31st Annual Graduate Research Conference

31st Annual UMB Graduate Research Conference

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Foreword by GSA Executive Board

Welcome to the 31st Annual Graduate Research Conference (GRC)! The Graduate Student Association of the University of Maryland, Baltimore (UMB) has been dedicated to this project since the beginning of the school year. Each year, the GRC familiarizes graduate students with preparing for scientific meetings, as well as the opportunity to present results of their ongoing research in an interdisciplinary setting to peers, faculty members, and the UMB community at large. Approximately 50 students from across the UMB campus will present their work as either poster or oral presentations at this GRC, and we would like to thank each of the presenters for their time and effort to formally communicate their achievements – we commend your hard work and devotion to your science.

This year we will continue to take an interdisciplinary approach to the conference which highlights research across fields and even schools. We hope this will continue to enrich the students' experience as well as challenge them to apply their work to a new and broader audience. This year, GRC participants also have the opportunity to be considered for two special awards. The Geriatrics and Gerontology Education and Research Program (GGEAR) will be sponsoring awards in aging research to graduate students who have either completed research or have research in progress in Social science/behavioral/clinical research or Bio-medical/basic science research in the field of aging. The Office of Commercial Ventures & Intellectual Property (CVIP), in association with the Graduate Research Conference, is delighted to announce the first annual CVIP Translational Graduate Research Award. The award is made in recognition of important translational research performed by a UMB graduate student and encompasses a wide array of disciplines with biomedical or other practical applications.

The Graduate Student Association Executive Board would like to thank everyone who has contributed to this year's conference. Specifically, we would like to acknowledge Dr. Malinda Orlin, Vice President for Academic Affairs and Dean of the UMB Graduate School; Dr. Erin Golembewski, Associate Dean of the UMB Graduate School; and all the members of the UMB Graduate School Office. Additionally, we would like to thank the faculty members who have volunteered their time to serve as judges and mentors – your dedication to the advancement of your students here today, and everyday, is greatly appreciated. Thank you to the GSA Program Representative volunteers for your dedication, energy, and initiative. Finally, we would like to acknowledge the Graduate Program in Life Sciences (GPILS) and Dr. McCarthy for sponsoring our keynote speaker Mr. Merrill Goozner, who we are privileged to have here today.

We hope that you enjoy your experience at this year's GRC. We have worked hard to make the day as enjoyable and informative as possible. We invite you to participate fully in this year's conference and we look forward to welcoming you back next year. It has truly been a privilege and honor to provide a colloquium for all graduate students of the UMB community to present their achievements.

Graduate Student Association Executive Board
Maya Matheny, President
Jade Bernstein, Vice-President
Melissa Hayes, Treasurer
Khandra Sears, Secretary
Jim Nicholson, Graduate Council Representative

31st Annual Graduate Research

Conference Keynote Speaker

MR. MERRILL GOOZNER

Director, Integrity in Science Project

MERRILL GOOZNER spent more than 25 years in the news business as a foreign correspondent, economics writer and investigative reporter for the Chicago Tribune and other publications. He reported from over a dozen countries while posted in Chicago, Tokyo, New York and Washington. His jobs included Chief Asia Correspondent (1991-95), New York Financial Correspondent (1996-1998) and Chief Economics Correspondent (1998-2000). Like Benjamin Franklin, he spent his early years in the printing trade.

His freelance writing in recent years has appeared in numerous national publications including the New York Times, Washington Post, The Scientist, Columbia Journalism Review, The Nation, The American Prospect and the Washington Monthly. He's won six Peter Lisagor awards; a Michigan Journalism Fellowship (1995-96); and a Kaiser Media Fellowship (2001-02), and in 2008 was named a Distinguished Alumni of the University of Cincinnati College of Arts and Sciences. In April 2004, the University of California Press published his first book, "The $800 Million Pill: The Truth Behind the Cost of New Drugs," an exposé of the pharmaceutical industry's research and development practices.

Goozner left daily journalism in June 2000 to teach journalism at New York University. In December 2003, he joined the Center for Science in the Public Interest as director of the Integrity in Science project, where he continues his research and writing.

SCHEDULE OF EVENTS

31st Annual Graduate Research Conference

Friday, April 3, 2009

University of Maryland, Baltimore

8:00-9:00am	BREAKFAST & REGISTRATION
9:00am-12:00pm	PRESENTATIONS
12:00-1:30pm	LUNCH & KEYNOTE ADDRESS: MR. MERRILL GOOZNER *DIRECTOR, INTEGRITY IN SCIENCE PROJECT*
1:30-2:30pm	AWARDS RECEPTION

Abstracts

1. **SMALL MOLECULES BOUND TO UNIQUE SITES IN THE TARGET PROTEIN BINDING CLEFT OF CALCIUM-BOUND S100B AS CHARACTERIZED BY NUCLEAR MAGNETIC RESONANCE (NMR) AND X-RAY CRYSTALLOGRAPHY**

Thomas H. Charpentier, Paul T. Wilder, Melissa A. Liriano, Kristen M. Varney, Edwin Pozharski, Alexander D. MacKerell Jr., Andrew Coop, Eric A. Toth,* and David J. Weber*

Oral Presentation; Law/SSW Building 205

Structural studies are part of a rational drug design program underway to inhibit the S100B-p53 interaction and restore wild-type p53 function in malignant melanoma. To this end, structures of three compounds (SBi132, SBi1279, and SBi523) bound to Ca2+-S100B were determined here by X-ray crystallography at 2.10 Å (Rfree = 0.246), 1.98 Å (Rfree = 0.279) and 1.90 Å (Rfree = 0.228) resolution, respectively. Upon comparison, SBi132, SBi279, and SBi523 were found to bind in distinct locations/orientations within the hydrophobic target binding pocket of Ca2+-S100B with minimal structural changes observed for the protein upon complex formation with each compound. Specifically, SBi132 binds nearby residues in loop 2 (His42, Phe43, Leu44) and helix 4 (Met79, Ala83, Cys84 and Phe87); whereas, SBi523 interacts with a separate site defined by residues only within loop 2 (Ser41, His42, Phe43, Leu44, Glu45 and Glu46). The SBi279 binding site on Ca2+-S100B overlaps the SBi132 and SBi523 sites and contacts residues in both loop 2 (His42, Phe43, Glu45) and helix 4 (Ala83 and Phe87). NMR data, including saturation transfer difference (STD) and 15N backbone and 13C sidechain chemical shift perturbations, were consistent with the X-ray crystal structures and demonstrated the relevance of all three small molecule-S100B complexes in solution. The discovery that SBi132, SBi279, and SBi523 bind to proximal sites on Ca2+-S100B could be useful for the development of a new class of molecule(s) that interacts with one or more of these binding sites simultaneously. Such a molecule could provide a tight binding inhibitor that is specific for blocking protein-protein interactions involving S100B.

