

2008 UCSD Amgen Scholars Program- Abstracts Report

1) Abdi Farah- Amgen Scholar

Dr. Brinda Rana- Faculty Mentor

Title: Gene Expression in the Hippocampus of Isolation Reared Mice

Abstract: The majority of previous studies done on schizophrenia have emphasized prenatal developmental exposure to harmful environmental elements. Our research, however, focuses on the effects of rearing. After a precisely controlled experiment of raising two groups of mice, one in isolation and the other in a social setting, phenotyping analysis displayed that the mice reared in isolation exhibited the distinct hallmarks of schizophrenia. The socially reared mice displayed normal fear learning patterns whereas the isolation reared mice displayed deficiencies, a process facilitated by the hippocampus. We hypothesize that this may be due to differential gene expression in the hippocampus of the mice from the two groups. In this study we are isolating the RNA from the hippocampus of both the isolated and socialized mice in order to compare their gene expression patterns. It is expected that they will show remarkable differences and more insight into the genomic contributions to the onset of schizophrenia.

2) Abdi Ridwa- Amgen Scholar

Dr. Brinda Rana- Faculty Mentor

Title: Gene Expression in the Hippocampus of Isolation Reared Mice

Abstract: The majority of previous studies done on schizophrenia have emphasized prenatal developmental exposure to harmful environmental elements. Our research, however, focuses on the effects of rearing. After a precisely controlled experiment of raising two groups of mice, one in isolation and the other in a social setting, phenotyping analysis displayed that the mice reared in isolation exhibited the distinct hallmarks of schizophrenia. The socially reared mice displayed normal fear learning patterns whereas the isolation reared mice displayed deficiencies, a process facilitated by the hippocampus. We hypothesize that this may be due to differential gene expression in the hippocampus of the mice from the two groups. In this study we are isolating the RNA from the hippocampus of both the isolated and socialized mice in order to compare their gene expression patterns. It is expected that they will show remarkable differences and more insight into the genomic contributions to the onset of schizophrenia.

3) Abeyta Antonio- Amgen Scholar

Dr. Carl Ware- Faculty Mentor

Title: Function of Erythrocyte Expressed Herpesvirus Entry Mediator

Abstract: Herpesvirus Entry Mediator (HVEM) is a member of the Tumor Necrosis Factor (TNF) family of receptors, and is typically expressed on many cells of the immune system. HVEM serves as a receptor for multiple cytokine ligands, playing a role in the inflammatory signaling response of the immune system. HVEM also facilitates entry of the Herpes Simplex Virus-1 (HSV) through envelope glycoprotein gD. Through studies utilizing flow cytometry, we have shown that HVEM is expressed in mouse erythrocytes. Based on this data, we believe that erythrocytes may serve as an entry point for HSV. However, erythrocytes cannot serve as a host for HSV as they lack the machinery needed to replicate DNA. Using viral plaque assays, we will determine whether or not erythrocytes have the ability to bind HSV. We propose that mouse erythrocytes serve as a mechanism to block the dissemination of HSV through the blood. Therefore, predicting that HVEM wild-type erythrocytes will bind HSV while erythrocytes from mice genetically deficient in HVEM will lack this ability to bind HSV. By conducting viral plaque assays and binding of gD to erythrocytes, we plan to determine the function of mouse erythrocyte expressed HVEM.

4) Emily Albrecht- Amgen Scholar

Dr. J. Enrique Cometto-Muniz- Faculty Mentor

Title: Concentration-Detection Functions for Human Ocular Chemosensory Perception

Abstract: Along with an increasing concern about chemical pollution in the air of urban areas, and in workplaces, there is a developing need for adequate literature on chemical irritation (chemesthesis) in humans. With these applications of occupational health in mind, we measure concentration-detection functions for ocular chemesthesis by using a 2-channel vapor delivery device (VDD-2). The VDD-2 initially heats a liquid chemical into a vapor stimulus, and then mixes it with nitrogen, which acts as an inert dilution gas. For testing subjects, this gas mixture is delivered into one of two eyepieces while the other eyepiece receives plain nitrogen. Thus, participants are tested under a two-alternative forced-choice procedure with an ascending concentration approach. We selected three chemicals (1-propanol, heptanal, and trans-2-heptenal) for presentation in acute (4 second) exposures. Five nominal concentrations (in percent volume/volume) are tested per chemical. Ultimately, we quantify the vapor stimuli via gas chromatography, using a compound-specific calibration curve. The outcome produces, for each chemical, the concentration-detection function for eye irritation. This function ranges from chance detection to perfect detection. The eye irritation threshold is defined as the concentration producing detection at halfway this range.

