

MS / BS / Misc.

Associations of Posttraumatic Stress with C - Reactive Protein and Interleukin-6 Among Urban Police
Araia, Sewit

Advisor: Cecil Burchfiel

Our aim was to examine the association between posttraumatic stress disorder (PTSD) and the inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6) in a sample of police officers. Traumatic stress may alter the hypothalamic- pituitary-adrenal (HPA) axis response, possibly increasing the production of CRP and IL-6. Therefore, we hypothesized that PTSD symptomatology in police officers is associated with elevated levels of the inflammatory markers CRP and IL-6. 115 police officers were randomly selected from 934 sworn, active duty officers. PTSD symptomatology was measured using the Impact of Event Scale (IES) and defined as an IES score ≥ 26 . Blood samples were obtained after an overnight fast for CRP and IL-6 analyses. Univariate and multivariate analysis was performed to evaluate the association between PTSD, CRP and IL-6 among 114 officers with complete information. PTSD was identified in 34 participants (29.8%). The mean level of CRP was slightly lower in officers with PTSD symptoms compared to those without (1.32 $\mu\text{g/ml}$ vs. 1.65 $\mu\text{g/ml}$; $p=0.44$). In contrast, mean levels of IL-6 were slightly higher in officers with PTSD compared to those without PTSD (2.53 pg/ml vs. 2.22 pg/ml ; $p=0.78$). There were no significant differences in other covariates (medication use, physical activity, smoking history, alcohol consumption) between PTSD categories. Mean levels of CRP and IL-6 did not differ significantly across PTSD status in multivariate adjusted models ($p=0.55$ and 0.32 respectively). We found no association between PTSD and the inflammatory markers CRP and IL-6. This study was limited by sample size and its cross-sectional study design. The lack of association may be because CRP and IL-6 are elevated only during a particular time point of PTSD symptomatology, which we were unable to evaluate. However, further research examining these relationships in a larger population may be worthwhile.

A Timescale Analysis Of The Interleukin-12 And Epidermal Growth Factor Cell Signaling Pathways
Dolly, Brandon

Advisor: Klinke, David

Cells sense and respond to their external environment through a network of protein-protein interactions. These protein-protein interactions that direct the flow of information within cells are called cell signaling pathways. These signaling pathways ubiquitously appear in the literature as signaling cartoons. These cartoons represent the proteins and interactions that play putative important roles in controlling the flow of information within specific cells. However, it is unclear how well a specific cartoon applies to a specific cell type. Timescale analysis is an engineering technique that can be used to infer what interactions within these signaling cartoons control the dynamics of cell signaling. The objective of this work was to apply timescale analysis to two particular signaling networks calibrated to primary cell data: the Epidermal Growth Factor (EGF) signaling network to primary rat hepatocytes and the Interleukin-12 signaling networks to primary naïve CD4+ T cells. The timescale analysis was used to assess the relative speed at which each interaction takes place (e.g. very fast, very slow) in order to gauge the contribution of each interaction to the overall pathway. Some may happen very fast and therefore, experimentally, appear to be at equilibrium while others may be very slow and not be seen at all in the lab. To estimate the uncertainty in our analysis, we applied the timescale analysis in conjunction

with an empirical Bayesian approach. An empirical Bayesian approach is a way to state what we believe to be true based on experimental data. These two techniques were helpful to state at some probability the correlation of a specific protein-protein interaction with a certain timescale. We can then use these timescales to form a more complete picture about how cell signaling occurs and identify key protein-protein interactions that control how intracellular information flows in specific cell types.

Unmasking the Benefit of a Probiotic, *Lactobacillus reuteri*, in a Triphasic Biofilm Wound Model

Motlagh, Homed

Advisor: Thomas, John

Introduction Chronic wounds have always been a concern for medical professionals, yet demands on patient health and cost of healthcare dwarfs the knowledge base that exists. The role of biofilms in the healing process is, however, becoming increasingly apparent. Certain probiotic lactic acid bacilli, particularly various *Lactobacillus* and *Bifidobacterium* species have proven beneficial to chronic diseases and offer unique therapeutic modalities in wounds. **Hypothesis & Objective** The purpose of this study was to further improve a triphasic wound model incorporating mixed biofilms and diffusible products reported previously. We evaluated benefits of the oral probiotic, *Lactobacillus reuteri* (Lr) in reversing the activity of proven wound pathogens. Our hypothesis was that detrimental diffusible products of a three member biofilm could be altered via the presence of a proven probiotic. **Materials & Methods** Biofilms were made using singular, paired, and three species (*Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa), and *Candida albicans* (Ca)) suspended in 30% poloxamer F127. Mouse fibroblast cells were maintained using tissue culture media with 10% fetal bovine serum and standard antibiotics. The biofilms were separated by a filter (Whitman #3) to determine diffusible products transferred between the biofilm and fibroblasts. Lr was obtained from G.U.M®-Star. The completed triphasic model was incubated at 37°C for 24 hours in 5% CO₂; measure of cell death against an uninoculated control was used as a marker for the effect of the different biofilm communities. Samples were trypsinized, stained with Trypan blue and counted. **Results** The model optimized individual growth of eukaryotic and prokaryotic cells with the mouse fibroblasts well characterized in the UPrep spin column tubes. The impact upon fibroblasts and positive trypan blue dead cells increased with the number of biofilm prokaryotes (1-3) ranging from 2x10⁴ to 9x10⁴ after 24 hours. Interestingly, the monospecies toxicity was observed the most with Sa and least with Pa. A 24 hour biofilm of Lr decreased the toxicity by ≥40% for each of the pathogenic combinations (1-3) **Conclusion** The triphasic wound model highlighted one possible pathogenic role of biofilms via diffusible products; the model also highlighted the potential role of healthy bacteria and we are presently developing a hypothesis that these bacteria may replace and out-compete pathogenic biofilms.

Identification of a smoking-associated gene signature for lung cancer

Wan, Ying-Wooi

Advisor: Guo, Lan

This study presents a novel computational approach to identifying a set of smoking-associated genes, with implications for refined diagnosis of lung cancer and prognosis for smokers. First, genes significantly associated with survival in 256 lung adenocarcinoma patients were selected. From these genes, 217 genes showed significant differential expression in smokers versus non-smokers. Second, pair-wise gene coexpression networks based on prediction logic were constructed for smokers and non-smokers for these 217 genes and six major signaling hallmarks of lung cancer, including EGF, EGFR, MET, E2F3, E2F5, and KRAS. By comparing the coexpression networks associated with smokers and non-smokers, differential components, i.e., the unique gene coexpression patterns specific to each group, were identified. From the differential components, a set of seven genes having direct coexpression relations with all six signaling hallmarks were selected for constructing a prognostic model. The expression profiles of the identified seven genes were fitted into a Cox proportional hazard model as covariates of lung cancer survival. Based on this model, a classifier was built to predict post-operative survival in lung cancer patients on the training cohort. This model accurately (log-rank $P < 0.05$, Kaplan-Meier analysis) predicted survival in three validation sets of non-small cell lung cancer patients ($n=289$). The model's prediction strongly related to smoking association and smoking cessation ($P < 0.02$) based on Pearson's Chi-squared analysis. Furthermore, this 7-gene signature could accurately (accuracy $> 74\%$ in test sets) identify lung cancer patients ($n=100$) from normal patients ($n=92$), indicating that this signature could be used for screening of lung cancer risk in smokers. The interactions obtained from the coexpression networks were validated with multiple pathway databases and the results reported in Ingenuity Pathway Analysis and Pathway Studio. The experimental validation is being carried out by collaborators (Dr. Laura Gibson).

Caveolin-1 Scaffolding Domain Induces Leukocyte Adhesion Without Increasing Microvessel Permeability

Xu, Sulei

Advisor: He, Pingnian

We have demonstrated that perfusing microvessels with caveolin-1 scaffolding domain (CAV) inhibits inflammatory mediator-induced nitric oxide (NO) production and permeability increases. In addition to mediating permeability increases during inflammation, NO has been indicated to play anti-inflammatory role in preventing leukocyte adhesion under resting conditions. This study aims to investigate the roles of exogenously applied CAV in endothelial basal NO production, leukocyte adhesion, and adhesion-induced changes in microvessel permeability, as well as their potential mechanisms. Experiments were conducted in rat mesenteric venules. Hydraulic conductivity (Lp) was measured to determine microvessel permeability. NO was quantified in DAF-2 loaded vessels using fluorescence imaging. Changes in endothelial junctions were evaluated by immunofluorescent staining with confocal images.

Perfusing individual venules with CAV reduced basal NO at a rate of $1.3 \pm 0.1\%$ per min without affecting basal Lp. After resuming blood flow in CAV perfused vessels for 10 min, the adherent leukocytes increased from 1.3 ± 0.2 to 25.3 ± 3.7 per 100 μm of vessel length. However, the firmly adherent leukocytes altered neither basal Lp nor VE-cadherin distribution. Increases in Lp (15.3 ± 1.6 times baseline control) occurred only upon addition of formyl-Met-Leu-Phe-OH (fMLP) to vessels with CAV-induced adherent leukocytes, which is attributed to fMLP-stimulated release of reactive oxygen species (ROS) from adherent leukocytes. Similar results were observed with the application of NO synthase (NOS) inhibitor. Our results indicate that the exogenously applied CAV not only inhibits stimulated NO production during inflammation, but also reduces basal NO and promotes leukocyte adhesion. The CAV-induced leukocyte adhesion did not increase microvessel Lp, indicating the uncoupling of adhesion process from leukocyte oxidative burst and vascular barrier function. Additional stimuli are necessary for ROS-mediated permeability increases. The similar results of NOS inhibitor to those of CAV-mediated adhesion support the reduced basal NO as the mechanism of CAV-mediated leukocyte adhesion, though NO-independent mechanisms remain to be investigated.

Optimization of an in vitro 3-D microenvironment to reprogram synovium-derived stem cells for chondr
Li, Jingting
Advisor: Pei, Ming

Adult stem cells gradually lose their stemness once they are removed from their in vivo niche for plating in plastic flasks. Synovium-derived stem cells (SDSCs) are a tissue-specific stem cell for chondrogenesis. Our previous study indicated that SDSC-derived extracellular matrix (ECM) can serve as an in vitro three-dimensional (3D) niche, greatly enhancing SDSC propagation and allowing restoration of stem cell stemness toward chondrogenesis [1]. Considering articular chondrocytes reside in a hypoxic condition [2] and mitogens such as FGF-2 are involved in mesenchymal stem cell chondrogenesis [3], some important microenvironmental factors such as oxygen tension and mitogens are assumed to contribute to the regulation of these seeded cells. In this study, we hypothesized that in vitro microenvironment can be optimized by modulating oxygen tension and mitotic signal in ECM deposited by SDSCs to rejuvenate expanded SDSCs' proliferation and chondrogenic differentiation capacity.

M.D.

Stress Related Induction of Painful Bladder Syndrome in Mice: A New Murine Model
Jessop, Morris
Advisor: Stanley Zaslau

Painful Bladder Syndrome (PBS) is a chronic, painful inflammatory condition of the bladder wall. The cause of PBS is still unknown. Although there are many treatments available to relieve symptoms, there is no cure at this time. The lack of curative treatment is further hampered by the lack of representative in vivo models. In an effort to establish an in vivo model, the effect of chronic mild stressors on normal mouse bladders was evaluated for its impact on mast cell number and urothelium thickness. Forty mice were exposed to a series of random stressors daily (Cage Tilt, Damp Sawdust, No Sawdust, Social Stress, and Varying Light/Dark Cycles). After sixteen weeks, the mice were sacrificed and their bladders

removed, formalin-fixed, paraffin-embedded, and evaluated using routine light microscopy with hematoxylin/eosin, giemsa, and PAS stained sections. Urothelial and detrussor muscle mast cell numbers were evaluated by averaging ten representative 200x fields in the Giemsa section. Urothelial thickness was evaluated by averaging six representative regions in the PAS section. The control and test groups were statistically compared using the non-parametric Mann-Whitney method. Sequence of data for each category: Mean \pm Standard Deviation, Median, Minimum, Maximum Urothelial Mast Cells (per field; $p = 0.0041$): Control ($n=10$): 0.03 ± 0.09 , 0.00, 0.00, 0.30 Test ($n=28$): 0.31 ± 0.43 , 0.20, 0.00, 2.10 Detrussor Mast Cells (per field; $p = 0.5731$): Control ($n=10$): 0.45 ± 0.64 , 0.16, 0.00, 1.90 Test ($n=28$): 0.53 ± 0.58 , 0.30, 0.00, 2.00 Urothelium Thickness (micrometers; $p = < 0.0001$): Control ($n=10$): 583 \pm 114, 570, 473, 840 Test ($n=29$): 389 \pm 66, 393, 267, 513 The absence of effective and representative models of PBS has severely hindered the pursuit of curative treatment modalities for this disease. The significant increase in the number of urothelial mast cells and reduction of urothelial thickness suggest that chronic multiple stressors may be effective in inducing PBS in mice. This data may provide investigators with an effective in vivo mouse model of PBS

TNF-alpha Induces Transcription Factor E2F1 Binding Activity In The Pancreas

Johnson, Thomas

Advisor: Linda Vona-Davis

Background: Pancreatitis can be viewed as a process that is critically dependent on transcription factor activity in response to proinflammatory stimuli. We have previously reported that TNF-alpha stimulates transcription factors NF-kappaB and p53 in the pancreas. However, the extent to which this involves their signaling partner E2F1 is unknown. We hypothesized that TNF stimulation would increase E2F1 transcription factor binding activity in the pancreas. Methods and Results: Pancreatic acinar AR42J cells treated with recombinant TNF-alpha (200ng/mL) exhibited increased amylase production after 2 hr ($p < 0.05$). Higher levels of proinflammatory cytokines TNF-alpha, IL-6, IL-1-beta, and anti-inflammatory IL-4 and IL-10 were found in cell supernatants taken from treated cells ($p < 0.05$). Using array technology, we isolated nuclear protein from cells after TNF-alpha treatment and hybridized the bound protein/DNA complexes to an array with known consensus binding sequences for E2F1 and other transcription factors. Array analysis assay showed specific binding of protein to E2F1; signal density in the array from treated cells was increased by 2-fold above control at two different dilutions. Electrophoretic mobility shift assay confirmed specific E2F1 protein binding in competition experiments. Flow cytometric analysis using Annexin V staining revealed a 4-fold increase in apoptosis (3.7 vs 15.4 %) in TNF-treated cells ($p < 0.01$). In addition, treatment with TNF-alpha resulted in activation of downstream effector caspases 3 and 7, both known to be regulated by E2F1. These effects were reduced by the addition of the inhibitory gut peptide Peptide YY. Conclusions: Our data provide new insight in the regulation of E2F1 by cytokines in the pancreas. Further investigation linking E2F1 to pancreatic cell death or survival may represent a potential therapeutic intervention for ameliorating TNF-induced pancreatic dysfunction. Presented at Digestive Disease Week 2009

Behavioral Outcomes in Aged Female Rats Following Middle Cerebral Artery Occlusion

Mnatsukanova, Diana

Advisor: Charles Rosen

Despite data from the Framingham heart study indicating that age is the single greatest risk factor for stroke, the majority of stroke research is conducted using young animal models. The behavioral outcomes of aged animals undergoing experimental stroke are unclear. In the present study, we assessed reference and working memory errors using the 8-arm radial maze with young adult and aged rats following transient middle cerebral artery occlusion (MCAO) and reperfusion. We hypothesized that aged rats undergoing MCAO would have higher numbers of errors compared to young adult rats following MCAO. Behavioral data indicate that aged rats undergoing MCAO have greater reference memory errors compared to both young adult rats undergoing MCAO and sham-operated, aged rats. We corroborated behavioral data with Fluor Jade B labeling of damaged neurons and found differences in hippocampus between young adult and aged rats following MCAO. These findings indicate that aged rats suffer greater hippocampal damage following MCAO than young adult rats and that this neuronal loss may translate to increased reference memory errors on the 8-arm radial maze task.

Optimizing the Periosteal Flap for Lateral Eyelid Reconstruction

Neusch, Rebecca

Advisor: Clinton McCord

Full thickness lateral eyelid defects following Moh's surgery can be challenging to repair. Complex anatomy, defect variability, and inadequate security make these eyelid reconstructions prone to cicatricial ectropion. The use of a simple horizontal oriented periosteal flap was first described in 1981 by Byron Smith and later elaborated on by others^{1,2}. However no literature describes the design of a flap which attempts to optimize post-operative lateral eyelid position. A standardized flap design is needed in order to harness the many natural benefits of the flap and reduce complications. We present two fresh cadaveric dissections and 10 consecutive cases to illustrate to flap concepts. A traditional Tenzel flap incision is created to expose the periosteum of the lateral orbital rim as well as the edge of the temporalis fascia³. We then draw a central meridian from the upper puncta, through the pupil and reflect that line onto the lateral orbital rim. The superior aspect of the flap is started at the intersection of the central meridian and lateral rim and continues at a 40 degree angle superiorly out a distance X (which is the length needed to repair the defect). The width of the flap should measure 6-7 mm which compensates for primary contracture while maintaining strength of the periosteum. The periosteum is trimmed to fit and then secured to the lateral edge of the remaining tarsal plate with enough tension that the lid approximates the globe. If the flap is extended onto the temporalis fascia up to 50% of the posterior lamella of the lower eyelid can potentially be closed. Once the flap is secured, the Tenzel flap can simply be rotated to repair the anterior lamellar defect. The periosteal flap is an ideal flap to repair the lateral canthus due to its simplicity, reliability, and low risk. It can be designed to match the native eyelid contour, originates within the lateral orbit, is strong, and highly vascular². Additionally if the flap loosens post-op it can be easily detached and better secured within the lateral orbit. We present a more standardized method of flap harvest in order to improve outcome and reduce ectropion.

Could Platelet-Rich Plasma Be An Effective Treatment For Bone Infections?

Regal, Steven

Advisor: Bingyun Li

Platelet-rich plasma (PRP) gel has been used in orthopaedic surgery because of its high concentration of various growth factors released from platelets that enhance bone regeneration and soft tissue maturation. PRP-gel has recently been proposed to have antimicrobial properties. To our knowledge, the exact mechanisms of its antimicrobial activity are unknown, but we hypothesize it is due to a combination of factors such as the increased concentration of differentiated, unactivated leukocytes, which have been reported to be two to four times greater in PRP than in whole blood. The concentrated leukocytes in PRP may play an important role in the immune defense against bacterial infection. In addition, recent research has discovered that platelets release antimicrobial peptides upon activation. As a result, a combination of osteoinductive and antimicrobial activities may make PRP-gel effective in the treatment of trauma-associated bone infections. Osteomyelitis, a bone infection, is a serious complication in orthopaedic surgery and has been reported to reoccur 80 years after “effective” treatment. A possible explanation for recurrent osteomyelitis is that bacteria can be internalized into human cells (e.g. osteoblast) and can evade antibiotic treatments and the host immune system. The aim of this study was to test the in vitro antimicrobial activity of PRP-gel against six common bacterial strains associated with bone infections as well as the intracellular bactericidal activity of PRP-gel against osteoblasts with intracellular *S. aureus*. We found that PRP-gel has a time-limited antimicrobial activity against *Staphylococcus aureus* (Methicillin sensitive *S. aureus* or MSSA and Methicillin resistant *S. aureus* or MRSA), Group A. streptococcus, and *Neisseria gonorrhoeae*. PRP-gel used prophylactically for intracellular *S. aureus* infection of osteoblasts showed a significant decrease in invasion. PRP-gel as a treatment for intracellular infection failed to show any therapeutic effects. PRP-gel might represent a useful new alternative strategy in preventing postoperative bone infection.

A Comprehensive Management Algorithm for the Mangled Forearm
Schessler, Matthew
Advisor: William McClellan

The mangled forearm remains a challenging case for the hand surgeon despite significant advances in repair techniques, technology, and rehabilitation protocols. Regardless of injury mechanism, basic upper extremity wound management principles can be applied algorithmically to optimize patient outcome. Unfortunately, little literature exists that unifies management strategies into a comprehensive treatment algorithm. Early comprehensive evaluation by a trauma team and hand surgeon is crucial despite the appearance of an isolated upper limb injury. After stabilization, the upper extremity team can further evaluate the injury and determine limb salvageability. At this point a surgical plan that is cognizant of the time between debridement and rehabilitation can be developed. Sequential debridement will enhance wound stability. Maintaining length, restoring anatomical alignment, and achieving soft tissue coverage are reconstructive hallmarks. One must also assess the socioeconomic, vocational, and avocational demands on the patient to develop a rehabilitation plan that optimizes return to daily activities. We present a comprehensive management algorithm for the mangled upper extremity. The critical points include the global patient evaluation, intra-operative decisions regarding debridement, reconstruction, and tissue coverage, and finally rehabilitation protocols. Hand surgeons may use this algorithm to guide treatment of upper extremity wounds to optimize patient outcome.

