IUPUI Life-Health Sciences Internship Program

Spring 2010 Poster Session

Friday, April 16, 2010
3:30 PM—5:30 PM
VanNuys Medical Science Building Atrium
**Vision**

The Life-Health Sciences Internship Program seeks to connect talented IUPUI undergraduate students in the life and health sciences with enriching experiences in laboratories, research projects, and other professional experiences on the IUPUI campus and in campus-affiliated locations.

**Mission**

To educate, engage, and enlighten IUPUI life and health sciences undergraduates through on-campus internship experiences. We seek to achieve this through the following means:

1. Seeking out and arranging high quality internship opportunities in relevant fields.
2. Nurturing mentor and intern relationships through structured meetings and gatherings.
3. Providing opportunities and support to present work.

The IUPUI Life-Health Sciences Internship Program is funded by the Indiana University Commitment to Excellence Grant.
Intern Name: Tyler Zimmerly
Intern Major: Chemistry Pre-med
Mentor Name: Dr. Zollinger
Mentor Dept: Family Medicine, Bowen Center
Poster Title: Defining Race/Ethnicity for the State Master Research Program

The poster summarizes the work that the Bowen Research Center has been doing to develop a new standard for collecting and reporting data on race and ethnicity. At the center, we have been collaborating with several other state agencies to investigate the standards of several major government agencies, both at the national and state level, for collecting race and ethnicity data. The reason this is needed is to better improve the quality of healthcare in the United States by being able to target specific race and ethnic groups that show disparities in certain health trends. Right now since different agencies collect and report the data differently, the results can not be compared, which limits the ability to assess how great the disparity is between different races. This in turn slows down the process of targeting a certain group of people who are more likely to get a certain health problem.
Welcome to the IUPUI Life-Health Sciences Internship Program Spring 2010 Poster Session.

The Life-Health Sciences Internship program connects IUPUI life and health sciences undergraduates with research internships on and near the IUPUI campus. This program allows students to explore their career objectives and future career pathways, while also fostering valuable professional connections between students and faculty and staff. The students belong to a community of interns and mentors who support one another throughout the research experience and beyond. This program is funded by an IUPUI Commitment to Excellence grant to Dr. Doug Lees, Chair of IUPUI Department of Biology and Dr. Simon Rhodes, IU School of Medicine Associate Dean for Graduate Studies.

Life-Health Sciences Internship students represent 23 different majors and minors spanning six schools on the IUPUI campus. Many of these undergraduates have career goals involving research, medicine, dentistry, occupational therapy, physical therapy, and pharmacy. These internships are an excellent stepping stone for future research and graduate study. Mentors represent the Indiana University School of Medicine, the Indiana University School of Dentistry, the Indiana University School of Health and Rehabilitation Sciences, the Indiana University School of Nursing, Methodist Research Labs, and the pharmacy department of Indiana University hospital (Clarian Health). These professionals are providing invaluable experiences for undergraduate students and mentoring the next generation of scientists, researchers, and health professionals.

This program includes summaries of the posters presented and work completed by our interns. Thank you for joining us today!

Intern Name: Rachael Wojnowski
Intern Major: Pre-med: Biology
Mentor Name: Dr. Daniel Sliva
Mentor Dept: Cancer Research at Methodist Hospital
Poster Title: Pectin inhibits invasiveness of breast and prostate cancer cells by down-regulation of urokinase plasminogen activator (uPA) secretion

Breast and prostate cancers are the most prevalent cancers among women and men in the United States. Pectin is a heteropolysaccharide found mainly in citrus fruits. In the present study, we evaluated the effect of pectin on highly invasive breast [MDA-MB-231] and prostate [PC3] cancer cells. We have found that pectin significantly reduced the migration of these cells, however, had no effect on proliferation. Interestingly, when pectin was combined with ProstaCaid & BreastDefend, breast and prostate dietary supplements respectively, it markedly further enhanced inhibition of invasive behavior of breast and prostate cancer cells. These effects were mediated through the down-regulation of urokinase plasminogen activator (uPA) secretion. Further microarray analysis demonstrated that this treatment affected expression of cell-cycle regulatory genes e.g. GADD45-α which was confirmed by Western blot analysis. Our data suggests potential use of pectin with ProstaCaid or BreastDefend for the prevention and treatment of breast and prostate cancers, respectively.
Thymosin beta 4 (Tβ-4) and vascular endothelial growth factor (VEGF) are important growth factors in angiogenesis, a process critical to regeneration. Axolotl (*Ambystoma mexicanum*) is a unique animal model capable of regenerating their tissue parts after amputation, while short-toes (a mutant axolotl) and African clawed frog (*Xenopus laevis*) are considered as regeneration-deficient since they cannot replace their lost parts. However, we lack sequence information for VEGF from axolotl and short-toes, and that of Tβ-4 from all three animals, which has impaired our study of their roles in regeneration. In this study we partially sequenced and compared the mRNA of VEGF and Tβ-4 in Axolotl, short-toes, and African clawed frog. VEGF primers were designed based on *Xenopus* cDNA sequence while the Tβ-4 primers were designed based on human cDNA sequences. The total RNA was extracted utilizing RNeasy kit (Qiagen Sciences Inc., Germantown, MD) following the manufacturer’s instruction. One-step reverse transcriptase polymerase chain reaction (RT-PCR, Qiagen) was performed following manufacture’s instruction. The RT-PCR products were run on 0.9% agarose gel and then sent for two direction sequencing analysis (ACGT, Inc., Wheeling, IL). After comparing the RT-PCR products sequences, the results suggested that VEGF and Tβ-4 are highly conserved (>90%) among *Xenopus laevis*, axolotl and short-toes.
Proline-rich tyrosine kinase 2 (Pyk2) deficient mice have a high bone mass phenotype. While most studies focus on effects of Pyk2 in osteoclastic bone resorption, here we show that Pyk2 is also important in osteoblast proliferation and differentiation. With respect to proliferation we demonstrate that while in vitro wild-type and Pyk2-/- osteoblast numbers are identical, differences exist in cell cycle progression (significant greater than 8% increase in cells in S-G2M phase), with no differences detected in apoptosis. With regard to differentiation, Pyk2-/- osteoblast cultures exhibited a striking increase in alkaline phosphatase activity as well as calcium deposition as a marker of mineralization. Consistent with these findings, real-time PCR studies showed that Pyk2-/- osteoblasts expressed higher levels of alkaline phosphatase, type I collagen, and osteocalcin, compared to wild-type controls. Interestingly, it is known that alternative splicing of the Pyk2 gene can result in the production of two distinct isoforms that differ in length by 42 amino acids. We confirmed, by reverse-transcription PCR, that the smaller Pyk2 protein (106 KDa) was an alternatively spliced variant, which for clarity, we have designated as Pyk2-S. Importantly, as osteoblast differentiation increased, the expression of Pyk2-S RNA increased relative to full-length Pyk2. Taken together, these data suggest that Pyk2 is a critical regulator of osteoblast function and understanding the mechanism of its role will facilitate the development of novel anabolic therapies to treat bone loss associated with osteoporosis and other bone-related disorders.