5) Amin Khadija- Amgen Scholar

Dr. Palmer Taylor- Faculty Mentor

Title: Reversible Acetylcholinesterase Inhibition of Novel Oxime Reactivator Compounds and Their Reactivation Rate Kinetics

Abstract: The purpose of this study was to determine the inhibitory potency of different oxime compounds for the catalytic activity of the human enzyme, acetylcholinesterase (hAChE). Oximes are generally used to treat organophosphate poisoning, where the active site (serine) of hAChE is blocked. Organophosphates prevent the enzyme's main activity: to rapidly catalyze the hydrolysis of the neurotransmitter, acetylcholine, in the synaptic space between neurons. Oximes can act as antidotes by effectively breaking the covalent bond of organophosphate with the serine, thereby reactivating the enzyme. However, an oxime can also show reversible inhibition of the enzyme, as it can compete with the substrate for the active site within the enzyme, which the focus of this project. In this study, several oximes were tested for their inhibitory properties using absorbance spectrophotometry measurements, and analyzed using double reciprocal Lineweaver and Burke plots. It is hypothesized that a higher concentration of oxime will cause further inhibition, and slow down enzymatic activity, thereby decreasing the kinetic rate at which the enzyme would normally work. Oxime reactivators tested in this study to date were significant hAChE inhibitors with K_i (inhibition constant) values in the low μM concentration range.

6) Bhatnagar Akrita- Amgen Scholar

Dr. David W. Rose- Faculty Mentor

Title: Estrogen Antagonists and Inflammation

Abstract: In endothelial cells, inflammation can be linked to the expression of the NF- κ B dependant adhesion molecule VCAM-1. This leads to the binding and activation of macrophages, which produce inflammatory cytokines such as TNF α and IL-1 β . This in turn leads to the de-repression of certain cell signaling pathways, and thus the overproduction of NF- κ B dependant regulatory genes. This pathway also reduces the effectiveness of estrogen antagonists such as Tamoxifen, an anti-breast cancer drug. Conversely, the cytokine IL-6 induces the expression of the transcription factor Oct-1. It has been shown that higher expression levels of Oct-1 lessen the inflammatory response by directly reducing the TNF α stimulated expression of VCAM-1. Therefore, I intend to measure the level of Oct-1 expression in cancerous and normal mammary cells via biochemical assays such as SDS-PAGE and Western Blots.

7) Chen Hannah- Amgen Scholar

Dr. Paul Price- Faculty Mentor

Title: The *E. coli* cell wall calcifies in human serum

Abstract: A significant number of Gram-negative bacterial infections occur in hospitals each year, with *Escherichia coli* causing the greatest number of these infections. Thus, we wanted to understand the mechanisms present in human serum that defend the body against *E. coli*. We propose that one such mechanism may involve serum-driven calcification of the *E. coli* cell wall. Calcification in serum is directed by the activity of at least two proteins: one generates mineral crystals, which can diffuse into suitable matrices, such as the bacterial cell wall. The other, fetuin, selectively inhibits the growth of crystals remaining outside a matrix. Earlier experimentation showed that the cell wall of *Staphylococcus aureus*, a Gram-positive bacteria, is vulnerable to mineralization in human serum via the aforementioned mechanism. The present experiments were carried out to determine if Gram-negative bacteria such as *E. coli* are also a target for mineralization by this mechanism. After incubation in human serum, fluorescent microscopy of calcein-stained *E. coli* cells revealed that 85-95% of the cells exhibited mineralization. Calcium and phosphate assays determined the extent to which the cells mineralized, and scanning electron microscopy demonstrated that calcification most likely occurred in the cell wall. The ability of human serum to mineralize *E. coli* gives us insight into ways to combat bacterial infections and the innate defenses of human serum against *E. coli*.

