Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 241, Easel 135

11:00 AM to 1:00 PM

Characterizing the Role of CD82 in the Pathogenesis of Rhabdomyosarcoma

Phuong Van, Junior, Biology (Molecular, Cellular & Developmental)
Mentor: Eleanor Chen, Pathology
Mentor: Thao Pham, Pathology
Mentor: Terra Vleeshouwer-Neumann, Pathology
Mentor: Michael Phelps

Pediatric rhabdomyosarcoma (RMS) is a rare and aggressive cancer that arises from skeletal muscle precursors in muscle and connective tissues. The molecular mechanisms underlying RMS progression, relapse and metastasis remain poorly characterized. CD82, a novel metastasis suppressor gene, has been shown to decrease tumor progression in a subset of human cancer types when expressed. Recently, two scientific publications investigated the role of CD82 in cellular differentiation and proliferation in muscle precursor cells. Uezumi et. al (2016) claimed that loss of CD82 results in premature differentiation and a depletion of muscle precursor cells. In contrast, Alexander et. Al (2016) discovered opposing results in which loss of CD82 in fetal muscle cells impairs the capacity of myogenic precursor cells to differentiate into mature muscle cells. In RMS, tumor cells keep on proliferating and have lost the capacity to differentiate into mature muscle cells. The role of CD82 in regulating cell proliferation and differentiation in RMS is unknown. In order to investigate the function of CD82 in differentiation of RMS, I used the CRISPR/Cas9 genome engineering system to target the gene in RMS cell lines. To characterize the knockout phenotype, I performed various cell-based assays to characterize the effects of CD82 gene knockout on the cellular phenotypes in tumor cell differentiation, proliferation, and self-renewal. Investigating the role of CD82 in regulating cellular differentiation and proliferation in RMS would provide important insight into the pathogenesis of cancer.

CRISPR/Cas9-Mediated Gene Therapy of Rhabdomyosarcoma

Colton Heechang (Colton) Yang, Senior, Biochemistry
Mary Gates Scholar
Mentor: Eleanor Chen, Pathology

Rhabdomyosarcoma (RMS) is a soft tissue cancer that most likely develops from skeletal muscle stem cells. Like other cancers, RMS is a disease driven by critical mutation(s) in the patients' genome, causing otherwise normal cells to divide uncontrollably without differentiating. Current treatments of RMS include chemotherapy, radiation therapy and surgical removal of the tumor. However, these treatments are limited in that they are toxic to both cancer and normal cells and are frequently ineffective in killing cancer cells because they do not directly target the genetic mutations causing cancer. One promising strategy to treat cancer is to develop therapies targeting the essential genetic mutations causing cancer. By utilizing the novel Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR Associated Protein 9 (Cas9) gene editing tool that is more accurate and reliable than its predecessors, my project aims to target and knock-out the deleterious genes that promote growth and progression of RMS cancer cells. The two major subtypes of RMS are embryonal (ERMS) and alveolar (ARMS) with which they are defined largely by either activation of the RAS pathway (ERMS) or the presence of a fusion oncogene between PAX3 and FOXO1 (ARMS). To demonstrate the viability of the CRISPR-mediated gene therapy, I first targeted critical exons in the oncogenes of both RMS subtypes using inducible or viral CRISPR/Cas9 delivery systems. Targeting these essential genes resulted in significant regression of tumor xenografts. To further demonstrate that tumor-specific CRISPR/Cas9 gene therapy can also be achieved, precise targeting of only the mutation site(s) of the oncogene followed. This strategy disrupts the essential cancer oncogenes with minimal impact to normal cells that do not exhibit the unique cancer mutations. This novel approach to cancer therapy provides a promising alternative for traditional treatments while establishing a foundation for further development of CRISPR/Cas9 gene therapy for cancer treatment.

