



The 2020 University of Chicago Undergraduate Research Symposium Proceedings: Abstract

Analysis of Health Care Workers Opinions on Developing a National HIV Network

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Mentor(s): Professor John Schneider, Medicine: Infectious Disease and Global Health, Chicago Center for HIV Elimination

Currently the rate of transmission of HIV in Egypt demonstrates exponential curves approaching epidemic levels. As the majority of the world sees a steady decline in the prevalence of HIV/AIDS, Egypt is left behind and outside of an international network aimed at combating its presence. This research aims to understand how healthcare workers in Egypt envision Egypt's domestic development of an HIV network towards inclusion into the international HIV prevention network. This study interviews Healthcare Workers (HCW) because they have a panoptic perspective of the situation: they are at the intersection of the interaction between patients, the healthcare system, the government — and above all they can see the lack of HIV literacy at all ends. Egypt's HIV situation is not fully documented because of a cyclic lack of literacy that effectively prevents it from breaking through to the larger international network working towards AIDS eradication. The cycle prospers through the machinations of developing HIV institutions. HCW reveal what Egypt's current crisis looks like, how an ideal future would operate, and how it can be included in the international HIV network in a manner that preserves Egyptian ideals and is sensitive to the intricacies caused by stigmatization. Thus this paper postulates a blueprint for future development based on HCW input with the goal of augmenting HIV literacy to a level compatible with the international community.



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De Novo Arginine Synthesis is a Metabolic Requirement for Pancreatic Cancer Cell Proliferation

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Mentor(s): Professor Alex Muir, Biological Sciences, Ben May Department for Cancer Research

Pancreatic adenocarcinoma (PDAC) is one of the deadliest cancers with only 9.3% of people surviving five or more years after being diagnosed. Because of a PDAC tumor's dense stroma, traditional cancer treatments such as chemotherapy have a hard time reaching cancer cells and are limited in effectiveness. There is an urgent need for new methods to combat PDAC. The same dense stroma that limits chemotherapy effectiveness also creates a nutrient poor environment. In particular, arginine, an amino acid important for growth and proliferation, is depleted in the tumor microenvironment by almost 50-fold. But despite this depletion, PDAC cells are still able to survive and proliferate. We hypothesize that PDAC cells compensate for the low arginine concentration in the tumor microenvironment by using the urea cycle to synthesize arginine de novo. In this pathway, cells take up environmental citrulline, then use the enzymes argininosuccinate synthase 1 (ASS1) and argininosuccinate lyase (ASL) to convert citrulline into arginine. In agreement with our hypothesis, immunohistochemistry staining of ASL and ASS1 reveals that PDAC tissues highly express these arginine synthesizing enzymes, whereas healthy pancreatic tissue shows almost no expression. By perturbing de novo arginine synthesis through knocking down ASS1 expression using CRISPRi or withdrawing citrulline from culture media, we found loss of de novo arginine synthesis slows the growth rate of PDAC. Our results suggest that de novo arginine synthesis plays an important role in how PDAC cells survive and proliferate despite arginine deprivation. Currently we are working to determine if inhibiting de novo arginine synthesis limits tumor growth in orthotopic mouse models of PDAC. Overall, our results identify a potential protein target for more effective PDAC treatments.



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Nocte is Required for Motor Neuron Terminal Development

Katherine **DeLong**, 3rd-Year, Biological Sciences

Mentor(s): Meike Lobb-Rabe, Molecular Genetics and Cell Biology; Professor Robert Carrillo,
Molecular Genetics and Cell Biology