2. **BORDETELLA PERTUSSIS AND PERTUSSIS TOXIN INCREASE PATHOGENICITY OF A SUBSEQUENT INFLUENZA VIRUS INFECTION**

Victor Ayala

Oral Presentation; Law/SSW Building 205

Pertussis, an acute respiratory disease, is frequently complicated by coinfection with one or more respiratory viruses. The contributing factors for this condition, however, are not yet known. In this study, we performed influenza virus infections in mice previously infected with Bordetella pertussis (WT) or pretreated with pertussis toxin (PT), a virulence factor of B. pertussis. Intranasal inoculation with WT but not a mutant strain deficient for pertussis toxin (ΔPT) significantly increased lung viral titers and tissue damage caused by the virus infection. Pretreatment with PT was able to replicate the effects of the WT infection and was also shown to increase mortality by more than 50%, suggesting that the toxin mediated exacerbation of disease. Since lung viral titers were highest early after virus infection, we hypothesized that PT suppresses innate immune defenses such as type I interferons (IFN). Tests measuring the production of type I IFNs in-vitro showed that PT treatment suppressed IFN bioactivity early after influenza infection, however, normal levels were restored by 48 hrs. In vivo studies using mice demonstrated that PT treatment had no effect on IFN-γ or IFN bioactivity released into the airways. In mice solely infected with the bacterial strains WT or ΔPT, the presence of PT actually increased IFN bioactivity. To further investigate the effect of PT in our mouse model we performed a whole-transcript gene array on whole lung tissue from animals pretreated with PT and infected with influenza. From the 28,000 genes analyzed, we found over 1000 genes were differentially expressed. Type I IFNs were unaffected but many interferon

stimulated genes were, including genes involved in pathogen recognition, antigen presentation and virus control. Together these data suggest that B. pertussis through PT is targeting the type I IFN response to exacerbate subsequent viral infections.

3. IL-4 RECEPTOR ± (IL4R±) AND STAT6 REGULATE THE SEVERITY OF INFLAMMATION BUT ARE NOT ABSOLUTELY REQUIRED IN A MURINE MODEL OF ASTHMA

Preeta Dasgupta, Svetlana Chapoval, Elizabeth Smith, Nancy Noben-Trauth, Ann Kelly-Welch, Achsah D. Keegan

Oral Presentation; Law/SSW Building 205

T helper type-2 (Th2) cells, their cytokines (IL-4, IL-5, IL-13) and STAT6 are critical in inducing allergic lung inflammation, eosinophilia and mucus hypersecretion. It has been shown recently that IL-4 and IL-13 drive alternative activation of macrophages as well. However, the precise roles of IL-4-activated signaling molecules in modulating specific features of airway inflammation remain unclear. In this study, we adoptively transferred in vivo-primed CD4+ T cells into RAG2 KO, IL4RαxRAG2 KO or STAT6xRAG2 KO mice to examine the role of IL-4 and STAT6 signaling pathways in inflammation, using an ovalbumin-induced asthma model. We observed that although the level of airway inflammation and eosinophilia was significantly lower compared to RAG2 KO mice, eosinophils were still recruited to the lungs even in absence of STAT6 and IL4Rα. In mice deficient of STAT6 and IL4Rα, eosinophils preferentially accumulated in the lung parenchyma instead of around airways and blood vessels. Surprisingly, the decrease in eosinophil numbers was countered by an increase in mononuclear cells. However, mucus production by epithelial cells and expression of alternative activated macrophage genes such as FIZZ1 and YM1 were completely dependent on signaling downstream of IL4Rα through STAT6. These results suggest that IL4Rα and STAT6 contribute to inflammation but are not absolutely required. Signaling through the IRS-2 pathway or other IL-4-independent pathways may be responsible for this increase in disease severity. Further experiments using in vivo-primed T cells are required to elucidate the role of IL-4 signaling pathways in asthma.
[Supported by PHS Grant AI38985]

4. NURSE SHARK IGNAR PHAGE DISPLAY CLONES SPECIFIC FOR HIV GLYCOPROTEIN, GP120 S CD4-INDUCED CONFORMATION

Doremus C, Dooley H, Guan Y, DeVico AL, Lewis GK, Flajnik MF

Oral Presentation; Law/SSW Building 205

HIV entry into cells requires interaction of the virus surface glycoprotein, gp120, with CD4, causing gp120 to undergo a conformational change and exposing its chemokine receptor binding site. The structure of gp120 is thought to hinder production of neutralizing antibodies to the virus: the protein can be highly mutated and display diverse epitopes even in single infections, and conserved regions are either masked by heavy glycosylation or require conformational changes to be exposed. Even if conventional virus specific antibodies are generated, their large size may prevent them from accessing the exposed regions during viral entry. Cartilaginous fish possess a unique class of antibody, IgNAR (new antigen receptor), which consists only of two dimerized heavy chains that do not associate with light chains. IgNAR single-domain V regions are highly mutated by antigen-driven somatic hypermutation and are capable of recognizing antigen. These V domains, if expressed alone, may be able to fit into the space between the gp120 CD4 induced exposed epitope and the chemokine receptor, and have access to regions that typical antibodies containing heavy and light chains do not. In order to examine this, a phage display library was made of NARV regions from a nurse shark immunized to a fusion protein consisting of gp120 in its CD4-induced conformation (CD4i), coupled to the first two domains of CD4. The

antigen-reactive V regions examined fell into several families based on CDR3 signatures, each with various levels of mutation. Some Vs recognized native gp120, while others were specific for CD4i. The CD4i-specific V regions had long, diverse CDR3s enriched in Asp/Glu residues, and phages displaying these regions showed competitive binding with conventional CD4i-specific antibodies by ELISA. Fusion proteins of CD4i specific V regions displayed on human IgG Fc are currently being made to test their ability to block HIV infection.

5. **THE GROWTH COMPROMISED HSV-2 MUTANT DELTA-PK MEDIATES MELANOMA ONCOLYSIS THROUGH ACTIVATION OF MULTIPLE NON-REDUNDANT PROGRAMMED CELL DEATH PATHWAYS.**

Aric G. Colunga, Jennifer M. Laing, and Laure Aurelian

Oral Presentation; Law/SSW Building 205

Naturally occurring and molecularly developed oncolytic viruses are increasingly recognized as a novel therapeutic modality, that functions through selective replication and direct lysis of cancer cells (virotherapy). Despite its promise, cumulative data indicate that virotherapy has limited efficacy, underscoring the need for strategies that function through distinct molecular mechanisms. We report that the growth compromised HSV-2 vector PK kills melanoma cells in culture and in xenograft models through a potent bystander effect that consists of the activation of multiple non-redundant programmed cell death (PCD) pathways. PK is deleted in the kinase domain of the large subunit of ribonucleotide reductase (ICP10PK) that activates Ras and its downstream signaling pathways (Raf/MEK/ERK and PI3-K/Akt). Because these pathways are activated in melanoma, they should compensate for the loss of ICP10PK thereby providing for selective virus growth in these, as compared to normal cells. This interpretation was confirmed by growth studies. Thus, PK was replication compromised in Vero cells both temporally (growth began at 12hrs pi) and in terms of its burst size (1.1 0.1pfu/cell). Ras signaling in melanoma removed the temporal restriction (growth began at 4hrs pi), but the maximal burst size was similar to that in Vero (1.1 0.2pfu/cell). The % cells staining with antibody to the major capsid protein VP5 (indicative of virus growth) was significantly lower (16 1%) than the % dead cells (85-95% trypan blue or EtHD+ cells). The ratio of dead cells (trypan blue or EtHD+) to cells supporting virus growth (VP5+) was 1.8-4.1 for the different cultures at 24-72hrs p.i, with an average of 2.8, indicative of a robust bystander effect. Only a small percentage of the bystander effect was due to canonical apoptosis determined by TUNEL (12.4 1.1% TUNEL+ cells), suggesting that PK-induced oncolysis is primarily through alternate PCD mechanisms. Western blotting indicated that PK activated calpain and caspases-7 and -3 in a sequential and apparently independent manner. Indeed, cell death was partially reduced by treatment with the calpain inhibitor PD150606 (30-50% EtHD+) or pancaspase inhibitor zVAD-fmk (35-50% EtHD+) but it was fully abrogated by their combination, indicating that the two protease families contribute additively towards cell death. Strong inhibition of tumor growth was observed in A2058, A375, and LM xenografts after four once-weekly intratumoral injections of PK (10^6-10^7 pfu). Fifty days after treatment termination, LM xenografts evidenced nearly complete eradication (80% tumor free), and 100% survival (Kaplan-Meier survival analysis). This was associated with activation of calpain and caspases-7 and -3, upregulation of the apoptotic protein H11/HspB8 and the autophagy protein Beclin-1 that also functions as a tumor suppressor and the activation of caspase-1 related inflammatory processes. The data indicate that PK mediates melanoma oncolysis through activation of multiple non-redundant death pathways and is a promising anticancer virotherapeutic.