8) Cigan Alexander- Amgen Scholar

Dr. Robert Sah- Faculty Mentor

Title: Chamber for Mechanobiology Analysis of Articulating Cartilage

Abstract: Moderate levels of mechanical stimulation promote cartilage maintenance and matrix metabolism while excessive levels can result in tissue damage and loss. Surface defects in human articular cartilage disrupt normal joint function and subject cartilage to elevated strain levels. To further elucidate how these strains, particularly shear, influence cell fate, a mechanical testing chamber will be developed and fitted to an existing video microscopy and actuator system. The chamber will be designed to cyclically compress and shear apposing osteochondral blocks (12x3x8 mm³, LxWxH) within physiologic range, while maintaining environmental conditions such as pH, CO₂, and temperature necessary for cell viability. In addition, the chamber will be capable of applying displacements at slow (10-150 μ m/s) and relatively faster rates (1-10 mm/s),

while measuring the resultant forces in both the lateral and axial directions. The model will first be constructed virtually using Solidworks, and then a prototype will be fabricated and assessed for its achievement of design goals. The completed chamber will ultimately be utilized for tests designed to elucidate how chondrocytes respond to elevated shear strains that result from the presence of focal defects during joint articulation.

9) Cullen Lisa- Amgen Scholar

Dr. Michael Croft- Faculty Mentor

Title: The Role of 4-1BB in Immunology to Vaccinia Virus

Abstract: Smallpox is a contagious and sometimes fatal disease that can only be prevented by vaccination. Although smallpox was eradicated in the 1970s, it is currently being studied in the U.S. to better prepare the country against the potential use of the virus as a bioterrorism agent. Our laboratory seeks to improve the efficiency of the vaccination by using an antibody to the protein 4-1BB, expressed on T lymphocytes, to boost the immune response of mice to vaccinia, the “live virus” used in the smallpox vaccine. A model of lethal infection will be used whereby mice normally die within 1 week following intranasal exposure to the virus. Immunization of animals with a peptide that stimulates CD8 T lymphocytes can afford some protection against the virus but it is weak and limited. We will investigate whether targeting 4-1BB with a stimulatory antibody will generate a better immune response to the virus by generating more virus-specific T cells. These studies might suggest that antibodies to 4-1BB could potentially be used as an adjuvant to make smallpox vaccination more effective in humans.

10) Deming Janise– Amgen Scholar

Dr. Gourisankar Ghosh- Faculty Mentor

Title: Investigation of self-assembly of NF-kappaB protein p100

Abstract: The NF-kappaB family of proteins is an important group of transcription factors involved in innate and adaptive immunity. The NF- kappaB family consists of five members: RelA, c-Rel, RelB, p50, and p52. p52 is generated from its precursor protein p100 through an event called processing. p100 processing is induced by infectious agents. The purpose of this study is to determine what prevents p100 from being processed under normal cellular conditions. The current model is that p100 prevents processing by associating with itself. My focus is to test if p100 self-associates and if so, how to disturb the self-association.

Finally, I will test if non-oligomeric p100 undergoes processing in the absence of an infectious agent. I will do this by expressing p100 and comparing it to the expression of a p100 mutant to see if the oligomeric state of wildtype p100 is different from that of the mutant.

11) Haus Daniel- Amgen Scholar

Dr. Stuart Lipton- Faculty Mentor

Title: Nitric Oxide Downregulates Adult Neurogenesis and Neuronal Development by Reducing MEF2 Activity

Abstract: The transcription factor Myocyte Enhancer Factor 2 (MEF2) promotes neurogenesis and regulates diverse aspects of neuronal development. Nitric oxide (NO) is involved in critical physiological functions of the central nervous system (CNS) such as neurotransmission and synaptic plasticity. Interestingly, NO also functions to negatively regulate adult neurogenesis and neuronal development. However, the underlying molecular mechanism has yet to be studied. Here we report that adult neural progenitor cells (aNPC) express NO synthase 1 (NOS-1). Knockdown of NOS-1 using ribonucleotide interference (RNAi) increases MEF2 activity as well as increases neurite outgrowth in aNPC. These results suggest that NO downregulates neurite development by reducing MEF2 activity.