Complex neural circuits are required for all aspects of behavior and cognitive function. Although the mechanisms underlying the formation of these circuits has been intensely studied for several decades, we still lack a complete understanding of how neurons wire with precision and how synaptic growth is coordinated during development. The *Drosophila melanogaster* neuromuscular system provides an ideal model to uncover molecules and mechanisms that regulate these developmental programs due to the nearly invariant and well conserved circuitry along the larval body axis within each repeated segment between motor neurons and their muscle targets. One molecule, Dpr10, has been implicated in synaptic connectivity and growth. In a previous biochemical screen searching for proteins that bind to Dpr10, we identified Nocte, which is expressed in muscles, glia, and several other cell types. Prior studies showed that Nocte is required for circadian rhythm entrainment. However, Nocte has not been previously characterized during larval development. We utilize genetic manipulations, dissections, immunohistochemistry, and fluorescent imaging to delve into Nocte expression and function in the *Drosophila* neuromuscular system. Loss of nocte results in aberrant terminal morphology and axon pathfinding errors, suggesting that it is required for normal neuromuscular circuit development. The mechanisms underlying these Nocte functions are currently under investigation. Many neurological diseases have an underlying synaptic dysfunction etiology; thus, unraveling the mechanisms that control synaptic function may allow for identification of novel therapeutic targets.



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Improving Diabetes Care and Outcomes on the South Side of Chicago

Christine **Egede**, 3rd-Year, Biological Sciences

Mentor(s): Dr. Monica Peek, Medicine: Internal Medicine; Dr. Marshall Chin, Medicine: Internal Medicine

Over the summer of 2019, I performed research under the supervision of Dr. Monica Peek and Dr. Marshall Chin. During my rotation, I worked on the South Side Diabetes Project, a multi-faceted program that seeks to raise awareness about the prevalence of diabetes and ways to improve diabetes outcomes in the South Side of Chicago. The program targets the South Side because these communities suffer from ethnic and socioeconomic disparities that make getting access to clinical care and fresh food difficult. As a result, these communities have poorer health outcomes due to complications from diabetes, such as leg and foot amputations, blindness, kidney failure, and heart disease. The goal of the program is to empower patients to manage their diabetes through physical fitness, shared decision-making, and medication when needed. I performed research at the 61st Street Farmer's Market, where we set up a booth and provided educational resources, health screenings, and a tour of the 26 vendor booths offered at the market, emphasizing the variety of fruits and vegetables available. We administered pre- and post-knowledge surveys to assess the efficacy of the program. We gave all participants who completed the surveys and tour \$7 in Market Bucks to encourage them to purchase fresh produce. Market Bucks are coupons that can only be used to purchase fresh produce. We interacted with 269 community members and welcomed 42 new participants. We collected 265 surveys and data analysis of the surveys is in progress. Through this program, we have successfully engaged with South Side communities and offered individuals a resource for accessing fresh fruits and vegetables. The program has also raised awareness for better ways to manage one's diabetes through Diabetes Empowerment classes. Other facets of the program include health screenings, health education, and cooking demonstrations at the KLEO Community Center, nutrition tours at four Save-A-Lot grocery store locations across the South Side, and collaborations with six clinics on the South Side of Chicago.



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Minimally Invasive Surgery (MIS) Does Not Compromise Outcomes in NSCLC Patients with Clinical N1 Disease

Najya **Fayyaz**, 1st-Year, Biological Sciences

Mentor(s): Dr. Sai Yendamuri, Thoracic Surgery, Roswell Park Cancer Institute, Buffalo, NY, Surgery, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY

Minimally invasive surgery (MIS) offers potential advantages compared to open thoracotomy for patients with clinical N1 disease. Additionally, there have been conflicting studies on whether adjuvant or neoadjuvant chemotherapy produces the best long-term survival. The National Cancer Database was queried for patients with clinical N1 NSCLC who underwent surgical resection between 2010 and 2014. An intention-to-treat analysis was performed to compare patients receiving MIS to patients who underwent open thoracotomy. Comparison was also done between neoadjuvant, adjuvant chemotherapy, and only surgery. Proportional hazard models were used to assess the effect of surgical approach and timing of chemotherapy to overall survival. The results included a total of 1,440 and 3,942 patients who underwent MIS and open thoracotomy respectively. MIS achieved better surgical margins and shorter length of stay compared to open thoracotomy. There were no differences in 30-day and 90-day mortality, or readmission rates. Neoadjuvant and adjuvant chemotherapy was administered to 13.5% and 57.2% of patients respectively. The remaining 29.3% did not receive any chemotherapy. There was no significant difference in 5-year overall survival between MIS and open thoracotomy (46% vs 46% $p=0.08$). There was significantly better 5-year overall survival in neoadjuvant and adjuvant chemotherapy versus only surgery, but no difference between neoadjuvant and adjuvant chemotherapy (48% vs 47% vs 44%, $p<0.01$). In conclusion, in clinical N1 NSCLC, MIS does not compromise oncological quality or overall survival when compared to open thoracotomy. Overall survival improved in patients treated chemotherapy, but there is no difference based on the timing of chemotherapy.