Excessive alcohol consumption during pregnancy causes many adverse developmental deficits to the fetus that are represented as a spectrum of cognitive, behavioral, and structural abnormalities termed Fetal Alcohol Spectrum Disorder (FASD). Effective clinical diagnosis of FAS, the most severe form of FASD, depends in part on a distinct set of abnormal facial features (smooth philtrum, short palpebral fissures, thin upper vermillion, flattened face). However, FASD cases that demonstrate none or only a small subset of facial dysmorphology are difficult to diagnose with little or no clinical intervention. Recent human studies to improve diagnosis of partial FASD involve anthropomorphic measures coupled with discriminant analysis to assess multiple alterations in facial dysmorphology and similar studies using C57BL6 mice suggest an animal model for dysmorphic analysis. More recent studies have used mouse models of FASD with varied alcohol doses and timing of exposure to study developmental defects distinct in structural head and face development. However, a comprehensive understanding of the mechanisms that underlay alcohol induced facial alterations is unknown. We suggest that a C57BL/6 mouse model of FASD will be able to assist in understanding a comprehensive range of facial dysmorphology and assist in clinical diagnosis of FASD. We also suggest that developing a model that controls for timing and dose of exposure and assures consistent control of maternal factors will aid in understanding the mechanisms of alcohol induced abnormalities. We propose the use of two alcohol doses of 4.8 and 3.6 v/v % alcohol in pregnant dams during developmental periods of embryonic days 7 (E7) to E12 and E7 to E17. Alcohol exposure at E7 in know to induce alteration in development during critical stages of neurulation and neural stem cell proliferation, migration and differentiation.
In response to Acute Kidney injury (AKI), it has been shown that cells which have an appearance of a monocyte or macrophage morphology were involved in the chronic effects to kidney function. Several studies have also shown that AKI results in salt sensitive hypertension and this was indicative in this experiment. This research study looks at kidney injury at different experimental conditions. This study particularly looks at the effect of direct against the effect of indirect kidney injury through humoral/inflammatory effects or mediators. This study was carried out on three groups, namely; sham control, injury with non injured kidney (i.e. indirectly injured) and injury with injured kidney (i.e. directly injured). Blood Pressure, Urine Albumin/Urine Creatinine ratio, and Histological data were collected in analyzing kidney injury. In the course of this study, it was found that the directly injured and the indirectly injured groups gave a higher level of blood pressure compared to the sham control. The directly injured group gave a higher Albumin/Creatinine excretion ratio compared to the indirectly injured and sham control groups.

Telomeres function as the protective caps that prevent the ends of chromosomes from being recognized as double-strand breaks (DSB). Many important proteins in the DNA damage response (DDR), especially in the non-homologous end-joining (NHEJ) pathway, are also important in telomere maintenance. Two important protein kinases in the DDR are ataxia telangiectasia mutated protein kinase (ATM) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs). It is not understood which proteins are phosphorylated by these kinases, or what affect these phosphorylated proteins have on the telomere and other cellular processes. This research focuses on TRF2, an essential mammalian telomeric protein, which coordinates the assembly of the proteinaceous cap on the telomere. Our recent studies suggest that human TRF2 phosphorylation by ATM in response to DNA damage is critical for DNA repair. This along with the work of other groups suggests the sharing of protein components from the DDR to the telomere, and vice-versa. Therefore, our results suggest a functional role for TRF2 in DNA repair that connects the telomere with the DDR via a specific signaling pathway. Using over expression of mutant forms of TRF2, immunoprecipitation, and mass spectrometry the binding partners of TRF2 will be identified.
Dicalcium phosphate dihydrate (DCPD) typically prepared using monocalcium phosphate monohydrate (MCPM) and β-tricalcium phosphate (β-TCP). Hydroxyapatite (HA) can be substituted for β-TCP, but a prior study showed that this leads to an increased tendency of the DCPD formed to convert back to HA during degradation. Thus, the objective of this study was to directly compare the degradation characteristics of MCPM:HA and MCPM:β-TCP cements. MCPM:β-TCP cements were prepared with 1:1, 1:1.5, 1:2, and 1:3 MCPM:β-TCP molar ratios and a powder to liquid ratio of 0.97. MCPM:HA cements were prepared with MCPM:HA molar ratios of 4:1, 4:1.5, 4:2, and 4:3 and a powder to liquid ratio of 1.0. To evaluate the degradation characteristics, cement cylinders (3 mm diameter, 7 mm height) were subjected to static degradation in phosphate buffered saline (pH=7.4, 37°C) for 14 days. The MCPM:HA cements had lower pH and higher mass loss due to conversion of the DCPD to HA, as determined by powder x-ray diffraction. These results are important for the clinical application of DCPD cements for bone repair because conversion of DCPD to HA will limit resorbability of the cement. The lower pH may also have a negative affect if the cement is implanted.
Intern Name: Nancy Tanjung  
Intern Major: Biomedical Engineering  
Mentor Name: Dr. Joseph R. Dynlacht  
Mentor Dept: Department of Radiation Oncology  
Poster Title: The Effect of Mre11 Exonuclease Inhibitor MIRIN on U1 Cells

The M/R/N complex (Figure 1) which consists of MRE11, Rad50, and Nbs1 is homogeneously distributed in human cells’ nuclei. This complex acts as a DNA damage detector and contains DNA repair checkpoint factors. In this experiment, we are focusing on the protein MRE11, which functions as a 3’-5’ double-strand DNA exonuclease and endonuclease of U1 human melanoma cells. Hyperthermia and irradiation are known to cause DNA double strand damage; therefore, radiation and heat shock were used to see how the MRE11 exonuclease inhibitor, Mirin, affects the survival of U1. We hypothesized that once the DNA double strand is damaged, Mirin will inhibit the MRE11 from performing DNA double strand break repair and thus decrease the survival of the cells.

The concentration of Mirin chosen for this experiment was 50 µM and the length of drug treatment was 18 hours at 37o Celsius for each flask. The toxicity of 50 µM Mirin toward the U1 cells over several different lengths of time is shown in Figure 2. Two experiments were done, one which involved only Mirin and radiation, and another which involved heat shock as well. Four different radiation doses were chosen for the treatment: 0 Gy, 1.5 Gy, 3 Gy, and 6 Gy. For the experiment which involved heat shock, each sample was heated for 2 hours at 41.5o Celsius. Some samples were treated with only DMSO to serve as a control to be compared to the cells treated with Mirin. In the end, by comparing survival results from the different radiation and heat treatment schemes, we shall determine whether Mirin would sensitize U1 cells to the effects of radiation.