12) Hernandez Bryan- Amgen Scholar

Dr. Clodagh O'Shea- Faculty Mentor

Title: pAd-Syn

Abstract: The overall objective of this proposal is to develop new technologies that will enable the systematic engineering and cellular re-targeting of therapeutic viruses. The tools we create will also have the potential to radically change current methods for delivering and expressing multi-component systems in almost any mammalian cell. There is a desperate need to identify new classes of drugs and therapeutic modalities that conclusively ablate cancer cells but leave normal cells unharmed. Viruses that hone in on tumor cell receptors and replicate selectively within the tumor mass (oncolytic viruses) have enormous potential as lytic cancer therapies. Unfortunately, current methodologies to engineer such viruses fall short of this goal. In the first aim, we will pioneer a novel application of multi-site recombination that will enable the *de novo* assembly of viral genomes *in vitro* from genomic component parts and heterologous elements. This will allow multiple compound modifications and properties to be combined overnight to create new and improved viral vectors and therapies. The tools

we create will also enable multi-protein complexes and entire pathways to be assembled, delivered and co-expressed in any cell type via adenoviral infection. We will apply these technologies to develop 'next generation' oncolytic viruses that specifically kill p53 mutant tumors and pre-malignant breast cancer cells, and which have the potential to save the lives of many cancer patients.

13) Kim Seung Hyun (Chris)- Amgen Scholar

Dr. Robert Sah- Faculty Mentor

Title: Design of Articulating Biomaterials with Spatially-Varying Properties

Abstract: Focal defects in articular cartilage affect cartilage mechanics by elevating local tissue strain near the defect during articulation, and as a result, likely predispose the joint to the development of osteoarthritis. One therapeutic approach garnering attention is the tissue engineering of implantable constructs to fill focal defects and recapitulate native cartilage. The concept is that the construct provides a temporary structure for the encapsulated chondrocytes to produce native matrix, and over time, the construct degrades away as native tissue is produced. Agarose is a biomaterial that is biodegradable, biocompatible, and exhibits enough mechanical stiffness and integrity for implantation, making it a possible ideal material to create implantable constructs with. Stiffness of mature cartilage in both compression and shear is depth-varying, being lowest at the surface and becoming highest near the bone. As a result, studies to not only create homogenous, but also depth-varying agarose constructs have been recently attempted. While these studies elucidated relative compressive deformation and stiffness with depth as well as between homogenous and non-homogenous agarose constructs, its shear deformation remains to be elucidated. Since mechanical shear modulates matrix metabolism of cartilage, the relative shear deformation experienced by the encapsulated chondrocytes likely dictates its metabolic rates in the agarose construct. Therefore, the objective of this study is to determine the local and overall shear deformation of homogenous and depth-varying agarose hydrogel when compressed and articulated against normal articular cartilage.

14) Knoedler Joseph- Amgen Scholar

Dr. Samuel Pfaff- Faculty Mentor

Title: Expression of evolutionarily conserved enhancer regions in the chick spinal cord

Abstract: It is becoming increasingly clear that the complexity and diversity of eukaryotes is due not only to the variety of genes in the genome but also to how the expression of these genes is regulated – where, when and how strongly genes are

expressed. However, the expression patterns of the genomic regulatory elements responsible for controlling this intricate developmental program remain to be characterized. In this study we are using comparative genomics and immunofluorescent labeling to investigate the expression of evolutionarily conserved enhancer regions in the embryonic spinal cord. Specifically, we are cloning enhancer regions from the human genome that have homologues in chick, inserting them into plasmid vectors, electroporating the vectors into the neural tubes of ~60 hr old chick embryos, and using cryosectioning and immunofluorescent staining to determine where in the spinal cord these enhancers are active. This research will help build a foundation for investigating how evolutionarily conserved enhancer regions regulate the development of the vertebrate spinal cord.

15) Lau Lawrence-Amgen Scholar

Dr. David A. Holway- Faculty Mentor

Title: Linking nutrition and behavioral dominance: carbohydrate scarcity and territorial aggression in Argentine ants

Abstract: Argentine ants (*Linepithema humile*) are a well known dominant invasive insect, displacing native species in many areas where they are introduced. They form supercolonies (a web of interconnected and cooperative colonies), each genetically distinct and antagonistic towards another. This study focuses on two supercolonies in San Diego, the large supercolony and the Lake Hodges supercolony. Previous work on Argentine ant supercolonies determined that a high carbohydrate diet increased colony activity and aggression. Furthermore, increased contact and familiarity with antagonistic conspecifics elevates intercolony hostility. Building on ongoing research, this study examines behavioral effects of imposed carbohydrate scarcities in laboratory cultured colonies over a several month period. We hypothesize that carbohydrate restriction will hinder the ability of colonies to defend territories against aggressive conspecifics. To test this hypothesis, replicates (experimental colonies) from both supercolonies were connected in antagonistic pairs in multiple trials, with sucrose advantages given to one colony in each pair. Behavioral assays will clarify whether a carbohydrate scarcity decreases combat effectiveness and aggression in intercolonial conflicts. Activity levels, willingness to engage in aggressive behavior, and other variables will be measured to evaluate their performance. This is a novel attempt to understand how macronutrient availability affects behavioral changes and invasive success.