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Rancho La Brea Weasels as a Special Case of Response to Pleistocene Disturbance

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Mentor(s): Professor Stephen Pruett-Jones, Ecology and Evolutionary Biology, Biological Sciences

Mesocarnivores are a guild that possesses higher diversity than large carnivores on measures of diet, body size, and other traits that affect interactions with biotic and abiotic factors in an ecosystem. Thus, different mesocarnivore species' responses to disturbance are highly variable. The Pleistocene megafaunal extinctions at the end of the last Ice Age ~11 kya were accompanied by observed decreases in body size in four other mesocarnivore taxa (grey fox, american badger, striped skunk, and bobcat). Weasels (genus *Mustela*) may not have followed suit. We found that weasels in California today are not significantly bigger or smaller than they were 11 kya (and overall not as temporally dimorphic as the other four taxa), though their bodies are not the same. Using linear morphometrics and geomorphometrics on fossil specimens from Rancho La Brea (RLB) and modern specimens from the LA County Museum of Natural History, we examined three species of the genus *Mustela* to determine the species status of the fossils at RLB and how they compare to modern weasels. Based on dental and skeletal analyses, weasels today display greater omnivory but not decreased carnivory compared to their fossil counterparts. Accompanied with more robust shoulders and limbs, this can be interpreted to mean that weasels post-extinction began to rely more upon prey larger than themselves (ex. rabbits), all while supplementing their diet with more plant matter. Thus, weasels demonstrate that the effects of the Pleistocene megafaunal extinctions on the ecology of their survivors were not uniform across surviving taxa. Further research will expand the sample size of the study as more specimens are excavated from the Pit 91 collection and expand the scope of the geometric morphometric analyses by including more landmarks.



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The Unique Structure Hybrid Proline Rich Proteins: A Novel Bipartite Chloroplast Targeting Mechanism and Involvement in Arabidopsis and Staple Crop Stress Defense

Rachael **Filzen**, 3rd-Year, Biological Sciences (Endocrinology Specialization)

Mentor(s): Professor Jean Greenberg, Molecular Genetics and Cell Biology

Hybrid Proline Rich Proteins (HyPRPs) are a novel family of proteins implicated in stress response and pathogen defense in *Arabidopsis thaliana* and several staple crops. As climate change progresses, these proteins represent an important point of research in understanding how plants combat environmental stresses. *Arabidopsis* and staple crops face abiotic stresses such as soil salinity arising from scarce freshwater resources as well as biotic stress in the form of pathogens owing to agricultural practices like monoculture. AZI1 is one *Arabidopsis* HyPRP that localizes to the chloroplast, a key organelle in plant defense. AZI1 contains a proline rich region (PRR), a unique characteristic of the HyPRP family, which is essential to its chloroplast outer envelope membrane (OEM) localization and likely extends to other HyPRPs. The foundation of this project is composed of two parts: the first identifying the role of the PRR in HyPRP chloroplast OEM localization and the second determining the general involvement of HyPRPs in stress response. The role of HyPRPs in the defense mechanisms of staple crops also has yet to be studied. *Oryza sativa* HyPRPs that are homologs of *Arabidopsis* HyPRPs of interest will also be studied to ascertain the extent to which conclusions in *Arabidopsis* may be applied to staple crops.