6. **MATERNAL HYPOXIA GENERATES REACTIVE OXYGEN SPECIES IN FETAL GUINEA PIG LEFT VENTRICLES**

LaShauna Evans and Loren P. Thompson, Ph.D.

Oral Presentation; Law/SSW Building 205

Recent studies suggest that intrauterine hypoxia can predispose the offspring to cardiovascular disease through fetal programming mechanisms. We have previously shown that by exposing the fetus to low oxygen levels nitric oxide (NO) synthesis in fetal left ventricles is increased via upregulation of inducible nitric oxide synthase. Other synthetic pathways, such as the generation of reactive oxygen species (ROS), may also be activated by conditions of chronic hypoxia. Excessive ROS levels have been shown to cause injury to the heart by mutating DNA, inactivating proteins, and inducing lipid peroxidation. The aim of this study is to investigate the effect of intrauterine hypoxia on ROS generation in the fetal heart. Malondialdehyde (MDA), a marker for lipid peroxidation, and aconitase activity, an enzyme inhibited by ROS, are used as indices of ROS production. Methods: Pregnant guinea pigs were exposed to room air (NMX) (N=5) or 10.5%O2 (HPX) (N=5) for 14d prior to term (term=65d). At 60d gestation, near term fetuses were removed via hysterotomy from anesthetized sows. Fetal hearts were excised, left ventricles were frozen in liquid N2, and stored at -80C. MDA and aconitase activity were measured with commercially available assay kits. Results: Chronic HPX increased fetal cardiac (P=0.024) MDA levels by 42% compared to NMX controls (0.0012+0.0001 vs 0.0017+0.0002 umol MDA/mg protein). In the same tissues, hypoxia decreased (P=0.034) aconitase activity by 38% compared to NMX controls (9.0+0.8 vs 5.6+0.9 nmol/min/mg protein). These results demonstrate a hypoxia-induced increase in ROS generation in fetal ventricles. This suggests that, under low oxygen conditions, the fetal heart may be susceptible to injury when reactive molecules such as ROS and NO are elevated. This is likely to have both an immediate impact on fetal cardiac function, as well as, lasting effects on the offspring due to programming. [(NIH HL90044/LE) & NIH HL49999/LT)]

7. **COORDINATING THE INITIAL STEPS OF BASE EXCISION REPAIR: AP ENDONUCLEASE 1 ACTIVELY STIMULATES THYMINE DNA GLYCOSYLASE.**

Megan E. Fitzgerald and Alexander C. Drohat

Oral Presentation; Law/SSW Building 302

DNA glycosylases initiate base excision repair by removing damaged or mismatched bases, producing an abasic (AP) site in the DNA. Many glycosylases bind the AP-DNA product tightly, impeding enzymatic turnover. Human thymine DNA glycosylase (hTDG), which recognizes G•T mispairs and other mutagenic lesions, exhibits severe product inhibition, precluding the use of steady-state kinetics to study its catalytic mechanism. To overcome this problem, we developed a coupled enzyme assay, monitored by fluorescence, where the second enzyme in the BER pathway, human AP endonuclease (hAPE1), stimulates the turnover of hTDG. We determined the steady-state kinetic parameters for hTDG, and its catalytic core (hTDG-core, Phe111 to Val308) against a G•U, G•T, and G•U5Fsubstrate. We have also obtained steady state rates of hTDG using burst kinetic experiments, accurately quantifying kcat in the absence of hAPE1 for the first time. In most cases, hTDG turnover is greatly stimulated by hAPE1. For example, using the uracil substrate, we find that hAPE1 enhances the steady state turnover (kcat) of hTDG and hTDG-core by about 26-fold and 77-fold, respectively. But, there are differences in kcat for each substrate, indicating the excised base remains trapped in the active site complex by AP-DNA. Also, we have concluded that hAPE1 actively displaces AP DNA from hTDG and hTDG-core.

8. **THERMODYNAMIC AND KINETIC CHARACTERIZATION OF BINDING STOICHIOMETRY AND MISMATCH REPAIR ACTIVITY BY HUMAN THYMINE DNA GLYCOSYLASE**

Michael T. Morgan and Alexander C. Drohat

Oral Presentation; Law/SSW Building 302

Human thymine DNA glycosylase (TDG) is involved in efficiently repairing G:T and other mismatches through the base excision repair pathway. Our recent crystal structure revealed the catalytic core of TDG bound to abasic DNA in a 2:1 (protein:DNA) complex. The N-terminal domain of TDG promotes the 2:1 complex and enhances the activity for G:T lesions. Here we investigate the relationship between 2:1 binding and T removal. Our work seeks to understand the mechanisms of this dimerization event, the substrate characteristics that favor 1:1 or 2:1 binding, the role of the N-terminus in mediating such an event, and how the second subunit affects activity of the productively bound subunit. We find that, as compared with U, T removal is more sharply diminished when the substrate is only capable of binding a single copy of TDG. Furthermore, we find that both subunits are catalytically active, and the activity depends on the number of bases between two mispairs. ITC experiments show that TDG binding is significantly less favorable for DNA that forms a 1:1 versus a 2:1 complex , and this effect is greater for G:T versus G:U substrates.

9. **ALTERED HEMATOPOIESIS IN THE TS65DN MOUSE MODEL OF DOWN SYNDROME**

Laureanne Pilar E. Lorenzo, Sarah Clark, Paul Yarowsky and Mark S. Williams

Oral Presentation; Law/SSW Building 302

Down Syndrome is a genetic disease primarily caused by a triplication of chromosome 21. It occurs in approximately 1 in 700 births and is characterized by certain facial features, impairment of cognitive ability, congenital heart disease, hearing deficits, Alzheimer's disease, and hematologic abnormalities. The hematologic alterations in individuals with Down Syndrome include increased red cell volume, thrombosis in infancy, transient myeloproliferative disease, and leukemia. These hematologic abnormalities imply that the hematopoietic system is altered in individuals with Down Syndrome, therefore our overall goal is to determine how changes in hematopoietic stem cells (HSCs) or other hematopoietic progenitors may be involved in the pathogenesis of this disease. Several studies have linked elevated levels of reactive oxygen species (ROS) and oxidative stress to the pathology of Down Syndrome. It is hypothesized that overexpression of the antioxidant protein superoxide dismutase 1 (SOD1) by the triplication of chromosome 21 paradoxically results in increased ROS levels and oxidative stress, by an increase in the production of the ROS hydrogen peroxide (H202). The Ts65Dn mouse model of Down Syndrome is trisomic for a segment of mouse chromosome 16 that is syntenic to human chromosome 21, including the gene encoding SOD1. We hypothesize that there are increased ROS levels in HSCs and hematopoietic progenitors from the Ts65Dn mouse, and that altered redox balance leads to changes in proliferation, self-renewal, differentiation, and survival of progenitor cells. Hematopoietic progenitor phenotype was analyzed by flow cytometry. Hematopoietic progenitor differentiation potential was assessed by colony forming assays and proliferation was determined with 5-bromo-2-deoxyuridine (BrdU) and CFSE. Apoptosis was measured by active caspase 3 and p53 expression. We have found that Ts65Dn mice have fewer HSCs, common lymphoid progenitors (CLPs) and megakaryocyte-erythroid progenitors (MEPs), but more granulocyte-monocyte progenitors (GMPs) in comparison to wild-type mice. This may correlate with myeloproliferative disease observed in individuals with Down syndrome. When cultured in vitro, Ts65Dn bone marrow cells exhibited decreased proliferation and increased apoptosis in comparison to wild-

type bone marrow cells. Future directions include the assessment of ROS levels and self-renewal ability of Ts65Dn hematopoietic progenitors and analysis of SOD1-deficient mice to determine if elevated SOD1 levels are responsible for the pathology observed in Down Syndrome.