A Potential Mechanism For The Increased Hypothalamic Microglial Number During High-Fat Feeding
Spears, Christopher
Advisor: Joshua Thaler

High-fat diet consumption results in obesity partly due to the development of hypothalamic leptin and insulin resistance. One mechanism by which this resistance develops involves the generation of hypothalamic inflammation which disrupts signaling downstream of the leptin and insulin receptors. Our group has hypothesized that microglia are the primary mediators of this inflammation based on an observed increase in microglial cell number in the hypothalamus of high-fat fed rats. To address the functional relevance of the increased microglial number, we have sought to identify the source of these additional cells, postulating three potential underlying mechanisms: 1) increased proliferation of precursor cells 2) migration from neighboring brain regions or 3) decreased cell turnover. To test the first of these possibilities, we analyzed brain sections from chow or 60% fat fed rats that received a 1 week lateral ventricle infusion of bromodeoxyuridine (BrdU), an indelible marker of DNA synthesis. To optimize the staining technique we also generated slides of spleen, jejunum and hypothalamus from animals injected systemically. We performed double label immunofluorescence using an anti-BrdU antibody and an antibody to the microglial marker Iba1. In single-label control experiments, we determined that anti-Iba1 antibody was most effective at a dilution of 1:500; however the DNA denaturation step required to ensure the detection of BrdU resulted in a loss of Iba-1 specific staining in colabeling studies. The anti-BrdU antibody at a dilution of 1:200 detected abundant cell proliferation in both spleen and jejunum, but did not show immunoreactivity in the hypothalamus at all tested dilutions. The optimal treatments for exposing the BrdU epitope were determined to be the use of DNase (1:100) or pretreatment with 2N HCl (10 minutes at 37° C). Current efforts are aimed at improving the detection of BrdU in the brain to allow quantification of microglial proliferation during high-fat feeding.

Health Assessment-I

Factors Associated with Discharge Against Medical Advice from the Emergency Department

Geiger, Sarah

Advisor: Mary Carter

Background: Despite representing 12.7% of the population, older adults accounted for nearly 17% of all emergency department (ED) visits nationally in 2006. In 187,276 (.72%) of these visits, the patient chose to discharge against medical advice (DAMA). The limited existing literature on DAMA consistently demonstrates that patients who self-discharge exhibit increased mortality, morbidity, and comorbidity. However, research on DAMA in older adults is almost nonexistent. Methods: Data from the 2006 National Emergency Department Sample was used to investigate individual and hospital-level predictors of DAMA among older adults. After imposing age-restriction inclusion criteria, the study sample included 4,366,907 adults aged 65 and older. Descriptive statistics were used to examine patient characteristics, and bivariate statistics were used to examine age and gender differences among older adults who DAMA and those discharged for all other reasons, and to guide model development for logistic regression analyses. Results: Results indicated that older adults who DAMA from the ED had fewer procedures and diagnoses, but incurred costs approximately \$200 higher on average than those who do not DAMA. Logistic regression results suggest that adults 85 years and older were less likely to DAMA in comparison with their otherwise similar counterparts, while males were more likely to DAMA than otherwise similar females. Alcohol use was also positively associated with increased odds of DAMA. Conclusion: Although a small segment of the older adult population, uninsured elders are at greater risk of DAMA, suggesting that costs may be a barrier to patient care. Moreover, because those who left DAMA were more often less than 85 years of age, male and had alcohol involvement, these elders may incur many of the same access to care problems as those too young for Medicare services. This study provides an inroad for further research, with implications in both federal healthcare costs and policy.

Incremental Healthcare Expenditure Due To Cvd Among Patients With Copd

Rane, Pallavi

Advisor: Suresh Madhavan

To estimate incremental healthcare expenditure associated with comorbid cardiovascular disease (CVD) among patients with chronic obstructive pulmonary disease (COPD) in the hospital setting. Retrospective data derived from the 2007 National Inpatient Sample (NIS) of the Healthcare Cost Utilization Project (HCUP) was analyzed. Hospital inpatient discharge records for patients with a primary diagnosis of COPD and who were ≥ 40 years of age; were extracted to form the study sample. The sample was then categorized into two groups based on whether there was a secondary diagnosis of CVD. Incremental healthcare expenditure due to comorbid CVD was then determined by comparing hospital charges among the two groups. In addition to hospital charges, national estimates of hospital length of stay (LOS) and in-hospital mortality were also examined and compared between the two groups. For patients with COPD and comorbid CVD, the outcomes were compared across the different

CVDs (hypertension, heart failure, ischemic heart disease, pulmonary heart disease, cerebrovascular disease, and cardiomyopathy and dysarrhythmia). Of the 114,812 COPD-related hospital discharges in 2007; 78.23% (n=89,822) had CVD listed as a secondary diagnosis. Mean hospital charges were \$15,691(95% CI \$14,943 \$16,439) for patients with COPD and no comorbid CVD, and were \$23,986 (95% CI \$23,012.59 \$24,959.22) for patients with COPD and CVD. Among COPD patients with comorbid CVD, the mean charges were highest for those with cardiomyopathy and dysarrhythmia, at \$25,359(95%CI\$24,052.96 \$26,664.45). The mean LOS was also higher among COPD patients with comorbid CVD as compared to those without comorbid CVD (4.59 days vs. 4.16 days). The mean LOS was also longest for patients with cardiomyopathy and dysarrhythmia at 5.75 days. Health care expenditure due to CVD is substantial among hospitalized patients with COPD.

Correlates of Weaker Grip Strength Among Older American Indians: The Native Elder Care Study

Pignataro, Rose

Advisor: Turner Goins

BACKGROUND: Hand grip strength is an objective robust measure of frailty in the older adult population (Sayer, 2005) and is predictive of chronic illness, disability, nursing home placement, and mortality (Sasaki, 2007). Previous research has shown an elevated prevalence of chronic disease in American Indian elders (Okoro et al., 2007) and some of the highest rates of disability (Goins et al., 2007).

OBJECTIVES: Thus, the purpose of this study was to examine the association of demographic characteristics and measures of health with grip strength in a population of older American Indians.

METHODS: We used cross-sectional data collected from a sample of 505 community-dwelling American Indians aged 55 years or older. Hand grip strength was measured using standard dynamometry of the dominant hand. Participants performed the grip strength test three times and the average recorded strength in pounds was used for this analysis. **RESULTS:** Results from an ordinary least squares regression model indicated that increased age, female sex, lower educational attainment, not being a smoker, lower body weight, increased comorbidity, and increased number of limitations with activities of daily living were associated with weaker grip strength. **DISCUSSION:** Implications of these findings include evidence for use of hand grip testing as a simple, non-invasive screening measure to identify persons at risk and target interventional strategies to reduce health disparities and promote healthy aging. Research has demonstrated the effectiveness of physical exercise in enhancing function in older adults (Binder, 2002). Future efforts should focus on interventions that reduce the onset as well as the management of chronic disease and thus contributing to the preservation of autonomous function in older populations, especially in those individuals who are most susceptible to adverse outcomes.

Metabolic Syndrome And Carotid Intima Media Thickness Among Urban Police Officers

Hartley, Tara

Advisor: Cecil Burchfiel

Police officers, a known high stress occupational group, have higher rates of cardiovascular disease (CVD) compared to other occupations. The relationship between work stress and CVD may be mediated by the metabolic syndrome (MetSyn) and this relationship may differ by gender. The purpose of this study was to examine separately in male and female urban police officers, the association between MetSyn and carotid intima media thickness (IMT), a measure of subclinical CVD. A cross-sectional study was conducted in 2004-2009 (279 men, 104 women). MetSyn was defined using the NCEP ATP III guidelines with recent AHA/NHLBI modifications. MetSyn was considered present if ≥ 3 components were present: abdominal obesity, hypertension, glucose intolerance, elevated triglycerides or low HDL cholesterol. B-mode ultrasound was used to measure the mean and maximum carotid artery thickness. Analysis of covariance was used to compare mean IMT values across the number of MetSyn components adjusting for age, education, smoking, and marital status. Adjusted mean IMT was significantly and positively associated with the number of MetSyn components for women (0: 0.578mm \pm 0.014, 1: 0.607mm \pm 0.019, 2: 0.615mm \pm 0.017, ≥ 3 : 0.633mm \pm 0.025; p-trend=0.028). A similar association was found for the adjusted maximum IMT (p-trend=0.011) in women. In contrast, the number of MetSyn components was not associated with mean (p-trend=0.318) or maximum IMT (p-trend=0.601) in men (p-interaction by gender=0.267 for mean IMT, 0.166 for maximum IMT). Among female officers, mean and maximum IMT values were inversely associated with low HDL cholesterol and directly associated with hypertension. For male officers, hypertension was significantly and positively associated with maximum IMT. MetSyn was significantly and positively associated with IMT in female but not in male officers. Further assessment is warranted to understand the mechanisms underlying these gender-specific associations. To our knowledge, this is the first study to examine the relationship between MetSyn and carotid IMT using the 2005 MetSyn modifications and to examine this association in a high stress occupation.

Beyond Reach And Effectiveness: Evaluating The N-O-T Program In West Virginia From 2000-2005

Anesetti-Rothermel, Andrew

Advisor: Kimberly Horn

Even though the majority of teenage smokers want to quit, the likelihood that they do resembles that of flipping a coin. However, evidence-based cessation programs, like the American Lung Association's Not On Tobacco (N-O-T) program, have proven to be effective. Therefore, evaluation of the dissemination of these "model" programs is critical. The current study uses the RE-AIM framework to evaluate the N-O-T program in West Virginia from 2000-2005. RE-AIM components were composed of four measures, while regional dissemination was measured by the comparative differences between Regional Educational Service Agency regions (RESAs). Significant associations were found between RESAs for numerous baseline characteristics. Among the RE-AIM components, only two measures of Implementation were found to be significantly different between RESAs. However, the variability between RESAs provided valuable descriptive evidence of N-O-T program dissemination in West Virginia. We concluded that N-O-T dissemination in West Virginia was greatly influenced by place. Therefore, geographical tailoring grounded in community-based participatory research could increase the N-O-T program's overall dissemination.

Strategies to Promote Adoption of Clinical Practice Guidelines

Cotton, Sandra

Advisor: Susan McCrone

Abstract Background: Stage 3 chronic kidney disease (CKD), defined as glomerular filtration rate (GFR) of 30-59 mL/min, is a major co-morbidity in many rural states. Chronically ill patients are often managed in primary care. Literature indicates advanced CKD management in primary care may be suboptimal. The adoption of clinical practice guidelines (CPG) may facilitate improved patient care, but there is a challenge in how to promote the utilization of guidelines in practice. The question posed by this systematic review was: Does the use of a prompt or clinical management tool (electronic or paper) and educational sessions for primary care practitioners increase CPG adoption? **Methods:** Five major electronic data bases were searched, the National Guidelines Clearinghouse, Cochrane Library, Full-Text Databases, PubMed, and CINAHL using the following criteria: Published within the last 10 years, English language, systematic reviews, and randomized controlled trials (RCTs). As there were no published studies on CPGs for CKD, the search was expanded to include the following key words: CPGs in adult, outpatient care. Nine relevant documents were found. **Results:** Reminders and prompts may play a role in CPG adoption and implementation. Literature indicates that electronic prompts seem to be more effective than paper prompts, but findings are inconsistent. Educational strategies alone were not found to enhance practice change. Combined translational strategies were more effective. Challenges remain in combined approaches. **Discussion:** Coupling strategies with longer patient visits such as preventive exams and targeting specific aspects of disease management may increase CPG adoption. Further research examining combinations of methods, exploring provider differences in communication, effect on patient outcomes, and process of provider learning and change are needed to further understand successful CPG adoption.

Respirator Use and Practices (2000-present): Results of the BLS/NIOSH Survey, Focus Groups, and Worksite Observations and Interventions

Doney, Brent

Advisor: Eva Hnizdo

Through a mail-out survey, focus groups and site visits in road construction, NIOSH collected data of respirator use and practices to identify interventions to improve respirator use. Overall, 79% of respirator-using establishments in construction had at least three indicators of a potentially inadequate respiratory protection program (RPP). Factors adversely impacting elements of RPP included high worker turnover, short job duration, and worker low educational level. Results were disseminated including five articles published in 2009. New construction sites have been identified in West Virginia for RPP evaluation. Recommendations for enhancements to RPP throughout the construction industry will be formulated.

Association Between Pre-hypertension And Cancer In NHANES 2007-2008 Study

Bhandari, Ruchi

Advisor: Ian Rockett

Studies have demonstrated association between hypertension and different types of cancer. However, there is negligible research on the relationship between pre-hypertension and cancer. The Seventh Report of the Joint National Committee (JNC-7) defines pre-hypertension as not being on antihypertensive medication and having systolic blood pressure (BP) of 120–139 mmHg or diastolic BP of 80–89 mmHg. This cross-sectional study examines the association between pre-hypertension and cancer of any type (excluding skin cancer) using the latest data from the National Health and Nutrition Examination Survey (NHANES) 2007-2008. The NHANES survey design is a stratified, multistage probability sample of the civilian U.S. population. Using complex sample weights on 5858 observations (2875 males, 2983 females), the unadjusted odds ratio (OR) between pre-hypertension and cancer was 1.81 (95% CI: 1.11 - 2.97). Therefore, people with cancer were 81% more likely to have pre-hypertension than those without cancer. In subpopulation analyses, the odds ratio between pre-hypertension and cancer was significant for whites (OR: 1.81; 95% CI: 1.04 - 3.17) but not for people from other races, significant for people with annual family income less than \$25,000 (OR: 1.89; 95% CI: 1.17 – 3.06) but not for people with higher income, and significant for people who had smoked 100 cigarettes or more in their lives (OR: 2.17; 95% CI: 1.11 – 4.23) but not for others. A weighted analysis of 2143 observations in multivariable logistic regression showed an adjusted odds ratio of 1.79 (95% CI: 1.02-3.16) between pre-hypertension and cancer. After controlling for gender, race, age, family income, education, body mass index, and smoking, people with cancer were 79% more likely to have pre-hypertension than those without cancer. Future research from prospective cohort studies can provide stronger evidence for the relationship between pre-hypertension and the risk of different cancer types, so that preventive interventions can be directed toward pre-hypertensive people.

Health Assessment-II

Associations of Discrimination And Violence With Smoking Among Sexual Minority Young Adults

Blosnich, John

Advisor: Kimberly Horn

Gay and bisexual people (i.e., sexual minorities) smoke more – up to 50 to 70 percent more - than the general population, but reasons for this disparity are largely unexplored. Studies with racial/ethnic minorities have documented a positive association between smoking and discrimination, suggesting that minority stress (i.e., stigma stemming from minority status) can affect health behaviors. Using secondary analysis of cross sectional data from the Fall 2008 National College Health Assessment, we (a) examined prevalence of past-year discrimination and victimization (i.e., threat; assault; fight; sexual assault) among young adult (ages 18-24) sexual minorities (n=1,416), and (b) tested associations between these factors and smoking frequency (i.e., never smoked; smoked, but not in last 30 days; smoked in last 30 days; smoked daily) in overall and gender-stratified models. Among sexual minorities, females reported more sexual assault than males, and males reported more discrimination than females; no gender differences occurred in smoking frequency. Among all sexual minorities, multivariate ordinal logistic regression analyses of smoking frequency, adjusted for age and race, showed that respondents who experienced discrimination had 30 percent increased odds of more frequent smoking (OR=1.30, 95 percent confidence interval = 1.02-1.66); those who were verbally threatened also had increased odds

of more frequent smoking (OR=1.36, 95 percent confidence interval = 1.08-1.74). Analyses stratified by gender, showed that among sexual minority males, only fighting was significantly associated with increased smoking frequency. Among sexual minority females, being verbally threatened and experiencing discrimination were associated with greater frequency of smoking. Although limited both in measurement of discrimination and statistical power due to sample size, these results are among the first to examine the relation between discrimination and victimization with smoking frequency in a sample of young adult sexual minorities. Smoking cessation efforts for sexual minorities may consider the unique effects of minority stress experiences as triggers for smoking behaviors. Future studies need to examine specific pathways and interactions of social stressors on risk behaviors among sexual minorities.

Alprazolam-Related Deaths: Analysis of Data Compiled by a Centralized State Medical Examiner System
Shah, Neel

Advisor: Joel Halverson

Purpose: We examined the characteristics of drug-induced or drug-related death cases caused or contributed by alprazolam along with comorbidities, other drugs, and mean alprazolam concentrations of the decedents. Methods. We extracted drug-related death data from the West Virginia Forensic Drug Database from January 1, 2005 to November 16, 2007. Data included demographic, blood drug concentration and disease state information for all drug-related death cases in West Virginia. Results. Of the 1,199 drug-related deaths, alprazolam was a contributing cause in 204 (17.0%) cases. At least one other drug was a contributor to death in 97.5% of the alprazolam cases, with opioids most commonly involved. The mean alprazolam blood concentration was significantly higher when taken alone than when identified with other drugs ($p < 0.001$). In more than half (52.5%) of the alprazolam cases the decedent had a prescription for the drug. Conclusion. Alprazolam-associated fatalities have increased steadily in West Virginia between 2005 and 2007. Controlled substances prescriptions monitoring programs, where available, should be routinely used by health professionals to detect drug misuse/abuse. Cooperative interstate and federal agreements to effectively share database information are suggested.

Ecological Analysis of the Impact of Arsenic, Cadmium, and Chromium on Lung Cancer Incidence
Putila, Joseph
Advisor: Lan Guo

Trace elements such as arsenic, cadmium, and chromium have been seen to have a negative impact on respiratory health in occupational and experimental settings, but the cancer link has been less clear for the relatively lower concentrations found in U.S. water sources. There is strong evidence to suggest that these elements directly influence carcinogenesis, raising the possibility that even low-level exposure may result in an excess of lung and other cancers in affected populations. Data on elemental levels of As, Cd, and Cr were obtained from the National Geochemical Survey and compared with the lung cancer incidence rates calculated from the NCI SEER database (N=270,848) as well as published rates for a total of 12 states representative of a wide range of exposures and containing the core of the Appalachian region. Poisson regression on the county age-adjusted lung cancer incidence rates showed that As and Cr were strong independent predictors of lung cancer incidence ($P < 0.0001$), and that these effects persisted after controlling for smoking rates and median income ($P < 0.0001$). Additionally, strong interactions were found between county smoking prevalence and the effect of As, Cd, and Cr on lung cancer incidence ($P < 0.0001$, $P < 0.0001$, $P = 0.02$), suggesting that exposure to these elements has a synergistic effect with smoking which is observable even at the population level. This is the first study of this scale to find a significant association between levels of these elements and lung cancer in a U.S. population, and forms the epidemiological framework for an ongoing analysis of molecular markers associated with arsenic, cadmium, and chromium induced carcinogenesis.

Patient Characteristics and In-Hospital Mortality Predictors after Lung Cancer Hospitalization in WV
Nadpara, Pramit
Advisor: Suresh Madhavan

The objectives of this study were to describe the characteristics of patients who were hospitalized for lung cancer in West Virginia during the year 2007 and to identify predictors of in-hospital mortality. Study data are drawn from 2007 hospital discharge records in the state of West Virginia, using the Agency for Health Research and Quality Healthcare Cost and Utilization Project State Inpatient Database (HCUP-SID). All patients who had a primary diagnosis of lung cancer were included in the study. Univariate and multivariate logistic regression analyses were used to identify patient characteristics that were associated with in-hospital mortality. In total, 1326 discharge records with lung cancer as primary diagnosis were identified. The average age of patients was 66 years, the mean length of stay in the hospital was 8 days, and the in-hospital mortality rate was 13%. Patients who had comorbid conditions and complications and patients who were grouped under the self-pay/no charge/ others category had greater odds of in-hospital mortality compared with patients who were covered by private insurance ($P < 0.05$). In conclusion, patients with comorbid conditions and complications and patients without adequate insurance coverage had greater odds of in-hospital mortality. One reason for this may be inadequate access to care because of the absence of insurance or underinsurance. Further studies controlling for disease stage will be required to determine whether insurance status and patient-related factors can influence outcomes from lung cancer in individual patients independent of their disease stage.

Advanced Age Alone as Criteria for High Level Trauma Activation Does Not Affect Mortality.

Lindauer, Steven

Advisor: Jennifer Knight

Objective: Trauma patients of advanced age have a higher mortality than younger patients with the same injury severity score (ISS). Therefore, it has been proposed that age alone be a criterion for the full trauma activation (P1). We hypothesize that this will not affect mortality in trauma patients of advanced age. **Methods:** All trauma patients over the age of 65 years, who presented to a level 1 trauma center over 2 years, were compared. For the protocol year (2005), patients were given P1 status based on advanced age alone. In the control year (2006), priority designation was based on criteria set by the American College of Surgeons (ACS). Differences in clinical characteristics and mortality were compared using the Wilcoxon rank sum and Pearson chi-square test with an alpha of 0.05 selected as the threshold of statistical significance. **Results:** 584 patients were evaluated. In 2005, of the 320 patients admitted, 158 (49.3%) required full activation by advanced age alone per protocol. In 2006, of the 264 patients admitted, 30 (11.4%, $p < 0.0001$) required full activation. Clinical characteristics were similar for both cohorts (table). The mortality overall was 8.75% in the protocol year and 8.37% in the control year ($p = 0.87$). When comparing the overall mortality of either year, listed above, to the mortality seen in patients receiving full trauma activation based on age alone and not ACS criteria (7.87%), there was no statistical difference ($p = 0.76$ and 0.86 respectively). **Conclusion:** Age alone as criteria for full trauma activation does not affect mortality and it significantly increases the cost of caring for patients of advanced age.

