MAXIMIZING ACCESS TO RESEARCH CAREERS (MARC)

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Sponsor: National Institute of General Medical Sciences-NIGMS/TWD Division GM 08718.

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ALEXANDRE CAVALCANTE

LINE-1 Activity in Developmental Cardiac EMT and its Consequences in Heart Morphogenesis

The University of Arizona, Physiology
Mentor: Todd Camenisch

Abstract

Congenital heart defects (CHD) are malformations in the structure of the heart that cause defective functioning of the cardiovascular system. CHD account for 18.5% of newborn deaths in the first year of life in United States. Some of the diseases induced by CHD are: coronary artery disease, valve disease, high blood pressure, congestive heart failure, arrhythmia, and stroke. A key process in cardiac morphogenesis is the epithelial-to-mesenchymal transition (EMT). Cardiac developmental EMT causes increased cellular motility and invasive phenotype, which are essential for proper formation of non-myocyte heart cells, which contribute to structures such as heart valves, interventricular septum, and coronary vessels. Developmental EMT in the heart is mediated by the transforming growth factor β2 (TGFβ2) signaling pathway through canonical SMAD activity. Long Interspersed Element-1 (LINE-1 or L1) is a retrotransposon that constitutes 17% of the human genome and is active during early stages of development before it is silenced. We found that, when aberrantly expressed in later stages of development, LINE-1 disrupts cardiac developmental EMT by preventing SMAD activity in the TGFβ2 signaling. The forthcoming consequences are disrupted EMT and loss of cardiac mesenchymal cells. In summary, LINE-1 reactivation in later stages of development is antagonistic to developmental cardiac EMT, predisposing the onset and progression of heart malformations.

JACOB CROFT

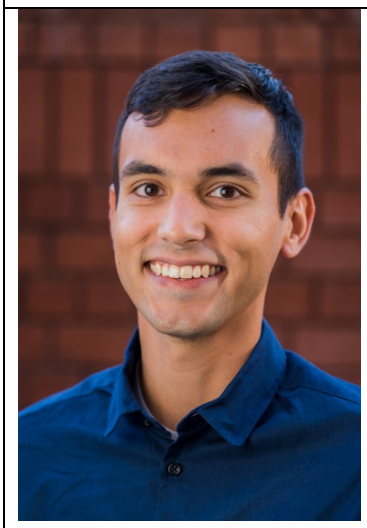
Role of the Coiled Coil Domain in Soluble Guanylate Cyclase

The University of Arizona, Biochemistry and Molecular & Cellular Biology
Mentors: William Montfort

Abstract

Nitric oxide (NO) signaling regulates numerous physiological processes including vasodilation, making the NO signaling pathways therapeutic targets for cardiovascular disease. The primary NO receptor is soluble guanylyl cyclase (sGC), which catalyzes the conversion of GTP to cGMP, causing a signaling cascade that leads to vasodilation. How binding of NO to heme in the β1 HNOX domain leads to catalysis remains unknown. A model developed by our lab suggests that signal transduction occurs through small changes in the coiled coil domain upon NO binding. In support of this model, increasing coiled coil length leads to decreased ligand binding affinity. We developed a system of expressing sGC truncations from *Manduca Sexta (Ms)* sGC, which is homologous to human sGC but expresses in larger quantities and has greater stability. We have created a series of double mutations that introduce a disulfide bond to link both subunits at the coiled coil. Currently, this double mutation has been introduced to a construct

containing the HNOX, PAS, and coiled coil domains. We intend to introduce this double mutation to another construct containing the catalytic and coiled coil domains, as well as full length *Ms* sGC. These mutations will allow investigation of the effects of restricting rotation of the coiled coil on NO/CO binding affinity, histidine release rates, and cyclase activity. These parameters will also be tested in the presence of an allosteric stimulator of sGC to determine whether rotation of the coiled coil is important in activation by stimulator compounds that bind the β1 HNOX domain.



TYLER ESPINOZA

Creating an Allosterically Regulated Lyn Kinase via BH3 Domain Insertion

The University of Arizona, Biochemistry

Mentor: Indraneel Ghosh

Abstract

Kinases play a critical role in all aspects of cell signaling and the dysregulation of kinase activity has been linked to various diseases, including cancer. However, studying the activity of a specific kinase in the presence of more than 500 other kinases, many of which share strong structural similarities, is challenging. Developing methods to modulate the activity of a specific kinase would help in understanding the roles that kinases play in cell biology. To modulate the activity of a specific kinase we used a domain insertion strategy to create a Lyn kinase that is regulated by allostery. We inserted the BH3 domain of Bad into a non-homologous region of Lyn kinase to allow for potential allosteric regulation of Lyn-Bad. The intrinsically unstructured BH3 domain of Lyn-Bad was hypothesized to become structured and form an α-helix upon binding Bcl-X_L. We have shown that the addition of Bcl-X_L, which binds Bad, decreases the activity of the Lyn-Bad kinase and that the addition of ABT-737, a small molecule that disrupts Bcl-X_L/Bad binding, restores the catalytic activity of the designed Lyn-Bad kinase