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Early Neural Tube Development during Chick Embryogenesis

Anne **Havlik**, 3rd-Year, Neuroscience & Biological Sciences

Mentor(s): Dr. Timothy Sanders, Pediatrics, Grossman Institute for Neuroscience

In partnership with Dr. Timothy Sanders, MD, PhD, this study seeks to reveal further the undefined mechanisms of neural tube closure in vertebrate embryos. As the neural tube develops, an array of complex morphogenetic movements leads to changes in cell morphology and cell fate. These changes result in epithelial fusion of opposing specialized structures known as neural folds, leading to the creation of two separate structures: a neural tube and a non-neural ectoderm. The communication between neural progenitor cells was studied through the use of both fixed and live embryo imaging of stained membranes in the chick embryo, a widely accepted model organism of the field of developmental biology. Through fixed embryo staining and live embryo injection of targeted membrane specific dyes followed by electroporation, pathways of injected progenitor cells were imaged during early embryonic development. Bright-field microscopy, stereomicroscopy fluorescence, and computational clearing, paired with the use of several different dyes and trials, allowed for the mechanistic imaging and delineation of the cellular architecture of the process of neural tube closure in chick development. A novel, reliable experimental paradigm was created to both collect and image the developing embryo morphology of the closing neural tube in chick embryos. This convenient developmental tool was established via exploration and optimization of several combined methods to be able to further delineate the process of neural tube closure both anteriorly and posteriorly throughout early nervous system development in vertebrate embryos. This information is useful for furthering the understanding of one of the most common birth defects in humans, neural tube defects (NTDs). NTDs, posteriorly identified as spina bifida, affect the human nervous system functions in over 300,000 live births per year. This experimental paradigm will allow us to advance our understanding of NTDs as well as improve treatment strategies for patients with NTDs.



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Voltage Mapping across Cell Membranes Using a DNA-based Probe

Katharine **Henn**, 2nd-Year, Neuroscience, Chemistry

Mentor(s): Professor Yamuna Krishnan, Chemistry, Grossman Institute for Neuroscience; Anand Saminathan, Chemistry

In order to understand how diseases in voltage-dependent cells such as neurons and cardiomyocytes can be prevented, diagnosed, and treated, it is first necessary to understand the core mechanism of signal transduction: the voltage itself. Currently, calcium, small molecule, and protein-based imaging methods that track voltage do not allow for absolute quantification of membrane potential changes that occur as voltage spikes propagate across cells. In this project, we propose the characterization of a DNA-based fluorescent reporter that allows for quantitative, dynamic study of individual voltage spikes in singular or multiple cells. The reporter contains a voltage sensitive fluorophore as well as a reference fluorophore for ratiometric quantification of membrane potential during action potential propagation. This project presents a probe that is targetable, adaptable, and able to be used for study of minute membrane potential changes on the millisecond scale across populations of cells at one time; we present the applicability of this probe to voltage-dependent cell types and use it to quantify voltage spikes. The long-term goal of the project is to look at the relationship between membrane potential changes and organelle voltage, paying particular attention to the voltage dependence or independence of diseases, including cancer, cardiac disease, and neurodegenerative disease.



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Dynamics of Adaptive Changes in Cortical Activity in the Primary Somatosensory Cortex Following Sensory Loss

Madison **Jewell**, 4th-Year, Neuroscience

Mentor(s): Professor Fritzie Arce-McShane, Arce-McShane Orofacial Cortex Lab

The primary somatosensory cortex (S1) is known to provide sensory input to the primary motor cortex. Many cranial nerves project to S1, including the trigeminal nerve (CN V) which conveys tactile sensations from the oral region, guiding natural motor behaviors such as eating and drinking. Damage to S1 can lead to profound deficits in these behaviors and little is known about the neuronal response to temporary, targeted paresthesia of nerves that project into this region of cortex. To investigate the cortical representation of temporary sensory loss in S1, a monkey (*Maccaca mulatta*) was anesthetized via lidocaine injection to the maxillary (V2) and mandibular (V3) branches of CN V. During the three-hour window of effective anesthesia, neural data (via chronically implanted microelectrode array in S1) and 3D kinematic data (via high-resolution bilateral videoradiography and an X-ray Reconstruction of Moving Morphology workflow) were collected as the subject engaged in natural feeding behavior. By analyzing the neural response coincidental with kinematically similar tongue and jaw movements occurring at different times during the effective period of the lidocaine nerve block, the way in which S1 neural populations adapt to the absence of sensation over time can be characterized. Using coherence analysis and mutual information between precise kinematics and neural activity we will determine the dynamics of cortical adaptation in S1 during sensory loss.