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Intern Name: Mason Anthony “Tony” Brown  
Intern Major: Biology  
Mentor Name: Dr. Brittney-Shea Herbert  
Mentor Dept: Medical and Molecular Genetics  
Poster Title: Effects of Donor Age and Media on Normal Mammary Epithelial and Stromal Cell Growth Characteristics

Normal breast epithelial and stromal cells derived from healthy women with no history of breast cancer were examined for their replicative lifespan and characteristics of mammary gland tissue. These unique cells were generated from tissue collected at the Komen Tissue Bank (KTB) of Indianapolis, Indiana, and the expansion of this repository will provide a valuable resource foundation for any cellular based research. Epithelial denotes a surface cell while stromal refers to the connective tissue, and this pairing aimed to study the environment of breast cancer. We tested the hypothesis of a relationship among age and the cells’ in vitro cellular growth as finite or infinite. Cells expressing finite growth, or a Hayflick limit, present clues to a given tissue’s life potential. This limited cell growth is due to the shortening of genetic information at the telomere, or capped end of DNA. Results concluded younger donor stromal cells to grow and divide for a longer duration than older donor stromal cells. When grown in varying media, stromal cells divided approximately twice as fast in DMEM/F12 as in DMEM. Within the field of regenerative medicine, telomerase immortalization and cell differentiation provide many promising benefits in both cancer and stem cell therapeutics.
The research conducted throughout this academic school year dealt with the analysis of select Indiana University School of Medicine class lectures and shadowing Dr. Willis in several clinics. The analysis of class lectures was done online from the IU School of Medicine's media recording catalog. Two classes were focused on: ICMI and ICMI of the Spring 2009 semester. Each lecture focused in on a different field of medicine, but almost all lectures incorporated in some way a focus point that is trying to be stressed more and more in medical school, a concept known as Competency VI. On several Fridays Dr. Willis was followed around and observed in a normal day in clinic to see the patient-physician, physician-physician, and physician-co-worker interactions and relationships, as well as the documentation side of medicine that would be nearly impossible to witness without the opportunity to shadow.

Osteogenesis imperfecta (OI) is a genetic disorder primarily due to mutations in the genes for type 1 collagen. Persons with OI have a high incidence of fracture. Therapy with bisphosphonate medications that decrease bone resorption has been shown to increase bone mineral density (BMD) assessed by dual x-ray absorptiometry (DXA), decrease measures of bone turnover, and decrease fracture rates in children with OI. However, a broad variety of treatment regimens are used and few data exist on the consequences of stopping treatment.

For this project, data were obtained from a long-term follow-up of a prospective, randomized study comparing the effects of alendronate and pamidronate therapies. Following a mean of 4 years of therapy, eight of the children stopped treatment. At the time treatment was stopped, children showed increased total body and lumbar spine BMD z-scores and lower markers of bone resorption compared to when they had begun therapy. After a mean of 822 days off therapy (range 230-1396), children showed decreases in BMD z-scores and increases in markers of bone turnover compared to the time treatment was stopped.

The effects of bisphosphonates attenuate with time after therapy cessation. Further data are needed to determine the optimal dosing and duration of treatment.
p53 is a tumor suppressor that regulates cell cycle arrest and apoptosis. Its activity and stability is regulated by Mdm2. While much work has been done to examine the role of Mdm2 in hematopoietic cells, its role in osteoblasts is not well understood. We hypothesized that osteoblast-specific Mdm2 overexpression would result in the down regulation of p53 and Rb, and a subsequent increase in osteoblast number, which in turn would increase bone mass in vivo. Using the osteoblast-specific type I collagen promoter, we generated osteoblast-specific Mdm2 transgenic mice. Offspring from two founder lines exhibited a 12% increase in bone mineral density compared to wild-type littermate controls in both male and female mice examined at 6 weeks–1 year. Similarly, the more sensitive micro-computed tomography showed a 20% increase in bone volume and 15% increase in number of trabeculae. Cortical bone histomorphometry revealed a significantly higher endocortical bone formation rate and a resulting decrease in endocortical area of Mdm2 overexpressing mice compared to controls. Trabecular bone histology remains to be completed. With regard to biomechanical properties, Mdm2 overexpression also resulted in an increase in the ultimate force (N, 14% increase), but no difference in stiffness (N/mm). Taken together these data suggest that osteoblast-specific overexpression of Mdm2 results in an increase in bone mass and in some biomechanical properties. Therefore, these findings may offer novel strategies to treat bone loss diseases such as osteoporosis.

Liver transplantation is a life-saving procedure that changes a person forever. In this study, the data from COPM interviews with 20 recipients of liver transplants was approached through a qualitative framework. The recipients were then assigned to 3 groups ranging from 2-6 weeks, 2-6 months and 6-12 months post-liver transplant. Common themes prevalent in each respective group are represented to provide a sense of what a recipient experiences within the first year post-transplantation. Pain and fatigue continue to be constant barriers for some patients, which hinders involvement in certain activities. Driving, active recreation, socialization, and employment are a few of the areas that seem to be most problematic throughout the first year. In some cases actual quotes are used to better reflect the liver recipient’s attitude towards their ability to participate in important activities. While certain activities prove challenging, the data reflects a certain degree of choice made by each patient regarding the activities he or she feels are important. This choice between what is important, what is necessary, and what can wait normally relay a recipients overall attitude toward their new lives as liver transplant recipients.
In recent years, more attention is being paid to tobacco usage and the complications that it can cause in the oral cavity and body. Tobacco has been connected to various types of cancers, as well as other systemic diseases. About 26% of Indiana’s population smokes or uses other forms of tobacco. The state is also ranked number 2 in the U.S. in number of tobacco users. Approximately 90% of oral cancers are squamous cell carcinomas and about 75% of these are in smokers. Alcohol enhances tobacco induced cancer. During this research study, the initial effects of tobacco (nicotine and cigarette smoke condensate) on human gingival fibroblasts were analyzed. Specifically, the study analyzed the effects of nicotine and cigarette smoke condensate (CSC) on cellular levels of two cell cycle proteins (p53 and p21) using immunoblotting. After one exposure to nicotine and CSC, the protein levels of p53 and p21 increased. This shows that even one cigarette can affect cells of the oral cavity in a negative manner.

Canonical transient receptor potential (TRPC) proteins form cation selective Ca2+ permeable plasma membrane channels which are implicated in numerous physiological and patho-physiological processes including neurite outgrowth, tumor progression, muscle dystrophy, secretion, contractions, etc. Thus far, the crystal structure of TRPCs is not known. This hampers efforts for developing the drugs that can specifically modulate the channel activity. TRPCs exhibit a substantial homology to bacterial K+ channels, such as KcsA. The crystal structure of KcsA is solved. We used computer modeling to generate a structure model of the TRPC5 channel using the KcsA crystal structure as template. TRPC5 was chosen because our laboratory has been studying TRPC5 for a decade and accumulated a large amount of functional data. TRPC5 can be activated only in the presence of extracellular Ca2+. We focused on establishing the molecular determinants underlying this property of TRPC5. We obtained several thousand models of the TRPC5 channel and identified one exhibiting the lowest potential energy. The model successfully predicted a mechanism that underlies the Ca2+/La3+ sensitivity of TRPC5. We propose that the generated TRPC5 model would be very useful during drug discovery process.
Elliot Smith
Intern Major: Biochemistry
Mentor Name: Nick Brustovetsky
Mentor Dept: Pharmacology & Toxicology
Poster Title: Elevated cytosolic $\mathrm{Ca}^{2+}$ is required for mitochondrial depolarization and leads to the uncoupling of oxidative phosphorylation

In ischemic stroke, formation of a blood clot restricts blood flow to a region of the brain leading to a rapid decline in oxygen and glucose. This oxygen/glucose deprivation results in an increase in glutamate concentration, $[\text{Glu}]$, a major excitatory neurotransmitter. This prolonged increase in $[\text{Glu}]$ can cause persistent stimulation of Glu receptors (GluR) including NMDAR and AMPA/kainate subtypes of ionotropic GluR. This results in an influx of $\mathrm{Ca}^{2+}$ and $\mathrm{Na}^{+}$ into the cytosol through the activated GluR. The neurons that experience this prolonged elevation of Glu, the change in cytosolic $\mathrm{Ca}^{2+}$ ($[\mathrm{Ca}^{2+}]_{c}$) occurs in three steps. Glu exposure causes (1) an initial jump in $[\mathrm{Ca}^{2+}]_{c}$, followed by (2) a transient decrease in $[\mathrm{Ca}^{2+}]_{c}$. Then after some delay, (3) a larger sustained increase in $[\mathrm{Ca}^{2+}]_{c}$ occurs. This delayed increase in $[\mathrm{Ca}^{2+}]_{c}$ is known as delayed Ca$^{2+}$ deregulation (DCD). This onset of DCD is essential for neuronal death in stroke. In this study, we are investigating how elevated Glu and sequential elevation in cytosolic $\mathrm{Ca}^{2+}$ results in the failure of the cellular bioenergetics. We used live-cell fluorescence imaging techniques with rat hippocampal neurons to examine the role $\mathrm{Ca}^{2+}$ plays on the depolarization of the mitochondria and the failure in cellular bioenergetics.