Overcoming Nutrition And Physical Activity Barriers: A Pilot Study

Leary, Janie

Advisor: Lesley Cottrell

West Virginia has one of the highest obesity rates in the nation. Further, many individuals express barriers to addressing their current health status. Focus groups were conducted in the spring of 2009 to better understand the barriers of individuals living in the state. Barriers included schedule constraints, cost, and having a program leader that understood the difficulty of maintaining a healthy lifestyle. This study offered a unique intervention focused on problem solving the barriers to health eating and being physically active. The program was offered as an 8-week healthy nutrition and physical activity intervention. Participants included men and women from a small community of approximately 17,000. Each session was offered twice a week, once in the morning and once in the evening. Participants were encouraged to change their usual session as necessary to better meet their schedules, thus encouraging a participant-friendly environment. Thirty one participants began the program and 25 completed (81% completion rate). Results will be presented from the decision-making pre-post surveys as well as participant feedback on the usefulness of the program.

Health Outcomes Associated With Excessive Lung Function Decline And Respiratory Symptoms
Baughman, Penelope
Advisor: Eva Hnizdo

In creating disease prevention strategies, a thorough understanding is needed of the relative contributions of lung function and respiratory symptoms to predict future morbidity and mortality. The objective of this study was to evaluate the risk of future morbidity and mortality associated with excessive lung function decline and with respiratory symptoms and disease in an aging community-based cohort using the Copenhagen City Heart Study data, a prospective cardiovascular study of 23,000 men and women. Lung function and self-reported respiratory symptoms and disease were collected at four examinations during the 28-year period from 1976 to 2003. Risks of chronic obstructive pulmonary disease (COPD) morbidity (hospital diagnosis of COPD), COPD and coronary heart disease mortality, and all-cause mortality were estimated with Cox proportional hazards models (95% confidence interval, CI) stratified by sex and smoking status. Adjusted for baseline age, baseline FEV1 divided by height squared, and height, the models included asthma, chronic bronchitis, shortness of breath, and one of three measures of longitudinal lung function decline (FEV1 slope; slope relative to baseline FEV1; or the Relative Limit of Longitudinal Decline [LLDr] which is designed to take into account within-person variability in FEV1, or the precision of the data). For a hospital diagnosis of COPD, quartiles of the slope were increasingly more predictive than the respiratory symptoms. The fourth quartile hazard ratio for the slope was 3.73 (CI 2.75-5.05) for males and 5.85 (CI 4.46-7.68) for females. The respective hazard ratios for the relative slope were 3.46 (CI 2.60-4.60) and 5.01 (CI 3.88-6.46). For the mortality outcomes, hazard ratios for the measures of longitudinal lung function decline were more similar to those for the symptoms. This study provides evidence that excessive lung function decline predicts future respiratory morbidity and mortality after adjustment for baseline lung function and respiratory symptoms.

Smoking Status and Educational Indicators: Correlates of High School Dropout
Jarrett, Traci
Advisor: Kimberly Horn

Cigarette smoking in youth and high school drop out cost billions in direct and indirect costs annually. Educational context is important for smoking initiation and maintenance, yet few studies investigate the relationship between cigarette smoking in youth and high school dropout at the aggregate level. The purpose of this study is to explore the association between educational indicators, including dropout rates and smoking prevalence among high school students at the county level. Also, academic achievement and incidents of crime and violence were included in models to test for mediation in the association between smoking prevalence in high school youth and county level dropout. Data from the 2006 Florida Youth Tobacco Survey, the Florida Education and Community Profile Report 2006-2007, and the 2006-2007 Florida School Indicators Report were used in a series of multiple regression models. Smoking prevalence was measured for never smokers, experimental smokers, and current smokers (past 30 day use), and tested in three different models. Prevalence of high school current smokers were significantly ($p < .05$) associated with high school dropout at the county level even after including academic achievement and incidents of crime and violence in the model. The significant relationship

between current smoking prevalence and dropout rate provides opportunity for interventions targeted toward prevention and cessation. By monitoring both high school dropout and smoking, schools and students at risk can be identified, which may guide policies to provide more effective and targeted programs for youth that combine intervention components that target both dropout and smoking.

Association among cardiometabolic risk factor clustering, weight status and cardiovascular disease
Blake, Kimberly
Advisor: Carole Harris

Context: It has been suggested that within the traditional body mass index (BMI) categories there is a heterogeneous pattern of cardiometabolic risk factor clustering. Objective: To determine the association among obesity, cardiometabolic abnormalities and cardiovascular disease (CVD) in a large population-based study of Appalachian adults. Design: Cross-sectional survey of Appalachian adults residing in six communities in Ohio and West Virginia, who were aged ≥ 18 years ($n=14,783$, 50.9% women). Setting: General community Methods: We categorized BMI into normal weight (<25 kg/m²), overweight (25-29.9 kg/m²) or obese (≥ 30 kg/m²). Cardiometabolic abnormalities were defined as the presence of hypertension, elevated triglycerides (≥ 150 mg/dL), decreased HDL (<40 mg/dL [men], <50 mg/dL [women]), elevated fasting glucose (≥ 100 mg/dL)/ diabetes, insulin resistance (homeostasis model assessment >5.13) or elevated CRP (>3 mg/L). Results: We found that 25.6% of normal weight adults displayed clustering of ≥ 2 cardiometabolic abnormalities; in contrast, 49.2% of overweight and 24.4% of obese adults displayed no clustering. Compared to normal weight subjects without clustering of cardiometabolic abnormalities (referent), the odds ratio of CVD was 1.06 (0.84-1.34) among overweight/obese individuals without cardiometabolic clustering, 2.21 (1.74-2.81) among normal weight subjects with cardiometabolic clustering, and 2.45 (2.02-2.97) among overweight/obese individuals with cardiometabolic clustering. Conclusions: Our results suggest that within the traditional BMI categories, there may be heterogeneity of CVD risk depending on whether there is underlying clustering of cardiometabolic abnormalities.

Basic Science-08

VEGF Expression and Skeletal Muscle Capillarity in Mighty Mini Mouse
Audet, Gerald
Advisor: Mark Olfert

Selective breeding for high wheel running capacity in untrained mice has resulted in a “mini muscle” (MM) phenotype that has increased skeletal muscle capillarity compared with control mice. Because vascular endothelial growth factor (VEGF) is an essential mediator of skeletal muscle angiogenesis, and that elevated skeletal muscle capillarity following exercise training is thought to reduce VEGF response to exercise, we hypothesized that untrained MM mice with elevated muscle capillarity would have higher basal VEGF expression, and an attenuated VEGF response to acute exercise. We examined VEGF expression and skeletal muscle morphology in two untrained mouse lines selectively bred for high exercise capacity (MM and Non-MM) compared to control mice. In the gastrocnemius (GA), MM mice

had 38% and 30% greater muscle capillary-to-fiber ratio (C:F) than control ($p < 0.001$) and the Non-MM lines ($p < 0.001$), respectively. The MM mice also had a 58% increase in basal VEGF expression in the GA when compared to control mice ($p < 0.05$); however, there was no significant difference when compared to Non-MM mice (35%, $p = 0.07$). In the plantaris (PLT), the MM mice had a 13% greater C:F than the controls ($p < 0.05$) but no difference compared to the Non-MM mice. This corresponded with a 79% increase in VEGF protein in MM mice compared to the controls ($p < 0.05$), but no difference when compared to the Non-MM mice. After acute exercise the MM mice had a significant 41% and 28% increase ($p < 0.05$) in VEGF in the GA and PLT, respectively. Neither controls nor Non-MM showed a significant VEGF response to acute exercise. There was no response in the soleus muscle under any conditions, in any of the mice. This data suggests that elevated capillarity and VEGF expression in MM mice occurs preferentially in glycolytic skeletal muscle, and increased capillarity per se may not be the primary signal responsible for attenuating the VEGF response to exercise.

CD44 regulates characteristics that are associated with the breast cancer stem cell phenotype
Cieply, Benjamin
Advisor: Steven Frisch

CD44 is an adhesion receptor that binds hyaluronan which is an extracellular matrix component. Isoforms of CD44 can enhance a variety of signaling cascades including EGFR, c-Met, TGF- β , and FGFR. It is also a breast cancer stem cell (BCSC) marker and up-regulated in mammary epithelial cells which have undergone an epithelial to mesenchymal transition (EMT). Anoikis resistance and mamosphere formation are characteristics of BCSCs and are induced by EMT; and we hypothesize that CD44 is contributing to these phenotypes. The objective of this study is to investigate the functional role CD44 in anoikis resistance and mamosphere formation. shRNA knockdown of CD44 enhances anoikis in MCF10a and reduces the ability of HMLE+twist cells to form mamospheres. MCF10a cells form acinar structures with hollow lumens when cultured in matrigel; the hollowing out of the lumen is dependent on anoikis. By including Hermes 3 (CD44 agonistic antibody) in this assay, anoikis was inhibited. Also, over-expressing hyaluronan synthase 2 (HAS2) in MCF10a dramatically disrupted acinar morphogenesis, an effect that was CD44 mediated. Overall these data suggest that CD44 can suppress anoikis, enhance mamosphere formation, and facilitate HAS2 mediated atypical acinar morphogenesis. Further experiments will be aimed at characterizing the mechanisms involved in these phenotypes. Because the CD44 gene is highly alternatively spliced it will be insightful to identify which isoform(s) are responsible for these effects. We plan to rescue the expression of CD44 isoforms in the shRNA expressing cell lines and assay their effects on anoikis and mamosphere formation. We also plan to test the effect of CD44 knockdown and rescue expression on tumorigenicity using breast cancer cell lines in a xenograft model. The importance of this work is to identify CD44 as a breast cancer stem cell specific therapeutic target that can be blocked to restore anoikis sensitivity and prevent metastasis.

Hsp27 Overexpression Protects against Diabetic Cardiomyopathy by a Mitochondria-associated Mechanism

Croston, Tara

Advisor: John Hollander

Diabetic cardiomyopathy is the leading cause of mortality in patients suffering from diabetes mellitus (DM) and is characterized by mitochondrial dysfunction. The small heat shock protein 27 (hsp27) is a human protein that is cytosolic in nature and is known to protect against stresses, such as ischemia/reperfusion injury. The murine analog to this small heat shock protein is hsp25. The goal of this study was to determine whether overexpression of hsp27 preserves cardiac contractile function in a type 1 DM mouse model. Hsp27 transgenic mice and littermate controls were made diabetic through multiple low dose injections of streptozotocin. Five weeks following hyperglycemia onset, the mice were sacrificed and, using an isolated Langendorff perfusion model, cardiac contractile function assessed. Hsp27 overexpression preserved cardiac contractile function in the diabetic heart as demonstrated by an increase in developed pressures (DP) and enhanced rates of relaxation (-dP/dt) and contraction (+dP/dt) (*P<0.05 for all three). Cytosolic cytochrome c content and caspase activities (3 and 9) were increased in the diabetic heart as compared to controls, but decreased with overexpression of hsp27 (*P<0.05). Using an antibody that detects both hsp25 and hsp27, electron micrographs of left ventricular thin sections indicated enhanced translocation of hsp25/27 to the mitochondrion of hsp27 transgenic mice during DM, as compared to the diabetic and wild-type (WT) controls. These experiments were complimented by Western blot and flow cytometric analyses using an antibody specific to hsp25/27. These results demonstrate that the overexpression of hsp27 elicits a protective effect in the mouse heart following a type 1 diabetic insult by preserving cardiac contractile function and decreasing apoptosis, which may be due to an interaction with mitochondria and/or apoptotic constituents.

Eliminating Decreased Locomotor Activity As A Confounding Variable In Memory Tests Of Aged Mice

Hansen, Rolf

Advisor: Han-Ting Zhang

Decreased memory performance in aged animals often parallels memory deficits observed in aged humans. This correlation serves as a model for aging studies aimed at evaluating memory deficits acquired within aged animals and the effects that memory enhancing drugs might have on them. However, in current animal memory tests, decreased locomotor activity with age is often a confounding variable. Since most of the current hippocampal targeted memory tests require the movement of an animal, it is sometimes difficult to rule out the effect of decreased locomotor activity on the measurement and accuracy of memory tests. Because of this dilemma, there is a need for a protocol to accurately measure memory in aged rodents while excluding the potential influence of decreased locomotor activity. In a recent pilot study, we showed using the open field test that locomotor activity was indeed significantly reduced in aged mice (12-15 months) when compared to younger mice (2 months). Also, in the Morris Water Maze test, a highly accepted hippocampal targeted memory test, memory performance of aged mice was significantly reduced when compared to young mice. To exclude the effect of decreased locomotor activity, we also performed the step down passive avoidance test.

During training, it took the aged mice significantly longer to learn to stay on the platform. In addition we found that aged mice 1 week after training had a significantly decreased latency to step off the platform in comparison to younger mice. These results suggest that decreased locomotor activity is not a critical factor for the interpretation of results in memory tests, although utilization of multiple memory tests is necessary for evaluations of memory in aged animals.

Development of a model for SHP-2 specificity and substrate prediction

Hartman, Zachary

Advisor: Yehenew Agazie

SHP2 is a non-receptor tyrosine phosphatase that has been identified as an oncogenic protein. Overexpression of this enzyme correlates with enhanced tumor initiation and maintenance, and genetic downregulation in breast cancer cell lines leads to mesenchymal to epithelial transition. Thus, SHP2 is an attractive target for therapeutic intervention. Unfortunately, the discovery of an appropriate inhibiting compound has proven difficult, thanks to specificity and potency issues. In this study the long-term goal is to design a potent and specific inhibitor of SHP2 by using information derived from biological substrates. A model for substrate identification is to be elucidated that will allow for the prediction of unknown biological substrates and ultimately the design of a small molecule that mimics the substrate binding. HER2-derived peptide was shown by surface plasmon resonance and kinetics assays to bind and inhibit the activity of SHP2, and using computer-based molecular modeling techniques, the binding mode was predicted. In this model for substrate binding, substrate-enzyme interactions that have not yet been observed by other inhibitor studies were noticed, suggesting a role for a pair of lysines adjacent to the active site. The model was tested to predict binding of another putative substrate, focal adhesion kinase, by substrate trapping. Phosphorylated Y397 of focal adhesion kinase was found to bind SHP2 as opposed to the closely-related phosphorylated Y407 of focal adhesion kinase. These results suggest that a prediction model may be useful in exploring other putative substrates for SHP2. Future experiments will investigate the binding and predicted orientations of other substrates in order to design a small molecule that will specifically bind SHP2.

Pharmacological Evaluation Of Novel Agents With Mixed Mu/Delta Opioid Receptor Interactions

Healy, Jason

Advisor: Rae Matsumoto

The three opioid receptor subtypes mu, delta and kappa have long been associated with analgesia. Traditional opioid analgesics exert their effects through mu receptors located in the CNS. Unfortunately, side effects including tolerance and physical dependence are problematic. Therefore, there is a pressing need to identify a pharmacological agent that maintains potent analgesic properties while alleviating the side effects. Recent studies suggest that the development of an agent displaying dual properties of mu agonism and delta antagonism could potentially be of benefit to individuals who require constant administration of opioid analgesics. The design of novel agents is largely based on the message-address concept, which looks to analyze the structure activity relationships of endogenous peptides selective for their respective receptor subtypes. The lab of Dr. Andrew Coop (University of Maryland-Baltimore) has designed several synthetic compounds that address the issues herein. Specifically, compounds UMB375-378 have been tested for their affinity at the mu, delta and kappa receptors. Affinity data is obtained through radioligand binding assays using [3H]DAMGO, [3H]DPDPE and [3H]U69,593 for the mu, delta and kappa receptors respectively. Our data indicates that UMB375-378 display high to moderate affinity at the mu and delta receptors with moderate to low affinity at the kappa receptor. Affinities and mu/delta selectivity ratios obtained are encouraging, yet, further experimentation including in vitro [35S]GTPgammaS assays and in vivo animal models (i.e. hot plate and tail flick) need to be performed in order to characterize the agonistic/antagonistic properties of these novel agents.

SN79-A Novel Sigma-2 Receptor Antagonist, Attenuates Methamphetamine-Induced Neurotoxicity

Kaushal, Nidhi

Advisor: Rae Matsumoto

Methamphetamine (METH) is a psychostimulant drug of abuse, causing hyperthermia and neurotoxicity at high or repeated doses. The mechanism of these neurotoxic effects is not very clearly understood and there is still no effective pharmacotherapy to treat these effects. This necessitates the identification of potential novel therapeutic targets. Radioligand binding assays showed that METH interacts with sigma receptors which are present on dopaminergic and serotonergic neurons and can modulate their actions. From independent experiments, it has been shown that sigma-2 receptor activation causes cytotoxic effects in tumor cells (Crawford and Bowen, 2002) and blockade of sigma receptors can attenuate METH-induced neurotoxicity (Matsumoto et al., 2008). Taking all this into consideration, the hypothesis of this study is that METH mediates its neurotoxic effects via sigma-2 receptors. In order to test the hypothesis, a sigma-2 receptor selective antagonist, SN79, was developed. Using radioligand binding studies, SN79 was shown to have high nanomolar affinity and selectivity for sigma-2 receptors, with >10,000 nM affinity for sigma-1 receptors and 59 other binding sites. In this study, SN79 dose dependently attenuated METH-induced hyperthermia, dopaminergic and serotonergic neurotoxicity (measured as depletions in dopamine and serotonin levels using ELISA, in the striatum of male Swiss Webster mice). Together, the data suggest that antagonism of sigma-2 receptors can convey protective actions against the neurotoxic effects of METH to dopaminergic and serotonergic neurons.

Parkinson's disease and Possible Feedback Loop Mechanisms

Klabnik, Jonathan

Advisor: James O'Donnell

The pathogenesis of Parkinson's disease (PD) is characterized by dramatic cell death within the substantia nigra pars compacta (SNpc), a brain region consisting of cell bodies for dopaminergic neurons. However processes that lead to this cell death are poorly understood as many pathways have been hypothesized to be involved. In addition, many pathways that are presently being identified do not account for the late onset of disease in life and rapid progression once the patient is diagnosed. In this thesis study, the goal is to isolate and study the effects of two pathways that address these concerns, tetrahydrobiopterin (BH4) and thioredoxin (Trx); both of which are intertwined around one substrate, soluble guanylyl cyclase (sGC), which is responsible for the conversion of GTP into cGMP. BH4 is synthesized from GTP by GTP cyclohydrolase (GTPCH). Under normal conditions sGC and GTPCH compete for GTP but in the presence of peroxynitrate, sGC activity is dramatically reduced, possibly leading to increased BH4. Normally BH4 is essential in dopaminergic cells as it is an essential cofactor for the enzyme tyrosine hydroxylase. Recently though, it's been shown that slightly elevated levels can cause increased cell death under certain conditions. Trx, which is upregulated by cGMP-dependent kinase (PKG), has been shown to be a better antioxidant than glutathione leading to better control of reactive oxygen species. However, cGMP and subsequently PKG activity may be decreased by the oxidative stress model due to inhibition of sGC, which could lead to increased cell death. Increased PKG activity also has been shown to increase synthesis of BH4. We propose that phosphodiesterase-2(PDE2) inhibitors, which lead to increased levels of cGMP, may help prevent apoptosis in PD. We propose a central role for PDE2 over other PDE due to the prevalence of this enzyme within the SNpc compared to other cGMP PDEs.

Down-regulation of Serotonin and Norepinephrine Transporters After Chronic Antidepressant Treatment

Lueptow, Lindsay

Advisor: James O'Donnell

Depression has a lifetime prevalence of approximately 20% in the United States. Many of the antidepressants currently available work to inhibit the reuptake of neurotransmitters by serotonin, norepinephrine and/or dopamine transporters (SERT, NET, DAT, respectively), resulting in increased monoaminergic transmission. Despite this acute change, a therapeutic effect may not be seen for weeks. In order to examine the underlying mechanisms leading to a therapeutic effect, rats were treated systemically for 14 days with either 15 mg/kg/day desipramine (norepinephrine reuptake inhibitor) or 70 mg/kg/day venlafaxine (serotonin-norepinephrine reuptake inhibitor). Two days following the cessation of treatment, persistent antidepressant-like effects were assessed using the forced swim test. Consistent with previous findings, both treatment groups showed decreases in immobility time, a measure of antidepressant-like activity, as compared to non-treated animals. Furthermore, desipramine treated animals showed decreases in NET expression and binding, with no effect on SERT. However, despite persistent antidepressant-like activity, venlafaxine treated animals did not show functional changes in NET or SERT. Additionally, rats treated with beta-PMA, a protein kinase C (PKC) activator, showed antidepressant-like effects in the forced swim test, and a functional down regulation of NET, similar to those seen with desipramine treatment; these effects were reversed with the administration of a PKC inhibitor. While the regulation of these transporters seems to correlate with the onset and persistence of antidepressant-like behaviors in the rat model, the precise regulatory mechanisms are still unknown. In the future we plan to focus on the elucidation of these pathways, specifically, the role of post-translational modifications, internalization and/or degradation, through the use of PKC activators, and the possible differential regulation through the use of venlafaxine. Understanding the underlying mechanisms of both the pathogenesis and subsequent treatment of depression is essential for the development of faster acting and more efficacious treatment options.

