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Online Proceedings

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Using Modern Genetic Approaches to Investigate Development and Disease

Session Moderator: Celeste Berg, Genome Sciences

MGH 389

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Racial Differences in Genetic Determinants of Telomere Length

Andrew Michael (Andy) Zeiger, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar, UW Honors Program

Mentor: Sam Oh

We demonstrate that results from genetic association studies of telomere length performed in European populations are not generalizable to African Americans. Telomeres are repeated segments of DNA at the ends of chromosomes important for maintaining healthy chromosomes. Telomeres have been associated with a number of diseases and age-related conditions, however this relationship is complex because it is uncertain if an individual's telomere length increases their risk for disease, or if disease and other environmental exposures are modifying the individual's telomere length. Recent studies have scanned the entire genome for genetic variants that may be associated with telomere length. Nine highly significant genetic variants associated with telomere length have been identified, though the study populations have been of exclusively European descent. Since it is well known that the genetic architecture varies extensively across racial populations, we examined whether the nine genetic variants were associated with telomere length in 1,442 African American children (age 8-21) from the Study of African Americans, Asthma, Genes and Environments. We examined the genotypes for each variant using multivariable linear regression to test whether the nine genotyped variants were associated with telomere length. We found that the nine variants identified in European populations were not significantly associated with telomere length in African American children. In addition to these nine variants, we examined locations across the entire genome for association with telomere length. We found several genetic loci associated with telomere length that were specific to sex and asthma status. These loci map to chromosome positions 6p22.1, 13q21.1, 20p11.23 and genes *INPPB4* and *CFTR*. To our knowledge, this is the first study demonstrating that the nine variants associated with telomere length in European populations are not generalizable to African Americans. Our study underscores the necessity for including diverse populations in biomedical research.

Genetic Differentiation in Reproductive Gene of Pacific Cod Along Species' Geographical Range

Theodore Shumway (Teddy) Hartinger, Senior, Aquatic & Fishery Sciences

Mentor: Daniel Drinan, School of Aquatic & Fishery Sciences

Mentor: Lorenz Hauser, School of Aquatic and Fishery Sciences

Pacific cod (*Gadus macrocephalus*) is the target species of a large fishery in North America that ranges from Alaska to California. In a recent genetic sequencing study, very high divergence among Pacific cod from different geographic areas was observed in what appears to be the Zona pellucida sperm-binding protein 3 (ZP3) gene. In many other vertebrates, the ZP3 gene codes for proteins that are significant to reproduction, and this gene may be an important factor in the context of the species' life history and management. To increase resolution of how ZP3 varies across the Pacific cod range, this project investigates the entire coding sequence in Pacific cod from four geographic areas: Kodiak Island, Prince Williams Sound, Hecate Strait, and the Washington Coast using PCR and Sanger sequencing. Differences in amino acids and protein structure were identified. It appears that there are significant differences in the ZP3 gene between southern and northern populations of Pacific cod. Understanding these patterns is important for future studies of how differences in the ZP3 gene may affect reproduction and population connectivity.

A Novel Growth Factor Gene Family Mediates Tube Formation in *Drosophila*

Liesl Grace (Liesl) Strand, Senior, Biology (Molecular, Cellular & Developmental)

Levinson Emerging Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee

Mentor: Celeste Berg, Genome Sciences

From blood vessels to the small intestine to the spinal cord, tubes are an essential part of nearly all multi-cellular organisms. Errors in tube formation, called tubulogenesis, cause many of the birth defects that afflict infants, including congenital heart defects and spina bifida, a failure to close the neural tube. Our lab uses the fruit fly *Drosophila melanogaster* to study tube formation because of the highly conserved nature of this morphological process between our species. My project focuses specifically on a family of genes called Imaginal Disc Growth Factors (IDGFs), which are linked to tubulogenesis in *Drosophila* and are closely related to a human protein family (CLPs) that have been found to be dysregulated in numerous diseases, including arthritis, and in metastasizing tumor cells. While this homology indicates that IDGFs have a role in cell behaviour, the mechanisms by which these genes act remain unclear. Last year, I used the gene-editing technology CRISPR/Cas9 to investigate the function of one IDGF, the gene Idgf6, by deleting it entirely. Analysis of these knock-out mutants suggests that Idgf6 plays an important role in making and shaping tubes and that removal of Idgf6 results in branched structures instead of discrete tubes. This branching phenotype likely limits the flow of oxygen to the developing embryo, resulting in decreased survival of offspring. My current research explores this genetic pathway further by testing tube morphogenesis under hypoxia and other stress conditions such as crowding, with the aim of further defining the mechanism of tube dysfunction in these mutants.