Nadia I Chaidir
Intern Major: Biotechnology
Mentor Name: Dr. Stephen Kempson
Mentor Dept: Cellular and Integrative Physiology
Poster Title: Phosphorylation may mediate normal trafficking of the Betaine/GABA transporter during hypertonic stress in renal medullary cells

The betaine/GABA transporter (BGT1) mediates uptake of the osmolyte betaine in renal medullary cells. During hypertonic (Hyp, 550 mOs) stress BGT1 is synthesized and inserted in the plasma membrane of MDCK cells. The phorbol ester PMA down-regulates BGT1 by endocytic removal. To further test if PKC regulates BGT1, mutants of EGFP-tagged BGT1 were prepared by substituting alanine at consensus sites for PKC phosphorylation and were expressed in MDCK cells. All mutants behaved like native BGT1 except T40A that remained intracellular during Hyp stress. Substitution of aspartate or glutamate at T40 did not interfere with the normal trafficking response. Native MDCK cells were incubated in Hyp growth medium for 24 hr in absence (controls) or presence of PKC inhibitors (Staurosporine 0.3 mM or Go6976 10 mM), or PMA (50 nM) for downregulation of PKC. In Hyp controls GABA transport was increased 10-fold compared to isotonic groups, but the increase was only 3-4 fold when inhibitors or PMA were present ($p<0.001$, n=3). In contrast, amino acid transport system A was upregulated normally under all Hyp conditions. Western blots of cell lysates showed the abundance of BGT1 protein was not changed by drug treatment. Addition of okadaic acid or calyculin, to inhibit protein phosphatases, produced a significant stimulation of hypertonic upregulation of BGT1 transport activity, consistent with improved trafficking of BGT1 to the plasma membrane. Data suggest PKC-dependent phosphorylation at T40 may be required for normal trafficking of BGT1 to the plasma membrane during Hyp stress. AHA 09GRNT2010156.
**Intern Name:** Kaylie Cheesman  
**Intern Major:** Biomedical Engineering and Chemistry  
**Mentor Name:** Ryan Lau  
**Mentor Dept:** Methodist Hospital Neurophysiology Department  
**Poster Title:** Total intravenous anesthetic increases specificity and sensitivity for intra-operative neuromonitoring of evoked potentials

This research dealt with studying the effects of a total intravenous anesthetic (TIVA) versus a gaseous anesthetic regime on the monitoring of transcranial motor evoked potentials (MEPs). MEPs are monitored during high risk spine, brain, vascular, and peripheral nerve surgeries to prevent motor and sensory deficits post-operatively. A TIVA regimen does not significantly decrease waveform amplitudes, and therefore, increases the specificity and sensitivity of the interpretation of MEP monitoring. In contrast, a gaseous anesthetic technique reduces the amplitude and increases latency of the MEP waveforms and makes accurate interpretations difficult. Through this research project, 300 TIVA surgical cases will be analyzed to support the use of TIVA for MEP monitoring practice in the operating room.

**Intern Name:** Jeromy M. Shoup  
**Intern Major:** Nursing  
**Mentor Name:** Dr. Yuichiro Takagi  
**Mentor Dept:** Biochemistry and Molecular Biology  
**Poster Title:** Probing Human Mediator in Breast Cancer Cells

Mediator is a large multi-protein complex, which plays critical roles in gene expression in eukaryotes. Prompted by a series of observations suggesting a potential role of Mediator in breast cancer, the lab has begun investigating human Mediator in breast cancer cells. Probing human Med17 subunit of Mediator, the one of the key regulatory subunits, necessitates a recombinant hMed17 used as a positive control for western blotting. Therefore, my research project is to obtain the recombinant human Med17 subunits as GST-hMed17 fusion protein form. An expression vector construction started with a computational vector design, or called “paper cloning”, followed by primer design based on my paper clone. Subcloning of human MED17 gene into the expression vector was carried out using the latest cloning technique, SLIC method, which involves PCR, T4 pol reaction, and RecA annealing process followed by transformation. Identification of positive clones was done by the restriction digests. Validation was done by DNA sequencing. All 36 clones were tested but only 2 clones proved to be positive. Expression and purification of GST-hMed17 is the next step followed by testing the recombinant protein by western blotting with anti-hMed17 antibody.
Intern Name: **Mercy Shitemi**  
Intern Major: Informatics  
Mentor Name: Jennifer Baron  
Mentor Dept: Telemedicine  
Poster Title: Clarian Telemedicine Services

Using advanced videoconferencing technology, Clarian telemedicine offers live interactive consultations between providers and patients. Physician specialists from Clarian Health in Indianapolis can meet with patients and physicians hundreds of miles away without traveling to that location. This makes seeing patients in other cities easier for both patients and the clinicians.

Patients visit a clinic/hospital in their local community and have a live Tele-appointment with a specialist from Clarian Methodist Hospital, Indiana University Hospital or Riley Hospital for children.

Telemedicine not only connects patients to providers, but it also connects providers to providers. Clinical Providers also offer education/distance learning by use of the Clarian Telemedicine technology.

Clinical professionals at partner sites can complete their continuing medical education requirements, using the same video unit, to virtually attend clinical educational events from Clarian Health and Indiana University School of Medicine. Clarian Telemedicine has already offered more than 450 hours of educational programming qualifying for continuing medical education credit.