10. CELL-TYPE SPECIFIC PAR2 ACTIVATION DIFFERENTIALLY REGULATES TLR4 SIGNALING IN MUCOSAL EPITHELIAL CELLS AND MACROPHAGES

Quan M. Nhu, Kari Ann Shirey, Sarah Netzel-Arnett, Aiping Zhao, Toni Antalis, Terez Shea-Donahue, Alessio Fasano, Stefanie N. Vogel

Oral Presentation; Law/SSW Building 302

Proteinase-activated receptor 2 (PAR2), a 7-transmembrane G-protein-coupled receptor, contributes to inflammation positively or negatively in different experimental models. We hypothesized that during tissue injury and infection, coordinated activation of PAR2 in functionally distinct innate immune cell types might produce different inflammatory outcomes. To this end, we examined the functional effects of signaling cross-talk between PAR2 and Toll-like receptor 4 (TLR4), the cellular sensor for LPS derived from most Gram-negative bacteria, in two developmentally distinct innate immune cell types: mucosal epithelial cells and macrophages. Human colonic epithelial SW620 cells responded to PAR2 AP or the TLR4 agonist LPS with IL-8 expression; combined treatment augmented IL-8 production synergistically. Similarly, murine intestinal epithelial CMT-93 cells were responsive to stimulation with AP or LPS, and co-stimulation synergistically enhanced gene expression of the chemokines, KC and MIP-2α. In contrast, in thioglycollate-elicited murine peritoneal macrophages, AP enhanced LPS-induced IL-10 expression, yet suppressed LPS-induced expression of the pro-inflammatory cytokines TNF-□, IL-6, and IL-12. PAR2-/- macrophages exhibited diminished LPS-induced expression of IL-10, while displaying increased KC expression. PAR2-/- macrophages also exhibited decreased STAT-3 phosphorylation, a downstream target of IL-10. We propose a model in which PAR2 on the mucosal epithelium, when activated during tissue injury and infection, promotes the generation of a chemotactic milieu for the recruitment of immune cells, whereas PAR2 on macrophages maintains tissue homeostasis by dampening inflammation. Such differential functional utilization of PAR2 in mucosal epithelial cells and macrophages highlights the complex hierarchical organization of the innate immune system.

11. OVARIECTOMY SHIFTS THE SITE OF ESTRADIOL-INDUCED VASCULAR ENDOTHELIAL GROWTH FACTOR mRNA EXPRESSION IN THE MOUSE UTERUS FROM LUMINAL EPITHELIAL CELLS TO STROMAL CELLS

Kristin Happ Molitoris, Armina A. Kazi, Robert D. Koos

Oral Presentation; Law/SSW Building 302

In the uterine endometrium, 17β-estradiol (E2) is the master regulator of gene transcription, acting primarily through its receptor (ERα). The biological hallmark of E2's effects is a wave of luminal epithelial (LE) cell proliferation. We have previously shown that this is preceded by a large increase in vascular endothelial growth factor (VEGF) expression in the uterus, resulting in increased stromal microvascular permeability, and that this involves the simultaneous recruitment of hypoxia-inducible factor 1 (HIF-1) and ERα to the VEGF promoter. Recent work from our laboratory has confirmed that these events occur in the LE cells of the endometrium (Kazi et al., 2009). Some studies, however, have found that E2-induced expression of VEGF occurs in the stroma rather than LE cells. One difference is that we used intact immature rats whereas those studies were done using ovariectomized (ovx) rats or mice, which suggested that removing the ovaries might shift the site of E2-induced VEGF expression from the LE cells to the

stroma. Interestingly, the ovx mouse model was also used in some well-known studies that identified stromal cells as the site through which E2 acts to induce LE cell proliferation in target tissues. An ovariectomy-induced shift in the site of E2-induced VEGF expression, which we believe plays a central and essential role in E2-induced LE cell proliferation through its effects on microvascular permeability, could explain why stromal cells appear to mediate that proliferation when ovx animals are used. To address this question, we separated the LE cell fraction from the uterine stroma of both intact and ovx female rats and intact and ovx female mice at intervals after E2 treatment and examined VEGF expression in both compartments using quantitative real-time RT-PCR. Measurement of progesterone receptor (PR) mRNA, which is expressed only in the stroma in response to E2, was increased in residual tissue only, confirming the purity of the LE cell fraction. As expected, E2 strongly stimulated VEGF mRNA expression in LE cells in both intact immature mice and rats by 1.5 h, with a return to basal levels by 4 h. Interestingly, the identical response was observed in ovx rats. In ovx mice, however, LE cells were almost completely devoid of VEGF message before and at both times points after E2 treatment. A marked increase in VEGF mRNA was still seen, however, in the residual uterine tissue, which contains the stromal cells, by 1.5 h. In contrast to the normal transient induction in LE cells, this apparent stromal expression remained elevated at 4 h. Thus, removal of the ovaries in mice does shift the site of E2-induced VEGF expression from the LE cells to the stroma, which we believe explains why stromal cells, rather than the LE cells, have been identified as mediating E2-induced LE cell proliferation. In conclusion, we propose that VEGF, through its potent ability to increase microvascular permeability and expose LE cells to plasma growth factors, is the key “estromedin” mediating E2-induced LE cell proliferation. In rats and intact mice, the VEGF arises in the LE cells themselves, but in ovx mice it is produced by stromal cells. These results have significant implications for our understanding of how E2 induces the proliferation of both normal epithelial cells and transformed carcinoma cells. This work was supported by NIH/NICHD U54 HD36207, RO1 HD047275, and NHLBI/NIH Institutional Training Grant HL72751 (KHM).

12. GENETIC VARIATIONS IN THE 6P24-21 GENOMIC REGION THAT AFFECTS THE EYE TRACKING ABILITY IN SCHIZOPHRENIA PATIENTS

Nithin Krishna, Ikwunga Wonodi,Elliot Hong and Gunvant Thaker

Oral Presentation; Law/SSW Building 302

Smooth pursuit eye movement (SPEM) is one of the most established intermediate phenotypes linked to schizophrenia. Two linkage studies implicate chromosome 6p21-24 as a candidate locus for the oculomotor deficit; other linkage studies that used schizophrenia diagnosis as a phenotype have also implicated this region. This genomic region is of interest in developmental dyslexia, a disorder associated with motion processing and SPEM abnormality. In the current project we examined (i) phenotypic overlap between reading ability and the predictive pursuit gain (PPG) subcomponent of SPEM; and, (ii) effects of select candidate genes within 6p24-21 on PPG. We selected candidate genes based on findings of association with schizophrenia (DTNBP1), dyslexia (TTRAP, KIAA0319, DCDC2), or their role in GABA pathway (ALDH5A1). Schizophrenia (SZ) patients (n=175) and matched healthy control (HC) subjects (n=171) both between the ages of 15-58 years participated in the study. Eye tracking was performed using standard methods; PPG was measured when the moving target became briefly invisible. We selected 13 SNPs covering the 5 genes that were independent (not correlated) and resulting genotypes were frequent enough (i.e., > 5%) in our sample. Analyses used ANOVAs to examine the effects of the

genotypes on PPG; the p values were adjusted using the false discovery rate (FDR) adjustment.
Examination of reading ability in a small sample of schizophrenia patients showed a significant correlation between Nelson reading speed and PPG ($r=0.57$, $p<0.05$, n-17). The genetic analyses showed that, there was a significant ALDH5A1 rs2328824 genotype by diagnosis interaction; GG (minor genotype) was associated with poor PPG compared with AG in SZ, while AA showed significantly better PPG than AG in HC. Variation in a dyslexia related gene, TTRAP, significantly affected PPG such that the major homozygous was associated with worst PPG. Lastly, there was a trend for DCDC2 genotype by diagnosis interaction effect on PPG ($p=0.06$ after adjusting for FDR). Our data suggest that two genes within 6p24-21, ALDH5A1 and TTRAP are associated with SPEM deficit. ALDH5A1 encodes an essential enzyme required for degradation of gamma-aminobutyric acid (GABA). Whereas, TTRAP is a dyslexia related candidate gene. In our sample, preliminary data from a small subgroup showed that patients with poor PPG perform poorly on a reading test.