Attenuated Myocardial Effects of Prenatal Stress on Female Rats

Mullenax, Heather

Advisor: Mitchell Finkel

Third trimester maternal stress (prenatal stress; PS) has been demonstrated to result in behavioral changes in male offspring consistent with mood disorders in humans. Predominant effects on male offspring are consistent findings, regardless of the PS paradigm used. We have previously reported echo and hemodynamic evidence in vivo and cardiac myocyte evidence in vitro of myocardial dysfunction and adrenergic desensitization in male PS rats followed by restraint stress (R) (PS+R) compared with matched Control offspring of non-stressed dams (Control + R). Accordingly, we sought to determine if female PS rats were protected from myocardial effects of PS as they are from behavioral effects. Consistent with prior behavioral assessments, there were no significant differences between baseline hemodynamics (+dp/dt; -dp/dt) in awake female PS+R vs female Control+R ($p=NS$; $n=6-8$). However, more subtle effects of PS+R on contractility (+dp/dt) were demonstrable in female PS+R following beta

adrenergic stimulation with isoproterenol (ISO) ($p < 0.05$; $n = 6-8$) or isoflurane anesthesia ($p < 0.05$; $n = 6-8$). In addition, cardiac myocytes isolated from female PS+R also revealed a blunted inotropic response to ISO vs female PS+R ($p < 0.05$; $n = 15$). However, female PS+R were more responsive to ISO than male PS+R ($p < 0.05$; $n = 15$). These data support a protective effect of female sex on myocardial, as well as behavioral consequences of PS.

Assessment of Fibrogenic Potential of Carbon Nanotubes In Vitro

Mishra, Anurag

Advisor: Liying Wang

Carbon nanotubes (CNT) are one of the most widely used nanomaterials in various commercial and biomedical applications. However, their fiber-like structure, low specific weight and nanoscale dimension also raises concerns about possible adverse effects on human health. Previous in vivo studies in our lab using mice have shown that pulmonary exposed nano-sized carbon nanotubes can enter the lung interstitium and induce interstitial fibrosis in days while micron sized particles usually induce fibrosis in months. Mechanism of this unusual CNT-induced lung fibrotic response is largely unknown due to lack of knowledge of toxicity of these novel nanomaterials. With the aim to determine properties of CNT in response to their specific bio-activities, in this current study, physiologically relevant doses of single or multi wall CNT, were examined for their cellular toxicity and fibrogenic potential using human bronchial (BEAS-2B), alveolar (A549) epithelial and lung fibroblast cells. CNT were dispersed in natural lung surfactants and exposed to the cells in culture. Cellular toxicity was assessed over time by direct cell counting and by WST-1 proliferation assay. Fibrogenic biomarkers or mediators such as lung collagen, TGF-beta and Matrix Metalproteinase (MMP) production was determined by Western blot assay and ELISA. The results showed that: 1) CNT can be effectively dispersed using natural surfactants into nano-size which is similar to aerosol CNT; 2) Dispersed CNT caused a substantial decrease in cell viability and proliferation as compared to non-dispersed CNT or vehicle control treatment 3) Dispersed CNT induced collagen formation in fibroblast cells 4) Up-regulation of TGF-beta and MMP-9 in the CNT-treated lung epithelial cells were also observed. Our result shows the potential mechanism of fibrogenicity of single wall and multiwall CNT in in vitro model which is supported by our in vivo studies. Thus, the in vitro methods may potentially be used as a predictive model for in vivo toxicity assessment and to aid the mechanistic studies of nanomaterials induced pulmonary toxicities.

The antidepressant-like effects of ketamine in an animal model

Robson, Matthew

Advisor: Rae Matsumoto

Ketamine, an NMDA antagonist and dissociative anesthetic, has been shown to exert fast-acting antidepressant actions in human clinical populations. The exact mechanisms by which ketamine exerts these effects are unknown. Ketamine interacts with numerous receptors and transporters including sigma receptors. Sigma receptor ligands have been shown to display antidepressant-like effects in animal models and sigma receptors are believed to be novel targets for the formulation of a new class of antidepressants. The purpose of this study was to examine the antidepressant-like effects of ketamine in an animal model and to determine the extent to which sigma receptors modulate these effects. Ketamine was shown to have antidepressant-like effects in male Swiss-Webster mice, however the sigma receptor antagonist BD1047 failed to exert a significant effect on the antidepressant actions of ketamine. NE-100, another sigma receptor antagonist also failed to alter the antidepressant-like effects of ketamine. In human clinical populations, a single administration of ketamine produced a significant antidepressant effect for up to 2 weeks. The time-course of the sustained antidepressant effects of ketamine was also studied. These data provide evidence that ketamine does exert antidepressant-like effects in the forced swim test, however sigma receptor antagonists did not mitigate these effects. It was also shown that the forced swim test is not a valid model to study the sustained effects of a single administration of ketamine, as seen in human clinical populations. Future studies should be conducted to determine the mechanisms behind the rapid and sustained antidepressant effects of a single administration of ketamine utilizing behavioral analyses other than the forced swim test.

Identification of specific markers for photoreceptors during early mouse eye development

Rodgers, Helen

Advisor: Pete Mathers

Mouse retina is commonly used in studying mechanisms of cellular differentiation, retinal diseases/disorders, and retinal development. During eye development, the retina forms into a laminar structure with seven main cell types, six neuronal and one glial. In the mouse, retinal neurons are born in a temporal order starting with retinal ganglion cells at embryonic day 10.5, followed by horizontal cells, cones, amacrine cells, rods, bipolar cells and finally Müller glial cells. The cone and rod neurons comprise the photoreceptors where phototransduction occurs. In investigating the retina it is often important to be able to identify specific retinal neurons. Specific markers for photoreceptors in adult mouse retina are well characterized in immunohistochemical studies. However, there is a lack of literature on photoreceptor-specific markers during early mouse retinal development. We identified a battery of antibodies as candidates for being specific photoreceptor markers, with an emphasis on possible cone-specific markers. These antibodies include: mouse cone arrestin, cone phosphodiesterase (cone PDE), cone transducin, phosducin, retinoid X receptor gamma (RXR gamma), orthodenticle homeobox2 (Otx2), and aryl-hydrocarbon-interacting protein-like 1 (AHL1). We performed immunohistochemistry on cryosectioned mouse retina at different time points between embryonic day 10.5 and postnatal day 10. This study allowed us to characterize the temporal and spatial expression

pattern of these proteins, as well as the specificity of the antibodies. The earliest markers we identified were phosducin and cone transducin that are expressed in cones by embryonic day 12.5 and 13.5 respectively. We were able to designate them as either photoreceptor specific (phosducin, marking both rods and cones) or as cone specific (cone transducin). These results provide useful tools for researchers planning immunohistochemical studies in embryonic mouse retina that were previously not available.

The electrophysiological changes which occur in the adult Fisher 344 rat trachea upon infection with Shimko, Michael
Advisor: Jeffrey Fedan

The respiratory syncytial virus (RSV) is a member of the pneumovirus family, and primarily infects the intrapulmonary and extrapulmonary airways of infants and adults respectively. This infection is thought to alter the normal physiological state of epithelial cells lining the trachea which can lead to the generation of asthma later in life. Through the use of ussing experiments and cell culture, we will determine the acute and chronic electrophysiological alterations the epithelial cells lining Fisher 344 (F344) rat trachea undergo upon intranasal infection with RSV. Previous work has shown alterations in Na⁺ transport in mouse tracheas, and altered protein expression in cells infected with RSV (Kunzelman 2006, Chen 2008, Groskreutz 2007). F344 rats are considered a good model to study the RSV infection, and will be used in this experiment. They demonstrate a similar immune response as a immunocompetent patients, and are able to eliminate a RSV infection in approximately 30 days to allow for long term studies to be preformed. To better understand the mechanisms responsible for the increased risk of developing asthma after infection with RSV, several specific ion channel blockers will be used in ussing experiments on F344 rat tracheas and electron microscopy of cel cultured rat epithelial cells. Chen, L., et al., Inhibition of Na⁺ transport in lung epithelial cells by respiratory syncytial virus infection. *Am J Respir Cell Mol Biol*, 2009. 40(5): p. 588-600. Groskreutz, D.J., et al., Respiratory syncytial virus decreases p53 protein to prolong survival of airway epithelial cells. *J Immunol*, 2007. 179(5): p. 2741-7. Kunzelmann, K., et al., Inhibition of airway Na⁺ transport by respiratory syncytial virus. *J Virol*, 2007. 81(8): p. 3714-20.

Basic Science-07

Bisphenol A Activates Maxi-K Channels In Coronary Smooth Muscle
Asano, Shinichi
Advisor: Gregory Dick

Bisphenol A (BPA) is used to manufacture plastics, including food containers into which it leaches. Recently, environmental exposure to BPA became a potential public health hazard. High levels of exposure to this estrogenic endocrine disruptor are associated with diabetes and heart disease. Estrogen and estrogen receptor modulators increase the activity of large conductance Ca²⁺/voltage-sensitive K⁺ (Maxi-K) channels, but the effects of BPA on Maxi-K channels are unknown. We tested the hypothesis that BPA activates Maxi-K channels through a mechanism that depends upon the regulatory

beta1 subunit. Patch clamp recordings of Maxi-K channels were made in human and canine coronary smooth muscle cells as well as in AD-293 cells expressing pore-forming alpha or alpha plus beta1 subunits. BPA (10 uM) activated an outward current in smooth muscle cells that was inhibited by penitrem A (1 uM), a Maxi-K blocker. BPA increased Maxi-K activity in inside-out patches from coronary smooth muscle, but had no effect on single channel conductance. In AD-293 cells with Maxi-K channels composed of alpha subunits alone, 10 uM BPA did not affect channel activity. When channels in AD-293 cells contained beta1 subunits, 10 uM BPA increased channel activity. Effects of BPA were rapid (<1 min) and reversible. A higher concentration of BPA (100 uM) increased Maxi-K current independent of the beta1 subunit. These data indicate BPA increases the activity of Maxi-K channels and may represent a basis for some potential toxicological effects.

SRp20 and hnRNPs K and L Regulate RNA Splicing In Response To Changing Nutrient Availability
Cyphert, Travis
Advisor: Lisa Salati

Glucose-6-phosphate dehydrogenase (G6PD) provides a useful model for studying regulation of alternative splicing by intron retention. Expression of G6PD mRNA increases 15- to 17-fold during refeeding and is inhibited 80-90% by starvation and the addition of polyunsaturated fat to the diet. These nutrient stimulated changes occur exclusively through a post-transcriptional mechanism. The large changes in the accumulation of G6PD mRNA are due to changes in the rate of pre-mRNA splicing. In this regard, dietary carbohydrate enhances intron removal, which increases the accumulation of G6PD mRNA. Starvation and polyunsaturated fatty acids decrease the rate of intron removal, leading to intron retention and degradation of the pre-mRNA. A regulatory element within exon 12 of the G6PD pre-mRNA that contains both an ESS and an ESE mediates these changes in splicing efficiency. SR proteins along with hnRNPs bind to this regulatory region. UV crosslinking and proteomics approaches identified hnRNP K, L, and A2/B1 as putative regulatory factors. Deletion of the 30nt regulatory region abolishes binding of these hnRNPs. SRp20 binding to the regulatory element was detected using RNA Affinity and nuclear extracts from refeed livers. SRp20's increased binding corresponds to the increase in splicing that occurs with refeeding. Depletion of SRp20 from cells results in decreased splicing of a G6PD reporter as well as the endogenous G6PD mRNA. In contrast, addition of purified SRp20 to in vitro splicing assays enhances mRNA splicing. Loss of function experiments were next used to test the role of hnRNPs in regulated splicing of G6PD mRNA. Preliminary studies show that depletion of hnRNP K and L in cells results in an increase in splicing of an RNA reporter containing exon 12. Together, this data suggests that SRp20, hnRNP K and hnRNP L are opposing splicing regulatory factors involved in the nutrient regulated splicing of G6PD mRNA.

Anti-diabetic factor fibroblast growth factor-21 is regulated by multiple signaling pathways

Damron, Holly

Advisor: Brad Hillgartner

Fibroblast growth factor-21 (FGF-21) is a hepatic hormone that plays a role in mediating adaptive changes in fatty acid oxidation and gluconeogenesis caused by starvation and consumption of a high-fat, low-carbohydrate (ketogenic) diet. The FGF-21 signaling pathway is a novel target for treating metabolic syndrome, as administration of recombinant FGF-21 reverses diabetes and obesity in experimental animals. Hence, there is a strong interest in the physiological and molecular mechanisms controlling the hepatic expression of FGF-21. Starvation and high-fat consumption stimulate a 16- to 35-fold increase in hepatic FGF-21 mRNA levels and this effect is dependent on the presence of the peroxisome proliferator-activated receptor-alpha (PPAR α), a nuclear receptor that is activated by long-chain fatty acids. We have shown that incubating primary cultures of rat hepatocytes with long-chain monounsaturated and polyunsaturated fatty acids partially recapitulates the stimulatory effect of starvation and high-fat feeding on FGF 21 expression. We employed rat hepatocyte cultures to screen for additional blood-borne factors controlling FGF-21 expression. We found that the primary bile acid, chenodeoxycholic acid (CDCA), stimulated a 20-fold increase in FGF-21 mRNA abundance. This effect was associated with an increase in the secretion of FGF-21 protein into the culture medium. CDCA also increased the expression and secretion of FGF-21 in human HepG2 hepatoma cells indicating that bile acid regulation of FGF-21 is conserved in human cells. CDCA is a natural agonist of the farnesoid X receptor (FXR). Administration of a synthetic and highly selective FXR agonist (GW4067) increased hepatic FGF 21 expression in wild type mice but had no effect in FXR knockout mice. In conclusion, we have identified a new signaling pathway (CDCA-FXR) that activates FGF-21 gene expression. Activation of the FXR signaling pathway represents a new approach to stimulate endogenous FGF-21 production that may lead to enhanced glucose tolerance and reduced triglyceride accumulation in obese/diabetic individuals.

Digitoxin and a novel derivative show cytotoxic and cell cycle arrest activity against NSCLC cells

Elbaz, Hosam

Advisor: Yon Rojanasakul

Recent research findings concur with earlier epidemiological observations regarding the potential of cardiac glycosides as novel anticancer agents. The mechanism by which cardiac glycosides exhibit their antineoplastic effect is unclear. Understanding how cardiac glycosides exhibit their antineoplastic effect would significantly contribute to developing effective anticancer drug therapies with reduced side effects. The purpose of this study is to understand how digitoxin (DTX), a prototypical cardiac glycoside, and its novel synthetic analog, GJ-22 which has recently been developed at WVU, exert their antineoplastic effect in non-small cell lung cancer (NSCLC) cells. NCI-H460 cells were treated with various concentrations of DTX and GJ-22, and their effects on apoptosis and necrosis were determined by Hoechst 33342 and propidium iodide assays. Cell proliferation and cell cycle were studied using MTT assay and flow cytometry. Key G2/M phase regulators and checkpoint regulators were investigated by Western blot analysis. The results showed that DTX and GJ-22 were highly effective in inducing

apoptosis of lung cancer H460 cells with an IC₅₀ of ~40 nM and 10 nM, respectively. Apoptosis is the primary mode of cell death induced by the cardiac glycosides; however, at high doses (> 100 nM), necrosis becomes dominant. At very low doses (< 10 nM), the glycosides exhibited an anti-proliferative effect without causing cell death. Flow cytometric and Western blot analyses show that such inhibition was due to cell cycle arrest in the G₂/M phase and was associated with the down regulation of checkpoint proteins Chk1, and Chk2, as well as cyclin B1. Checkpoint abrogation by the cardiac glycosides appears to be independent of cell cycle. Together, our results indicate that DTX and GJ-22 exhibit their antineoplastic effect through different mechanisms depending on their doses with cell cycle arrest being a key mechanism at low doses and apoptotic/necrotic cell death being dominant at high doses. This study also indicates that sub-toxic doses of cardiac glycosides could be used as treatment modalities for lung cancer. Supported by NIH-R01-HL076340

In vitro models for intracellular *Staphylococcus aureus* infection of osteoblasts and macrophages
Hamza, Therwa
Advisor: Bingyun Li

Infections of bone (osteomyelitis) represent major pathologic and therapeutic challenges in the orthopaedic field. Despite advances in both antibiotics and surgical treatments, the recurrence rate of chronic osteomyelitis remains at approximately 20% to 30% (Conterno and da Silva Filho, 2009). *Staphylococcus aureus* (*S. aureus*), the most common pathogen in bone infections, can invade and survive within different types of cells in vitro and in vivo (Garzoni and Kelley, 2009). Intracellular bacteria are protected from most antibiotics and the immune system, which might explain the recurrent nature of these infections. The main objective of this study was to establish and verify in vitro *S. aureus*-osteoblast and *S. aureus*-macrophage infection models. Osteoblast and alveolar macrophage cell lines were infected separately with *S. aureus*. Different multiplicity of infection (MOI) ratios were tested to optimize the infection models. Results of viable osteoblast and macrophage cell counts after infection were determined by Trypan blue exclusion assay. The number of intracellular bacteria within osteoblasts and macrophages post infection was estimated, using colony forming unit (CFU) counts and flow cytometry tests, to determine the infection rates. In conclusion, in vitro co-culture models of chronic osteomyelitis were established in two different cell types and this may help us to better understand the mechanisms underlying recurrences of bone infections.

Isolation of microvascular endothelial cells from tissues using counterflow elutriation

James, Megan

Advisor: Robert Brock

The purpose of this study was to optimize the isolation of pure and viable microvascular endothelial cell (EC) populations from a variety of murine tissues. While several methods are reported in the literature, many of these are lengthy and result in low yields. Counterflow elutriation has been used to isolate a variety of cell types, including liver sinusoidal ECs. However, we are unaware of isolation of ECs from other tissues using counterflow elutriation. Therefore, we proposed to evaluate the conditions necessary for separation of ECs of various tissues. Previously described procedures for isolating liver sinusoidal ECs via counterflow elutriation were optimized using our equipment. Additionally, methods for isolating ECs from aortae and kidney were developed and optimized. All cells were isolated from mice overexpressing endothelial-specific GFP (Tg(TIE2GFP)^{287Sato/J}). Flow cytometry was used to determine purity of cell populations using staining of GFP, CD31 and Dil(3,3'-dioctadecylindocarbocyanine)-labeled acetylated LDL. The methods described herein result in EC populations of at least 98% purity. These cells have been successfully used for downstream applications, including molecular biological assays and cell culture. Counterflow elutriation can be employed to obtain high-yield, high-purity EC populations from a variety of murine tissues. This work was funded by: NHLBI T32 HL090610

The complex hook basal body structure of the Lyme disease spirochete *Borrelia burgdorferi*

Miller, Kelly

Advisor: Nyles Charon

The proto-oncogene Src tyrosine kinase (Src) is overexpressed in human cancers and is a current target of anti-invasive therapies. Src activation is essential to produce invadopodia, cellular structures that mediate extracellular matrix (ECM) proteolysis. Invadopodia assembly is a multistep process, first requiring the targeting of actin-associated proteins to form pre-invadopodia. Pre-invadopodia subsequently mature by recruitment and activation of matrix metalloproteases (MMPs) that facilitate ECM degradation. We demonstrate that active, oncogenic Src alleles require the presence of a wild-type counterpart to induce ECM degradation and phosphorylation of the invadopodia regulatory protein cortactin. Distinct phosphotyrosine-based protein binding profiles in cells forming pre- and mature invadopodia were identified by SH2-domain array analysis. These results indicate that while elevated Src kinase activity is required to target actin-associated proteins to pre-invadopodia, regulated Src activity is required for invadopodia maturation and matrix degradation activity. Our findings describe a previously unappreciated role for proto-oncogenic Src in enabling the invasive activity of constitutively active Src alleles.

