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.

A Distal Non-Coding Control Region Tunes the Timing of T-cell Commitment
Allan L. Wang, Senior, Biochemistry
Mentor: Hao Yuan Kueh, Bioengineering
Mentor: Nick Pease

In order for multipotent stem cells to properly differentiate into specialized cells, specific genes must be expressed at a specific time and amount during development. Many of the factors that regulate expression have been identified; however, it remains unclear how they work together to control the timing and amplitude of gene expression. Non-coding DNA elements, known as enhancers, can increase the the likelihood of transcription of a gene by integrating signals in the cell to provide regulatory logic for gene regulation. To understand how enhancers tune gene expression timing and amplitude during development, our lab has generated a transgenic mouse in which each of the two copies of the T-cell identity gene, Bcl11b, have been tagged with distinguishable fluorescent reporters, providing a sensitive readout for gene activity at the single locus level. Bcl11b turns on during T-cell development, and its activation executes a developmental switch from a hematopoietic stem cell to a T-cell committed progenitor. There is a non-coding region far downstream of Bcl11b which harbors a cluster of putative enhancers. To interrogate the function of individual candidate enhancers, we use CRISPR/Cas9 targeting to generate specific genomic deletions in T cell progenitors. From our preliminary experiments, we have shown that cutting off the entire enhancer region completely inhibits the expression of Bcl11b entirely compared to when we cut out only an individual enhancer peak which only partially inhibits it. This is promising because it shows that we have found an enhancer that controls the probability of activation while not being necessary for the activation of Bcl11b. This work will reveal the cis-regulatory logic that underlies the control of a master lineage-specifying gene. This better understanding will help us identify new strategies to control the expression of master regulatory genes for cellular reprogramming.

POSTER SESSION 2

Balcony, Easel 108
1:00 PM to 2:30 PM

Characterizing Stem Cell Specific-Kinases as Regulators of Self-Renewal in Rhabdomyosarcoma
Shelly Lin, Senior, Biology (General)
Mentor: Eleanor Chen, Pathology
Mentor: Thao Pham, Pathology

Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. The survival outcomes are poor for patients with relapsed and metastatic disease. Embryonal RMS (ERMS), a major subtype of RMS, is driven by genetic mutations in the RAS pathway. Cancer stem cells (CSCs) drive the process of self-renewal to recapitulate the heterogeneity of the cancer and are thought to be responsible for cancer relapse due to their resistance to conventional chemotherapy. In ERMS cells, a population of CSCs has been identified and is believed to play a critical role in tumor relapse and metastasis. A previous screen of all known human kinases identified candidates that played a role in regulating self-renewal of ERMS CSCs. I have prioritized two most promising candidates, ERN1 and MAPK10, for further characterization. I hypothesize that these kinases regulate the self-renewal capacity of CSCs but do not affect growth of non-CSC tumor cells. Using the gene-editing tool, CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats)/Cas9, I knocked out ERN1 and MAPK10 in ERMS cells and assessed their role in regulating ERMS CSC cell growth and self-renewal. To assess whether the effects of gene disruption are specific to the changes in the CSC population in vitro and in vivo, I used an ERMS CSC reporter cell line that specifically expresses Green Fluorescent Protein (GFP) in CSCs. The ERMS CSC reporter cell lines harboring ERN1 or MAPK10 gene disruption are subjected to self-renewal assays in cultured ERMS cells in vitro and ERMS xenograft model in vivo. My findings will provide new insights into the biological mechanism underlying as well as new potential targets against relapse and metastasis of ERMS.

POSTER SESSION 3

Commons East, Easel 50

2:30 PM to 4:00 PM

Using Patient Reported Outcome Measurements in Assessing Disease Activity of Chronic Non-Bacterial Osteomyelitis

Sumaya Aden, Junior, Pre-Sciences
Thuan Thi Bui, Sophomore, Pre-Sciences
Claire Yang, Junior, Environmental Health
Pola Soliman, Senior,
Mentor: Yongdong Zhao
Mentor: Chessie Snider, Center for Clinical Translational Research, Seattle Children's Research Institute