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Intern Name: **Melanie Day**  
Intern Major: Pre-med, Biology  
Mentor Name: Dr. Marc S. Mendonca  
Mentor Dept: Radiation Oncology, Medical & Molecular Genetics  
Poster Title: DMAPT Increases Radiation Sensitivity of Prostate Cancer Cells

DMAPT (dimethylaminoparthenolide) is a derivative of parthenolide, an anti-inflammatory sesquiterpene lactone that is derived from the feverfew plant that has been used in Europe for centuries. More recently, parthenolide has been studied as a chemotherapeutic drug and a radiation sensitizer in clinical oncology. Because DMAPT is water-soluble and bioavailable parthenolide analog, we are investigating it as a radiosensitizer in Du145, a p53 mutant advanced prostate cancer cell line. We show here that DMAPT enhanced radiation-induced cell killing. Further investigation showed that the radiation sensitization by DMAPT is due to the suppression of split-dose recovery, perhaps through the inhibition of double stranded DNA break repair. NF-kB is a transcription factor which is constitutively active in a large number of cancers. It is likely that DMAPT is suppressing repair by inhibiting the activation of NF-kB.
Past research has indicated that nicotine, the major addictive constituent in cigarette smoke, is directly related to the effects of smoking on the cardiovascular system such as the development of arterial diseases. The lining of vascular blood vessels is composed of endothelial cells, which are directly involved in the central functions of the cardiovascular system through regulation of blood flow and blood pressure. Research studies have demonstrated that nicotine causes various changes in the cellular behavior of human endothelial cells, including morphological changes and an increase in cell death. This study was performed to assess the effect of nicotine on the cytotoxicity and cell proliferation of human endothelial cells. Human umbilical vein endothelial cells (HUVECs) were obtained from American Type Culture Collection (ATCC) and cultured in Endothelial Basal Medium-2 (EBM-2) supplemented with 10% fetal bovine serum at 37°C in 5% CO₂. HUVECs were seeded in 6-well plates with 100,000 cells per well and were allowed to attach overnight. Each well was then exposed to a different concentration of nicotine (0, 50, 100, 200, 400, and 800 µg/mL) in serum-free EBM-2. Endothelial cell cytotoxicity and cell proliferation were measured by the lactate dehydrogenase (LDH) assay (Roche Diagnostics) and water-soluble tetrazolium-1 (WST-1) assay (Roche Diagnostics), respectively. LDH assay results indicated increasingly higher percentages of cytotoxicity from nicotine concentrations of 200, 400, and 800 µg/mL in comparison to the control. WST-1 assay results exhibited decreased cell proliferation at 400 and 800 µg/mL nicotine in comparison to the control. Morphological changes and endothelial cell death were observed at the 800 µg/mL nicotine concentration. According to data, nontoxic levels of nicotine that can then be utilized to analyze other cellular mechanisms include concentrations at 50 and 100 µg/mL. Results of this study indicate a relation between nicotine and human endothelial cells and the contribution of nicotine to various effects on the cardiovascular system.
Aurora kinases, particularly aurora A and aurora B, play a role in normal cell proliferation. Since an unusually high expression of these kinases has been reported in tumor cells, they have become the target of a variety of aurora kinase inhibiting anticancer drugs. One such drug, ENMD-2076, was studied in this translational research project involving a xenograft mouse model and a phase I clinical trial. The preclinical xenograft model evaluated multiple myeloma tumors in NOD/SCID mice treated with ENMD-2076, lenalidomide, and combinations of both drugs, then compared them with an untreated vehicle control group. Immunohistochemistry (IHC) was used to predict the best course of treatment for the myeloma tumors based on caspace-3, Ki67, phospho-histone H3 (p-H3), and CD34 biomarkers. For the human clinical trial, biopsy cores were taken just prior to treatment and again 28 days post-treatment. Using IHC, both p-H3 and CD34 were evaluated at both time points. While the clinical trials lacked the quantity of data needed to draw sound conclusions, the xenograft model clearly showed drug effect of the ENMD-2076, lenalidomide, and the combination against MM in vivo.

In the United States today, $1.3 trillion are spent each year on health care yet 46 million Americans lack basic health care coverage. Furthermore, evidence suggests a significant shortage of primary care physicians in the near future. Recent trends in medical school applications show a decreased interest in primary care. This creates “the perfect storm”- under supply of physicians, increasing medical expenses, and decreasing matriculation. In response to this looming crisis, we created a unique and innovative service learning experience for pre health professional undergraduate students. The goal of this course is to increase student awareness of the daily challenges faced by medically underserved populations.

The course activities include focused shadowing, weekly forum discussions, a service learning project, a summary presentation, and a reflective paper. Students describe these activities as interactive, engaging, and informative; an uncommon opportunity to experience the United State’s healthcare system in the real world classroom. This course empowers students to define their roles in bettering the healthcare system.

From analyzing pre and post course surveys, the data demonstrated that students recognize the looming crisis for this vulnerable population, reigniting their passion for community service.
Intern Name: **Heba Elantably**  
Intern Major: Pre-Pharmacy Biology  
Mentor Name: Derek McMichael  
Mentor Dept: Clarian Health IU Hospital Pharmacy  
Poster Title: IU Hospital Compounding Pharmacy Overview

Hospital pharmacy is one of the most diverse fields of pharmacy; it includes rounding with physicians and clinical teams as a clinical pharmacist, functioning as an inpatient staff pharmacist performing pharmacokinetic calculations, making dosing recommendations, entering practitioner orders, and checking IV’s. Another area of practice is nutrition support. The nutrition clinical pharmacist is able to advise the physicians and dieticians on parenteral and enteral nutrition therapy. IU Hospital is unique in the fact that it is a teaching institution and performs many clinical trials. There is an entire division of pharmacists that work in this area and oversee and coordinate more than 300 clinical trials. Another area that is unique to IU Hospital is the Clarian Compounding where pharmacists research and formulate recipes for different suspensions, creams, and capsules.

During my internship, I had the opportunity to closely work with pharmacists in all of the mentioned fields. During the first semester, I spent most of my time preparing prepackaged medications and compounding parenteral medications. I also delivered medications to the nursing units and performed the hourly pharmacy delivery. In addition, I obtained orders from the warehouse and refilled any prepackages. During my second semester, I had the pleasure of joining the compounding pharmacy, which I learned the most from. I had the chance to make different types of suspensions, suppositories, and creams.

The wide scope of activities I participated in through this internship helped develop an interest and passion for pharmacy and assisted me in deciding which field of pharmacy I would like to pursue. Additionally, I formed strong relationships with many of the pharmacists I interacted with. Also, this internship opportunity helped reinforce my interest for pharmacy. Most importantly, however, I understood the crucial role pharmacists play in the healthcare system and how I, as a future pharmacist, would contribute to that role.

Intern Name: **Irene Robinson**  
Intern Major: Anthropology  
Mentor Name: Arlene Schmid, PhD, OTR  
Mentor Dept: Roudebush VA, HSR&D; Indiana University School of Health and Rehabilitation Sciences  
Poster Title: Perceptions of Recovery after Stroke

Strokes are often devastating to those who experience them. Depending on the location of insult, a stroke may result in a wide variety of physical, and mental symptoms. Rehabilitation is a great asset in treating these difficulties. In designing a successful rehabilitation treatment program it is important to take patients’ own perceptions of their recovery into account. In this study we interviewed five male veterans who suffered stroke, completed inpatient rehabilitation, and still suffer some residual disability. Then we read through the transcribed interviews, identifying common themes in how the veterans described their perceptions of recovery. All of the survivors identified concrete physical and mental barriers that were still holding them back from living life as fully as they wished. Many commented on the emotional difficulty of depression. Several also noted a decrease in their pursuit of hobbies, and most, despite their difficulties, saw themselves as lucky or blessed not to have greater stroke-related limitations. The clinical implications of this study are that many of the factors participants identified that were still holding them back from hobbies and daily activities could be eliminated through continued rehabilitation. In addition, better access to psychiatric care might reduce the incidence of post-stroke depression.
Intern Name: **Mia Recupito**  
Intern Major: **Biology**  
Mentor Name: **Dr. George Sandusky**  
Mentor Dept: **Pathology and Laboratory Medicine**  
Poster Title: **Increased c-FLIP expression corresponds with decreased caspase 3 activation and increased plasma cell survival**

Cellular FLICE-like inhibitory protein (c-FLIP) blocks Fas-mediated apoptosis and confers drug resistance in various cancer models. In plasma cell myeloma (PCM), c-FLIP expression and function is unclear. Immunohistochemistry (IHC) and computer image analysis software were used to determine the status of c-FLIP expression in human clinical cases of plasma cell myeloma, normal bone marrow, normal tonsil, and in a mouse myeloma model where c-FLIP expression was controlled with doxycycline. IHC reveals that in seven out of seven patients with PCM, c-FLIP showed higher expression overall at both diagnosis and at relapse when compared to normal tissues. In the mouse model, low activated caspase 3 levels correlated with high c-FLIP in the control, and high activated caspase 3 correlated with low c-FLIP when doxycycline was administered. This model indicates that increased c-FLIP expression corresponds with decreased caspase 3 activation and increased plasma cell survival.