Scl1, of Group A Streptococcus, Promotes Biofilm Formation via Extracellular Matrix (ECM) Adhesion

Oliver-Kozup, Heaven

Advisor: Slawomir Lukomski

Group A Streptococcus (GAS) is a gram-positive, human-specific pathogen causing a myriad of diseases. Successful pathogenesis requires colonization and invasion of the host often mediated by GAS surface proteins and the host's extracellular matrix (ECM) components. We have recently reported that the streptococcal collagen-like protein-1, Scl1, of several GAS strains binds to the ECM proteins cellular fibronectin (cFn) and laminin (Lm). Whether this mechanism is used as a step toward biofilm formation has not been realized. Biofilm formation is an emerging field in GAS pathogenesis potentially responsible for antibiotic resistance and immune evasion. We hypothesize that the Scl1-ECM interaction initiates the formation and stabilization of GAS biofilm. *scl1*-inactivated mutants of M41- and M1-type GAS were compared to wild type parent strains for biofilm formation under static conditions in plastic tissue culture wells, wells coated with ECM, or wells coated with natural matrices (nECM) formed by cultured fibroblasts or epithelial cells. Assessment was done spectrophotometrically following crystal violet staining or microscopically using GFP-expressing strains. We identified decreased biofilm formation by both Scl1-negative mutant strains with M41 strains showing overall enhanced biofilm over M1 strains. Complementation of the M41 Δ *scl1* mutant restored the biofilm phenotype to WT levels, indicating the importance of Scl1 expression. ECM coated wells confirmed these results with cFN enhancing biofilm formation as compared to Lm for both M-type strains. Next, biofilm thickness, measured by microscopy of WT, GFP-expressing M41- and M1-type GAS, showed 24 micrometers and 11 micrometers average thickness, respectively. Finally, our preliminary data suggest that natural matrices formed by human cells may provide an alternative model for studying GAS biofilm formation in vitro. This work (i) identifies variations in biofilm formation ability among GAS strains investigated, (ii) highlights that the Scl1-ECM interaction provides an adherence mechanism to facilitate biofilm, and (iii) established that Scl1 surface protein is an essential determinant of GAS biofilm. In summary, contribution to biofilm development and stability represents a new role for Scl1 in GAS pathogenesis.

Regulation of anoikis by DBC1 (Deleted-In-Breast Cancer 1)

Park, Sun

Advisor: Steven Frisch

Metastasis is the cause of the death in the majority of patients with cancer. For metastasis to occur, cancer cells need to undergo epithelial-to-mesenchymal transition (EMT) allowing them to avoid anoikis, apoptosis induced by detachment from extracellular matrix. The mechanism of EMT mediated anoikis resistance is an area of ongoing research. The gene Deleted in Breast Cancer 1, DBC1, is found in chromosomal region 8p21, which is deleted in some breast cancers, while several other cancers show DBC1 overexpression. Several studies have shown that DBC1 regulates tumorigenesis both positively and negatively in a cancer context dependent manner. Large-scale mapping of human protein-protein interactions by mass spectrometry showed that DBC1 interacts with Neurotrophin Receptor-interacting MAGE domain protein (NRAGE). Co-Immunoprecipitation was performed to demonstrate the interaction between DBC1 and NRAGE. Our lab has already established that NRAGE is over-expressed in melanoma

as well as lung, breast and colon carcinomas. In addition, NRAGE was found to suppress anoikis. The objective of this study is to determine DBC1's ability to regulate anoikis in the context of EMT. The hypothesis is that through direct interaction, NRAGE might inhibit the function of DBC1 as an inhibitor of Sirtuin1 histone deacetylase, also known as SIRT1, and affect downstream molecules such as p14ARF or Bim, a known anoikis inducer. We are also able to show depletion of DBC1 in human mammary epithelial cells conveys resistance to anoikis. Sensitizing cancer cells to anoikis by targeting this novel pathway could be a vital area for cancer therapeutics.

Diesel Exhaust Particle Exposure Augments Arteriolar Mechanotransduction

Porter, Katrina

Advisor: Timothy Nurkiewicz

Pulmonary exposure to particulate matter (PM) is known to cause systemic cardiovascular dysfunction. While this laboratory has characterized microvascular dysfunction after PM exposure, the specific hemodynamic adjustments that follow PM exposure remain unclear. In addition, we have shown that exposure to diesel exhaust particles (DEP) alters microvascular endothelium-dependent responsiveness to ACh. The purpose of this study was to determine the effects of pulmonary exposure to DEP on the peripheral microvascular response to alterations in luminal blood flow. Rats were intratracheally instilled with DEP (NIST SRM 1650b, 10 or 100 micrograms/rat). The spinotrapezius muscle was prepared for intravital microscopy 24 hr after exposure. Arteriolar reactivity was assessed with the parallel occlusion technique. Briefly, using a micropipette, luminal flow in an arcade arteriole was increased by gently occluding the parent arteriole immediately downstream from the arcade arteriole origin. Center-line red cell velocity was measured to quantify the microvascular hemodynamic consequences of DEP exposure. At rest, diameter and volume flow were similar among groups (28-29 micrometers and 4-7 nl/s); however, WSR was significantly lower in the 100 µg DEP group (2600 ± 400 s⁻¹) compared to sham-controls (3600 ± 500 s⁻¹). In all groups, parallel occlusion increased volume flow compared to baseline measurements. Compared to controls, 100 µg DEP exposure significantly augmented arteriolar dilation ($17\% \pm 3$ vs $46\% \pm 14$) and wall shear rate ($19\% \pm 7$ vs $60\% \pm 13$) in response to increased flow. Preliminary data suggests the effect is present to a lesser degree in the 10 microgram DEP exposure group, in both dilation ($31\% \pm 5$) and WSR ($55\% \pm 20$). These results suggest DEP-exposure augments responsiveness to physical changes in luminal flow compared to sham-controls, as well as impaired ability to properly adapt to such changes, and that the effect is dose-dependent. We speculate that this augmented responsiveness is consistent with an adaptive response to upstream vasoconstriction. Support: NIH Cardiovascular & Pulmonary Diseases Training Grant 5T32HL090610-02 (KLP), NIH RO1-ES015022 (TRN).

Characterization of Optimized Sigma Ligands

Seminario, Michael

Advisor: Rae Matsumoto

Methamphetamine has detrimental effects on a wide array of neurological functions and has quickly become an international public health concern. With the absence of a proven pharmacological agent to counteract methamphetamine-induced complications, the development of a therapeutic treatment is essential. Methamphetamine has been shown to bind to sigma receptors at physiologically relevant concentrations, and sigma receptors have emerged as viable therapeutic targets. In particular, CM156, a selective sigma receptor ligand, displayed sub-nanomolar affinity at both sigma-1 and sigma-2 receptors while providing in-vivo protective effects against methamphetamine-induced neurotoxicity and locomotor hyperactivity. However, due to an exceedingly short half-life and poor metabolic stability, the compound was deemed inadequate for pursuing drug studies in humans. Our hypothesis is that CM156 can be structurally optimized to a more metabolically stable compound without detrimentally affecting its pharmacological profile. In order to test our hypothesis CM156 analogs were synthesized by our collaborators at the University of Mississippi and tested for their sigma receptor binding affinity. We determined that six of the newly synthesized "AZ" compounds maintained high nanomolar affinity at both sigma-1 and sigma-2 receptors. We will test the compounds at six other sites (5-HT₂, NMDA, opioid, DAT, SERT, NET) as well as determine their metabolic stability in vitro. Finally, based on binding affinity and stability, two compounds will be tested in vivo for their ability to attenuate the methamphetamine-induced neurotoxic and stimulant effects.

Humans mimicking animals: implications for species-specific vocalization processing in human auditor

Talkington, William

Advisor: James Lewis

The perception of conspecific (within-species) vocalizations is arguably the most salient auditory skill. Human fMRI studies have described "voice-sensitive" regions of cortex that preferentially process verbal (speech) and non-verbal human vocalizations with respect to those produced by other animals. These areas have been traditionally localized to "higher-order" auditory cortices along the superior temporal gyri (STG) and within the superior temporal sulci (STS). However, previous studies have not considered the effects of using non-stereotypical vocalizations to define species-specific vocalization processing pathways in humans. Here, we define regions of cortex that are preferentially responsive to human vocalization that are mimicked versions of real-world animal vocalizations, thus minimizing the acoustic attributes indicative of human vocalizations. These regions are spatially situated between tonotopically (pure-tone frequency)-defined regions and those that show greater preference to more typical human vocalizations, which may represent early and late stages of proposed auditory hierarchies, respectively. The current findings indicate that the neural mechanisms in place for extracting the acoustic and behaviorally relevant features unique to human voices are occurring at earlier processing stages than previously reported.

Conformational Effects of C8-Arylguanine Substitution on B/Z-DNA Equilibrium: A Comparison Study
Train, Brian

Advisor: Peter Gannett

We have shown that C8-arylguanine adducts bearing a para-substituent shift the B/Z-DNA equilibrium toward the Z form in oligonucleotide sequences with two modified bases. The para-substituent was found to have a significant and predictable effect on the B/Z-DNA equilibrium by altering base-pair stacking in the B form and modifying the minor groove widths of both the B and Z conformations. Here a series of C8-arylguanine adducts bearing para-substituents have been prepared in hairpin-turn oligonucleotide sequences containing only one modified base. The conformational effects of aryl substitution were determined by NMR spectroscopy, molecular modeling and circular dichroism (CD). CD was also used to quantify B/Z DNA ratios. Our results to date show that oligonucleotide sequences bearing only one C8-arylguanine modified base exhibit similar effects on B/Z DNA equilibrium as for oligonucleotides with two aryl substitutions. (Supported by WVEPSCoR STEM EPS2005-27)

Cortactin and Coronin 1B cooperate to promote tumor cell invasion in HNSCC
Walk, Elyse

Advisor: Scott Weed

Amplification of chromosome 11q13 in head and neck squamous cell carcinomas (HNSCC) is a common late-stage occurrence associated with poor patient prognosis. Within this region is the gene that encodes cortactin (CTTN), a filamentous (F-) actin binding protein and Src kinase substrate. HNSCC patients with CTTN gene amplification show increased lymph node metastases and decreased survival rate. In vitro, cortactin amplification in HNSCC cell lines promotes cell motility and invasion. Knockdown of cortactin expression in lines with CTTN amplification reduces these effects. Also located within the 11q13 region the gene encoding a second F-actin regulatory protein, coronin 1B (CORO1B). Coronin 1B has been identified as an antagonist of cortactin, promoting the breakdown of F-actin branches and counteracting the pro-migratory effects of cortactin in fibroblast model systems. Here, we show that coronin 1B is a potential mediator of HNSCC invasion. Coronin 1B localizes with cortactin within HNSCC invadopodia, ventral membranous protrusions responsible for degrading extracellular matrix to facilitate loco-regional tumor invasion. RNAi-mediated coronin 1B knockdown increases the number of invadopodia per cell while reducing the degree of matrix degradation per cell area. Conversely, overexpression of coronin 1B increases invadopodia matrix degradation activity. These results collectively indicate that coronin 1B is an important regulator of invadopodia function in HNSCC cell lines, and supports a potential diagnostic role for CORO1B amplification in identifying a previously unidentified invasive subset of HNSCC.

Effects of diacetyl and 2,3-pentanedione vapor inhalation on airway reactivity to methacholine (MCh)
Zaccone, Eric
Advisor: Jeffrey Fedan

RATIONALE. “Popcorn workers lung” is a fixed obstructive pulmonary disease caused by inhalation of artificial butter flavoring during the manufacture of microwave popcorn. Previous investigations have implicated one of the components of butter flavoring, the α -diketone flavoring, diacetyl, as an inhalation hazard in the workplace. Prior studies in rats demonstrated that inhalation of diacetyl vapor for 6 h elicited dose-dependent damage to the airway epithelium of the nose, larynx, trachea and intrapulmonary airways. A diacetyl substitute that is currently being used in the flavor industry is 2,3-pentanedione. We hypothesized that diacetyl or pentanedione-induced epithelial damage results in hyperreactivity to MCh in the isolated, perfused trachea (IPT). **METHODS.** Male rats were exposed by inhalation for 6 h to diacetyl (60, 100, 200, 300, 360 ppm) or 2,3-pentanedione (120, 240, 320, 360 ppm) vapor; controls were exposed to air. Eighteen hours after exposure, tracheas were removed and placed on holders for perfusion at a constant rate while measuring inlet minus out pressure difference (ΔP , cm H₂O) as an index of tracheal diameter. Methacholine was added in stepwise-increasing cumulative concentrations to the tracheal lumen to induce contractile responses. We hypothesized that diacetyl and pentanedione-induced epithelial damage results in hyperreactivity to MCh in the IPT. This could reflect the consequences of epithelial damage and the greater penetration of MCh across the airway wall to reach the smooth muscle. **RESULTS.** The IPT technique revealed butter flavoring-induced changes in reactivity to MCh at several concentrations of diacetyl (100, 200, 360 ppm) and 2,3-pentanedione (120, 320 ppm). **DISCUSSION.** Due to the inconsistency between functional data and changes in morphology, it is difficult to determine the No Observable Adverse Effect Level (NOAEL) for diacetyl and pentanedione vapor using IPT.

Basic Science-06

Arsenic Regulation of miR-190 in Human Carcinogenesis
Beezhold, Kevin
Advisor: Fei Chen

Arsenic is a well-studied human carcinogen. The mechanism by which arsenic induces cancer, however, is not well understood. It is known that as a general stress inducer, arsenic can activate kinases leading to the over activation of transcription factors including JNK and NF-kappaB. These transcription factors are known to regulate the expression of early response genes, and likely to regulate miRNAs. The expression of miRNAs is often altered in cancer and other proliferative disorders. It is highly probable then that miRNAs whose expression are altered by arsenic will play a significant role in carcinogenesis. To test this hypothesis, we investigated in-depth the role of arsenic in the transcriptional regulation of the miRNAs, determined targets of these miRNAs which cause carcinogenesis, and studied the overall cellular responses induced by arsenic that are attributable to these miRNAs. Data show that arsenic is capable of inducing the expression of many miRNAs, most remarkably, the miRNA-190. In silico analysis of possible miR-190 targets indicated that this miRNA may also be involved in tumor formation by targeting multiple proteins including PHLPP, an AKT phosphatase. Kinase activation analysis demonstrated that miR-190 is able to mediate arsenic-induced AKT activation, and that this may occur in a PHLPP dependant manner. Furthermore, overexpression of a miR-190 precursor can enhance the

expression of VEGF, a protein downstream of AKT signaling. These data suggest that arsenic is capable of inducing expression of miRNAs which may play a critical role in arsenic-induced carcinogenesis.

Ozone-Induced Nerve Growth Factor Expression in Rat Airway Epithelium During a Critical Period

Carrell-Jacks, Lynnsey

Advisor: Richard Dey

Airway infection or irritant exposure during early postnatal periods may contribute to the pathogenesis of asthma in children and adults. One mechanism for early life sensitivity may be enhanced airway innervation mediated by nerve growth factor (NGF) release following exposure to airway irritants. Previous studies have demonstrated that acute ozone (O₃) exposure at postnatal (PD) 6 increases NGF mRNA in the tracheal epithelium, but lacked changes at PD10, 15, 21, and 28. The purpose of this study was to examine if a critical period ozone exposure, PD6, leads to an increased NGF expression and release from airway epithelium upon a re-exposure in later life. Fischer 344 rat pups were exposed to O₃ (2ppm) for 3h on PD6, within a proposed critical period of development or on PD21, beyond the critical period. Both groups were re-exposed to ozone on PD28 and sacrificed 12h later and assayed for mRNA or 24h later for protein analysis in bronchoalveolar lavage fluid (BALF). Tracheal epithelial cells were isolated and total RNA was extracted and transcribed into cDNA. NGF mRNA expression was amplified using real-time PCR and analyzed via the comparative threshold method and expressed relative to endogenous Beta-actin. NGF protein levels were measured in BALF using the NGF Emax Immunoassay System. The relative NGF mRNA expression in the airway epithelium of the PD6 ozone group re-exposed to ozone on PD28 showed a significant two-fold increase in comparison to the group exposed at PD21 and re-exposed at PD28 and all other controls ($p \leq 0.05$, $n=5$). The NGF protein was also significantly increased in the PD6 O₃/PD28 O₃ group (77.0 ± 5.2 , $n=5$) over the PD21 O₃/PD28 O₃ group (52.0 ± 7.0 , $n=4$, $p \leq 0.05$). The results suggest that there is a critical period in early postnatal life during which an O₃ exposure leads to increased NGF expression following re-exposure to O₃ in adulthood. This supports the idea that NGF release following airway irritant exposure in early life mediates sensitivity to inhaled irritants in later life.

Progress on the Development of In Vitro Functional Assays for Screening Sigma-1 Receptor Ligands Fishback, James

Advisor: Rae Matsumoto

The sigma-1 receptor represents an attractive drug development target for a number of therapeutic indications including cancer, depression, psychostimulant abuse, and stroke. At the molecular level, sigma-1 receptors have been shown to modulate ion channels in the endoplasmic reticulum and at the plasmalemma. To date, no in vitro activity assay that is amenable to routine use has been developed for screening novel sigma-1 ligands. Such an assay would greatly aid in the development of sigma-1 targeted therapeutics both with regards to selecting compounds for in vivo evaluation and in correlating activities observed in vivo with those observed in vitro. A recently identified protein-protein interaction between sigma-1 and binding immunoglobulin protein (BiP/GRP78) appears to provide a relevant target for selectively evaluating the functional activity of sigma-1 receptor ligands. Two complimentary assay platforms that exploit this interaction as a read-out of sigma-1 activation will be investigated. Measurement of the activity of sigma-1 ligands in membranes isolated from animal tissues will be performed in an ELISA format; Alphascreen technology will be exploited for measurements in a cell free system. We believe the outcome of this work will yield robust, quantitative, high throughput methods for analyzing the functional activity of novel sigma-1 ligands.

Extracellular Matrix Improves Porcine Articular Chondrocytes Self-Renewal and Redifferentiation He, Fan

Advisor: Ming Pei

Articular cartilage is a unique and avascular tissue, mostly made of extracellular collagens and proteoglycans, but has a limited ability to heal after trauma and degenerative disease. The autologous chondrocyte transplantation technique has been used in clinical trials for cartilage repair, which relies on isolation of chondrocytes from the patient, proliferation in the laboratory, and injection into the cartilage damage area. However, the main disadvantage of this technique is that chondrocytes rapidly lost the capacity to regenerate cartilage tissue in the process of expansion ex vivo. We hypothesized that the extracellular matrix (ECM) made by synovium-derived stem cells (SDSCs) could improve articular chondrocytes expandability and the redifferentiation potential. Porcine articular chondrocytes were expanded on normal plastic flasks (Plastic) or on SDSC-derived ECM for 7 passages. Chondrocytes of passage 6 were induced to chondrogenesis in the pellet culture system for 14 days with the treatment of transforming growth factor beta-1 (TGF-beta1). ECM greatly improved the chondrocyte proliferative rate by expanding 4 times as much as the cell number of Plastic group. To evaluate the level of chondrogenesis, ECM group generated 110 µg per pellet of sulfated glycosaminoglycans comparing with 14 µg in Plastic group, confirmed by biochemistry and Safranin O staining. Immunohistochemistry and western blot showed that type II collagen was strong in ECM group but not detectable in Plastic group. The mRNA level of type II collagen in ECM was 384-fold higher than Plastic, and aggrecan in ECM was 30-fold more than Plastic. SDSC-derived ECM was able to dramatically improve chondrocyte proliferation capacity and effectively enhance the chondrogenic redifferentiation potential. These results demonstrated that SDSC-derived ECM can function as a new ex vivo expansion system to improve chondrocytes proliferation and redifferentiation potentials for further clinic applications.

SN79-A Novel Sigma-2 Receptor Antagonist, Attenuates Methamphetamine-Induced Neurotoxicity

Kaushal, Nidhi

Advisor: Rae Matsumoto

Methamphetamine (METH) is a psychostimulant drug of abuse, causing hyperthermia and neurotoxicity at high or repeated doses. The mechanism of these neurotoxic effects is not very clearly understood and there is still no effective pharmacotherapy to treat these effects. This necessitates the identification of potential novel therapeutic targets. Radioligand binding assays showed that METH interacts with sigma receptors which are present on dopaminergic and serotonergic neurons and can modulate their actions. From independent experiments, it has been shown that sigma-2 receptor activation causes cytotoxic effects in tumor cells (Crawford and Bowen, 2002) and blockade of sigma receptors can attenuate METH-induced neurotoxicity (Matsumoto et al., 2008). Taking all this into consideration, the hypothesis of this study is that METH mediates its neurotoxic effects via sigma-2 receptors. In order to test the hypothesis, a sigma-2 receptor selective antagonist, SN79, was developed. Using radioligand binding studies, SN79 was shown to have high nanomolar affinity and selectivity for sigma-2 receptors, with $>10,000$ nM affinity for sigma-1 receptors and 59 other binding sites. In this study, SN79 dose dependently attenuated METH-induced hyperthermia, dopaminergic and serotonergic neurotoxicity (measured as depletions in dopamine and serotonin levels using ELISA, in the striatum of male Swiss Webster mice). Together, the data suggest that antagonism of sigma-2 receptors can convey protective actions against the neurotoxic effects of METH to dopaminergic and serotonergic neurons.