Chronic non-bacterial osteomyelitis (CNO), or chronic recurrent multifocal osteomyelitis (CRMO), is an auto-inflammatory bone condition that causes persistent bone pain, poor growth, and other complications. Physical exams, laboratory tests and radiographs are not sensitive in detecting the active disease. MRI is the gold standard assessment for diagnosis. Patient reported outcome (PRO) measurements have not been commonly used to determine the disease impact on the physical health of affected children. Pain score is not accurate in assessing disease activity due to the confounding effects from other associated conditions such as amplified pain syndrome. Consensus treatment plans (CTPs) were developed by the Childhood Arthritis and Rheumatology Research Alliance (CARRA). In this study, a prospective patient registry based on CTPs is used to compare the effects of different treatment plans. This study was approved by the Seattle Children's Institutional Review Board (#1232). The center is the coordinating site of this multicenter observational study, Chronic nonbacterial Osteomyelitis International Registry (CHOIR). Inclusion criteria are: $\leq$21 years old at enrollment and CNO diagnosis. Consent and assents are obtained. Standard of care is provided to the subjects and no investigational intervention is performed. Detailed clinical information, laboratory, and imaging data along with patient/parent reported outcome measurements (PROMIS questionnaires) and childhood health assessment questionnaire (CHAQ) are collected at baseline and follow-up visits. We aim to enroll 2,000 subjects worldwide and follow up with subjects for at least 5 years. Our center has enrolled 74 subjects between June 2018-present and collected PROs at baseline visits. Data is being entered into REDCap database for further analysis. PROs collected throughout the course of the study will be correlated with imaging results and expectedly determine the effectiveness of commonly used medications for patients. The data will establish measurements for physicians to estimate disease burden and treatment responses in children with CNO.

POSTER SESSION 3

MGH 241, Easel 153

2:30 PM to 4:00 PM

Natural Variation of Reproductive Output in *Drosophila melanogaster*

Meghna Manoj, Senior, Biology (General)
Mentor: Daniel Promislow, Department of Lab Medicine & Pathology, University of Washington School of Medicine
Mentor: Xiaqing Zhao, Pathology

This project examines reproductive output across different wildtype genotypes in the fruit fly, *Drosophila melanogaster,* and how it correlates with lifespan, activity and age-specific metabolite levels. To answer this question, we used the Drosophila Genome Reference Panel (DGRP), a collection of ˜190 fully sequenced *Drosophila* strains that have been inbred to near homozygosity. Fecundity was measured as the *per capita* number of adult offspring produced by female flies over 48 hours when they are 4, 8, and 12 days old, using 3-5 replicate vials for each *Drosophila* strain. We have shown that there is highly significant genetic variation for reproductive output. Reproductive output declines with age in most but not all genotypes. The correlation between reproductive output and lifespan was not statistically significant, suggesting that there is not a direct trade-off between survival and reproduction in this population. A genome wide association study (GWAS) identified statistically significant single nucleotide polymorphisms correlated with variation in reproductive output, although most of them were located in genes of unknown function.

POSTER SESSION 4

MGH 258, Easel 192

4:00 PM to 6:00 PM

Generating Constructs for Synaptic Neuro-Proteomics

Taylor Moreno, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry
UW Honors Program
Mentor: Shao-En Ong, Pharmacology

The mammalian brain is composed of a wide variety of neurons, all of which signal through structurally and biochemically heterogeneous synapses. Understanding the proteomic makeup of these synapses is crucial for understanding brain function. However, biochemical isolation and characterization has proven difficult due to the morphological complexity of neurons. MS-based proteomics could be a useful tool for studying such diversity, but spatial information is lost because the analysis is performed post-lysis. In this study we proposed to overcome this limitation by using proximity biotin labeling of genetically specified neuronal subpopulations. I thus constructed APEX2-GFP fusion constructs with

post-synaptic proteins which could be expressed in vivo using an adeno-associated virus (AAV). Four post-synaptic proteins were chosen: LRRTM1 and LRRTM2 to localize excitatory synapses, and NGLN2 and SLITRK3 to localize inhibitory synapses. A pAAV-CAG (AAV plasmid with CAG promoter) backbone was digested using NotI and AscI, and an APEX2-GFP fragment with NotI and AscI ends was generated via Phusion PCR. Following NotI/AscI double digests, the pAAV-CAG backbone and APEX2-GFP fragment were ligated together in order to make a pAAV-CAG-APEX2-GFP vector. The pAAV-CAG-APEX2-GFP vector was opened with a NotI digest, and gene fragments of the four post-synaptic proteins with Gibson overlaps were then generated via Phusion PCR. Gibson assembly was then used to assemble the pAAV-CAG-APEX2-GFP vector with the gene fragments, and pAAV-CAG-gene-APEX2-GFP vectors were generated for LRRTM1 and LRRTM2. The vectors were then expressed in HEK cells and monitored for GFP expression and biotinylation activity. The pAAV-CAG-APEX2-GFP vector showed bioluminescence and biotinylation activity, but the vectors with gene inserts did not. Although we successfully cloned two of the target constructs, current investigations are still under way in order to optimize them.