found that upon pathogenic *P. aeruginosa* infection, the trachea that oxygenates the intestine is dramatically remodeled (neotracheogenesis), similar to pathological neoangiogenesis observed in mammals, and this remodeling is necessary for ISC-mediated regeneration upon infection. To identify molecules involved in neotracheogenesis, we assessed the conserved Hypoxia Inducible Factor 1α (HIF1α)/Similar (Sima) signaling, which is known to be involved in inflammation, angiogenesis and tumors. We found that Hif1a/Sima and its targets, the FGF/Branchless (Bnl) and the FGFR/Breathless (Btl), are induced in the damaged intestine and are necessary for ISC-mediated regeneration. To test if known upstream regulators of HIF1α/Sima, such as Reactive Oxygen Species (ROS) or hypoxia can trigger regeneration, HIF1α/Sima activation and neotracheogenesis in the adult intestine, we orally exposed flies to exogenous ROS, which induce inflammatory signaling and regeneration and we reared flies in hypoxia. In addition, we fed flies with Dextran Sulfate Sodium (DSS), which induces regeneration by disrupting the basement membrane. We found that ROS and DSS caused an ISC-mediated regenerative response in the intestine. However, only ROS-treated flies exhibited induction of intestinal neotracheogenesis (induction of HIF1α/Sima activity and expression of the FGF/*bnl* and the FGFR/*btl*). Interestingly, we found that hypoxia is a potent inducer of HIF1α/Sima and tracheogenesis in the *D. melanogaster* intestine, although it inhibits ISC mediated regeneration. Thus, our experiments show that, pathogenic *P. aeruginosa* infection is phenocopied by ROS and hypoxia with regards to intestinal tracheogenesis, and this response is independent of regeneration. Nevertheless, although hypoxia induces tracheogenesis, it suppresses the *P. aeruginosa*-induced ISC regenerative response indicating that oxygen (or a product of oxygen) from the trachea is necessary for ISC function. We demonstrate that ROS generation from the intestinal trachea and *P. aeruginosa* in the presence of oxygen (normoxia) facilitates the ISC-mediated regenerative response. Strikingly, elimination of trachea-derived ROS diminishes the infection induced ISC proliferation. Thus, trachea derived ROS are necessary for ISC mediated regenerative response to infection.

Overall, our work focused on the role of conserved signaling pathways: TNFα, FGFR, HIF1α in intestinal homeostasis and regeneration, not in isolation but in the context of the wider environment of the intestine and the tissues that surround it. It establishes for the first time *D. melanogaster* as a model for the study of pathological angiogenesis in the intestinal epithelium of the adult animal. The discovery of the cellular events and the signaling pathways that link the inflammation, with tissue regeneration and tracheogenesis in *D. melanogaster*, may lead to the discovery of new therapeutic approaches.

Tuesday, December 17, 2019 at 14:00
Building ΧΩΔ02, Room B109 (Panepistimioupoli Campus)

The presentation is open to the public.