POSTER SESSION 1

MGH 241, Easel 133

11:00 AM to 1:00 PM

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MGH 241, Easel 134

11:00 AM to 1:00 PM

Developing Gene Knock-in Technology in Zebrafish
Jessica Erin Gianopulos, Junior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar, UW Honors Program
Mentor: Eleanor Chen, Pathology
Mentor: Michael Phelps

Zebrafish are a valuable model organism in scientific research, however there are limited genetic tools available for inserting/integrating engineered DNA into precise locations in the zebrafish genome. This project has developed a new method of precisely integrating engineered genes into specific zebrafish genomic locations. The heat shock-70 like (hsp70l) gene in the zebrafish genome is an ideal location for new gene (transgene) integration because the hsp70l promoter enables temporal regulation of the transgene through a heat shock-inducible mechanism. CRISPR/Cas9 genome editing technology was used to insert genetic markers, called attP sequences, flanking the green fluorescent protein (GFP) gene into the hsp70l gene locus in zebrafish. I built a construct containing attB sequences flanking the red fluorescent protein (RFP) transgene. These attP and attB markers are recognized by viral integrase proteins, bxb1 and fC31, which cut and recombine the attP and attB sequences causing directional recombination replacing GFP with RFP. I performed a proof-of-principle experiment by injecting zebrafish embryos with this new attB-RFP construct and the old attP-GFP construct with both the bxb1 and fC31 integrase enzymes to show functional integration in zebrafish. I optimized injection techniques to maximize the recombination frequency of the attB-RFP integration at the hsp70l gene in the zebrafish genome. This system allows for the rapid insertion of any transgene precisely into the endogenous hsp70l locus. I used this system to create heat-shock inducible Cas9 transgenic zebrafish as a tool for characterizing the function of novel genes essential for promoting or suppressing cancer growth in zebrafish tumor models. Inducing Cas9-mediated gene knockout is allowing us to determine the specific role a gene plays in cancer progression which helps us identify viable targets for the development of new cancer therapies.

POSTER SESSION 1

MGH 241, Easel 132
11:00 AM to 1:00 PM

Activating and Repressing Genes in Zebrafish Using Cas9-VPR
Marilyn Erin Moelhman, Senior, Biology (Physiology)
Mentor: Eleanor Chen, Pathology

Cas9 is a versatile and effective genome engineering tool in characterizing biological effects of gene pathways due to its ability to activate and knock out genes with high efficiency and accuracy. When given truncated guide sequences 15 base pairs in length, Cas9 will bind to the specified site without cutting. By manipulating the binding sites of Cas9 in a gene, Cas9 can turn on or off gene transcription, thereby influencing gene activity. We constructed a Cas9-VPR system with the aforementioned features and have begun testing its ability to increase and decrease transcription of various cancer promoting genes. We quantified the level of each gene activity after introducing the Cas9-VPR system into the cells using quantitative Polymerase Chain Reaction (qPCR). Once we have optimized the system in modulating gene activity in cultured cells, we will move on to testing the functionality of Cas9-VPR in vivo using zebrafish as the model system. We plan to demonstrate Cas9-VPR's efficacy by increasing expression of the *Wnt* pathway, which should result in the formation of a secondary embryonic axis in developing zebrafish embryo. Demonstrating Cas9-VPR's ability to function in zebrafish will allow future research to examine the effects of manipulating multiple genes in order to understand the complex interaction of pathways driving a biological process.

POSTER SESSION 1

Commons East, Easel 61
11:00 AM to 1:00 PM

Home Automation Diagnostic System
Khe Tran La (Khe Bach) Bach, Senior, Electrical Engineering
Ennis Dakhil, Senior, Electrical Engineering
Yeojun Yoon, Junior, Electrical Engineering, Applied & Computational Mathematical Sciences (Engineering & Physical)
Mentor: Tai-Chang Chen, Electrical Engineering

With the expansion of the lighting industry, there has been an increase in LEDs being used in new infrastructure. Comparing with incandescent and fluorescent lights, LEDs are more energy efficient, require less maintenance, and are compatible with the internet of things. However, troubleshooting a failing LED is a tedious task for an electrician, and there are not good ways of diagnosing problems with a LED. A probable solution is a handheld device that is capable of measuring multiple lighting parameters such as temperature, luminosity, color temperature and flickering percentage. This project uses the principle of photometric testing and analysis, the theory of Black Body radiation in color temperature measurement to collect data from the luminosity, temperature, and RGB sensors. The data is used to compare with the manufacturer's performance standards and real time data of the sensors is displayed on LCD screen. Electricians can pinpoint the issues with an LED if one of the lighting parameter does not match with the manufacturer's standards. In the future, this system will expand to other types of smart devices such as switches, dimmers and will allow user access through an application on a smartphone.