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Intern Name: **Dillon Etter**  
Intern Major: **Biology & Spanish**  
Mentor Name: **George Sandusky, DVM PhD**  
Mentor Dept: **Department of Pathology and Laboratory Medicine**  
Poster Title: **Effects of Circulating Hematopoietic Stem & Progenitor Cells on Angiogenesis in the C32 Human Melanoma Xenograft**

Tumor angiogenesis is a term used to describe the proliferation of blood vessels into a tumor and is an essential process in the metastasis of a tumor. Recent studies suggest that endothelial cells involved in angiogenesis could be derived from bone marrow. These CD31^+CD34^{bright}CD45^{dim}AC133^+ circulating hematopoietic stem and progenitor cells are commonly known as pro-angiogenic circulating progenitor cells (PA CPCs) and can be isolated using polychromatic flow cytometry. CD31^+CD34^{bright}CD45^{dim}AC133^+ non-angiogenic circulating progenitor cells (NA CPCs) express similar markers but can be distinguished from PA CPCs by their lack of AC133 expression. Previous findings show that injection of human PA CPCs into immunodeficient mice with the C32 Human Melanoma Xenograft causes a significant increase in tumor growth. This experiment utilized immunohistochemistry techniques to investigate angiogenesis at 7 days and at 30 days in tumors of mice injected with PA CPCs, NA CPCs, and CD34^+ cells. At 7 days, there was a significant increase in angiogenesis of mice injected with PA CPCs compared to those injected with NA CPCs, CD34+ and Media. This data suggests that the increased growth of tumors in mice injected with PA CPCs is due to increased angiogenesis in the early stages of tumor development.
The CRC is a part of the Indiana CTSI and is located in the IU Hospital. Its purpose is to provide high quality space, nursing, and sample processing support to conduct academic and industry sponsored clinical research studies for both inpatient and outpatient visits. It is in collaboration with the Schools and Pharmaceutical companies within the Indianapolis area. The CRC is capable of conducting research in many fields such as neonatal and cancer. A clinical research process begins with a submission to the IRB, following submission to the CRC. After the appropriate review, the research begins. Orders are issued for the research and policies are reviewed. Then, patients are recruited for the study and the protocol orders are carried out by the CRC’s highly qualified nursing staff. Once a protocol is finished, the data is given to biostatisticians for analysis. After all data is collected and analyzed, the study is closed thorough the IRB. The PI, based on his research, submits his finding to a medical journal in hopes that it will be published.

Osteoglophonic dysplasia (OGD) is characterized by rhizomelic dwarfism, telecanthus, and craniosynostosis. This disorder is associated with mutations in a tyrosine kinase like receptor, fibroblast growth factor receptor 1 (FGFR1). One of the mutations (C381R) located in the transmembrane domain of FGFR1 was found in three of the six unrelated patients that have been sequenced to date. This represented a “common” mutation in the disorder. Our goal was to determine the level of cellular signaling by the mutant FGFR1. Wild-type and mutant FGFR1 were cloned in an expression vector and transfected into HEK293 cells. The level of FGFR1 signaling was determined by quantitative RT-PCR. Compared to wild-type FGFR1, mutant FGFR1 had approximately 20 fold higher signaling. We conclude that the “common” mutation in FGFR1 is an activating mutation, which results increased cell signaling downstream of the receptor. This inappropriate signaling leads to the phenotypes observed in patients with OGD.
Intern Name: **Chelsea Quirk**  
Intern Major: Pre-Med Forensic Science  
Mentor Name: Sandra Kinsella  
Mentor Dept: Anesthesiology  
Poster Title: Analysis of Inhaled Anesthetic Agents in Clinical Liver Transplantation

Recent anesthesia research suggests that certain inhaled anesthetic agents have an improved capacity to ameliorate ischemia-reperfusion injury in transplanted organs. This study compares three inhaled agents in liver transplantation. Outcomes included early graft loss, perioperative serum AST and ALT levels and late graft survival.

Data were extracted by reviewing deceased donor liver transplant recipients between 2001-2009. The choice of anesthetic agent was strictly at the discretion of the anesthesiologist. Early graft loss included loss of graft function within 7 – 30 days of transplant. Serum AST, ALT and total bilirubin levels were measured daily in the immediate post-transplant period.

There were 1013 transplants analyzed, with three inhalation agents: isoflurane (84%), desflurane (9%), and sevoflurane (6%). In the first week post-transplant, the isoflurane group had the highest AST and ALT levels, but all groups were similar by post-operative day 7. No difference occurred among the groups in total bilirubin levels within 30 days. The risk of 7-day and 30-day graft loss did not differ, nor did 1-year graft survival.

The results suggest that desflurane and sevoflurane abrogate the severity of ischemia-reperfusion injury better than isoflurane in deceased donor liver transplantation. The groups do not differ in early or late graft survival.

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Intern Name: **Megan Feustel**  
Intern Major: Biology, Spanish  
Mentor Name: Dr. Natalie Hamrick  
Mentor Dept: No longer works in Indianapolis- affiliation now is Healing Journey Ministries  
Poster Title: Piloting a Latino Faith-Based Cancer Survivor Support Program

Many people diagnosed with cancer turn to religion or spirituality to cope with difficult issues such as guilt and anger, to face their own mortality, and to find meaning in their illness. Latino cancer survivors may especially search for answers in religion and spirituality because these elements compose such an integral part of Latino culture. While several programs offering secular support and a handful of faith-based supportive care programs are available to English-speaking cancer survivors in the Indianapolis area, language barriers limit accessibility for Latino cancer survivors. The purpose of this study was to determine interest in and the effectiveness of a faith-based cancer support program offered in Spanish for Latino cancer survivors and their families.

To date, we conducted two four-hour retreats (Christian and Non-Religious components) based upon the major strengths of our successful faith-based program developed for English-speaking cancer survivors: Letting go of uncontrollable aspects and finding meaning and growth. Thirteen individuals participated. Results suggest that Spanish-speaking faith-based support is well received (satisfaction mean 3.70/4.00, SD=0.31) and could be beneficial to many Latino cancer patients. Therefore, further steps have been taken to organize monthly meetings for interested participants and to eventually form regularly-meeting support groups conducted in Spanish.
Intern Name: **Tyler Foxworthy**  
Intern Major: Applied Mathematics  
Mentor Name: Ernesto Levy M.D.  
Mentor Dept: Division of Rheumatology  
Poster Title: 3-D Data from 2-D Photos: A Novel Approach To The Quantification of Synovitis In Individuals With Inflammatory Arthritis

In the treatment of individuals with Rheumatoid Arthritis counts of tender and swollen joints often serves as a key indicator of disease progression and severity. In clinical practice the assessment of patients synovitis is most often made subjectively by a physician, whose assessment may differ significantly from that of other physicians. Current methods for the quantification of joint volume and inflammation (MRI, Ultrasound, Cat Scan) are not cost-effective for many patients or most clinical studies. By combining computational methods from statistics and geometry we were able to develop a computer algorithm that could find the volume of any solid (i.e., an inflamed joint) using only a handful of multi-perspective digital photographs from a standard digital still camera. This method is both highly accurate as well as extremely cost-effective and could be applied to numerous problems such as gauging drug efficacy, remote monitoring of disease progression, and numerous measures for clinical studies.