From Genotype to Phenotype: Unraveling the Developmental Effects of *vent* and *eve1* Mutations Using CRISPR-Cas9

Alice Xin (Alice) Dong, Senior, Biochemistry

Mary Gates Scholar, UW Honors Program

Mentor: David Kimelman, Liberal Arts

During development, the repression of transcription factor *tbx16* allows embryos to dynamically allocate neural and muscle tissue in the correction proportion as the body axis is formed. Understanding how *tbx16* is regulated is crucial for identifying the underlying mechanisms of neuromuscular developmental defects. Currently, we are investigating how two genes of interest termed *eve1* and *vent* interact to regulate the expression of *tbx16* in zebrafish embryogenesis. Previous studies have relied on Morpholino anti-sense oligonucleotides (MOs), but MOs often create off-target effects and misleading phenotypes that don't reflect genetic mutants. Using the CRISPR-Cas9 system in zebrafish, we have created true genetic knockouts for *eve1* and *vent*. By analyzing the difference in phenotypes between *eve1* mutants, *vent* mutants, and *eve 1/vent* double mutants, we can determine if *eve1* and *vent* act redundantly to repress *tbx16*. Our goal is to uncover the mechanisms behind these mutant phenotypes in order to identify the role that *eve1* and *vent* play during embryogenesis. Our research aims to further our understanding about how serious birth deficits arise from gene deficiencies and complications in development.

Engineering Zebrafish to Model Human Genes Related to Congenital Heart Defects

Kimia Imani, Senior, Biochemistry

Mary Gates Scholar, NASA Space Grant Scholar

Mentor: Lisa Maves, Pediatrics

Congenital Heart Disease (CHD) is the most common inborn defect in babies and is responsible for 1/3 of all major congenital anomalies. With its continuous prevalence in the human population, the genetic causes remain poorly understood. Previous findings have shown that *Pbx* transcription factors control gene expression during development. Studies in zebrafish shows that *pbx4* has significant requirements for heart muscle differentiation. Our hypothesis states that mutations in human *Pbx* genes can lead to heart defects. The purpose of this study is to acquire further insight about the role of *Pbx* genes in the context of CHD and analyze cardiac dysmorphic patterns in *pbx* gene mutants. By using the genomic editing system CRISPR/Cas9 (Clustered regulatory interspaced short palindromic repeats), we have been able to make exact genetic changes in *Pbx* genes in zebrafish (*Danio rerio*) animal models. In particular, we have engineered a *Pbx* gene mutation found in humans with heart defects, known as *Pbx4* SNP (single nucleotide polymorphism). For our experiments, samples of zebrafish DNA are obtained and genotyped with various assays for mutations. We use microscopy and transgenic markers to analyze heart defects in mutant embryos. *Pbx4* mutant zebrafish display distinct phenotypessuch as precardial edema and bulged ventricles, but it is not clear at what stages these morphogenesis defects occur. To further investigate the dysmorphic phenotypes, our next step is to conduct cardiomyocyte counting analysis in *Pbx4* SNP and *Pbx2/Pbx4* double mutant fish and gather data at 24 and 48 hours of development. This experiment will assist in better understanding the heart defects previously seen in the single *Pbx4* mutant samples. Understanding the mechanisms and discovering the genes of CHD, as well as cardiac morphogenesis patterns, will contribute to finding therapies and improving genetic screenings for patients with lifelong heart defects.

RNA-Targeting CRISPR/Cas9 to Visualize RNA Trafficking in Differentiating Fibroblasts in Response to Disease Stimuli

Jasmine Jay (Jasmine) Fuerte Stone, Senior, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Jennifer Davis, Bioengineering & Pathology

Myocardial infarctions (MI) are ischemic events which cause heart tissue to die and scar tissue (fibrosis) forms to maintain integrity of the walls of the organ. Fibrosis is crucial in the initial healing process but continued formation can compromise the elasticity of the tissue and lead to heart failure. Fibrosis is formed by myofibroblasts. These cells differentiate from fibroblasts after MI. Currently, the differentiation process is not well understood. The RNA binding protein muscleblind like (MBNL1) is known to be critical in this process, however. This project aims to use fluorescent imaging to track the localization of mRNA bound by MBNL1, particularly serum response factor (SRF) which has also been shown to play a role in myofibroblast differentiation. We aim to use regularly interspaced short palindromic repeats (CRISPR) and nuclease-null, RNA-targeting CRISPR associated protein 9 (RCas9) to specifically bind to the desired sequence of SRF mRNA. Enhanced green fluorescent protein (EGFP) is bound to the RCas9 in order to be able to visualize the RNA. Two target sequences on SRF mRNA were identified and protospacer adjacent motif presenting oligonucleotides (PAMmer) and single-guide RNA (sgRNA) were designed to bind to these sequences. PAMmer and sgRNA allow Cas9 to bind to the target sequence. This system was transfected into mouse embryonic fibroblast (NIH 3T3) cells and differentiation induced using transforming growth factor beta. During differentiation, live cell imaging was used to visualize RNA dynamics and localization. Results are expected to show colocalization with MBNL1 in the cytoplasm. This data will allow for the correlation of RNA trafficking and cell differentiation. It will also allow for a better understanding of myofibroblast differentiation and fibrosis formation after a myocardial infarction.

