SESSION 1C

MOLECULAR CONTROL OF THE CELL

Session Moderator: Hannele Ruohola-Baker, Biochemistry

MGH 171

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Role of ATF5 in the Regulation of Diapause-Like State in Mouse ESCs *In Vitro*

Rufuto Rahman, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Abdiasis Hussein, Biochemistry

During embryonic development, a dormancy-like state known as diapause arises during the transition from pre to post implantation. This state of suspended development is a reproductive strategy which favors newborn survival in mammals during nutritional deprivation or stress. Studies from the Ruohola-Baker lab found potential candidate regulators of diapause by establishing an in-vitro diapause model using pluripotent mouse embryonic stem cells (mESC). One of the genes is Activating Transcription Factor 5 (ATF5) which encodes a protein capable of survival-mediated functions such as maintaining mitochondrial activity during stress, modulating cell differentiation, preventing apoptosis and regulating cancer pathway. ATF5 has been known to transcriptionally target mTOR, a mechanistic target of rapamycin. Energy stress in the form of starvation and pharmacological inhibition of mTOR has shown to induce diapause-like state in mESCs in vitro. Our hypothesis is that upregulation of ATF5 under energy stress will reestablish diapause-like state in naïve mouse embryonic stem cells in vitro. We will test our hypothesis by loss-of-function and overexpression experiments. We test if ATF5 gene knockout using CRISPR-Cas9 prevents the mutant lines from entering diapause-like state from energy stress. Using western blots, we will quantify phospho-mTOR levels and its downstream targets in the ATF5 KO lines and compare them with the wildtype lines. For the overexpression of ATF5, we will make rescue lines for the ATF5 KO cells. We predict that overexpressed ATF5 in rescue lines will enter diapause-like state, and have reduced mTOR and its downstream target signals compared to KO lines. Our discoveries of ATF5 function in diapause can be useful in understanding how early-staged cancer stem cells enter a diapause-like state or quiescent state which enables them to escape chemotherapy detection. We can potentially contribute to the development of therapies to target ATF5 mechanism so that these undetected cancer stem cells can be detected.

POSTER SESSION 2

Balcony, Easel 94

1:00 PM to 2:30 PM

Examination of Molecular Mechanisms in Zebrafish Heart Regeneration

Gargi Sivaram, Senior, Biochemistry
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Elisa Clark

Neonatal mammalian heart tissues possess regenerative capabilities after injuries like myocardial infarctions that are mostly lost in adult mammalian tissues but conserved through adulthood in other vertebrates like zebrafish. Previous studies have shown that regeneration in ventricular cardiomyocytes (CM) occurs through de-differentiation and proliferation, but the underlying mechanisms that cause cardiomyocytes to enter the primed cell-cycle are unknown. Here we show that amino acid and metabolite levels in injured cardiomyocytes result in a primed state for regenerating cells. In chemically ablated zebrafish, it is shown that the amino acid profile activates the mTOR pathway to drive regeneration. Amino acid activation of mTOR is a result of high glutamine and leucine levels post-injury and in early heart regeneration in adult zebrafish, which is lost in adut mammals. Inhibition of the Wnt/β-catenin signaling pathway upstream of mTOR shows down regulation of mTORC1, showing that mTOR is necessary for CM proliferation in regenerating heart tissue. How Wnt signaling gets activated upon injury is unknown, and this study aims to understand the pathways upstream of Wnt signaling for activation. It is known that scarring needs to occur before regeneration occurs in heart tissue. This study also investigates why macrophages are essential for scar formation in ablated heart tissue and its underlying mechanisms. Further, single cell RNA sequencing one-week post injury is used to determine cell fates of the heart tissue. Cardiac cell types like CMs, endocardial and epicardial cells, and bulbus arteriosus (BA) cells were activated post-injury, with epicari-

dal cells promoting CM regeneration and BA cells activating signaling pathways during heart regeneration. This study demonstrates the signaling and metabolic pathways that activate cardiomyocyte regeneration in zebrafish hearts.