13. CHARACTERIZATION OF THE ESSENTIAL ACTIVITIES OF SACCHAROMYCES CEREVISIAE MTR4P

Jade Bernstein, Dimeka Patterson, Jeff Ballin, Gerald Wilson, Eric Toth

Poster Presentation; Westminster Hall

Accurate processing of precursor RNA and timely degradation of aberrant RNAs is crucial for proper cell function. Ribosomal, small nuclear and small nucleolar RNAs are all initially synthesized as long precursors, which must then be trimmed to form functional RNAs[1]. Any byproducts of this trimming as well as any defective RNAs must be rapidly degraded. These processing events are mediated by the 3′→5′ single stranded exonucleolytic RNA degradation machinery consisting of an exonuclease complex called the exosome and the helicase Mtr4p. Mtr4p is a critical partner of the exosome that presumably maintains the momentum of exonucleolytic decay/processing by removing secondary structures of the target RNAs. We have shown that Mtr4p is in fact a bona fide helicase with 3′→5′ polarity and that this activity is dependent on hydrolysis of (d)ATP. Our studies also examined the RNA binding parameters of Mtr4p showing that Mtr4p binds single stranded RNA in a length and nucleotide-dependent manner. These studies also showed that Mtr4p has a unique interaction with polyA RNA substrates. Taken together, these studies offer an initial characterization of the essential activities of Saccharomyces cerevisiae Mtr4p and provide insight into how it might function within the context of the nuclear exosome.

14. CASEIN KINASE 2 (CK2) INTERACTS WITH AND REGULATES TEF-1 (TEAD1) DNA BINDING AND TRANSCRIPTIONAL ACTIVITY

William M. Mahoney, Jr., Melody F. Francis, Mark K. Lafferty and Iain K.G. Farrance

Poster Presentation; Westminster Hall

TEF-1 (TEAD) family members are key transcriptional regulators in cardiac muscle during development, under normal physiological conditions, and during the hypertrophic response. TEF-1 proteins require cofactors for activity. Therefore, we undertook studies to identify TEF-1 cofactors active in cardiac transcription. We found that a DNA binding-deficient, carboxy-terminal fragment of rat TEF-1 (aa 111 to 430) inhibits endogenous TEF-1 in neonatal rat cardiac myocytes, presumably by titrating cofactors from endogenous TEF-1. Therefore, we performed a yeast 2-hybrid screen with aa 111 to 430 of rat TEF-1. We isolated the regulatory subunit of Casein Kinase 2 (CK2beta). CK2 is a ubiquitous ser/thr protein kinase consisting of a heterotetramer (two regulatory beta and two catalytic alpha subunits). CK2beta interacts with many proteins in addition to CK2alpha. CK2 activity, once thought to be unregulated, is now known to be controlled by targeting CK2 holoenzyme or CK2beta

and binding partner(s) to intracellular locations.
Here we show that TEF-1 interacts with CK2beta in vitro, by GST-pulldown assays, and in vivo, by co-immunoprecipitation assays. Furthermore, TEF-1 interacts the active CK2 holoenzyme in vivo. In previous work we showed that TEF-1 is phosphorylated near the amino terminus in cardiac and skeletal muscle. However, the effect of phosphorylation on TEF-1 activity was not known. We found that CK2 phosphorylates TEF-1 in vitro and that phosphorylation of TEF-1 at S11 inhibits the binding of TEF-1 to DNA. CK2 also regulates TEF-1 activity in vivo as a CK2 inhibitor activates TEF-1 dependent promoters.
We propose that the TEF-1:CK2 association may regulate transcription by three mechanisms: (1) CK2 phosphorylation directly regulating binding of TEF-1 to its regulatory elements in promoters; (2) TEF-1 recruiting CK2beta and associated proteins to DNA, regulating the activity of nearby transcription factors or chromatin structure; and (3) TEF-1 recruiting CK2 holoenzyme to muscle promoters, where it can regulate the activity of other transcription factors that are CK2 targets, such as MEF2C.

15. FEBRILE RANGE TEMPERATURE MODIFIES CYTOKINE GENE EXPRESSION IN LPS-STIMULATED MACROPHAGES WITHOUT AFFECTING UPSTREAM SIGNALING EVENTS

Zachary Cooper, Arundhati Ghosh, Aditi Gupta, Tapan Maiti, Ivor J. Benjamin, Stefanie N. Vogel, Jeffrey D. Hasday, and Ishwar S. Singh

Poster Presentation; Westminster Hall

We have previously shown that exposure to febrile-range temperatures (FRT, 39.5-40°C) reduces LPS-induced TNFα expression, in part through the direct interaction of heat shock factor-1 (HSF1) with the TNFα gene promoter. However, it is not known whether exposure to FRT also modifies more proximal LPS-induced signaling events. Using HSF1-null mice, we confirmed that HSF1 is required for FRT-induced repression of TNFα in vitro by LPS-stimulated bone marrow derived macrophages and in vivo in mice challenged intratracheally with LPS. Using the RAW 264.7 mouse macrophage cell line we showed that exposure to FRT exerted opposing effects on TNFα and IL-1β, two cytokines activated through the same MyD88-dependent pathway, and had no effect on interferon-βIFN-β) expression, which is activated through the MyD88-independent pathway. TNFα mRNA levels 1h after LPS stimulation were reduced by 57% while IL-1β mRNA levels were increased 2.6-fold by co-exposure to FRT. Exposure to FRT failed to alter LPS-induced activation of NFκB, ERK and p38 MAPK, but chromatin immunoprecipitation (ChIP) analysis demonstrated that exposure to FRT reduced LPS-induced recruitment of NFκB p65 to the TNFα promoter while increasing its recruitment to the IL-1β promoter. These data suggest that FRT exerts its effects on cytokine gene expression through distal effects on promoter activation rather than proximal receptor activation/signaling events. Finally, we showed that exposure to heat shock temperature (HST; 42°C) and FRT exerted different effects on expression of IFN-β, IL-1β, and RANTES, suggesting distinct cellular responses to the two temperatures.

16. UNDERSTANDING THE STABILITY OF PROHORMONE CONVERTASE 1

Hoshino A., Kowalska D., Lindberg I.

Poster Presentation; Westminster Hall

The prohormone convertase 1 (PC1/3) is a member of a family of serine proteases that are involved in the processing of prohormones. It is found in the regulated secretory pathway of neuroendocrine tissues and participates in the production of bioactive peptide hormones by cleaving important peptide precursors such as proinsulin and proopiomelanocortin after dibasic residues. Along the secretory pathway, PC1/3 is synthesized as a 97 kDa zymogen and is processed itself at the N-terminal to yield a mature 87 kDa enzyme.