Functional Engraftment of Murine Pre-Osteoblastic Cells in a Zebrafish Model of Epimorphic Bone Regeneration

Barrie Sue Sugarman, Senior, Biology (Physiology)
Mary Gates Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Ronald Kwon, Orthopaedics and Sports Medicine, UW School of Medicine/Institute for Stem Cell and Regenerative Medicine

Urodeles such as newts and salamanders have the capacity to regenerate limbs following amputation through epimorphic regeneration, a process characterized by the formation of a proliferative mass of partially dedifferentiated cells called the blastema. The blastema is formed through three steps: migration of cells to the amputation site, dedifferentiation, and re-entry into the cell cycle. It is unknown whether mammalian cells possess competence to respond to blastema formation and process the inductive signals that drive migration, dedifferentiation, and proliferation. To explore this question, our goals were twofold. First, we aimed to develop a xenograft model of epimorphic regeneration by introducing mammalian pre-osteoblastic cells into the regenerating zebrafish tail fin (a tractable model of epimorphic bone regeneration that molecularly resembles amphibian limb regeneration). Next, we sought to compare the behavior of mammalian cells to embryonic zebrafish cells by developing an allograft assay. Adult zebrafish were subjected to caudal fin amputation and housed in ˜33C water. Three days post amputation, CM-DiI-labeled MC3T3-E1 murine pre-osteoblastic cells or fluorescent-dextran labeled embryonic zebrafish cells extracted from the blastula period were injected into the proximal blastema of the adult fish. Fish were subjected to daily *in vivo* imaging. Injected murine cells exhibited migration to the blastema, engraftment, and stability for up to 48 hours post injection (hpi); decreased fluorescence was observed at 2-3 days post injection potentially due to fish immune system activity. Allograft comparisons showed distal migration, engraftment, and stability for 48-72 hpi in embryonic zebrafish cells in a matter nearly identical to the behavior observed in murine cells. Because MC3T3-E1s and embryonic cells exhibit similar migration behavior, our data suggest that murine cells are able to process inductive signals driving localization to distal tissue. Provided the correct conditions, mammalian pre-osteoblastic cells may be capable of engrafting and proliferating in an epimorphic bone regeneration process.

Redundancy of Heterochromatin Protein 1 Family Members Preserves Normal Cardiac Myocyte Function After Gene Ablation

Chen (Joe) Fang, Senior, Biology (General), Biochemistry
Mentor: Kyohei Oyama, Cardiology
Mentor: Robb MacLellan, Medicine, Cardiology

Mammalian cardiac myocytes (CMs) decrease proliferation soon after birth. This is caused by silencing of cell cycle gene expression. Gene activity is epigenetically regulated by physically changing chromatin structure. Generally, gene silencing is mediated by heterochromatin because of its tightly packed structure, which limits accessibility for transcription factors to bind to the gene promoters. Silenced chromatin is believed to form when heterochromatin protein 1 (HP1) binds to histone H3 lysine 9 tri-methylation (H3K9me3) to condense chromatin into heterochromatin. We previously found that demethylation of H3K9me3 inhibited cell cycle gene silencing in CMs and resulted in hyperplastic heart growth. This suggests that H3K9me3/HP1 pathway is an important regulator for cell cycle gene silencing.

There are three isoforms of HP1 (alpha, beta and gamma) expressed in mammals, but we found that HP1 gamma was most highly expressed in CMs. Therefore, we created a cardiac specific HP1 gamma knockout (KO) mouse and tested our hypothesis that loss of HP1 gamma would reactivate cell cycle gene expression and induces cardiac growth. Using RT-PCR we confirmed deletion of critical exons of HP1 gamma mRNA. Western blot demonstrated significant reduction of HP1 gamma protein in KO CMs. Immunofluorescence staining showed HP1 gamma expression was reduced specifically in CMs. HP1 gamma KO mice showed normal heart growth and no significant difference in heart mass was observed compared to control hearts. Consistent with this result, there were no significant differences in cell cycle or cardiac gene expression between HP1 gamma KO and control CMs. Interestingly we found an increase in protein level of HP1 beta, but not HP1 alpha, in HP1 gamma KO CMs. These data demonstrate that HP1 gamma's function is redundant in cell cycle gene silencing and cardiac growth, which could be due to HP1 beta compensating for the loss of HP1 gamma in CMs.