16) Mubin Madiha- Amgen Scholar

Dr. Phill Bourne- Faculty Mentor

Title: Podcasts as Multimedia Teaching Tools in Structural Biology

Abstract: The concept of creating Podcasts, by combining a particular scientific paper with a video to elaborate key points, is fairly new and growing. The objective of this research is to develop multimedia content around structure – function relationships of protein molecules, by integrating information from prior research conducted in this discipline. This multimedia presentation will synchronize various scientific publications with a corresponding video related to adrenergic receptors. It aims to serve as a tool, which can be used for different molecules, to aid the scientific community in the search for desired research material and facilitate readership of scientific work.

17) Ozdemir Ege- Amgen Scholar

Dr. David W. Rose- Faculty Mentor

Title: A possible role for Bcl-3 in inflammation and its effects upon nuclear receptors

Abstract: Possible Role for BCL-3 in Inflammation and its Effect upon Nuclear Receptors

A deep understanding of the mechanism behind hormone mediated cancer growth is a critical precursor in its prevention. In prostate and breast cancer, it is known that the cytokine IL-1B secreted by macrophages serves a role in the regulation of growth through the dismissal of transcription factors such as the N-COR/HDAC3 corepressor complex. The dismissal of these factors allows the derepression of the hormone's target genes. However, the inflammatory response signal IL-1B plays a role in another pathway, NF-kB, which leads to the eventual production of BCL-3. BCL-3 shares the selective N-terminal L/HX₇LL motif with the receptors for the reproductive hormones, testosterone and estrogen. This paper explores any interaction between the BCL-3 and nuclear factors via co-immunoprecipitation techniques.

18) Pascua Mary Rose- Amgen Scholar

Dr. Dwayne Stupack- Faculty Mentor

Title: The Role of Caspase 8 and Integrins in Neuroblastoma Progression.

Abstract: Loss of caspase 8 appears to be a negative prognostic indicator of neuroblastoma, a solid tumor of the peripheral nervous system, which develops in young children. However, 10-30% of aggressive neuroblastoma retain caspase 8. Caspase 8 plays a critical role in apoptosis. Recently, the Stupack lab has uncovered a link between the activation of caspase 8 and integrin signaling. Integrins are cell-

surface receptors for the extracellular matrix and commonly known for their “positive” signaling, which facilitates adhesion, migration and survival. Conversely, antagonized (unligated) integrins on cells can promote activation of caspase 8 and apoptosis, inhibiting tumor metastasis. So the question is how can cells which maintain caspase 8 go on to a malignant disease? In vitro, decreased integrin expression permits increased survival among caspase 8–expressing cells. I will test whether clinical samples that express caspase 8 and are malignant will express decreased levels of integrins. We will obtain at least 50 sample specimens from the Rady Children’s Hospital in San Diego and will test these samples via immunofluorescence staining. These studies will determine whether there is an inverse connection between integrins and caspase 8 expression in patients; these results may be useful when considering future clinical management of neuroblastoma.

19) Ramos Yamil- Amgen Scholar

Dr. Robert Sah- Faculty Mentor

Title: Characterization of Human Femoral Head Cartilage

Abstract: The overall objective of this proposal is to develop new technologies that will enable the systematic engineering and cellular re-targeting of therapeutic viruses. The tools we create will also have the potential to radically change current methods for delivering and expressing multi-component systems in almost any mammalian cell. There is a desperate need to identify new classes of drugs and therapeutic modalities that conclusively ablate cancer cells but leave normal cells unharmed. Viruses that hone in on tumor cell receptors and replicate selectively within the tumor mass (oncolytic viruses) have enormous potential as lytic cancer therapies. Unfortunately, current methodologies to engineer such viruses fall short of this goal. In the first aim, we will pioneer a novel application of multi-site recombination that will enable the *de novo* assembly of viral genomes *in vitro* from genomic component parts and heterologous elements. This will allow multiple compound modifications and properties to be combined overnight to create new and improved viral vectors and therapies. The tools we create will also enable multi-protein complexes and entire pathways to be assembled, delivered and co-expressed in any cell type via adenoviral infection. We will apply these technologies to develop 'next generation' oncolytic viruses that specifically kill p53 mutant tumors and pre-malignant breast cancer cells, and which have the potential to save the lives of many cancer patients.