MARIAJOSE FRANCO

Deregulation of Long Non-coding RNAs is Vital for Rhabdomyosarcoma Tumorigenesis

The University of Arizona, Molecular and Cellular Biology

Mentor: Justina McEvoy

Abstract

muscle, is the most common soft tissue sarcoma in children. Yet, the molecular events that lead to RMS tumorigenesis remain unknown, making therapeutic development challenging. Two major histological RMS subtypes exist: ERMS, which displays several somatic mutations, and ARMS, characterized by a translocation that produces the fused *PAX3/7-FOXO1* gene. Published work demonstrate that RMS malignancies overall have lower mutation rates than adult solid tumors, therefore, we propose that non-genetic mechanisms potentially contribute to RMS formation. From our preliminary analyses of RMS patient-derived xenografts, we discovered a group of novel long non-coding RNAs (lncRNAs) that are unique to RMS and are aberrantly expressed in both or either subtype. This is exciting because deregulation of lncRNA activity is often associated with the promotion of cancer hallmarks such as cell survival. Indeed, preliminary knockdown of one of these lncRNAs, lnc *19_31*, led to rapid cell death in RMS cell lines and increased expression of pro-apoptotic genes such as *TP53*. Surprisingly, this knockdown produced

	a complete loss of *PAX3-FOXO1* expression, indicating lnc *19_31* may be a major driver of RMS oncogenesis. In addition, the expression of the adjacent transcription factor *ZNF536* decreased, thus suggesting potential cis-regulatory activity for lnc *19_31*. *ZNF536* knockdown also induced cell death. Together, these data led us to hypothesize that lnc *19_31* is essential for cell survival through expression of *ZNF536*. These findings could translate into therapies targeting lnc *19_31*, which we expect will improve the outcomes of RMS patients.
	**NADIA INGABIRE** *Characterization of a Novel Integrin β4 Variant in Human Prostate Cancer* The University of Arizona, Biochemistry Mentors: Anne Cress **Abstract** While localized prostate cancer is curable, the survival rate of patients with prostate cancer is lower as cancer cells become metastatic. Here, we study adhesion of PC3N cells expressing a unique integrin *β*4 isoform, *β*4E to laminin 332. Laminin binding integrins (LBI) are important in the development and progression of cancer. The LBI *α6β4* has both cancer promoting and suppressing roles and is known to form strong adhesions mediated by stable hemidesmosome formation. All splice variants of *β*4 conserve the transmembrane and extracellular domains but variants A-D retain the same cytoplasmic domain as *β*4. Interestingly, Variant E (*β*4E) consists of a unique cytoplasmic domain with 114 amino acids. The novel cytoplasmic domain of *β*4E results in the loss of major protein motifs that may alter cell anchorage mechanisms such as adhesion. Since integrin activation can be dependent upon the cytoplasmic domain, it was important to determine whether cells containing the isoform *β*4E had reduced adhesion with laminin 332 as compared to cells expressing *β*4C isoform. In our study, adhesion and crystal violet assays were used to measure absorbance of adherent *β*4-null PC3-N cells that were transfected with *β*4C- RFP and *β*4E- GFP plasmid constructs. The results showed no significant difference in cell adhesion to laminin between the cell groups. These data suggest that *β*4E isoform is equally capable of supporting adhesion to laminin as compared to *β*4C isoform. Future experiments will be to test the significance of the unique cytoplasmic domain for supporting integrin signaling and cancer invasion.

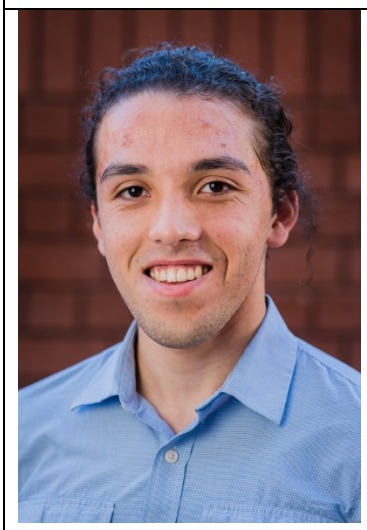
JAYME JACKSON

Detecting Papillomavirus Recombination in vivo

The University of Arizona, Microbiology
Mentor: Koenraad Van Doorslaer

Abstract

Human papillomaviruses (HPVs) are the most common sexually transmitted disease (STD), with certain types causing cervical cancer and a significant amount of head and neck cancer.The vaccine is based on virus like particles that reconstitute the viral epitopes. This approach raises highly specific antibodies. With 64 different types of genital HPV, and vaccines only protecting against 9 types deemed as “high risk”, the vaccinated population is still susceptible to the majority of HPV types.
While rare, when multiple types of HPV are present in a cell, there is evidence that HPV can recombine in vivo. It is unclear whether papilloma genomes readily recombine, and whether the offspring genomes would be sufficiently fit to become established in the population. Our experiments test, whether under ideal lab conditions, inter-type recombination occurs. To investigate this, we introduced wild type and mutant viral genomes into primary keratinocytes derived human foreskin tissue. Cells will be transfected with a wild-type genome and a mutant genome unable to replicate. The mutant genome also contains an antibiotic resistance marker (allowing for selection with G418). When grown under constant selective pressure, the presence of colonies indicates that viral occurred in the cell. The recombination breakpoints will be analyzed and specific recombinant viruses will be used in competition assays to determine relative fitness.