The 87 kDa form can be further intermolecularly cleaved at the C-terminus into two much more active but highly unstable 74 kDa and 66 kDa species. It is not yet understood why the 66 kDa form of PC1 has such a short activity life. We found that protein aggregation can be observed within an hour following the production of 66 kDa PC1 from the 87 kDa form. Furthermore, these aggregates contain mixed disulfide bonds, as determined using non-reducing SDS-PAGE. Because the time frame of aggregation is slower than the loss of enzyme activity, aggregation may represent a secondary step that follows a local unfolding event which represents the initial cause of the loss of enzyme activity. We speculate that this primary event may involve the exposure of hydrophobic surfaces.

17. RESTORATION OF DEACTIVATION IN N-TRUNCATED AND LQTS HERG K+ CHANNEL MUTANTS BY A RECOMBINANT N-TERMINAL REGION FRAGMENT

Gustina AS, Gianulis EC, Trudeau MC

Poster Presentation; Westminster Hall

The Human Ether-á-go-go Related Gene (HERG) encodes a voltage-activated K+ channel, which is a primary component of the cardiac delayed rectifier K+ current (IKr). Cardiac IKr contributes to the repolarization of the ventricular action potential by conducting an outward K+ current whose amplitude is determined, in part, by the closing (deactivation) rate of the channel. Deletion of the HERG N-terminus (amino acids 2-354) leads to a channel (N-truncated) with very rapid deactivation kinetics compared to wild-type HERG channels. To investigate how N-terminal interactions regulate gating, we constructed a genetically encoded N-terminal region fragment, and co-expressed cRNAs encoding N-truncated HERG and this fragment in Xenopus oocytes. Electrophysiological recordings from these cells showed channels with a significant increase in the time constant for deactivation over that of the N-truncated channels alone. The time constant was not significantly different from that of wild-type HERG channels. These results demonstrate a restoration of slow deactivation to the N-truncated channel by the N-terminal region fragment. Förster resonance energy transfer (FRET) experiments showed that the N-terminal region fragment and the N-truncated channel are in proximity at the cell membrane. Interaction of the N-terminal fragment with the channel was also demonstrated using biochemical techniques. We next co-expressed the N-terminal region fragment with HERG channels bearing N-terminal mutations which have been shown to underlie Type II Long QT Syndrome (LQTS), a cardiac arrhythmia characterized by a prolongation of the ventricular action potential. The N-terminal region fragment recovered slow deactivation in these mutant channels. In summary, a recombinant N-terminal region interacted directly with N-truncated and LQTS mutant HERG channels to restore slow deactivation gating.

18. TARGETED DELIVERY OF ELECTRON PARAMAGNETIC RESONANCE (EPR) IMAGING PROBES TO HER-2 OVEREXPRESSING BREAST TUMORS

Burks, SR; Macedo, LF; Barth, ED; Brodie, AM; Martin, SS; Halpern, HJ; Rosen, GM; Kao, JPY.

Poster Presentation; Westminster Hall

With recent advances in electron paramagnetic resonance imaging (EPRI) technology, in vivo localization of single tissue types based on unique physiology has become a real possibility. For example, EPRI could be a powerful imaging modality to define tumor dimensions and boundaries, as well as to detect metastasis. We previously developed biologically-compatible nitroxides as EPRI probes, and demonstrated that encapsulating them in liposomes allows accumulation of cellular EPR signal through endocytosis. The mechanism of concentration-dependent signal-quenching, exhibited by both

fluorophores and nitroxides, allows liposomes encapsulating high (mM) concentrations of nitroxides to have minimal spectral signals and appear spectroscopically "dark". Only inside tissue, where liposomal lysis and dequenching occur, can signal become visible. Furthermore, liposomal surfaces can be decorated with Fab' antibody fragments specific to the Human Epidermal Growth Factor Receptor 2 (Her2). This permits tissue-specific nitroxide accumulation (~750 μM) in breast tumor cells overexpressing Her2 in vitro, while immunoliposomes are excluded from non-targeted tissues. We show through the use of EPRI tissue-phantoms, if these concentrations are achievable in vivo, Her2-overexpressing breast tumors should be visible by EPRI. We also provide evidence for the feasibility of this mechanism in vivo through the use of Her2-overexpressing tumor models. These results lay the foundation for using EPRI to visualize tumors in animal models.

19. **HIGHER EXPRESSION OF P63 AND LOWER INDUCTION OF P53 AND APOPTOSIS LED TO BENZO(A)PYRENE AND DIMETHYLBENZ(A)NTMHRACENE INDUCED SKIN TUMORS IN NQO1-/-/NQO2-/- DOUBLE KNOCKOUT MICE**

J. Shen, R. Barrios, A.K. Jaiswal

Poster Presentation; Westminster Hall

Quinone oxidoreductases (NQO1 and NQO2) are cytosolic proteins that detoxify quinones, prevent redox cycling and protect cells against oxidative stress and neoplasia. NQO1-null and NQO2-null mice were generated in our laboratory. Earlier, we have shown that NQO1-null and NQO2-null mice demonstrate increased susceptibility to dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BaP)-induced skin carcinogenesis.

We crossed NQO1-null mice with NQO2-null mice and generated double knockout mice (DKO) deficient in both NQO1 and NQO2 proteins. C57BL/6 wild type and DKO mice were used to investigate the relative susceptibility of DKO mice to DMBA and BaP induced skin carcinogenesis and metastasis, as compared to wild type and single knockout mice. The dorsal skin of wild type and DKO mice were shaved and exposed to a single dose of dimethylbenz(a)anthracene (DMBA) or benzo(a)pyrene (BaP) followed by twice weekly application of phorbol 12-myristate 13-acetate (TPA) for twenty weeks. Mice were analyzed for development of skin tumors. In related experiments, wild type and DKO mice were exposed to acetone (control) or BaP or DMBA for 6, 12 and 24 hours and analyzed for alterations in growth, differentiation and proliferation factors by Western blot and immunohistochemical analysis.

DKO mice exposed to DMBA showed significantly higher skin tumor frequency and tumor multiplicity per mouse as compared to wild type and single knockout mice. One hundred percent DKO mice showed DMBA-induced tumor incidence and average tumor multiplicity was greater than 15 per mouse. In contrast, only 30% of wild type mice showed tumor incidence and average tumor multiplicity was less than 3. DKO mice also showed significantly higher skin tumor multiplicity than single knockout mice that showed tumor multiplicity lower than 4 per mouse. BaP showed 100% incidence of tumors in DKO mice, as compared to none in wild type mice. Western blot and immunohistochemical analysis revealed that the treatment with BaP and DMBA induced higher levels of p63 but failed to significantly increase p53 and apoptosis in the skin of DKO mice, as compared with wild type mice. The results led to the conclusion that BaP and DMBA-mediated higher expression of p63 and lower induction of p53 and apoptosis led to significantly increased sensitivity of DKO mice to BaP and DMBA induced skin carcinogenesis, as compared with wild type and single knockout mice.