20) Rivas Maria- Amgen Scholar

Dr. Francisco Villarreal- Faculty Mentor

Title: Short and Long-Term Effects of (-)-Epicatechin on Myocardial Ischemia-Reperfusion (I/R) Injury on the Rat Heart

Abstract: Studies have shown a relationship between cardiovascular outlook and flavonoid-rich diets. Flavonoids are found in cocoa, specifically the flavonols epicatechin and catechin, and may protect tissue against injury. We studied the long-term effects of epicatechin on myocardial ischemia-reperfusion (I/R) injury. Epicatechin treatment (1mg/kg/day) was given via oral gavage to male rats 10 days prior to 45 min coronary artery occlusion and continued during 3 weeks of reperfusion. The control group received water. The parameters measured are myocardial infarct size, myeloperoxidase (MPO) activity, and matrix metalloproteinase-9 (MMP-9) activity. The infarct area of the control group vs. epicatechin group are $50\pm6\%$ vs. $34\pm4\%$, respectively. MPO levels in the right ventricle, left ventricle border region, and left ventricle infarct region in the control group (n=6) vs. epicatechin group (n=6) are 53 ± 17 vs. 24 ± 5 , 67 ± 15 vs. 82 ± 17 , and 63 ± 12 vs. 36 ± 11 . MMP-9 levels in the right ventricle, left border region, and left ventricle infarct region in control group (n=6) vs. epicatechin group (n=6) are 61 ± 4 vs. 60 ± 4 , 72 ± 7 vs. 68 ± 5 , and 76 ± 7 vs. 67 ± 2 . These results suggest that (-)-epicatechin reduces tissue markers of myocardial (I/R).

21) San Pedro Matthew- Amgen Scholar

Dr. David W. Rose- Faculty Mentor

Title: The Role of Oct-1 and Inflammation in Prostate Cancer

Abstract: The inflammatory cytokines secreted by macrophages are known to derepress crucial gene regulatory networks, leading to the hormone resistance and the proliferation of tumor cells. Macrophages present in prostate tumors may adhere to the vascular cell adhesion molecule-1 (VCAM-1). One way to inhibit the macrophage/prostate cancer cell interaction is to block the expression of VCAM-1 in tumor cells. The Oct-1 protein mediates repression of a select group of inflammatory genes activated by $TNF\alpha$ and is dose responsive to decreasing the VCAM-1 content. Therefore, gauging the expression levels of Oct-1 in prostate cancer cells and normal prostate cells is vital to understanding the role of Oct-1 during an inflammatory response.

22) Sharma Aditi- Amgen Scholar

Dr. Jason H. Haga- Faculty Mentor

Title: Endothelial cell remodeling induced by mechanical forces: Imaging the effects of directional shear flow and tensile stretch on endothelial actin filaments and focal adhesions.

Abstract: Vascular endothelial cells are an important interface between blood vessel walls and blood flow, and play an important role in physiological functions within the body. Endothelial cells experience different physical forces including shear stress due to blood flow, tensile stretch due to the wall deformation, and normal stress hydrostatic pressure due to blood pressure. The physiological functions of endothelial cells change in response to the mechanical environment and these changes are correlated with the localization of atherosclerosis and cardiovascular disease. While experiencing fluid shear stress, cultured endothelial cells have shown marked elongation and orientation in the direction of flow. In addition, thick stress fibers of actin filaments appear and align along the cell long axis. Thus, the aim of this project is to analyze the rearrangement of endothelial cells in response to shear stress and tensile stretch in cultured endothelial cells through the use of live cell imaging techniques. The results from this study will help better understand the correlation between physical forces and endothelial cell function in relation to the progression of cardiovascular disease.