POSTER SESSION 2

Commons East, Easel 52

1:00 PM to 2:30 PM

Plugable Smart USB Charger

Roy Raeyeon (Roy) Jang, Senior, Electrical Engineering
Long Hei (Ringo) Wong, Senior, Electrical Engineering
Yangming Ke, Senior, Applied & Computational Mathematical Sciences (Discrete Mathematics & Algorithms), Electrical Engineering
Bolun (bolun) Yan, Senior, Electrical Engineering
Mentor: Bernie Thompson
Mentor: Tai-Chang Chen, Electrical Engineering

We are UW Smart USB Charger Team supervised by Plugable Technology and a UW EE faculty mentor. The main objective of this project is to design and implement a Smart USB charger that has five USB ports. It is called "smart" because the charger will be able to charge the higher priority phones at almost full rate before moving on to lower priority phones. With that function, this USB charger will fully charge several phones much faster than traditional USB chargers having only a 5V 3A power supply. However, there is no such five ports USB charger in the current electronic device market, so we see the important implications of solving this problem. What our team will have done by the day of our Symposium presentation is to implement the priority charge behavior module using C language and an Arduino Mega Board which has a microcontroller inside. The challenges of the task are that the actual charging behavior of different devices is often unpredictable or sometimes disappointing and the devices themselves decide how much current to draw and manufacturers have not settled on a single standard. Therefore, our module should be able to select the best charging mechanism for each of different devices while supporting priority charge. To do this, it requires us to test our module with as many different devices as possible and to collect data for future references. The other goal is to make the product as low-cost as possible: We will use lowest-cost microcontroller with sufficient I/Os to control all ports and lowest-cost external components for port power control. Finally, if more time is allowed, we will try to design and implement a PCB.

SESSION 2S

MODULATION OF CELL BEHAVIOR AND ITS COMPONENTS

Session Moderator: Valerie Daggett, Bioengineering

JHN 175

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Immunostaining Histone acetyltransferase p300 (also known as p300) and cAMP response element-binding protein (CBP)

Noah Schlenk, Junior, BBMB (Biochemistry, Biophysics, Molecular Biology), Whitman College
Mentor: Paul Yancey, Biology, Whitman College
Mentor: Samuel LaBarge, Orthopaedic Surgery, University of California, San Diego
Mentor: Simon Schenk, Orthopaedic Surgery, University of California, San Diego

My presentation focuses on research I conducted using immunohistochemistry to identify proteins in insulin stimulated pathways. My research contributed to the lab's ability to analyze p300 and CBP proteins for their use of acetylation. p300 and CBP often are talked about hand in hand as two proteins that function together to induce and carry out acetylation of other proteins. Generally, you can think of them as a messenger, carrying the information from one source to the next in a long line of messangers. Phosphorylation has long been seen as the primary driving force of downstream signaling. Our lab examined the importance and underestimation of acetylation through these two proteins. Initially, the lab used Western Blotting to look for protein presence, but the results we obtained were faint and difficult to analyze. I was assigned the task of trouble-shooting a protocol for the staining of these two proteins. I sliced tissue, plated it, stained it, and calculated the changes between knockout- and wild-type tissue. I was ultimately successful in identifying p300 but statistically unsuccessful in identifying CBP due to a technical error. My research showed a much more reliable way of identifying at least 1 of the proteins rather than Western Blotting techniques. This has a broader implication that there will possibly be more accurate or reliable research done on these p300 and CBP proteins, and acetylation as a primer for downstream pathway messaging done in the future.