20. **IDENTIFYING THE EXPRESSION PATTERNS OF Γ- AND Β- SUBUNITS OF G- PROTEIN IN THE OLFACTORY EPITHELIUM OF MUS MUSCULUS**

Abhinav Parikh, Julie Wolf, Weihong Lin

Poster Presentation; Westminster Hall

Olfaction is one of the five major senses. Smells are recognized by the olfactory system using chemical signals which are perceived by the olfactory sensory neurons (OSNs) and amplified using a signal transduction pathway. The signal transduction pathway is activated when the odorant molecule binds to the olfactory sensory receptor causing a conformational change which dissociates the heterotrimeric Golf protein into Gα, and heterodimer Gβγ subunits. The role of the Gα is well understood but the functional significance of Gβγ heterodimers is not clear. Recent studies have shown involvement of Gβγ in the upstream recognition of receptors and downstream regulation of effectors in other chemosensory transduction pathways. Our study focuses on identifying and localizing the γ- and β- subunits in the olfactory pathway to further understand their contributions to olfaction. Previously, our lab identified six γ- subunits (γ2, γ3, γ5, γ8, γ12, γ13) and four β- subunits (β1, β2, β4, β5) present in the main olfactory epithelium (MOE) of the Mus musculus using RT- PCR Analysis. These γ- and β- DNA inserts were cloned into the pGEM-T-Easy vector. Restriction digestions and sequence analysis confirmed the identity of γ and β insert in the RT-PCR clones. To further study the localization pattern of the γ- and β- mRNA within the nasal epithelia, RNA in situ hybridization was conducted to map the distribution of γ2, γ3, γ5, γ8, γ13 and β2, β4, β5 subunits in the MOE of the Mus musculus. The result suggests that γ2 is found in the vomeronasal orgran (VNO) of the olfactory system while γ3, γ5, γ13 and β2 were found in the MOE. Thus, our data shows that previously unidentified γ- and β- subunits are present in the MOE and seems to transduce responses within the OSNs in the olfactory system.

21. **PROTEIN DYNAMICS OF THE CALCIUM-BINDING PROTEIN S100A5 IN THE PRESENCE AND ABSENCE OF TARGET PEPTIDE**

Melissa A. Liriano, Kristen M. Varney, Nathan Wright, Paul T. Wilder, Thomas H. Charpentier, and David J. Weber

Poster Presentation; Westminster Hall

The S100 family is a class of small, homodimeric proteins that are often characterized by their Ca2+-dependent biological effects, which is typically the result of a calcium-dependent conformational change. The majority of S100 proteins have a low μM binding affinity for Ca2+, but in the presence of a target, this affinity can increase dramatically like we have recently found with S100B. Although human S100A5 shares approximately 50% sequence homology with all the other S100 proteins, it binds to Ca2+ with an affinity 10-100 times greater then any other in the S100 protein family. Our preliminary data suggests that the increase in Ca2+-affinity, as seen with S100B in the presence of its target, is due to an elimination of protein dynamics upon target binding. We hypothesize that S100A5 inherently lacks the dynamic characteristics found in other S100 proteins, resulting in its high affinity for Ca2+ even in the absence of a target. To test this hypothesis, we will first solve the molecular structure of an S100A5-target complex using NMR (Aim #1). Furthermore, we intend to study the protein dynamics of S100A5 in the absence and presence of a target and compare it to other S100 proteins (Aim #2). We propose to also solve the structure of Ca2+-bound S100A5 using X-ray crystallography (Aim #3) to determine if there is an additional Ca2+ ion ligand in the coordination sphere (at position 9 of the EF-hand) that is observed in parvalbumin but not in other S100 proteins. Elucidating the basis of S100A5's high affinity for Ca2+ can give us a better understanding of S100A5's, and other S100 family members, interaction with molecular targets and thus their individual role in normal cells and disease processes.

22. **MEASURING INTRA-CELLULAR AND INTRA-MITOCHONDRIAL ZINC CONCENTRATIONS FOLLOWING HYPOXIA/HYPOGLYCEMIA WITH AN EXPRESSIBLE RATIOMETRIC FLUORESCENCE BIOSENSOR**

Bryan McCranor, Linda Bambrick, Rebecca Bozym, Gary Fiskum, Richard Thompson

Poster Presentation; Westminster Hall

Zinc is a “trace” metal necessary for proper cellular function. Studies have shown that the intra- and extra-cellular concentrations of labile zinc increase dramatically in models of cerebral ischemia (1, 2). Substantial evidence indicates that mitochondrial dysfunction plays a significant role in neuronal death following ischemia. Both mitochondrial dysfunction and increased intracellular zinc concentrations have been associated with increased reactive oxygen species (ROS) production and ultimately apoptosis (3, 4). We modified our fluorescent zinc biosensor (5) to be selectively expressed in the mitochondria of PC12 cells, enabling us to ratiometrically image the intra-mitochondrial concentration of labile zinc even at resting (picomolar) levels. We used this expressible biosensor and our previous sensor in cells which have undergone oxygen/glucose deprivation (OGD). Our initial results indicate that the concentration of labile, intra-mitochondrial zinc may not increase to the degree that we observed in the cytoplasm during hypoxic/hypoglycemic conditions, and may be lower than the concentrations observed in cells in more physiological conditions

23. **LOCAL DETECTION OF INTRACELLULAR REACTIVE OXYGEN SPECIES IN SINGLE INTACT CONTRACTING SKELETAL MUSCLE FIBERS**

Luke Michaelson, George Rodney, Chris Ward

Poster Presentation; Westminster Hall

Skeletal muscles produce low levels of reactive oxygen species (ROS) under resting conditions but during contractile activity, the rate of ROS production increases. Low levels of ROS production may act to stimulate adaptive responses in skeletal muscle, while increased ROS dependent oxidation at sarcoplasmic reticulum, myofilaments and other EC coupling components likely lead to decrements in contractile function. Multiple sites exist for ROS production, including mitochondria and NADPH oxidase; however, the contribution of each of these and the factors that regulate the increased production of ROS during contractile activity remains to be determined. Here we use repetitive field stimulation of single FDB myofibers as a model of ROS secondary to repetitive activity. In FDB’s loaded with the cytosolic, nonspecific ROS probe DCFH, we have imposed intermittent (0.25 Hz) trains (150msec, 1.5msec sq. pulse @, 80Hz) of tetanic stimulation to establish a reliable in vitro model for activity dependent ROS production. In recent studies with this model, we have begun to explore site dependent generation of ROS with a redox sensitive variant of green fluorescent protein (roGFP) that is targeted to the mitochondria (mito-roGFP). Following cDNA electroporation in vivo, expression of mito-roGFP, and FDB isolation, we report evidence of the fidelity and specificity of this probe in mitochondria and the activity dependent mitochondrial redox status during our stimulation paradigm.

24. **CYTOPLASMIC HEME BINDING PROTEIN PHUS IS ESSENTIAL FOR HEME HOMEOSTASIS IN PSEUDOMONAS AERUGINOSA**

Maura O'Neill, Angela Wilks

Poster Presentation; Westminster Hall

Iron is an essential element for the survival and virulence of bacteria. As such pathogenic bacteria have evolved several sophisticated mechanisms to acquire iron including siderophore uptake systems and directly utilizing iron and heme containing proteins. Hemeproteins are a particularly rich source of iron for pathogens as 95% of the iron within the body is complexed as

heme. PhuS is a cytoplasmic binding protein (CBP) in Pseudomonas aeruginosa which is required for the efficient utilization of heme as an iron source. The CBP has been shown to traffick heme to the iron-regulated heme oxygenase (paHO) which enzymatically degrades the heme to release iron, biliverdin and carbon monoxide. Recent studies examining the role of PhuS in vivo have further confirmed the importance of PhuS in heme trafficking and efficient heme utilization.