Intern Name: **Nora Mossa-Basha**  
Intern Major: Biology, Pre-Med  
Mentor Name: Dr. Dennis Ang and Ms. Janna Hilligoss  
Mentor Dept: Department of Medicine  
Poster Title: Fibromyalgia and Pain Clinical Research Center

Fibromyalgia is a medical syndrome affecting more than 6 million people in the United States alone, with a female to male frequency ratio of approximately 9:1. People with fibromyalgia have chronic widespread pain. Symptoms include tenderness, soreness, fatigue, problems sleeping, headaches, morning stiffness, and difficulty performing daily functions.

As an intern under the direction of Dr. Dennis Ang and Ms. Janna Hilligoss, I was privileged to be a part of a research study aimed at better understanding the causes and therapies used to help alleviate the pain of fibromyalgia. The purpose of the Drug and Talk Therapy Study is to better understand how talk therapies can improve the therapeutic benefits of drug for fibromyalgia. The drug used, Savella (milnacipran), is an FDA approved drug for fibromyalgia. The safety and efficacy of Savella has already been established, and because of this, the study was conducted in order to determine whether combination treatment of both Savella and talk therapy is more efficacious than just Savella or talk therapy alone. Volunteers of the study are randomized on two different levels. Each participant will either receive Savella or a placebo and will be assigned to receive either educational instruction or cognitive behavioral therapy (CBT).
**Amanda Meyer**
**Intern Major:** Biomedical Engineering  
**Mentor Name:** Dr. Hyun-Suk Lim  
**Mentor Dept:** Biochemistry and Molecular Biology  
**Poster Title:** An Encoding-Free Cyclic System: A Simple Strategy for the Construction of Combinatorial Cyclic Peptoid Libraries

Cyclic peptoids are of great interest in drug discovery and chemical biology. Their properties as protein-binding molecules, including increased conformational rigidity, make them preferential to their linear counterparts. Unfortunately though, their utility in high-throughput screening (HTS) is restricted by difficulties in sequencing the “hit” compounds. While other methods which involve extra synthetic steps for encoding have been created, we have produced a simple and “encoding free” method that employs cyclic peptoids as a model system. The key idea in this strategy is a post-screening “ring-opening” reaction to convert cyclic peptoids selected from a screen into sequenceable linear peptoids. Specifically, oxidation of a thioether linkage on cyclic peptoids, followed by a ring-opening reaction lead to linear peptoids, which can be sequenced by tandem mass spectrometry. Because of the simplicity and efficiency of this strategy, it can be readily applicable to peptides and other peptidomimetics. We believe that our encoding-free method could serve as a general platform with which to screen combinatorial cyclic peptide/peptidomimetic libraries.

**Anna R. Gaddy**
**Intern Major:** BS Biology  
**Mentor Name:** Robyn K. Fuchs  
**Mentor Dept:** Physical Therapy  
**Poster Title:** Investigating the Role of SFRP4 in Osteoporosis

Osteoporosis is a skeletal disorder characterized by low bone mass and compromised bone quality that afflicts over 10 million Americans. Currently available treatments function either by reducing bone loss or adding new bone by targeting the bone cells osteoclasts or osteoblasts, respectively. Osteoclasts function to break down bone, while osteoblasts function by depositing non-mineralized matrix. Drugs targeting osteoclasts are referred to as anti-resorptive agents, while drugs targeting osteoblasts are referred to as anabolic agents. Recently, investigators have focused on developing new therapeutic targets aimed at increasing new bone formation. The aim of our work is to evaluate the role of secreted frizzled-related protein 4 (sFRP4) in regulating bone formation through canonical Wnt signaling. sFRP4 is part of a family of five sFRP’s, and in the skeleton, sFRP4 may function by reducing the responsiveness of canonical-Wnt signaling. This would result in lower bone mass by impairing osteoblast proliferation. To accomplish our aim we will characterize the skeletal phenotype of mice deficient in the gene encoding for SFRP4 using histological, 3-D imaging and biomechanical testing. In addition, we are evaluating the therapeutic potential of SFRP4 as a target for increasing bone mass as a means to treat osteoporosis.
The research involved investigating DLC1’s (deleted in liver cancer 1) location within both ischemic and normal mouse kidney cells. Ischemia is a restriction in the blood supply and consequently in nutrients and oxygen. Ischemia leads to ATP depletion, which activates the pathway in figure 1, resulting in inactivation of RhoA and actin disregulation. Preliminary experiments suggest that DLC1 is relevant to ischemia because siRNA knockdown of DLC1 prevents some of the actin cytoskeletal derangements resulting from ATP depletion of S3 cells. After exposing the kidney cells to LMB or resveratrol, the collected proteins from the cells were probed with antibodies and analyzed to see where DLC1 is localized. The results showed that DLC1 was localized mostly with the cellular membrane in both normal and resveratrol treated cells. However, resveratrol treated cells also had some DLC1 in the cytosol at a slightly lower molecular weight.

Excessive alcohol consumption during pregnancy causes many adverse developmental effects that are now recognized as a spectrum of cognitive, behavioral, and structural abnormalities termed Fetal Alcohol Spectrum Disorders (FASD). The most severe form termed FAS. Effective clinical diagnosis of FASD depends, in part, on a distinct set of alterations in growth, facial dysmorphism, and central nervous system abnormalities. However, many FASD patients demonstrate only a small subset of dysmorphic features, with varying degrees of expression, and to date clinical diagnosis of less obvious dysmorphism is not reliable. Diagnosis of facial dysmorphism, encompass all or a partial set of distinct facial features; including smooth philtrum, short palpebral fissure, thin upper vermilion, and a flat mid-face. The most effective diagnostic methods for human facial features in FAS/FASD use direct clinical observations, scored assessment of two dimensional photographs, and or anthropomorphic craniofacial measures combined with multivariate analysis to assess sets of facial alterations that may be used to classify FASD, but to date is not highly accurate. Recent studies using mouse models of FASD have demonstrated distinctive structural alterations in head and face development. Studies using C57BL/6 (B6) mice demonstrate some distinct facial dysmorphology that parallels human dysmorphology and it has been suggested that craniofacial dysmorphology correlates with CNS abnormalities. Mouse models provide a means to study how alcohol exposure alters morphogenetic processes to produce a specific set of abnormal facial features, and to assess the extent to which the altered development of the face is associated with alterations in CNS structure and function. Mouse models in FASD facial dysmorphology provide the crucial experimental capability of controlling and manipulating the dose and developmental timing of the alcohol exposure, thereby allowing for dose-effect analyses and differences associated with the developmental stage of exposure. Information from mouse studies can then inform efforts to improve clinical diagnosis of FASD when the phenotype is only partially expressed, and may define a quantitative means to identify FASD in a quick and cost-effective manner.
Calcium/Calmodulin-dependent protein kinase (CaMKII) is a serine/threonine protein kinase that is highly enriched in the brain. The kinase makes up roughly 1-2% of the total protein in the brain, where it regulates synaptic function and plasticity in response to altered levels of calcium in the cell.