POSTER SESSION 3

Commons East, Easel 65

2:30 PM to 4:00 PM

Ticket Classification via Data Science and Machine Learning

Huy Phuong (Huy) Nguyen, Senior, Electrical Engineering
Zehao (Hubert) Hu, Senior, Electrical Engineering
Ruolan (iris) Wei, Senior, Electrical Engineering
Mentor: Xiang Chen
Mentor: Sreeram Kannan
Mentor: Payman Arabshahi
Mentor: Swetha Kannan

Companies care of their image in the customers' mind, then offer customer service to understand and take care of customers' need. However, customer services seem to be overwhelmed, while they have to serve an increasing huge num-

ber of customers. Companies have to pay a lot of expenses for employing labor, while customers are not taken care of well enough. Customer Ticket Classification project offered by Tupl Inc. delivers an algorithm that takes in customer queries, then categorizes and generates responses to those queries. In detail, the algorithm classifies thousands of customers' messages into different categories and automatically responds to these messages based on the detected categories by using data science and machine learning. In detail, the project employs new data science tools such as Spark and Zeppelin to improve effectiveness. Thus, this algorithm can help companies save man power or labor hours and expenses in operating customer services.

POSTER SESSION 3

Balcony, Easel 122

2:30 PM to 4:00 PM

Mechanism of Monosodium Titanate Inhibition of Cell Growth *in Vitro*

Joelle N. (Joelle) Moussi, Senior, Microbiology
Mentor: Yen-Wei Chen, Restorative Dentistry
Mentor: Jeanie Drury, Restorative Dentistry
Mentor: John Wataha, Restorative Dentistry

The ion exchanger monosodium titanate (MST) has been proposed to therapeutically deliver calcium ions in dental situations that require calcium. However, previous results suggest that MST limits cell growth *in vitro* by mechanisms that remain obscure. It is critical to understand these mechanisms for any successful therapeutic use of MST. The current study tested the hypothesis that MST limits cell growth *in vitro* by binding to culture polystyrene, reducing attachments sites available to cells, thereby limiting cell culture growth. MST was added to culture wells (8 concentrations between 0 and 200 mg/L) either 24 h after addition of L929 fibroblasts/OSC2 osteoblasts or prior to cell addition. Cell cultures (n = 8/condition) were left in contact with the MST for 72 h, after which cell growth was assessed using Cell TiterBlue®. Control groups received no MST; statistical differences were detected using ANOVA with Tukey post-hoc tests ($\alpha = 0.05$). When MST was added after plating of the cells, cell growth was inhibited in a dose-dependent fashion to a maximum of 40% ($p < 0.05$ compared to no-MST controls). Yet when MST was added prior to the cells, the maximal inhibition of growth was 80% vs. controls. ($p < 0.05$). The addition of MST before cells significantly decreased growth in the cultures (relative to conditions where cells were established first). Microscopic inspection of the culture wells revealed more attachment of the MST to the culture polystyrene than when cells were added first. These results and observations are consistent with our hypothesis that the MST attaches the polystyrene and reduces the ability of cells to attach and proliferate. Further testing with other cell types and additional cell densities is in progress.

POSTER SESSION 3

Commons West, Easel 22

2:30 PM to 4:00 PM

Optimization of CRISPR-Activation to Engineer Metabolic Pathways

Anika Smita (Anika) Patel, Senior, Biochemistry
UW Honors Program
Mentor: Jesse Zalatan, Chemistry
Mentor: Chen Dong, Chemistry

Bacteria are widely used in metabolic engineering because they can synthesize chemical products from cheap, renewable carbon sources. A major current problem, however, is that toxic intermediates can build up when bacteria express heterologous biosynthetic pathways. In principle, we can control metabolic gene expression levels so that downstream enzymes quickly consume toxic intermediates. To achieve this goal, we use a programmable gene regulatory tool called the CRISPR-Cas system. To optimize gene activation activity of CRISPR-Cas in bacteria, we sought to identify modular transcriptional activation domains that could be recruited to specific genes to activate transcription. This strategy has been previously used successfully in eukaryotic cells, but with only limited success in bacteria. We used CRISPR-Cas to recruit a number of potential synthetic transcriptional activators to specific loci in *E. coli*, and identified candidates that can activate a fluorescent reporter gene. We also used protein-engineering approaches to optimize the gene activation activity of our CRISPR-Cas system. The transcription factor screening and protein engineering we did gave an 80-100-fold increase in activation compared to basal transcription levels. To make this new tool useful for practical metabolic engineering, we plan to use this system to dynamically respond to metabolic stresses. This tool could potentially be used to optimize biosynthesis of useful polymer precursors such as lactate and p-aminostyrene. Production of these types of cheap, biosynthetic precursors will help us move towards a greener industrial future.