HEBER LARA

The function of the CD47-Thrombospondin-1-soluble Guanylyl Cyclase signaling axis in regulating the Tumor Microenvironment

The University of Arizona, Biochemistry, Molecular and Cellular Biology
Mentor: William Montfort

Abstract

Cluster of differentiation 47 (CD47), a trans-membrane protein, emerged recently as a promising target for cancer immunotherapy. Upregulation of CD47 by tumor cells correlates with poor survival in multiple cancer types. The interaction between CD47 and the signal-regulatory protein alpha (SIRP-α) on macrophages and dendritic cells relays a “don’t eat me” signal that blocks phagocytosis and subsequently inhibits cross-presentation allowing tumor cells to evade immune surveillance. [3,5] In relation to these known pathways, a humanized CD47 antibody is undergoing phase I clinical trials for cancer treatment.
Soluble guanylyl cyclase (sGC) is a key mediator in nitric oxide (NO) signaling which is known to play a significant role in regulating the tumor microenvironment (TME). [1] In endothelial and vascular smooth muscle cells, NO can bind to sGC to catalyze the formation of cyclic-guanosine monophosphate (cGMP). cGMP product initiates a molecular cascade which can lead to vasodilation along with other physiological effects. sGC activity is influenced by changes in cytosolic calcium concentrations [4] and its inhibition can be attributed to CD47 bound to thrombospondin-1 (TSP-1), an

extracellular glycoprotein. [2] Inactivation of sGC by CD47/TSP-1 occurs in non-cancerous cells, however, this interaction in cancerous cells continues unverified.
We will explore the signaling mechanism and function of the CD47/TSP-1/sGC signaling axis using human breast cancer cells and primary immune cells. With these models, we will also determine if the signaling axis has an influence on tumor metastasis and evasion of immune surveillance.

MARIANNE MADIAS

Paper Based Organ on a Chip for Angiogenesis Assay

The University of Arizona, Biomedical Engineering
Mentor: Jeong-Yeol Yoon

Abstract

Angiogenesis, the formation of new blood vessels, plays a critical role in many biological processes and is a mechanism that remains in need of further study. In vivo methods of studying angiogenesis are expensive and time-consuming, and traditional two-dimensional in vitro assays lack physiological relevancy. The use of organ-on-a-chip (OOC) platforms enables improved in vitro study of three dimensional morphogenetic processes relevant to angiogenesis. Polydimethylsiloxane (PDMS) based OOC's have been used as the conventional platform for such studies, however paper based systems have been shown to be a promising new platform for cell-based assay. Paper is advantageous as a platform because it is inexpensive, accessible, and most importantly flexible, presenting the opportunity for a dynamic OOC, as opposed to the rigid and unchangeable form of PDMS based OOC's. Here we demonstrate a paper based cell culture OOC, fabricated by wax printing hydrophobic barriers enclosing hydrophilic channels on nitrocellulose paper. Rat vascular endothelial cells (RVEC's) were used to study cell sprouting within collagen coated hydrophilic channels, using vascular endothelial growth factor (VEGF) and the signaling lipid sphingosine-1-phosphate (S1P) to induce sprout formation on the nitrocellulose OOC. The effect of shear stress was also studied by inducing pulsatile flow on the chip using servo motors run by an Arduino Mega 2560 microcontroller. We demonstrate angiogenic sprouting on a paper based OOC, showing that paper-based platforms can provide a simple, inexpensive, and accessible method of studying angiogenesis in a physiologically relevant in vitro environment.

BRITTANY WILLIAMS

The Effects of Aging on Stress Granule Formation

The University of Arizona, Molecular and Cellular Biology
Mentor: Daniela Zarnescu

Abstract

Aging is a process all living organisms inevitably undergo. As the aging population continues to grow, the incidence of age-related neurodegenerative diseases has become more evident. A common factor among neurodegenerative diseases is the presence of protein aggregates. While the protein aggregates have been labeled as the hallmark of age-related neurodegenerative diseases, their connection to aging is still unknown. The purpose of this study is to analyze the effects of aging on stress granule formation in Drosophila melanogaster. The presence of stress granules is ascertained using Drosophila Fragile X Mental Retardation Protein (DFMRP) as a marker. To determine age dependent changes in stress granules we examined the distribution of DFMRP in Drosophila brains at five different age points. The anticipated outcome of this project is the identification of variance in stress granule formation amongst the different age points. The findings may be useful in understanding if the cell's ability to handle environmental stress decreases with age.