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Use of a Point-of-Care Screening Tool to Identify Depression and Anxiety in Patients with Inflammatory Bowel Disease

Jordan Karpin, 4th-Year, Psychology, Creative Writing

Tina Rodriguez, 4th-Year, Biological Sciences

Mentor(s): Dr. David T. Rubin, Gastroenterology, Hepatology, and Nutrition

Depression and anxiety are comorbidities of inflammatory bowel disease (IBD). Recent guidelines from the ACG recommend screening for depression and anxiety in IBD patients, but the most effective and efficient way to do this is not established. We used a novel computerized adaptive testing technology to screen IBD patients for depression and anxiety and compared the screening results to disease activity. Consecutive patients at our tertiary IBD clinic were asked to complete the validated CAT-MH™ survey from Adaptive Testing Technologies (Chicago, IL). This tool is provided as a text or email link and takes 3-5 minutes to complete. We reviewed disease and patient characteristics. Categorical variables were assessed using Fisher's exact test. Clinical remission status was determined by the senior author, blinded to the CAT-MH™ results. 134 patients (75 women, 112 Caucasian, 84 Crohn's disease) participated in the study, 85 of whom had no prior history of psychiatric disorders. We identified 51 patients with depression (46 mild, 3 moderate, 2 severe); 32/51 (62.7%) were previously undiagnosed. 36 subjects tested positive for anxiety (24 mild, 10 moderate, 2 severe); 20/36 (55.6%) were previously undiagnosed. 2/134 patients were positive for suicidal ideation in the past month. Sex, race, type of IBD, surgical history, and number of discontinued medications were not significant (Table 1). Patients with active disease had a significantly greater relative risk for having depression (RR 2.26, 95% CI 1.50-3.39) and anxiety (RR 1.88, 95% CI 1.09-3.24) (Figure 1). We demonstrate the utility of a novel screening tool for depression and anxiety in IBD patients. Further, we illustrate the positive association between clinically active disease status and the presence of depression and anxiety. Physicians should consider patients with clinically active IBD at risk for depression and anxiety and treat or refer them accordingly.



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A Systems Biology Approach to Lamin-A Related Cardiomyopathy

Sunny Yang (Sunny) Liu, 4th-Year, Chemistry, Psychology

Mentor(s): Dr. Kohta Ikegami, Moskowitz Lab, Knapp Center for Biomedical Discovery; Professor Ivan Moskowitz, Pediatrics, Pathology and Human Genetics

LMNA encodes lamin-A, a nuclear lamina protein in the nuclear envelop. LMNA mutations lead to a wide array of diseases, collectively termed as "laminopathies," including cardiomyopathy. They cause 5-10% of all familial dilated cardiomyopathies, of which 20% of patients require heart transplants. Aside from promoting nuclear stability, lamin-A affects gene regulation. It was thought that nuclear structural defects, caused by LMNA mutations, result in gene expression changes that in turn lead to pathology. However, this hypothesis has been unsuccessful in explaining the lack of nuclear structural defects in some LMNA mutants. Recent evidence indicated that phosphorylated lamin-A, localized away from the nuclear envelop, associates with actively transcribed genes in the euchromatin, a region of open chromatin structure. Interestingly, lamin-A typically binds to the heterochromatin, a region of silent gene expression. This suggests that phosphorylated lamin-A may act as a transcription factor. Using an *in vivo* LMNA deletion model, the project's overall objective is to investigate the functions of phosphorylated lamin-A in cardiomyocytes under the context of cardiac dysfunction in LMNA-related cardiomyopathy. I am approaching this aim by two directions. Firstly, by quantification of phosphorylated and non-phosphorylated lamin-A, I have found that phosphorylated lamin-A is depleted faster than non-phosphorylated lamin-A in cardiomyocytes. This coincides with large-scale changes in the transcriptome. Secondly, by genomic analysis of transcriptome changes in other laminopathy-related cardiomyopathy models, I identified candidate genes that drive the cascade of gene expression changes from LMNA deletion to cardiomyopathy. Future experiments will isolate cardiomyocyte-specific gene expression changes and identify direct phosphorylation site in lamin-A to better understand mechanisms of LMNA-related cardiomyopathies.