POSTER SESSION 3

Balcony, Easel 106

2:30 PM to 4:00 PM

Development of a Doxorubicin-Loaded Cyclic Polymer Drug Delivery System

Karl Theodore (Karl) Manner, Senior, Bioen: Nanoscience & Molecular Engr
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering
Mentor: Yilong Cheng, Department of Bioengineering

The most common form of cancer treatment is the use of small-molecule chemotherapeutic agents. However, due to non-specific distribution and uptake, these drugs have a host of complications that limit their efficacy and usage. Polymer nanocarriers have been demonstrated to improve drug pharmacokinetics, enhancing therapeutic benefit of the drugs. The feasibility of cyclic micelle-like polymer nanoparticles for use as a delivery vehicle for doxorubicin (abbreviated as DOX), a potent chemotherapeutic drug, is discussed here. It is hypothesized that cyclic polymers will afford a more efficacious pharmacokinetic release profile than their linear counterparts. A linear polymer is functionalized with "click"able moieties at each end, which are then joined to create a single cyclized polymer. The polymer is decorated with poly(ethylene glycol) (PEG) chains to reduce immunogenicity, and carboxylic acid groups to electrostatically complex with the positively-charged DOX molecule. Upon addition of DOX, the polymer self-assembles into unimolecular micelle-like structures. When the construct accumulates in a tumor microenvironment due to the enhanced permeability and retention (EPR) effect, the low pH of the tumoral interstitium protonates the carboxylic acid groups, releasing DOX locally. Using a unimolecular micelle-like approach, less polymer can be used to deliver the same therapeutic payload, relative to other similar approaches that utilize pluralities of potentially toxic polymers to form micelle structures. The polymer's average molecular weight is around 11530 Da and the polydispersity of the reaction is 1.067. Gel Permeation Chromatography, Nuclear Magnetic Resonance, Fourier-Transform Infrared Spectroscopy, Ultraviolet-Visible Spectroscopy and Dynamic Light Scattering are used to confirm chemical identity, drug loading efficiency, particle size, and polydispersity. Cytotoxicity studies conducted with MDA-MB-231 invasive ductal carcinoma cells *in vitro* demonstrate the feasibility of this construct.

POSTER SESSION 4

MGH 206, Easel 177

4:00 PM to 6:00 PM

Microfluidic Yeast Replicative Lifespan

Toby Nathan Ven, Junior, Bioengineering
Mentor: Kenneth Chen, Genome Sciences
Mentor: Matthew Crane, Pathology

MSN2 is an environmental stress response transcription factor that is activated in yeast by multiple different stresses—e.g. glucose deprivation, heat, oxidative stress, misfolded proteins among many others—and in turn activates a collection of downstream transcriptional programs. Interestingly, while MSN2 becomes increasingly activated with age, deletion of MSN2 increases the replicative lifespan of the budding yeast. We show that in a glucose-rich environment, MSN2 misreads the environment and drives a pathological glucose starvation response that limits lifespan. Our experiments are facilitated by a novel budget microfluidic/microscopy method rather than the golden standard of microdissection. This innovative system analyzes data through time-lapse images to develop replicative lifespan curves rather than picking off daughter cells with a needle. Microfluidics drastically increases the throughput of experiments and allows for whole-lifespan monitoring of aging yeast cells. The system allows us to efficiently further the research of MSN2 and other transcription factors so that we can potentially translate this to other complex organisms that have similar types of transcription.