23) Shin Janice- Amgen Scholar

Dr. Toshiaky Kawakami via Dr. Michael Croft- Faculty Mentors

Title: Function of PLC β 2 in mast cell activation

Abstract: Allergy is one of the most common disorders of immune system in the industrialized countries. Mast cell is one of the key role players in allergic reactions of human body. When multiple IgEs are bound to the high affinity receptors (FceRI) on mast cells, mast cells are "sensitized". As antigens bind to the IgEs on the cell surface, IgEs are cross-linked and trigger mast cell activation, and that eventually leads to the activation of various signaling molecules. PLC β -isoforms have been known to play important roles for activation of cells downstream of G-protein coupled receptors. In this research project, we are investigating the function of PLC β 2 in the mast cells stimulated by IgE plus antigens and its role for allergic reactions using molecular and cellular biological technique.

24) Tian Yuan- Amgen Scholar

Dr. Celsa Spina- Faculty Mentor

Title: Use of inhibitory RNA (iRNA) to suppress histone deacetylase (HDAC) function in human primary T cells

Abstract: Several prior studies have shown that resting memory CD4 T cells serve as

one of the major cell reservoirs for latent HIV infection that is untouchable by current treatment. The central goal of our research is to understand the influence of chromatin structure in maintaining a latent viral state. HDAC enzymes function to cause chromatin condensation and gene silencing. Inhibitory RNA is being used to knockdown specific HDAC gene transcription to try to coax HIV out of latency. This project involves optimization of conditions for TaqMan PCR quantification of each HDAC gene transcription level to provide reliable baseline values to which iRNA knockdown effects can be compared. Total RNA is extracted from fresh CD4 T cells, and a reverse transcriptase (RT) reaction is run to generate the cDNA, needed for TaqMan PCR quantification. Then, by varying the amount of input cDNA and the concentration of primers and probe, conditions for amplification of the target HDAC gene sequence are optimized. Understanding the requirements to maintain viral latency serves as a stepping stone for the design of new treatments to completely eradicate HIV infection.

25) Tran Tuan- Amgen Scholar

Dr. Varghese Shyni- Faculty Mentor

Title: Electrically Sensitive Hydrogels

Abstract: The design and engineering of functional tissues must take into account the mechanical and chemical signals present in native tissues during homeostasis and repair. Smart hydrogels, with their ability to respond to external stimuli, offer great potential for engineering tissues as they can mimic both the structural and functional aspects of native tissues. In particular, chemomechanical hydrogels can respond to changes in pH, ionic strength, temperature, or an electric potential by converting the chemical energy directly into mechanical work. This study intends to develop environmentally sensitive hydrogels that can respond to the electric field while supporting cell growth. The hydrogel can apply strain and electrical potential simultaneously on cultured cells. The resulting behavior of the cells will be studied, which will aid in the development of engineered cartilage tissues that can better mimic the mechanical and electrical responses of native tissues.

26) Uche Peace Ozioma- Amgen Scholar

Dr. Chris Kintner, via Dr. Jennifer Stubbs- Faculty Mentors

Title: Functional Analysis of Cilia Proteins

Abstract: Cilia are hair-like projections of the plasma membrane that are capable of beating, thereby generating fluid flow across the surface of many vertebrate tissues. Proper formation of these cilia and the flow they generate are required for organ

function. Mutations in ciliary genes have been linked to various human diseases such as Primary Ciliary Dyskinesia (PCD). Better understanding of the molecular basis of ciliogenesis could lead to treatments for such diseases. Previous studies performed on ciliated epithelia have shown that FOXJ1, a forkhead transcription factor, is involved in controlling ciliogenesis. In this particular study various gene products, which are upregulated by FOXJ1, were cloned and fused in frame to fluorescent proteins followed by subsequent expression in ciliated cells of *Xenopus laevis*. Analysis of the localization of these fluorescent proteins allowed us to determine the subcellular location in the ciliary apparatus and hypothesize about their function. Expression of gene products were blocked by injection of antisense morpholinos into *Xenopus* embryos. Ciliary phenotypes were then analyzed specifically for defects in cilia elongation, cell polarity, basal body formation, and ciliary beat.

27) Vo Anh- Amgen Scholar

Dr. Richard Firtel, via Lee, Shwu-Ching "Susan" - Faculty Mentors

Title: Regulation of Ras Function

Abstract: Ras is a required small GTPase that controls a wide range of cellular processes including cell motility. What is not known is how these are regulated. The object of this study is to understand one of the key upstream regulators, the RasGEF (guanine exchange factor) complex containing RasGEF-I/J and RasGEF-F. For this study, we use *Dictyostelium discoideum*, an ideal organism for the dissection of Ras pathways as it can be easily manipulated and whose genome encodes multiple Ras genes that seem minimally redundant. GFP fusion proteins of the RasGEF and a scaffolding protein are being created and transformed into cells and the real-time spatial localization of the RasGEFs will be examined during chemotaxis. Time-lapse chemotaxis assays will be used and quantified to examine the kinetics of the response.