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Seizure Foci Localization Using Post-ictal FLAIR MRI

William J. Liu, 4th-Year, Neuroscience

Mentor(s): Dr. James X. Tao, Neurology; Dr. John Collins, Radiology

For focal epileptic patients, their post-ictal magnetic resonance imaging (MRI) images may contain transient reversible imaging abnormalities. These structural alterations within the brain are generally understood to be physiological or pathologic alterations due to seizure activity, but it is not clear when and how these changes occur, when and how they disappear, and why some turn into permanent abnormalities. In the present preliminary neuroimaging study, three temporal lobe epilepsy patients at the University of Chicago Medical Center were retroactively screened and selected such that their post-ictal MRI scans could be used to investigate whether these reversible MRI abnormalities can potentially be used to localize seizure foci in the brain. Using masks of the temporal lobes based on the Harvard-Oxford cortical and subcortical probabilistic atlases, we found that the difference in the mean T2 FLAIR intensity between the temporal lobes was statistically significant. Moreover, the hemisphere of the hyperintense temporal lobe corresponded with the location of the T2 FLAIR abnormality and the seizure lateralization as determined by electroencephalograph (EEG) and PET images. Because of our small sample size, we cannot definitively conclude that post-ictal MRI abnormalities can be used to lateralize the location of epileptogenic zones. However, if this method is viable, this approach may be useful as a complement to intracranial EEG and other functional imaging approaches, such as PET or SPECT, to localize seizure foci.



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Studying Molecular Development of Fish Fins May Lead to Mechanism behind the Fin-to-Limb Transition

Atreyo Pal, 4th-Year, Physics and Biological Sciences (Genetics Specialization)

Mentor(s): Professor Anindita Basu, Section of Genetic Medicine; Professor Neil Shubin, Organismal Biology and Human Anatomy

The homologous structures in tetrapod limbs and fish fins exhibit a range of shape diversity and have a shared evolutionary history. However, our understanding of fin diversity and the fin-to-limb transition remain hampered by a lack of genomic and developmental studies of fins outside of model taxa. We have performed a comparative developmental and transcriptomic study using high-throughput, droplet-based single cell RNA-sequencing (scRNA-seq) called Dropseq and have leveraged morphological diversity between fin types across the two species, zebrafish (*Danio rerio*) and little skate (*Leucoraja erinacea*) to study fin development. Implementation of trajectory inference in conjunction with scRNA-seq have given us cell type specific clusters in the fin tissue, which has been used to create a “developmental tree” or “Devtree” that combines information about genetic changes during cellular differentiation in a single figure. Based on these trees, we plan to use common developmental patterns to draw conclusions about how disparate morphologies are built and identify the molecular mechanisms behind the fin-to-limb transition.



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Y2O3:Eu Nanoparticles for Improving Treatment of Ovarian Cancer

Ariel Pan, 3rd-Year, Chemistry

Mentor(s): Professor Chin-Tu Chen, Medical Physics

Often diagnosed in the late stage due to its vague symptomatology (e.g., bloating, abdominal pain) and a lack of reliable screening tests, ovarian cancer is considered the fifth deadliest cancer for women, with 14,000 deaths estimated in the U.S. alone this year. Although treatment usually begins with cytoreductive surgery and chemotherapy, metastatic ovarian cancer is prone to relapse and chemoresistance, making radiotherapy a common second-line treatment. However, whole abdominal radiation (WAR) is quite toxic at the necessary therapeutic levels, leading to serious chronic side effects, such as small bowel obstructions, that decrease patient quality of life. Generally, lower radiation dose leads to lower toxic side effects; but lowering x-ray dose in WAR drastically reduces its efficacy. To counter this problem, we have developed silica-coated europium-doped yttrium oxide (Y2O3:Eu@SiO2) nanoparticles for use in low-energy x-ray activated photodynamic therapy (X-PDT). These nanoparticles have several advantages over conventional PDT, which uses photosensitizers activated by visible light to form cytotoxic reactive oxygen species (ROS): 1) nanoparticle ROS generation enhances radiotherapy, so lower x-ray doses can be applied, leading to fewer toxic side effects, 2) x-rays, as opposed to visible light, allow deep tissue penetration, and 3) the nanoparticles give off a luminescence directly proportional to the x-ray dose, allowing an accurate monitoring of tissue dose. To determine the efficacy of this treatment, two groups of female mice inoculated with a radiation sensitive strain of ovarian cancer cells underwent four consecutive days of radiotherapy, receiving 2 Grays (Gy) of x-ray per day. One group also received the nanoparticles, injected intratumorally before the treatment. Tumor volume and 18F-fluorothymidine uptake (marks cell proliferation) were measured 1, 4, 7, and 14 days after treatment using PET/CT scans. Ultimately, the group treated with nanoparticles showed an overall decrease in tumor size while the control group showed a slight increase in tumor size, suggesting that this nanoparticle-mediated treatment is a promising improvement to ovarian cancer radiotherapy.