The goal of our research is to identify key amino acid residues essential for CaMKII substrate phosphorylation. While some CaMKII substrates contain the previously established phosphorylation motif, others do not contain key residues thought to be necessary for phosphorylation. We used high-throughput peptide synthesis to elucidate what residues in these non-canonical sequences are important for CaMKII targeting. Using a robotic peptide synthesizer, we created an array of six hundred peptide sequences containing known substrates of CaMKII. Scanning mutagenesis of these peptides showed other residues, not thought to be necessary for CaMKII targeting, played a role in CaMKII phosphorylation.

Collapsin response mediator protein-2 is a protein crucial to neurite outgrowth during neural development and regeneration. CRMP-2 is the target of a novel anti-epileptic drug called Lacosamide. Lacosamide targets voltage-gated sodium channels in order to produce its anticonvulsant effect. This then raises the question of the significance of lacosamide binding to CRMP-2. In attempts to answer this question, the Khanna laboratory is evaluating the structure-function of CRMP-2 in neurons. Using computer docking/modeling, Dr. Meroueh predicted the binding of lacosamide to five pockets within CRMP-2. Mutations of single or multiple residues of such sites were created to test if the efficacy of lacosamide would be altered. My project involved (1) expression of wildtype and mutant CRMP-2 proteins in a model neuronal cell line, (2) expression of wildtype and mutant CRMP-2 proteins in neurons, and (3) ability of these neurons to maintain axon growth and dendritic complexity.
Recent studies suggest that megakaryocytes (MKs) play a significant role in skeletal homeostasis, as evidenced by the presence of osteosclerosis in multiple MK related diseases \(^1-^3\). We previously reported a novel interaction whereby MKs enhanced OB proliferation up to 6-fold by a mechanism that requires direct MK-OB cell-cell contact and the engagement of integrins \(^4-^6\). Here we show that within the first 18 hours of co-culture, MKs dramatically alter OB cytoskeleton morphology including focal adhesions. Since the tyrosine kinase Pyk2 is associated with cytoskeletal remodeling, we examined Pyk2 expression in MK-stimulated OBs. We found that MKs enhance Pyk2 expression in OBs by 2-3 fold following 1-4 hours in co-culture. Using OBs from Pyk2\(-/-\) mice, we further showed that Pyk2 is required for the MK-mediated increase in OB proliferation. The stimulation of OBs with MKs for 4 hours was sufficient to enhance OB number up to 5 days later. Next, we began to further dissect the MK-mediated signaling cascade regulating OB proliferation. We found that the proto-oncogene Mdm2 and tumor suppressor protein p53 were significantly activated in OBs following incubation with MKs and that this activation was reduced in Pyk2\(-/-\) OBs. Taken together, our data suggests that Pyk2 is a positive regulator of cell cycle progression in MK-stimulated OBs by regulating the activities of Mdm2, p53 and Rb. Further clarifying the mechanisms by which MKs enhance OB proliferation will facilitate the development of novel anabolic therapies to treat bone loss associated with osteoporosis and other bone-related diseases.

Bisphosphonates are a class of drug routinely used to treat osteoporosis, cancer, and other diseases that cause bone loss and fragility. A serious side effect of bisphosphonate treatment is osteonecrosis of the jaw (ONJ) – where dead bone becomes exposed in the oral cavity. This often occurs in patients who are immunosuppressed and who undergo dental surgery. The cause of ONJ is unknown and there is no treatment. The goal of this study was to 1) try to produce ONJ in an animal model and 2) determine if bisphosphonates and immunosuppressive drugs interact to affect dental extraction healing. Female beagle dogs were treated for 9 months with high doses of bisphosphonate, with or without an immunosuppressive agent (dexamethasone), and then underwent dental extraction. Extraction sites were evaluated after four or eight weeks of healing using high-resolution imaging. We found that bisphosphonates have subtle effects on extraction healing and the addition of dexamethasone does not alter this effect. There was no exposed bone in any animals – therefore no animals were considered to have ONJ. However, two bisphosphonate-treated animals had extraction site morphologies with similarities to ONJ in humans.
Josh Horton
Intern Major: Chemistry (B.S.) Pre-med, Math minor
Mentor Name: Dr. Simon Rhodes
Mentor Dept: Cellular and Integrative Physiology
Poster Title: A Novel Form of the Lhx4 (lim4) Neuroendocrine Transcription Factor in Zebrafish

LHX3 and LHX4 are LIM homeodomain transcription factors which have been shown to play critical roles in pituitary and motor neuron development in mammals. Anterior pituitary cell specificity and lineage are partly determined by the genes which code for these transcriptional regulators. Mutations in these genes have been linked to subjects exhibiting Combined Pituitary Hormone Deficiency diseases, making it necessary to further understand how these factors function. The zebrafish is a well-established genetic model and its LHX3 homolog, Lhx3, has been shown to be highly conserved in both sequence and expression pattern upon comparison with mammalian equivalents. Lhx4 has been sequenced by database projects but has not been analyzed. Lhx3 and lhx4 cDNAs, obtained from zebrafish embryos at 28 hours past fertilization, were cloned into an expression vector and sequenced. Sequence for lhx3 was found to exhibit 100% conservation with reported sequence for this gene. The acquired lhx4 sequence was analyzed in comparison with both putative zebrafish Lhx4 and accepted mammalian LHX4 sequence. It contained four silent mutations which did not affect the primary structure of the protein and a G>C nucleotide point mutation resulting in a proline for alanine amino acid replacement. This substitution is currently under investigation.

Elizabeth Hazlehurst
Intern Major: Biology
Mentor Name: Frank Yang PhD
Mentor Dept: Microbiology and Immunology
Poster Title: Regulation of Outer Surface Protein A (OspA) in the Lyme Disease Pathogen

Lyme disease is a poorly understood infection that is becoming increasingly common throughout the US. Borrelia burgdorferi is the pathogenic bacteria responsible for Lyme disease, and is transmitted to humans by a tick bite. In nature, B. burgdorferi is cycled between an arthropod vector (Ixodes scapularis) and a mammalian host. B. burgdorferi encodes virulence factors that allow it to progress undetected by the mammalian immune system. Two of these virulence factors are the major outer surface proteins OspA and OspC. Reciprocal regulation of these virulence factors is required for B. burgdorferi to be transmitted between the tick and mammal: elevated OspA levels are found during tick colonization whereas OspC increases upon infecting the mammal. OspC is regulated via the Rrp2-RpoN-RpoS pathway. OspA is regulated by an unknown mechanism. Our study was to elucidate the regulation of OspA expression. The ospA promoter contains three regions that might have a key role in ospA regulation: an Inverted Repeat region, a Repeats region, and a T-Rich region. We created several shortened ospA promoters and placed a luciferase reporter under their control. By doing this, we may determine if any of the three regions of the ospA promoter are required for ospA expression.