28) Weiss Jessica- Amgen Scholar

Dr. Steven Gonias- Faculty Mentor

Title: uPAR and the Effect of Cytokine Expression in 231 Cells

Abstract: Natural immune responses are the body's first defense mechanism against cancer; however, recent studies show that the interplay between cancer cells and immune cells is more complex, and certain types of inflammatory cells are crucial to the proliferation and survival of cancer tumor cells. The cytokine, Interleukin-6 (IL-6) has been implicated in both pro-inflammatory and anti-inflammatory responses. It has recently been suggested that elevated levels of IL-6 in the serum of cancer patients are

associated with tumor malignancy. The serine protease, Urokinase Plasminogen Activator (uPA), activates plasminogen into plasmin. Plasmin, in turn, activates a cascade of metalloproteases. Together with its highly specific receptor, uPAR, uPA induces various intracellular signaling pathways, and extracellular matrix degradation, which are crucial for inflammatory cell migration in response to infection and cancer cell metastasis. We observed that highly metastatic breast cancer cells, MDA-MB 231, express elevated IL-6 level, compared to normal human mammary epithelial cells. Upon inhibition of uPAR signaling with ATN 658 antibody, IL-6 expression significantly decreased. Thus, we hypothesize that the increase in IL-6 expression in highly metastatic breast cancer cells is mediated by the uPA/uPAR signaling. In order to prove our hypothesis, we will investigate whether blocking uPAR signaling using antibodies or short hairpin RNA against uPAR will decrease IL-6 expression in other cancer cell lines as well. Furthermore, in order to elucidate the mechanism by which uPA/uPAR increases IL-6 expression, we will examine whether IL-6 receptor, gp130, is activated by uPAR signaling.

29) Yip Kathleen- Amgen Scholar

Dr. Pamela L. Mellon, via Dr. Anita Iyer-Faculty Mentors

Title: Characterization of Far-Upstream Enhancer Regions of the GnRH gene

Abstract: Gonadotropin-releasing hormone (GnRH) is a neuropeptide responsible for proper reproductive function. GnRH gene expression is limited to about 800 differentiated neurons in the hypothalamus of the mouse brain. It is not clear how this gene is expressed only in GnRH neurons; although the GnRH enhancer (GnRH-E) and promoter (GnRH-P) target the GnRH neurons exclusively, the two elements are not sufficient for complete expression, constituting only 50% of its total gene expression in the adult mouse. This suggests that there are additional regulatory elements for complete GnRH gene expression. Previously, two evolutionarily conserved far-upstream enhancers were determined to be involved in the regulation of GnRH gene expression. We further characterized these far-upstream enhancers of the GnRH gene to determine specific areas critical for activity. Through the technique of site-directed mutagenesis, specific areas of the far-upstream enhancers were deleted and activity of the remaining sequence was analyzed through a luciferase/ β -galactosidase reporter assay in model GnRH neuronal cell lines. Understanding GnRH expression may lead to understanding hypogonadotropic hypogonadism and Kallmann's Syndrome, which are forms of infertility and are the result of defects in the production of GnRH or development of the terminally differentiated GnRH neurons.

30) Rochelle Young- Amgen Scholar

Dr. Benjamin Yu- Faculty Mentor

Title: Identification of RAS antagonists in the skin and hair

Abstract: Ras GTPases are cellular catalysts that have the ability to alternate between an inactive GDP bound form and an active GTP bound form. These states regulate the differentiation, proliferation, or survival of a cell. Germline Ras mutations in humans are the cause of three congenital syndromes including Costello, Cardiofaciocutaneous, and Noonan. These mutations cause Ras to be constitutively active. In this project, we are investigating which genes directly regulate Ras in the skin. Using a candidate gene approach, we are studying expression patterns of Ras antagonists called GTPase activating proteins (GAPs). We found that at least nine GAP genes are expressed in the skin. We now report on the tissue-specific pattern of each of these GAPs.