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Single Cell RNA Sequencing Analysis of Human Induced Pluripotent Stem Cell Derived Spinal Spheroids

Nicholas **Thom**, 2nd-Year, Molecular Engineering (Biology Track)

Mentor(s): Dr. Sergiu Pasca, Psychiatry and Behavioral Sciences, Stanford University

The central nervous system is responsible for sensation, perception, and movement. Neurons in the cerebral cortex connect through descending motor pathways to hindbrain and spinal cord neurons to ultimately activate muscle and generate movement. Through innervation motor neurons from the spinal cord form neuromuscular junctions with skeletal muscle, which is responsible for all voluntary movement in the body. Injury to or degeneration of the spinal cord is associated with many diseases, such as amyotrophic lateral sclerosis and multiple sclerosis. Currently, there are no good human models for many of these disorders, so our goal was to generate a human cellular model to study these diseases. To do so, we generated three-dimensional spinal-like spheroids from human induced pluripotent stem cells (hiPS cells). Here, I will show the characterization of the human spinal spheroids (hSpS) using single cell RNA sequencing. This analysis is two-fold, illustrating the reproducibility of our method of hSPS generation and confirming the identity of our spheroids. The single cell RNA sequencing method we used involves the isolation of single cells in a droplet. This then yielded a dataset of cell-ID by gene expression which we could then analyze using the R package Seurat. Through dimensionality reduction techniques, we were able to determine cell clusters and genes that characterize each group of cells. These clusters were recognizable due to similar gene expression profiles that are found *in vivo*. In addition, we were able to show that our method of differentiation was reproducible through the use of three iPS cell lines, each of which was derived from healthy individuals. These spinal spheroids, when assembled with cortical and muscle spheroids, allow us to model the motor circuit with the intent of finding novel treatments and drug targets.



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An Abundance of Microbes: Studying the Impact of Microbiomes on Bull Kelp

Sativa **Volbrecht**, 4th-Year, Biology & Creative Writing

Mentor(s): Professor Catherine A. Pfister, Ecology and Evolution; Brooke Weigal, Ecology and Evolution

Kelp forests serve important ecological roles as food and shelter for many marine species near the coasts. The health of these forests is threatened by pollution, coastal development, and changes to marine food webs. Long-term studies of these kelp forests and what could be affecting their growth are still necessary to understand how to best conserve these habitats. For example, the area of kelp forests in Puget Sound, Washington are declining, but the causes are unknown. I am working on quantifying the microbial layers on the bull kelp *Nereocystis luetkeana* from a stable and healthy outer coast locale (Tatoosh Island, WA) to a declining population in Puget Sound (Squaxin Island, WA) to understand whether the microbe abundances differ geographically and whether the microbial layers on kelp samples change in abundance in response to stress. By staining the kelp samples with DAPI and then using a photo analysis to calculate the percent area of microbes in the photo, I can quantify the abundance of microbes on each sample. At this stage in the analysis, the data suggest there is a difference that the declining Squaxin has a lower abundance of microbes than the Tatoosh population, suggesting that the microbiome could be related to the health of these forests. In the future, I plan to look at abundances of microbial layers on stressed Tatoosh samples that have been exposed to heat to understand whether the kelp could be shedding their microbial layer in response to stress. If there are significantly fewer microbes on the stressed kelp, it could suggest that the kelp may shed their microbes in response to heat. In short, through my microscopic analysis of these huge kelp forests, I am finding evidence that the microbiome may be an important component.