CYP4A mediates exaggerated vasoconstriction through adenosine A1receptor in A1 WT compared to A1 KO

Kunduri, Swati

Advisor: Jamal Mustafa

Adenosine exerts its vasoconstrictor effect with the activation of A1AR through PLC- β III \rightarrow PKC α \rightarrow p42/44 pathway, and possibly arachidonic acid derived metabolite (20-HETE) generated through hydroxylation via CYP4A enzyme in vascular smooth muscle cells. 20-HETE plays an important role in the regulation of vascular tone in a number of vascular beds. We hypothesize that the A1AR-mediated vasoconstriction depends on CYP4A hydroxylation of arachidonic acid. Organ bath experiments with aorta from A1WT and A1KO mice were used in this study. The tissues were contracted with 10^{-6} M PE and the intactness of endothelium was tested with 10^{-6} M ACh. Tissues that relaxed $>40\%$ were used in this study. A1KO had significantly higher ACh relaxation ($+72.38\pm 14.07\%$) than A1WT ($52.76\pm 11.87\%$). CRC to a non-selective adenosine analog (NECA) and an A1AR selective agonist (CCPA, 10^{-6} M) were obtained with CYP4A inhibitors (HET-0016, 10^{-6} and DDMS, 10^{-5} M). HET-0016 changed the NECA ($-19.51\pm 3.68\%$) and CCPA-induced contraction ($-34.08\pm 5.68\%$) into relaxation ($60.36\pm 13.7\%$). DDMS changed CCPA (10^{-6} M) induced contraction ($-29.83\pm 7.19\%$) into relaxation ($18.79\pm 5.19\%$). CYP epoxygenase inhibitor (MSPPOH, 10^{-5} M) caused significantly higher contraction in the treated group ($-50.46\pm 6.25\%$) vs the control ($-20.46\pm 4.82\%$). These data support a role for CYP4A in A1AR-mediated vasoconstriction.

Estrogen Treatment Worsens Stroke Outcome in Aged Female Rats

Leon, Rachel

Advisor: Charles Rosen

The Women's Health Initiative (WHI) released reports on the effects of hormone replacement therapy (HRT) on stroke prevalence and outcome that conflicted with prior animal studies. While animal studies suggest estrogen to be neuroprotectant, randomized controlled trials from the WHI were terminated early due to increased risk of ischemic stroke following HRT. Meta-analyses of data from the WHI and other smaller clinical trials have also shown increased stroke severity with estrogen supplementation. This dichotomy has spurred a debate about the safety and necessity of HRT. We contend that age is a primary factor accounting for the contrast between animal studies and clinical trial findings. Studies on aging confirm that neuropoietic (interleukin 6-like) cytokines and their target receptor, glycoprotein 130 (gp130) play a primary role in the complex relationship between aging and pathology. Serum levels of soluble IL-6 and gp130 have been shown to increase in women as they age, while estrogen decreases expression of inflammatory products of gp130 signaling both in vitro and in vivo. Based on its anti-inflammatory activity, we hypothesized that estrogen supplementation would reduce infarct volume and functional deficit in aged female rats following transient middle cerebral artery occlusion (MCAO) and reperfusion. However, data demonstrate that aged female rats supplemented with estrogen suffer increased infarct size, higher mortality, and reduced functional recovery from ischemia/reperfusion compared to aged female rats treated with placebo. Despite its anti-inflammatory activity, estrogen may be detrimental to recovery from stroke in the aging brain, as activation of inflammatory pathways may be essential for the post-ischemic upregulation of beneficial downstream effectors of gp130 signaling. Estrogen also alters mRNA levels of neuropoietic cytokines and gp130 signaling modulators. These findings suggest that chronic estrogen treatment alters post-ischemic gp130 signaling pathways which may play a role in worsened stroke outcomes in women taking HRT.

Rescue of a trafficking defective human pacemaker channel via a novel mechanism

Lin, Yen-Chang

Advisor: Hangang Yu

Therapeutic strategies such as using channel blockers and reducing culture temperature have been used to rescue some long QT-associated voltage-gated potassium Kv trafficking defective mutant channels. A hyperpolarization-activated cyclic nucleotide-gated HCN4 pacemaker channel mutant (D553N) has been recently found in a patient associated with cardiac arrhythmias including long QT. D553N showed the defective trafficking to the cell surface, leading to little ionic current expression (loss-of-function). We show in this report that enhanced tyrosine phosphorylation mediated by Src, Fyn, and Yes kinases was able to restore the surface expression of D553N for normal current expression. Src or Yes, but not Fyn, significantly increased the current density and surface expression of D553N. Fyn accelerated the activation kinetics of the rescued D553N. Co-expression of D553N with Yes exhibited the slowest activation kinetics of D553N. Src, Fyn, and Yes significantly enhanced the tyrosine phosphorylation of D553N. A combination of Src, Fyn, and Yes rescued the current expression and the gating of D553N comparable with those of wild-type HCN4. In conclusion, we demonstrate a novel mechanism using three endogenous Src kinases to rescue a trafficking defective HCN4 mutant channel (D553N) by enhancing the tyrosine phosphorylation of the mutant channel protein.

Antibodies for immunodiagnostics of *Aspergillus terreus*

Nayak, Ajay

Advisor: Donald Beezhold

Aspergillus terreus is an emerging pathogen that causes morbidity and mortality in immunocompromised patients. The ability of the organism to grow at elevated temperatures (37°C-50°C) and its natural resistance to first line anti-fungal treatments make it a difficult mycotic disease to mitigate. Currently, no immunodiagnostic tests are available for early and rapid identification of the *A. terreus* in patients. We have approached this problem by developing strategies to produce species-specific monoclonal antibodies (mAbs) against *A. terreus*. Our first strategy involved using a 'shotgun' approach to develop mAbs against exoantigens from *A. terreus*. We immunized mice with a partially purified extract from *A. terreus* culture supernatants and generated mAbs using established protocols. We obtained 23 antibodies that reacted with *A. terreus*. Sixteen antibodies were highly specific to *A. terreus* and did not cross-react with any of the other 45 tested fungi species. We observed that the antigens recognized by these mAbs were expressed in higher concentrations in mycelium (active growth stage) and culture supernatant than in conidia (dormant stage). Further investigation, identified expression in cultures at 37°C as early as 24 hours but peaked after 3 days. Importantly, most antibodies did not show any inhibition of the epitope detection by human serum components. In a second approach, we specifically developed antibodies to the cytolysin, terrelysin. Recombinant terrelysin was cloned, expressed, and used to immunize mice. We generated 19 anti-terrelysin mAbs of isotypes IgG1 (12), IgG2a (4) and IgG2b (3). mAbs showed some variations in their sensitivity towards rTerrelysin in direct ELISAs and Western blots. Investigation of native terrelysin expression kinetics using an inhibition ELISA demonstrated that terrelysin expression peaked by day 4 in mycelium grown at room temperature and by day 2 at 37°C. Native terrelysin expression was also identified in culture supernatants suggesting it may be a useful biomarker for the detection of *A. terreus*. In summary, these antibodies can be potentially used in immunodetection of *A. terreus* in clinical and environmental samples.

Evidence for the role of A2B adenosine receptor in the regulation of vascular tone using A2B KO mice

Sharifi Sanjani, Maryam

Advisor: Jamal Mustafa

Adenosine, an endogenous nucleoside, exerts its effects through its four receptor subtypes (AR): A1, A2A, A2B, and A3. Adenosine is involved in the regulation of vascular tone where its effects are subtype-dependent. The contribution of ARs needs to be further investigated in order to better understand the heterogeneity of vasculature responses. In mice aorta, A2A AR is known to be involved in vascular relaxation, however, the role of A2B AR has not been studied in this tissue. To investigate this, we performed organ bath experiments. Tissues with <40% ACh relaxation to PE (10-6M) contraction were excluded (endothelial integrity). NECA, a non-selective AR agonist, induced a significantly higher contraction in A2B KO compared to WT (21.8±3.2% contraction vs 21.9±8.5% relaxation at 10-5M, p<0.01) showing that activation of A2B AR may induce relaxation. CCPA, a selective A1 agonist, induced contraction (38.9±6.3% at 10-6M) was significantly reduced in A2B KO (5.9±7.8% at 10-6M, p<0.01)

suggesting the down-regulation of A1 AR. Cl-IBMECA, a selective A3 agonist, showed significantly lower contraction in A2B KO compared to WT ($14.6\pm 3.3\%$ vs $42.9\pm 5.3\%$ at 10^{-6} M) respectively, further suggesting the down-regulation of A3 AR in A2B KO. These data show a relationship between A2B and A1 and A3 ARs which must be taken into account in the control of vascular tone. Supported by HL027339, HL094447, HL071802, and T-32 HL090610.

Involvement of neuronal pathways in A1 adenosine receptor-mediated airway hyperresponsiveness

Young, Ernest

Advisor: Jamal Mustafa

Rationale: The ability of adenosine to induce airway hyperresponsiveness in asthmatic mice through its effect on four extra-cellular adenosine receptors is well documented. Specifically, the A1 adenosine receptor has been shown to have a pro-inflammatory effect and to induce hyperresponsiveness in an allergic rabbit model of asthma (Nadeem, A, et al. 2006, *Eur J Pharmacol*, 551, 116-24). Recently, the A1 adenosine receptor has been implicated in inducing airway hyperresponsiveness through neuronal pathways in naïve, non-allergic mice (Hua, X et al., 2007, *Am J Physiol Lung Cell Mol Physiol*, 293(1):L25-32). It remains unknown whether such a mechanism is involved in the allergic mouse model of asthma. Methods: In our study, C57 mice were sensitized to allergen by 200 ug injections of ragweed extract in alum on days 1 and 6 and challenged with 1% ragweed aerosol on days 11-13 of a two week allergen protocol (Fan, M, and Mustafa, SJ, 2002, *Pulm Pharmacol Ther* 15: 147-155.). On day 14, pulmonary resistance in response to aerosolized adenosine, 6 mg/ml was recorded with or without 20 μ mol/kg atropine pretreatment. Results: Control animals received only alum injections and saline aerosol. Pretreatment with atropine significantly decreased ($p<0.05$) the pulmonary response to adenosine in the sensitized and challenged mouse ($176\pm 60\%$ of the response to vehicle in the untreated allergic mouse versus $110\pm 10\%$ in the atropine treated allergic mouse, $n=5-6$). Conclusion: These data indicate a cholinergic route for the activity of adenosine in allergic asthma, supporting a role for vagal neuronal pathways in this allergic mouse model of asthma. This research is funded through NIH grants: HL027339, HL094447, and T-32 HL090610.

Mechanisms of Hydrogen Peroxide-induced Increase in microvessel Permeability: Role of Nitric Oxide
Zhou, Xueping
Advisor: Pingnian He

H₂O₂ is implicated in the pathogenesis of various cardiovascular diseases. It has been reported to induce eNOS activation in cultured endothelial cells and contribute to vasodilation in arterioles. However, whether it induces eNOS activation and NO production in intact venules and plays a role in venular microvessel permeability remains unknown. We previously showed that H₂O₂ induced delayed but progressive increases in endothelial (EC) [Ca²⁺]_i, microvessel hydraulic conductivity (L_p), and cell apoptosis in intact venules. This study aims to explore the signaling mechanisms of H₂O₂-induced L_p increases with focus on H₂O₂-induced NO production in ECs of venular walls. NO and EC [Ca²⁺]_i were measured in DAF-2 and Fura-2 loaded vessels and permeability was determined by L_p measurements in rat venules. H₂O₂ (10 micromole) induced an immediate increase in NO and reached a plateau (9.1±0.4 times that of control) after 45 min, which occurred prior to the increases in EC [Ca²⁺]_i and not affected by Ca²⁺ influx inhibitor LaCl₃ (50 micromole). Immunostaining for nitrotyrosine using confocal microscopy revealed extensive tyrosine nitration in vessels exposed to H₂O₂ for 2h but not in untreated vessels. Blocking H₂O₂-induced NO production with L-NMMA not only abolished H₂O₂-induced delayed increases in EC [Ca²⁺]_i and tyrosine nitration, but also prevented the L_p increases. Our results indicate that H₂O₂ activates endothelial NOS via a Ca²⁺ independent pathway and the excessive NO production and subsequent peroxynitrite formation-mediated protein nitration may be the key for EC Ca²⁺ overload, resulting in cell injury and increases in microvessel permeability. The signaling mechanisms of H₂O₂-mediated vascular cell injury and barrier dysfunction in intact venules may provide potential therapeutic strategies for H₂O₂-associated cardiovascular diseases.

Basic Science-05

VE-cadherin and PECAM-1 enhance ALL migration across brain microvascular endothelial cell monolayers
Akers, Stephen
Advisor: Laura Gibson

Infiltration of the central nervous system (CNS) by leukemic cells is a problematic disease manifestation of acute lymphoblastic leukemia (ALL). Current understanding of the mechanisms by which leukocytes interaction with brain microvasculature and enter the CNS is derived from models of inflammation. Based on these models we determined that exposure of HBMEnd to ALL cells does not alter the expression of endothelial cell surface adhesion molecules VCAM-1 or ICAM-1 and does not impair endothelial barrier function. These data suggest that ALL invasion of the CNS may deviate from classical dogma. While characterization of ALL cell lines demonstrates VE-cadherin and PECAM-1 expression in Ph⁺ ALL cell lines, evaluation of primary ALL samples reveals that VE-cadherin and PECAM-1 are expressed by ALL regardless of Ph-status. We hypothesized that these proteins may mediate interaction between ALL cells and HBMEnd. Lentiviral-mediated expression of VE-cadherin and PECAM-1 and antibody based neutralization of these proteins demonstrate that VE-cadherin mediates ALL adhesion to HBMEnd and PECAM-1 mediates ALL adhesion to and migration through HBMEnd. ALL co-expression of VE-cadherin and PECAM-1 may position them to interact with HBMEnd and provides further insight into the mechanisms by which ALL cells cross vascular barriers to invade the CNS.

Examination of Protein Import in Mitochondria Influenced by Diabetes Mellitus in the Heart
Baseler, Walter

Advisor: John Hollander

Cardiac complications, such as diabetic cardiomyopathy, are the leading cause of morbidity and mortality in diabetes mellitus worldwide. An essential defect associated with diabetic cardiomyopathy is mitochondrial dysfunction due to hyperglycemic conditions. Two mitochondrial subpopulations exist in the cardiomyocyte, the subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM), which differ spatially, structurally, and functionally. Currently there are an estimated 1500 proteins in the human mitochondrion, with only 13 transcribed and translated in the organelle itself. The vast majority of proteins (99 percent) are nuclear encoded and imported into mitochondria through a complex mechanism of translocation. The goal of this study was to determine whether protein import is dysfunctional in cardiac mitochondrial subpopulations during a diabetic insult and whether dysfunction occurred in a spatially distinct manner. i-TRAQ proteomic analyses highlighted significant decreases in the majority of nuclear encoded mitochondrial inner membrane (IM) and matrix proteins, including glucose regulated protein 75 (GRP75), in the diabetic IFM relative to controls ($P < 0.05$). In contrast, there were minimal mitochondrial IM and matrix proteomic alterations in the SSM. Mitochondrial protein import was significantly decreased in the diabetic IFM relative to controls, with no change in the SSM ($P < 0.05$). IFM membrane potential was also significantly decreased relative to controls, with no change in the SSM ($P < 0.05$). Finally, Western blot analysis showed significant decreases in GRP75 content in the diabetic IFM relative to controls, with no change in the SSM ($P < 0.05$). Together, our results indicate type 1 diabetic insult causes dysfunction of the mitochondrial protein import process, leading to a loss of key proteins required for adequate mitochondrial function and structure. Further, dysfunction associated with mitochondrial protein import is most pronounced in the IFM. Ultimately, the research offers insight into a novel mechanism of mitochondrial dysfunction in diabetes mellitus.

Host and bacterial factors that influence *Pseudomonas* type III secretion translocon function
Bridge, Dacie

Advisor: Joan Olson

Bacterial type III secretion (T3S) is integral in the establishment of infections by a large number of Gram-negative pathogens. We study T3S in the opportunistic pathogen *Pseudomonas aeruginosa* (Pa), and through comparisons of cell culture models that are sensitive or resistant to Pa-T3S, we recognized a role of eukaryotic cell leading edge (LE) adherence properties and the underlying host cell architecture involved in migration in Pa-T3S translocon function. These findings led us to hypothesize that actin-induced forces on membranes are a contributing factor in translocon function. To test this, we used immunofluorescent staining to examine the cellular architecture that underlies Pa during T3S translocation into T24 epithelial cells. We used PA103deltaUT expressing WT ExoS, an effector-null PA103deltaUT strain, and translocon defective PA103deltaPopB strain and found that the ExoS effector and translocon proteins were influencing the Pa binding site to T24 cells. ExoS includes both GTP-ase activating protein (GAP) and ADP-ribosyltransferase (ADPRT) activities, which target proteins that affect actin polymerization and cell migration, and based on our hypothesis, should influence T3S translocon

function. Consistent with this notion, T3S translocon membrane insertion and effector translocation were more efficient in Pa strains expressing ExoS with mutations in its GAP and ADPRT activities, with the ADPRT mutation leading to greater effector translocation. Our conclusions are: 1) T3S translocon proteins influence the targeting of Pa to LE regions; 2) more efficient T3S translocation occurs in association with Pa binding to the LE and areas of dense actin staining; and 3) T3S translocation is down-regulated by effector ExoS ADPRT activity, and to lesser degree by ExoS GAP activity. Our studies of Pa-T3S are consistent with the opportunistic nature of Pa infections, which target wounded epithelial cells undergoing cell migration.

Mechanisms Of Arsenic-Induced Angiogenesis And Tumorigenesis

Carpenter, Richard

Advisor: Bing-Hua Jiang

Arsenic is a heavy metal widely distributed throughout the earth's crust. Epidemiology studies show significant associations between arsenic exposure, through drinking water, mining, metal smelting, cigarette smoke, and others, and incidence of lung carcinoma. The cellular mechanisms by which arsenic induces lung tumorigenesis, requiring both cell transformation and angiogenesis, are not well understood. Therefore, the purpose of this study was to assess the role of reactive oxygen species (ROS), PI-3K, and MAPK growth signaling pathways in arsenic-induced angiogenesis and tumorigenesis. Results show that arsenic promotes generation of ROS, at least partly due to an increased activity of NADPH oxidase. Arsenic treatment also increased phosphorylation of EGFR, Akt, and ERK1/2 and activation of the HIF-1 signaling pathway, all of which can promote cell proliferation. Increased activation of these signaling pathways required the formation of ROS as the hydrogen peroxide scavenger catalase prevented their activation. Furthermore, inhibition of EGFR phosphorylation prevented activation of ERK1/2 and HIF-1 signaling. Long-term arsenic exposure (6 months) to lung epithelial cells increased ROS generation and phosphorylation of Akt and ERK1/2. These chronically-treated cells also demonstrated anchorage-independent growth in soft agar and increased proliferation compared to control cells. Anchorage-independent growth and proliferation were prevented with inhibition of PI-3K and MAPK signaling pathways. Arsenic was also shown to induce epithelial cells to promote angiogenesis in vivo, which was prevented with inhibition of ROS, Akt, ERK1/2, HIF-1, and VEGF. Lung epithelial cells chronically treated with arsenic were able to form a greater number and larger tumors when injected into nude mice compared to control cells. When tumors were analyzed, arsenic-induced tumors showed increased phosphorylation of Akt and ERK1/2 compared to control epithelial cells. These results indicate arsenic promotes cell transformation and tumorigenesis in lung epithelial cells by increasing ROS generation, PI-3K and MAPK signaling, and promoting angiogenesis.

Post-Prenyl Processing Of Proteins Is Essential For Survival And Function Of Photoreceptor Neurons.
Christiansen, Jeff

Advisor: Vishy Ramamurthy

Post-prenyl processing (PPP) of proteins resulting in carboxyl methylation of their C-termini is part of a post translational lipid modification referred to as prenylation. The importance of PPP is underscored by embryonic lethality observed in mice defective in PPP. While it is clear that the first step of prenylation, the addition of the lipid prenyl group is needed for the association of proteins with membranes, the significance of the additional smaller methyl group is not obvious. It is thought that methylation, a reversible modification, may enhance the hydrophobicity of proteins modulating their association with membranes. Alternatively, methylation has been proposed to assist in assembly of multimeric signaling proteins. Several proteins in photoreceptor neurons, particularly in the outer segments, involved in light signal transduction are methylated. These include heteromeric proteins such as transducin and phosphodiesterase or monomeric rhodopsin kinase and a group of small GTPases. To evaluate the role of this protein modification in the survival and function of the retinal neurons, we specifically disrupted PPP in the developing mouse retina at embryonic day 9. Surprisingly, retina lacking PPP are able to develop normally up through postnatal day eight (P8) with no observed cell death. However at P10, when ciliated photoreceptor outer segment development begins, apoptosis is evident in the photoreceptor layer of retina lacking PPP. Even though photoreceptor nuclei can be observed in P14 sections, rod photoreceptor neurons do not function as measured by light evoked potentials recorded by electroretinogram (ERG). Our studies conclusively demonstrate that methylation is critical for proper functioning and stability of photoreceptor neurons.

Mitochondrial Overexpression of PHGPx Provides Cardioprotection from Type 1 Diabetes Mellitus Insult
Dabkowski, Erinne

Advisor: John Hollander

Mitochondrial dysfunction plays a critical role in the pathogenesis of type 1 diabetes mellitus (DM). Evidence suggests that the inner mitochondria membrane (IMM) and its constituents are specific targets for the deleterious effects associated with type 1 DM. As a result, maintenance of IMM integrity represents a specific locus for testing directed prophylactic intervention. We previously reported that type 1 DM imparts differential effects on spatially distinct mitochondrial subpopulations, subsarcolemmal (SSM) and interfibrillar (IFM). The goal of this study was to determine whether overexpression of a mitochondrially-targeted antioxidant enzyme, mitochondria phospholipid hydroperoxide glutathione peroxidase 4 (mPHGPx), which scavenges phospholipids in the IMM, could elicit protection to mitochondria during type 1 diabetic insult, and if the protection was subpopulation-specific. MPHGPx transgenic mice and littermate controls were made diabetic through multiple low dose injections of streptozotocin (STZ). Five weeks following hyperglycemia onset, in vivo analysis of cardiac contractile function revealed significantly decreased ejection fraction and fractional shortening in the diabetic heart which was reversed with mPHGPx overexpression. MPHGPx overexpression in the diabetic heart was associated with increased state 3 respiration and mitochondrial electron transport chain complex I, III, and IV activities in IFM as compared to diabetic controls, with no differences on SSM. Hydrogen peroxide production and lipid peroxidation were significantly attenuated in the diabetic IFM with overexpression of mPHGPx as compared to controls, with no changes in the SSM. Further,

Cardiolipin, a phospholipid unique to the inner mitochondrial membrane and necessary for proper mitochondrial function, was rescued with overexpression of mPHGPx in the diabetic heart. These results indicate that mPHGPx overexpression provides cardioprotective benefit to the diabetic heart, which is associated with mitochondrial subpopulation-specific protection.

Responses to Sound Features in the Auditory Midbrain are Shaped by the Superior Paraolivary Nucleus
Felix, Richard

Advisor: Albert Berrebi

The superior paraolivary nucleus (SPON) is a major source of GABAergic inhibition to the auditory midbrain. The ability of SPON neurons to signal the occurrence of discontinuities within ongoing stimuli makes them well-suited to encode temporal features of sound. In particular, SPON neurons detect silent gaps in pure tone stimuli with great sensitivity, and synchronize to low modulation rates of sinusoidal amplitude-modulated (SAM) tones with high vector strengths. Despite our growing knowledge of SPON unit responses to various stimuli, it remains unclear how this nucleus contributes to the processing of acoustic information in its main synaptic target, the inferior colliculus (IC). One approach to examine the influence exerted by the SPON on processing of temporal sound information in the midbrain is to compare single unit responses in the IC to gap and SAM stimuli, both in the presence and absence of SPON activity. Thus, we used iontophoretic delivery of the GABA_A receptor agonist muscimol to reversibly silence SPON activity while conducting simultaneous recordings in the IC. Inactivation of SPON inputs was accompanied by an increase in gap detection thresholds and a decrease in the synchronicity of responses to SAM stimuli in IC neurons. In addition, the range of SAM tone modulation frequencies over which IC neurons phase-locked was lower when the SPON was inactivated. These preliminary findings suggest that the SPON likely contributes to the encoding of gap and SAM stimuli by IC neurons.

Focal adhesion kinase and endothelial cell isometric tension

Miede, Kimberly

Advisor: Robert Wysolmerski

The vascular system is lined by a continuous sheet of endothelial cells (EC) that form a semi-permeable barrier that restricts solutes and macromolecules access to extravascular compartments. Inflammatory stimuli alter cell-matrix adhesion and trigger EC contraction, resulting in gap formation between adjacent EC and leading to increases in vascular permeability. Activation of EC contractile activity and/or alterations in cell-matrix adhesion(s) most likely causes inter-endothelial cell gap formation. However, how EC contraction and matrix adhesion(s) contribute to cytoskeletal rearrangement, gap formation, and increases in vascular permeability remains poorly understood. Cell-matrix adhesion occurs through multi-protein complexes called focal adhesions. These adhesions tether the cells contractile machinery (actin/myosin) to extracellular matrix proteins forming the structural link necessary for isometric tension generation. To investigate the role of focal adhesions in EC contraction, focal adhesion kinase (FAK), a

critical molecule within focal adhesions, was ablated. We hypothesized FAK is essential for isometric tension generation and is necessary for proper assembly and organization of the EC contractile machinery in order to transmit cellular tension. Infection of EC with a lentivirus expressing a FAK-shRNA caused a 90% reduction in endogenous levels of FAK protein. There was no up-regulation of Pyk2 detected. Loss of FAK results in a 50% reduction in basal EC focal adhesions compared to vector controls. Overexpression of FAK in EC results in altered cellular morphology, increased focal adhesions, and enhanced actin stress fiber formation. FAK knockdown modifies basal and LPA induced EC isometric tension generation. Loss of FAK alters nonmuscle myosin II distribution, suggesting a role for FAK in myosin II regulation. These data implicate a role for FAK in the regulation of EC isometric tension possibly through modification of the contractile machinery. Supported by NIH grants T32-HL090610, HL-45788, RR-16440.

The Potential Role of Kisspeptin in Puberty Onset in Sheep.

Nestor, Casey

Advisor: Bob Goodman

In peripubertal sheep, a decrease in inhibition by estradiol leads to an increase in gonadotropin releasing hormone (GnRH), and thus luteinizing hormone (LH), secretion, heralding the onset of puberty. Still, the neural mechanisms governing this process remain largely unknown. Kisspeptin, a recently discovered neuropeptide, is expressed in the arcuate nucleus of the hypothalamus of sheep and stimulates GnRH/LH secretion. We hypothesized that kisspeptin expression would be greater in postpubertal as compared to prepubertal sheep and that kisspeptin expression would be increased in the absence of steroid negative feedback. Four groups of females were used: prepubertal intact (n=3), prepubertal ovariectomized (n=4), postpubertal ewes in the early follicular phase (n=3), postpubertal ovariectomized (n=3). To examine potential sex differences in kisspeptin expression, 4 groups of males (n= 3 to 4 per group) that were similar in age to the females and either intact or castrated were also used. Blood samples were collected via jugular venipuncture at 12-min intervals for 4 hours prior to sacrifice, after which brains were perfused with 4% paraformaldehyde and hypothalami collected for immunocytochemical evaluation of kisspeptin expression. LH was measured to confirm the endocrine state of each animal. Mean levels of LH and LH pulse frequency were suppressed by the presence of the gonads in both males and females. In females, kisspeptin expression was greater in postpubertal ewes as compared to prepubertal ewes, and was increased by ovariectomy only in the prepubertal group. In the males, cell numbers did not differ with age, but were increased by castration in both age groups. Overall, females had a significantly higher number of kisspeptin-expressing cells in comparison to males. The increase in kisspeptin cell numbers in postpubertal females is consistent with a role for kisspeptin in puberty onset in the sheep. Further, the increase in kisspeptin expression with ovariectomy in prepubertal females indicates that decreased kisspeptin cell numbers in intact prepubertal females is due to the negative feedback effects of estradiol. The increase in kisspeptin expression in males with castration indicates that gonadal steroids regulate kisspeptin. Since ram lambs undergo puberty much earlier than ewe lambs the lack of age differences in the males suggests that differences in the females relate to puberty rather than age.

Differential Sensitivity Of Tumorigenic Cell Lines To Pseudomonas aeruginosa Type III Secretion.

Novotny, Matthew

Advisor: Joan Olson

The potential for microbial systems to act as chemotherapeutic agents against cancerous cell growth has been known for many years, whether it be the use of Coley's toxin to stimulate host immunity or the production of cytotoxic agents by Salmonella. The opportunistic pathogen, Pseudomonas aeruginosa, also has potential as a chemotherapeutic agent due to factors associated with its type III secretion system (Pa-T3S), which allows the direct translocation of bacterial toxins into the host cell cytoplasm. From two previous observations from our laboratory, the first that tumorigenic cell lines are more sensitive to Pa-T3S and the second that sensitivity to Pa-T3S is determined by host cell migrational dynamics, we hypothesize that the increased sensitivity of tumorigenic cell lines to Pa-T3S is due to their increased migrational capacity. To examine this, comparative studies between the related MTC line (non-metastatic) and MTLn3 line (metastatic) were developed. Initial studies confirmed that MTLn3 cells are more sensitive to Pa-T3S, as indicated by increased levels of translocated reporter toxin (ExoS) and increased levels of toxin mediated modification of host proteins. Also, insertion of the T3S-translocon channel, through which the toxins enter the host cell, is increased in the MTLn3s. Immunofluorescent analysis indicated that Pa binds with similar frequency to leading edge associated proteins in both cell lines, but that leading edge structures in the metastatic MTLn3 line are more numerous and pronounced. From these preliminary studies, we believe the more extensive leading edge structures of the MTLn3 line facilitate translocon insertion and function, resulting in more efficient translocation of the reporter toxin. It is our belief that dissection of the mechanism of differential sensitivity to Pa-T3S will allow potential development of both antimicrobial agents against this deadly opportunistic pathogen and a Pseudomonas based chemotherapeutic delivery system against cancer cell growth.

Adaptor Protein AFAP1L1: A Potential Cortactin Binding Partner

Snyder, Brandi

Advisor: Daniel Flynn

The actin-filament associated protein (AFAP) family of adaptor proteins consists of three members: AFAP1, AFAP1L1, and AFAP1L2 with AFAP1 being the best described as a Src binding partner and actin cross-linking protein. A homology search of AFAP1 done by us identified the third family member, AFAP1L1, which has a similar domain structure but is hypothesized to have distinct functions from other AFAP family members based on variations in sequence in the conserved domains. While AFAP1 has the ability to bind Src but not cortactin via an N-terminal SH3 binding motif, the SH3 binding motif of AFAP1L1 more closely resembles a cortactin SH3 domain binding site. Thus, we are seeking to determine if AFAP1L1 is a cortactin binding partner. AFAP1L1 has been shown by fluorescent microscopy to decorate actin filaments and, upon stimulation, move to punctate cytosolic actin structures, podosomes or invadopodia, and colocalize with cortactin in these structures. Unlike AFAP1, AFAP1L1 overexpression will strongly induce podosome formation. In addition, immunohistochemical analysis of AFAP1L1 shows a differential expression pattern from AFAP1 and different localizations with AFAP1L1 localizing strongly to muscle and sites of high actin dynamics in the brain. Due to its domain structure, cellular localization and potential to be a cortactin binding partner, we hypothesize that AFAP1L1 plays a role in podosome formation and actin dynamics, possibly through an interaction with cortactin.

C-peptide Prevents High glucose-Induced Mitochondrial Dysfunction in Renal Endothelial Cells

Vejandla, Himani

Advisor: Robert Brock

C-peptide has been shown to protect against renal dysfunction and other diabetic microvascular complications. High glucose-mediated microvascular damage, in endothelial cells, is based on excess generation of mitochondrial superoxide. The mitochondrial membrane potential ($\Delta\Psi_m$) and the amplitude of electrochemical proton gradient regulate superoxide production. The main objective of this study was to determine the effect of C-peptide on overall mitochondrial function as well as the levels of superoxide in renal microvascular endothelial cells exposed to high glucose (HG - 25 mM) for 24 and 48 h. Using JC-1 to assess $\Delta\Psi_m$, we observed that C-peptide (6.6 nM) restored the HG-induced hyperpolarization at 24 h, and depolarization at 48 h, back to the normal $\Delta\Psi_m$ measured with low glucose (5.5 mM). Respiratory control ratio, a measure of electrochemical coupling determined by polarography, was significantly higher in HG cells treated with C-peptide compared to HG alone ($p < 0.0002$ - 24 h and $p < 0.0155$ - 48 h). C-peptide also prevented HG-mediated superoxide ($p < 0.05$), as measured by mitosox red fluorescence using flow cytometry. In addition, C-peptide ameliorated HG-induced reductions in mitochondrial complex 1 activity. Together, these data demonstrate that C-peptide protects against endothelial mitochondrial dysfunction during hyperglycemia. (Funded by NIH Grant #RO1 DK067582 to RWB)

Gold-immobilized Cytochrome P450 constructs: A precursor to nanostructured metallic arrays

Wollenberg, Lance

Advisor: Peter Gannett

Cytochrome P450 Enzymes are an essential class of heme-containing proteins responsible for the metabolism of approximately 75% pharmaceutical compounds. Traditionally, reconstituted solution incubations are often used to elucidate the metabolic profile of a particular drug. To accomplish this, purified Cytochrome P450 enzymes are combined with additional lipid components that form an artificial lipid bilayer, thus creating a simulated microsome in both structure and activity. However this system is often problematic in that the solution based incubation tends to form protein aggregates, which often have profound effects on the catalytic activity of the enzyme. To counter this, we have designed an immobilized platform to minimize enzymatic aggregation and thus provide a mechanism to determine effects of enzyme aggregation as it occurs in-vitro. Furthermore, we have fabricated surface plasmon resonance based sensors for the real-time analysis of our Cytochrome P450 constructs, in hopes elucidating interactions between our enzymes of interest and our bio-mimetic gold-self assembled monolayer construct. Ultimately, we would like to use our proof-of-concept P450 construct to develop a nanostructured array which would unequivocally control the aggregation state of our Cytochrome P450 enzyme. In turn, we believe that the creation of this nanostructured Cytochrome P450 array will allow for better determination of in vivo – in vitro correlation as it pertains to drug metabolism.

Basic Science-04/03

Using Myoglobin as a Model to Study Electron Transfer in CYP450s on the Single Molecule Level

Jett, John

Advisor: Peter Gannett

Electron transfer is a fundamentally important process for proteins whose functionality relies on redox reactions with other proteins, cofactors, or the external environment. In heme-containing proteins—such as myoglobin and cytochromes P450—an iron atom changes between Fe⁺² and Fe⁺³ oxidation states as an electron is accepted by or donated to the protein. Most of the previous work to understand the mechanism of electron transfer has been based on statistical averages of protein ensembles measured by spectroscopic and electrochemical techniques. Here, we present the study of electron transfer in myoglobin on the single molecule scale by two techniques, nanometer-gap platinum electrodes fabricated by electromigration techniques, and conductive probe atomic force microscopy with proteins immobilized to a gold nanodot array. The development of these techniques with myoglobin as a model protein sets the path for future studies probing electron transfer in drug metabolizing cytochromes P450 at the single molecule scale.

Oncogenic Src Requires a Wild-type Counterpart to Regulate Invadopodia Maturation

Kelley, Laura

Advisor: Scott Weed

The proto-oncogene Src tyrosine kinase (Src) is overexpressed in human cancers and is a current target of anti-invasive therapies. Src activation is essential to produce invadopodia, cellular structures that mediate extracellular matrix (ECM) proteolysis. Invadopodia assembly is a multistep process, first requiring the targeting of actin-associated proteins to form pre-invadopodia. Pre-invadopodia subsequently mature by recruitment and activation of matrix metalloproteases (MMPs) that facilitate ECM degradation. We demonstrate that active, oncogenic Src alleles require the presence of a wild-type counterpart to induce ECM degradation and phosphorylation of the invadopodia regulatory protein cortactin. Distinct phosphotyrosine-based protein binding profiles in cells forming pre- and mature invadopodia were identified by SH2-domain array analysis. These results indicate that while elevated Src kinase activity is required to target actin-associated proteins to pre-invadopodia, regulated Src activity is required for invadopodia maturation and matrix degradation activity. Our findings describe a previously unappreciated role for proto-oncogenic Src in enabling the invasive activity of constitutively active Src alleles.

Lipopolysaccharide Hyperpolarizes The Guinea-Pig Airway Epithelium By Increasing Na⁺ Transport

Dodrill, Michael

Advisor: Jeffrey Fedan

The administration of lipopolysaccharide (LPS, 4 mg/kg, i.p.), which is involved in several airway inflammatory diseases, causes hyperpolarization of tracheal epithelium (Epi) at 18 h. This study set out to understand the mechanism(s) by which LPS causes hyperpolarization. In the isolated, perfused trachea preparation (IPT), LPS had no effect on epithelial ENaC or Na,K-ATPase transcription or ENaC abundance, but doubled Na,K-ATPase protein levels. In the presence of basolateral amphotericin B (AB, 7.5 μ M) to short-circuit the Na,K-pump, apical amiloride (10 μ M) inhibited transepithelial potential (Vt) to a greater extent in the LPS group compared to control. In the presence of apical AB to short-circuit ENaC, the resulting Vt, due to the Na,K-pump, was greater in the LPS group relative to control. In the Ussing chamber, readmission of K to the Epi Na-loaded by K omission led to a greater hyperpolarization after LPS compared to control. Apical trypsin (100 U/ml) added to activate ENaC, increased Vt to a similar degree in control and LPS-treated groups. Thus, LPS hyperpolarizes the Epi by increasing the activities of both ENaC and the Na,K-pump. This may reflect alteration in their regulation.

The effect of DISC1 mutation on PDE4 and cAMP/pCREB signaling

Lan, Xiao

Advisor: James O'Donnell

DISC1 and PDE4 are both high risk factors contributing to the pathogenesis and development of a variety of mental illnesses, such as depression and schizophrenia. Current studies demonstrate that DISC1 binds and modulates PDE4 as a scaffolding protein. However, the exact molecular mechanism underlying is poorly understood. This study determined whether DISC1 mutation affected the cAMP/pCREB signaling cascade, PDE4 expression or activity, especially the HARBS, which mainly contributes to the PDE4 inhibitor-mediated antidepressant effects. DISC1 mutation induced an increase in cAMP and pCREB levels in the homogenous mice. Simultaneously, PDE4 activity was decreased by 50% significantly in the brain. Analysis of PDE4 isoform showed the mutation did not change PDE4A variants (PDE4A1, PDE4A5), but decreased PDE4B variants (PDE4B1, B3, B4), as well as PDE4D variants (PDE4D3, D5) in the hippocampus of homogenous groups. Real-time PCR showed mRNA levels of PDE4B was not changed significantly in the hippocampus of homogenous mice compared with wild type. Binding assay revealed that HARBS was significantly decreased in the DISC1 mutated mice. Taken together, mutation of DISC1 decreased activity of PDE4, increased cAMP/pCREB signaling, and decreased PDE4B and PDE4D, but not PDE4A variants. Interestingly, HARBS was decreased by the mutation of DISC1. The study indicated that DISC1 strongly correlate with PDE4, especially HARBS, this provide more information for better understanding of mental illness and a new target for the psychiatric drug development.

Altered Airway Sensory Neuron Development in Rats Following Acute Early Postnatal Ozone Exposures Zellner, Leor

Advisor: Richard Dey

The environmental irritant ozone (O₃) plays a role in airway inflammation and is known to cause airway hyperresponsiveness especially in susceptible populations such as asthmatics and young children. Ozone exposures alter the expression of the inflammatory neuropeptide Substance P (SP) in sensory neurons of the vagal ganglia complex (VGC) that specifically project to the airways and innervate the tracheal epithelium. The effect of an acute ozone exposure on long-term postnatal development of VGC neurons has not been examined. The objective of this study was to determine if a single ozone exposure in early postnatal life would affect the number of sensory neurons in the VGC of Fisher344 rat pups. On postnatal day (PD) 5, green microspheres were instilled into the trachea to label nerve cell bodies in the VGC innervating the tracheal epithelium, and rats were exposed to either air or ozone (2ppm) for 3hrs. On PD10 and 15 (5 and 10 days post exposure) VGC were removed and dissociated. Isolated neurons were immunocytochemically stained for flow cytometry using the pan neuronal marker protein gene product 9.5 and SP, then counterstained with the nuclear stain DAPI to confirm whole neurons. The following cell populations were identified and quantified: total VGC neurons (TN), total airway neurons (TAN), and SP+ airway neurons (SP+AN). In PD10 rats the number of TN decreased from 9463 in air-exposed animals to 4673 in O₃-exposed pups while the number of TAN also decreased from 115 in air-exposed animals to 92 in O₃-exposed rats. SP+AN in PD10 pups decreased from 53 in air-exposed to 28 in O₃-exposed. In PD15 rat pups TN number decreased from 10107 after air exposure to 4938 following O₃. The number of TAN increased from 142 in air-exposed rats to 194 in O₃-exposed rats and SP+AN did not change between air (92) and O₃ (96) exposed rats. These results suggest airway neuron development may be regulated separately from non-airway sensory neurons following ozone exposures.

Postdoctoral Fellows

Inferring Predominant Pathways in Cellular Models of Breast Cancer Using Limited Sample Proteomic Profiling
Kulkarni, Yogesh

Advisor: David Klinke

The observation that onset and progression of many diseases arises from the interactions of a number of interconnected genes has shifted the drug discovery perspective from a molecule-centric to a network/pathway-centric approach. Clinical samples pose significant challenges for proteomic profiling, an attractive approach for identifying predominant pathways. The objective of this study was to determine if, in case of a sample that is insufficient to carry out a proteomic study with multiple replicates, the information obtained from a single gel replicate still provides insight into the predominant signaling pathway at work in a cell? A comparative proteomic analysis of total cell lysates was obtained from two cellular models of breast cancer, BT474 (HER2+/ER+) and SKBR3 (HER2+/ER-), using two-dimensional electrophoresis and MALDI-TOF mass spectrometry. Protein interaction networks and canonical pathways were extracted from the Ingenuity Pathway Knowledgebase (IPK) based on association with the observed pattern of differentially expressed proteins. Of the 304 spots that were picked, 167 protein spots were identified. A threshold of 1.5-fold was used to select 62 proteins used in

the analysis. IPK analysis suggested that metabolic pathways were highly associated with protein expression in SKBR3 cells while cell motility pathways were highly associated with BT474 cells. Inferred protein networks were confirmed by observing an up-regulation of IGF-1R and profilin in BT474 and up-regulation of Ras and enolase in SKBR3 using western blot. When interpreted in the context of prior information, our results suggest that the overall patterns of differential protein expression obtained from limited samples can still aid in clinical decision making by providing an estimate of the predominant pathways that underpin cellular phenotype.

Digitoxin synthetic analogs: a new hope for anti-cancer therapeutics?

Stueckle, Todd

Advisor: Yon Rojansakul

Recent epidemiological and in vitro research has shown that cardiac glycosides (CGs), normally used for congestive heart failure therapy, hold promise as anti-cancer therapy agents. Concerns remain, however, due to a narrow therapeutic window, low threshold for myocardia toxicity and few explanations for mechanism(s) of action at therapeutic doses. Our main objectives are to develop a new digitoxin analog for anti-cancer therapy and to identify a complete and rationale biological mechanism of action for CG anti-cancer activity. Using a de novo carbohydrate methodology, digitoxin analogs were synthesized and then tested for their anti-cancer activity using NCI multiple cell line screening and apoptosis structure-activity relationship analyses. For those analogs showing potent, broad spectrum cancer cell cytotoxicity, additional in vitro cell behavior assays including apoptosis, anoikis, angiogenesis, clonogenic survival, colony formation and migration were performed on NSCLC H460 cells to compare analog dose-response activity to digitoxin activity. To identify mechanism(s) of action for both digitoxin and GJ22, an identified potent synthetic analog, mRNA from unexposed and exposed H460 cells were subjected to a whole genome microarray analysis (~132K genes). GeneSpring GX followed by Ingenuity Pathway Analysis identified differentially expressed genes and significant changes in cancer cell signaling networks. Dose-response analysis of protein expression changes were performed using plasmid transfections, enzymatic inhibitors and Western Blot to further investigate anoikis cell signaling mechanisms. Our data suggests that GJ22, a Class III digitoxin analog, displays a 2 to 10-fold greater potency than digitoxin at increasing apoptotic and anoikis-related cell death. Similarly, GJ22 exhibits potent anti-angiogenic properties by decreasing capillary tube formation in both drug-exposed and H460 supernatant exposed HUVEC cells. Both digitoxin and GJ22 exposure at 25 nM caused differential gene expression in TRAIL, TNFR, PI3K, Ras/MAPK, p53 and inflammatory pathways. Moreover, GJ22 caused a greater number of significantly altered cell signaling pathways than digitoxin suggesting different mechanism(s) at the same dose. In conclusion, digitoxin and other cardiac glycoside analogs hold promise as novel anti-cancer therapeutic agents.

Enhanced Acute Responses to Inflammatory Stimuli at Early Stage of Microvessel Remodeling
Yuan, Dong
Advisor: Pingnian He

Vascular remodeling has been implicated in many chronic inflammatory diseases. This study aims to investigate the impact of chronic inflammation-induced vascular remodeling on the acute venular responses to inflammatory mediators. Experiments were conducted in mesenteric venules using a survival rat model. Microvessel permeability was determined by Lp measurements and changes in VE-Cadherin and F-actin were viewed with confocal images. In day 1, basal and platelet activating factor (PAF, 10 nM) stimulated Lp were measured in individually perfused venules under aseptic conditions. Then blood flow was resumed in the perfused vessel and rat was recovered from anesthesia. In day 4, the baseline Lp measured in the same vessel showed no significant changes from day 1, but vessel diameter increased $74 \pm 8\%$ ($n = 8$). When each vessel was exposed to PAF, the mean peak Lp reached 5.1 ± 1.2 times that of the response in day 1. VE-Cadherin staining at Lp peak showed frequent breaks with apparent gaps between endothelial cells (ECs), a pattern different from that observed in day 1. Phalloidin staining showed disorganized and fragmented F-actin in both ECs and pericytes. The venular intercellular junctions per circumference in day 1 showed no significant difference from the enlarged venules in day 4, indicating no increases in EC number. Our results demonstrated that the observed morphological changes 3 days after the initial stimulation did not change the basal Lp, but manifested augmented acute response to PAF, indicating that the cellular phenotype at early stage of vascular remodeling are capable of mediating acute exacerbated vascular dysfunction upon additional stimulation.

S1P Prevents PAF Induced Permeability Increases by Activation of Rac-1 Signaling in Intact Venules
Zhang, Gengqian
Advisor: He , Pingnian

S1P has been shown to enhance endothelial (EC) barrier function in vitro and prevent hyperpermeability in vivo. Our present study aims to identify the signaling mechanisms of S1P in regulation of microvessel permeability in intact microvessels using combined permeability measurements with confocal vascular structural analysis. Microvessel permeability was determined by measuring hydraulic conductivity (Lp). Rac-1-dependent signaling was examined using Rac 1 inhibitor NSC23766 (NSC, 200 μ M). NO was measured in DAF-2 loaded microvessels. EC gap formation and changes in VE-cadherin was examined with confocal microscopy using fluorescence microspheres or antibody staining. Results showed that preperfusing vessels with S1P abolished PAF-induced gap formation and VE-cadherin redistribution without affecting PAF-induced increases in EC $[Ca^{2+}]_i$ and NO production. Perfusing vessels with NSC did not affect basal Lp and VE-cadherin but abolished the inhibition of S1P on PAF-induced Lp increases, EC gap formation, and VE-cadherin disruption. In the presence of NSC and S1P, the peak Lp in response to PAF was restored to 7.2 ± 2 times that of the control and VE-cadherin showed no significant difference from that with PAF alone. These results provide the first evidence in intact microvessels that S1P inhibits permeability increases by Rac1 signaling on EC AJ, which is either downstream from or independent of the initial EC Ca^{2+} and NO signaling pathways. Supported by HL56237 and HL084338.

Residents/Clinical

A New Role for Community Pharmacists: InterACTS - An Interdisciplinary Approach to Colorectal Cancer
Demian, Cristina

Advisor:

Background: West Virginia has low colorectal cancer (CRC) screening rates and high morbidity and mortality. This NCI-funded pilot project utilized community pharmacists, often the most accessible community healthcare providers, to educate their non-compliant, average risk patients about the importance of undergoing guidelines-based screening. Currently, the health care system often does not allow sufficient time for patients and primary care providers to address primary and secondary prevention as well as acute and chronic care needs. Since community members have a high level of trust in their pharmacists, and community pharmacists are interested in expanding their practices to include more patient counseling services, beyond drug therapy management, this project proposed teaming with community pharmacists to develop, implement, and assess the feasibility of an innovative approach to educating patients and counseling them about CRC screening. Methods: The intervention strategy and the tools used in this project are based on the Theory of Planned Behavior (TPB). The literature suggests that patients' knowledge and beliefs, physician recommendation and the patient's ability to obtain transportation and overcome embarrassment (i.e., control beliefs) are predictive of screening uptake. These factors fit the construct of TPB. For the development of the counseling sessions and educational materials, the Transtheoretical Model (TTM) was utilized to explain and predict a patient's readiness to change behavior. In order to conduct counseling, pharmacists were trained in Motivational Interviewing (MI). By tailoring a counseling intervention that is appropriate to each patient's specific stage of readiness, the pharmacists would maximize the likelihood that the patient will progress from one stage to another, ultimately arriving at the action stage, resulting in the patient's decision to undergo CRC screening. Goals and Objectives: (1) To design, implement and establish the feasibility of a community pharmacist directed intervention to educate patients about CRC screening; (2) To determine if the intervention advanced patients' stage of change, as measured by improved knowledge about CRC screening and readiness to discuss screening with their primary care provider. Results: Twenty pharmacists were recruited to counsel 10 patients each. Two pharmacists withdrew before the start of the study. Eleven pharmacists counseled 80 patients. The poster includes a qualitative analysis of pharmacists' feedback about the intervention and preliminary quantitative analyses of patients' questionnaires. Conclusion: Unfortunately, only 3 pharmacists were able to meet the counseling goal. Reported recruitment barriers included lack of pharmacist's time, the surprising number of patients who were ineligible because they were compliant with screening guidelines, and the number of patients who did not keep appointments. Possible explanations will be discussed.

ADVANCED AGE ALONE AS CRITERIA FOR FULL TRAUMA ACTIVATION DOES NOT AFFECT MORTALITY

Engle, John

Advisor:

Objective: Trauma patients of advanced age have a higher mortality than younger patients with the same injury severity score (ISS). Therefore, it has been proposed that age alone be a criterion for the full trauma activation (P1). We hypothesize that this will not affect mortality in trauma patients of advanced age. **Methods:** All trauma patients over the age of 65 years, who presented to a level 1 trauma center over 2 years, were compared. For the protocol year (2005), patients were given P1 status based on advanced age alone. In the control year (2006), priority designation was based on criteria set by the American College of Surgeons (ACS). Differences in clinical characteristics and mortality were compared using the Wilcoxon rank sum and Pearson chi-square test with an alpha of 0.05 selected as the threshold of statistical significance. **Protocol year Control year P value Mean Mean Age 77.36 77.67 0.65 ISS 10.67 11.65 0.08 TRISS 0.914 0.907 0.05 GCS 13.8 13.6 0.27 LOS 6.03 5.4 0.85 ICU LOS 1.978 1.68 0.37 Vent Days 1.084 1.034 0.83 Results:** 584 patients were evaluated. In 2005, of the 320 patients admitted, 158 (49.3%) required full activation by advanced age alone per protocol. In 2006, of the 264 patients admitted, 30 (11.4%, $p < 0.0001$) required full activation. Clinical characteristics were similar for both cohorts (table). The mortality overall was 8.75% in the protocol year and 8.37% in the control year ($p = 0.87$). When comparing the overall mortality of either year, listed above, to the mortality seen in patients receiving full trauma activation based on age alone and not ACS criteria (7.87%), there was no statistical difference ($p = 0.76$ and 0.86 respectively). **Conclusion:** Age alone as criteria for full trauma activation does not affect mortality and it significantly increases the cost of caring for patients of advanced age.

Are Medical Professionals Aware of Quality Improvement Tools (QIT) currently used in medicine?

Jan, Azam

Advisor: Richard Vaughan

Introduction: New measures of improving Health care quality and safety are arising in field of medicine. These are being implemented by Managed Care Organizations claiming to reduce Medical care complications by at least 25% and more. We wanted to evaluate the knowledge and perceptions about QIT, of medical professionals who are responsible for delivering these measures. **Methods:** A questionnaire was distributed to physicians, students, nurses and others medical professionals. They were questioned about 3 QIT i.e. AHRQ (Agency for Health care Research and Quality), SCIP (Surgical Care Improvement Project) & NSQIP (National Surgical Quality Improvement Program). **Results:** Total participants were 60 with 29 males (48.33%) & 31 females (51.66%) with a mean age of 37. Attending physicians were 26.66% with mean age 45, Residents (35%) mean age 30, Nurses (15%) mean age 44, & 8 Students (13.33%) mean age 26.5. 36.6% were surgeons and performing surgeries used for AHRQ data collection. Only 40.5% of physicians had heard about AHRQ, and only 3 knew details about its 4 modules, use of data collection, and quality indicators. 4 physicians have been to AHRQ website and only 3 have used the data for research, none of them were attendings. 48% of the physicians had heard about SCIP and only 30% knew what outcome measures are included in it. 43.2% had heard about NSQIP and only 16% were aware of their institution's participation. 45.75% of physicians think patient outcome data should be public property. 40.5% were afraid they might be labeled as bad doctors if their outcome is below national average. 51.3% were worried that patient outcome data might be used against them by hospital/insurance. 21% of physicians first heard about it from formal teaching or colleagues and 8% from media. However, 91% of all participants think joining these agencies would help improve patient

care. 97% think they should share patient data for national comparison. 100% want to know more about these programs. 76% of all participants want QIT to be taught as part of surgery training curriculum and 16% were not sure. 74.5% would like to enroll in a program to provide them a 6 months comparison data for their patient vs. national outcome. Conclusion: QIT education should be part of education curriculum of medical training programs. Many doctors are anxious about misuse of the data.

New mutation in the PRP31 gene in a patient with dominant RP
Kresch, Zvi
Advisor: Vishy Ramamurthy

To report a new PRP31 mutation in a patient with dominant retinitis pigmentosa We describe the clinical and molecular findings in a patient with a history of early onset retinitis pigmentosa. She was first seen when she was 13 years old. Eleven other members of this family have been diagnosed with retinitis pigmentosa. An ERG, OCT and Goldmann visual fields were performed as part of her initial workup. DNA was sent to the eyeGENE network for molecular correlation. The patient's ERG showed non-recordable scotopic response and a severely decreased photopic response. Her OCT showed cystoid macular edema which improved with carbonic anhydrase inhibitors. Her Goldmann visual fields have shown progressive peripheral field loss. All results are consistent with a diagnosis of RP. The eyeGENE network examined 9 common dominant RP causing genes by amplified PCR. A new heterozygous mutation was identified in the PRP31 gene. PRP31 mutations are found in approximately 5.5 % of RP patients. This patient was diagnosed with an unreported mutation causing her disease. It has been previously reported that PRP31 mutations are often seen in familial mutation that skip generations as was seen in our patient's family. It is often observed that dominant RP is less severe than simplex RP and visual field and visual acuity are better preserved until later in life. However, our patient was affected with cystoid macular edema at a young age and at presentation had severe visual field constriction with a non-recordable scotopic ERG. She responded favorably to a combination of topical and oral carbonic anhydrase inhibitors. Phenotype-genotype correlation within this family may lead to better understanding of pathogenesis and retinal findings in patients with retinal degenerations. Supported by Research to Prevent Blindness

The Effects Of Directional Noise On Heading Precision In Age-Related Macular Degeneration (AMD)
Mali, Joshua
Advisor: Vernon Odom

The purpose of this study is to determine the efficacy of argon laser punctal stenosis in patients with contact lens-induced dry eyes. A retrospective review of 25 eyes of 13 patients who underwent argon laser punctal stenosis to improve their contact lens intolerance was performed. The mean age was 31

years (21-52) and 11 patients (85%) were female. The mean Schirmer I test was 15.2 (range, 3-35). All patients tolerated the procedure well. In 19 eyes, the treatment only involved the lower punctum, while in 6 eyes it involved both the upper and lower puncta. Eight patients required more than one treatment session (range, 2-6). At follow-up after 6 months, 10 of 13 (77%) patients reported a substantial improvement in their symptoms and contact lens wear time. Argon laser punctal stenosis provides a useful and titratable treatment for contact lens intolerance due to dry eyes.

Posterior Fossa Subdural Hematoma Resulting in Locked-in Syndrome

Sedney, Cara

Advisor: Julian Bailes

The Locked-in Syndrome (LIS) is a well-known and devastating clinical entity first described more than 200 years ago. It consists of complete inability to move or speak, but with intact consciousness. Stroke is known to be the most common cause of LIS, with trauma, usually from basilar artery occlusion, being a distant second. Recovery, more common in trauma patients than in stroke, is nevertheless a long process. This case report describes a 34 year old woman who suffered a fall with subsequent posterior fossa subdural hematoma. She was taken emergently for evacuation of the clot, but was found post-operatively to be locked-in. MRI of the brain demonstrated only a subtle area of hyperintensity in the posterior pons. Through the patient's convalescence in the hospital, she gradually regained function; by the time of discharge, she was able to move all extremities and mouth words. This represents a new etiology of LIS which was likely caused by direct compression of neural structures by the subdural hematoma. With prompt treatment of the underlying lesion, this patient demonstrated an accelerated recovery compared to other causes.

Development of an Animal Model for the Study of Keratoconjunctivitis Sicca

Saunders, Justin

Advisor:

Authors: Justin Saunders, M.D., Rachel L. Leon, Jason D. Huber, Ph.D., Charles L. Rosen M.D., Ph.D., H. James Williams, M.D., Jennifer Sivak-Calcott, M.D. Tear film is adversely affected by hormone replacement therapy (HRT), as evidenced by increased incidence of keratoconjunctivitis sicca (KCS) in women on HRT. Studies indicate that estrogen is the causative agent of HRT-induced KCS in combined estrogen-progestin HRT. Specifically, estrogen acts to decrease production of the aqueous tear component produced by serous cells in the lacrimal gland. In the present study, we hypothesized that chronic estrogen treatment would decrease tear production in aged female rats. We examined tear production in aged, ovariectomized female rats following nine months of estrogen or placebo treatment using a modified Schirmer's test. Results show that chronic estrogen exposure decreases tear production compared to placebo-treated rats. This difference in tear production was not accompanied by morphologic changes in lacrimal gland tissue. These findings confirm in an aged animal model, that estrogen supplementation may play a causal role in decreased tear production by the lacrimal gland. In conclusion, these results indicate that aged, chronically estrogen-treated female rats may prove a suitable model for the study of KCS and its treatment.

Impact Of Influenza Pandemic On Patients' Attitudes In Receiving Vaccinations In A Rural Pharmacy

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Objectives: To (1) characterize participants' interest in receiving vaccinations at pharmacies in West Virginia in 2009-10, (2) determine the effect of the H1N1 pandemic on patients' willingness to receive seasonal influenza vaccination at a rural community pharmacy in West Virginia, and (3) assess participants' satisfaction with pharmacist delivery of immunization services in a rural community pharmacy practice. Methods: A written survey has been developed and will be distributed to participants in two sections. The first half of the survey will be given pre-vaccination and will contain questions that would provide nominal data to determine participants' reasons for receiving vaccinations. The second part of the survey will be given immediately following vaccination and will utilize a Likert scale to measure participants' opinions of a pharmacist operated immunization service. The survey will be distributed at a rural, community pharmacy from October 2009- March 2010 to all participants (18 years of age and older) receiving the seasonal influenza and/or pneumococcal vaccine. Exclusion criteria include pregnancy, those with contraindication to the vaccines(s), and participants under the age of 18. The results of the survey will then be analyzed to assess the effect of the H1N1 virus on vaccination rates and characterize patient attitudes towards pharmacists as immunizers in rural West Virginia. Results: 25 participants completed the pre-vaccination survey and 23 participants completed the post-vaccination survey. Nearly half (48%) of participants receiving vaccinations responded that the H1N1 pandemic did not affect their decision to receive the seasonal influenza vaccine. Conclusion: Based on these results, it can be concluded that patients will continue to receive vaccines at the pharmacy even after the pandemic subsides This study provides valuable information

about the quality of care provided in a West Virginia rural pharmacy setting and patient's interest in receiving other types of vaccinations at their pharmacy

Predominance Of Brain And Lung Metastases In Triple-negative Breast Cancer Patients

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Background: Patients with triple-negative breast cancer have an increased likelihood of recurrence compared to other types of breast cancer, however, little is known about their pattern of metastatic spread. Our object was to evaluate the metastatic patterns of women diagnosed with triple-negative breast cancer compared to other subtypes. Methods: We studied a cohort of 572 white patients diagnosed with invasive breast cancer at West Virginia University Hospital between 1999 and 2004. Hospital registry, charts, and pathology records provided clinical data including tumor receptor status and biopsy-proven metastatic spread to bone, brain, liver and lung. Breast cancers that were negative for estrogen, progesterone, and HER2neu, otherwise known as triple-negative were compared with HER2neu-positive and HER2neu-negative (endocrine receptor positive) disease. Body mass index was calculated and a value of ≥ 30 considered indicative of obesity. Specimens of primary carcinoma were available for analysis of Ki67 mitotic index and expression of p53. Results: 134/572 (23.4%) had triple-negative breast cancer, while the frequencies were 108/572 (18.9%) and 330/572 (57.7%) in HER2neu-positive and HER2neu-negative (endocrine receptor positive) groups. Women with triple-negative disease were more likely to have brain-metastasizing breast cancer; 10.5% versus 4.6% for HER2neu-positive and 3.3% for HER2neu-negative ($P < 0.05$). They were also more likely to have metastasis to the lung; 10.5% versus 2.8% for HER2neu-positive and 7.0% for HER2neu-negative ($P < 0.05$). Triple-negative breast cancer patients who developed brain and lung metastases were younger < 50 years and significantly more obese ($P = 0.0236$). High Ki67 labeling index and p53 expression were associated with more advanced disease indicating an aggressive phenotype for this group. Conclusion: The excess risk of brain and lung metastasis in women with triple-negative breast cancers versus other subtypes needs further validation. The unique biology of triple-negative tumors may explain this pattern of metastatic spread. Presented at the 2009 AACR San Antonio Breast Cancer Symposium