25. **EFFECTS OF UNILATERAL LIMB LOADING ON GAIT CHARACTERISTICS IN SUBJECTS WITH CHRONIC STROKE**

I. Khanna, A. Roy, M. M. Rodgers, R. F. Macko, H. I. Krebs, L. W. Forrester

Poster Presentation; Westminster Hall

The purpose of this study was to assess the effects of a novel impedance controlled ankle robot mass on gait parameters, interlimb symmetry and joint kinematics of chronic stroke survivors (n=9) during overground (OG) and treadmill (TM) walking. This ankle robot device was developed to improve hemiparetic ankle motor control and gait after stroke. However, for this study the robot was not powered. The added mass of the ankle robot had no significant effect on selected gait parameters, including paretic and nonparetic step time and percent stance, in both OG and TM conditions. Interlimb symmetry of relative stance duration was greater on the TM than OG regardless of loading conditions. The robot mass reduced peak nonparetic knee flexion on the TM and peak paretic dorsiflexion OG ($p<0.05$). These findings suggest that subjects with mild to moderate hemiparetic gait can accommodate the addition of the passive robot mass without major disruption of these metrics. The enhanced symmetry observed on the TM suggests that it may provide a better gait training modality than using the ankle robot OG.

26. **AN AUTO-REGULATORY LOOP BETWEEN NRF2 AND CUL3-RBX1 CONTROLS THEIR CELLULAR ABUNDANCE**

James W Kaspar, Anil K. Jaiswal

Poster Presentation; Westminster Hall

Nrf2:INrf2 acts as a sensor for oxidative stress. When a cell encounters any form of stress, Nrf2 dissociates from the INrf2/Cul3-Rbx1 complex and translocates into the nucleus. In the nucleus, Nrf2 activates a myriad of antioxidant genes that protect cells. Nrf2 is then exported out and degraded. INrf2 serves as a substrate adaptor to link Nrf2 to Cul3 and Rbx1. Cul3 and Rbx1 make up the ubiquitin ligase complex that is responsible for the ubiquitination and degradation of Nrf2. Previously we have shown a feedback auto-regulatory loop between Nrf2 and INrf2 indicating that Nrf2 regulates INrf2 by controlling its transcription. Here we are extending this research by demonstrating the presence of another feedback auto-regulatory loop between Cul3-Rbx1 and Nrf2. Experiments using Hepa1 and HepG2 cells indicate that Nrf2 controls its own degradation by regulating expression and induction of Cul3-Rbx1 genes. Treatment with the antioxidant tert-Butylhydroquinone (tBHQ) leads to induction of Cul3/Rbx1 genes. Mutagenesis and transfection experiments identified an antioxidant response element in the forward and reverse strands of the proximal Cul3 and Rbx1 promoters, respectively, that Nrf2 binds and regulates expression and antioxidant induction of the Cul3/Rbx1 genes. In addition, short interfering RNA inhibition or overexpression of Nrf2 led to a respective decrease and increase in Cul3/Rbx1 gene expression. These data suggest that Nrf2 regulates Cul3/Rbx1 by controlling regulation of expression and induction of Cul3/Rbx1. The induction of Cul3/Rbx1 control Nrf2 by decreasing expression and increasing degradation.

27. **14-3-3 PROTEINS AND REGULATION OF INTRACELLULAR SIGNALING IN AIRWAY SMOOTH MUSCLE CONTRACTION**

Manoj Tyagi

Poster Presentation; Westminster Hall

14-3-3s are chaperone proteins that control multiple signal transduction cascades that modulate cell proliferation, migration and cell phenotype. 14-3-3 binds to its targets through serine/threonine phosphorylated motifs present on the target proteins (RSXPSXP or RXXXPSXP). Phosphorylated proteins bound to 14-3-3 are not available to other binding partners thus modifying the action of proteins required for regulating cellular functions. We examined the protein complexes interacting with 14-3-3s by co-immuno precipitation of 14-3-3 followed by separation of proteins by SDS-PAGE. Putative binding partners were isolated from gels and digested with trypsin prior to mass spectrometric LC-MS/MS analysis. Human bronchial smooth muscle cells were treated with salmeterol, a beta adrenergic agonist, and forskolin to induce phosphorylation of target proteins. Some of the proteins identified by MS/MS analysis included annexin1, alpha actinin 1&4, HSP27, plectin1 and vimentin. Several other unknown phosphoproteins were also found to associate with 14-3-3s. Annexin 1, a known adaptor protein, regulates the ERK and p38 MAPK pathways which are involved in cell proliferation and migration. Vimentin, an intermediate filament protein, is known to provide the framework for smooth muscle cell shape. Plectin 1 plays a role in cellular processes involving actin dynamics. Association of these proteins depends on their phosphorylation and require phosphopeptide domain of 14-3-3 to modulate the intracellular signaling mechanism. Our results support that idea 14-3-3 proteins are sequestering proteins that have a potent role in modulating cytoskeletal dynamics. Because the cytoskeleton in smooth muscles is highly dynamic we propose that 14-3-3s may participate in airway smooth muscle contraction by associating with key cytoskeletal proteins. Thus, phosphorylated partners such as vimentin, annexin, actinin alpha 1 &4, plectin 1, HSP27 and other unknown phosphoproteins, by sequestering 14-3-3, might also contribute to dysfunctional intracellular signaling mechanism involved in airway smooth muscle contraction in asthma.

28. **DIFFERENTIAL EXPRESSION OF AU-RICH ELEMENT-BINDING PROTEINS IN ONCOGENESIS**

Sarah E. Brennan and Gerald M. Wilson

Poster Presentation; Westminster Hall

AU-rich elements (AREs) are potent cis-acting determinants of mRNA decay and translational efficiency that function through interactions with diverse trans-acting factors, collectively termed ARE-binding proteins (ARE-BPs). Transcripts targeted by these proteins often encode factors that directly impact critical processes such as cell division, apoptosis, angiogenesis, and inflammation, raising the possibility that altered expression of one or more ARE-BPs could drastically influence cellular phenotypes associated with oncogenesis. In this study we evaluated changes in the expression of four well characterized ARE-BPs (AUF1, TIA-1, HuR, and TTP) across a variety of human neoplastic syndromes using three principal methods: (i) cDNA arrays comparing expression in 154 tumors from 18 different tissue types versus patient-matched non-transformed tissues, (ii) meta-analyses of gene chip studies comparing expression in normal versus primary and metastatic tumors across diverse tissue types, and (iii) comparing EST and SAGE frequency between normal versus cancerous cells derived from many tissue sources. For three ARE-BPs surveyed; AUF1, TIA-1, and HuR, expression was not systematically disregulated in cancers; however, in selected tissues expression of some proteins was frequently up- or down-regulated to a significant extent. For example, HuR expression was dramatically increased in many leukemias and

moderately induced in most melanomas and bladder cancers. AUF1 expression increased or decreased in tumors depending on tissue type, including modest increases in AUF1 mRNA levels as a function of tumor grade in breast cancer. More dramatically, expression of TTP was significantly decreased in many tumor types, and was robustly repressed in aggressive cancers of the breast and prostate. These data provide evidence that disregulated expression of one or more ARE-BPs may contribute to oncogenesis or tumor progression, and that evaluation of ARE-BP expression may ultimately contribute to molecular characterization of selected tumor types for prognostic and/or diagnostic purposes.

29. CHARACTERIZATION OF WILD TYPE AND MUTANT ATP13A2 PROTEINS

Janet Ugolino and Mervyn Monteiro

Poster Presentation; Westminster Hall

Mutations in ATP13A2 (Park9) are linked to juvenile parkinsonism with dementia, called Kufor-Rakeb syndrome. ATP13A2 is highly expressed in the brain and encodes a protein with high homology to P-type ATPases, which are thought to function as ion pumps. Interestingly, several ATP13A2 transcript variants exist and not much is known about the encoded ATP13A2 isoforms. A previous study indicated that wild type ATP13A2 isoform 1 protein localizes to lysosomes whereas three different ATP13A2 mutant proteins appeared to be retained in the ER. One speculation is that the mutant ATP13A2 proteins are retained in the ER by the quality control system possibly because they are misfolded. Misfolded proteins retained by the ER are usually retrotranslocated to the cytoplasm for rapid degradation by the ubiquitin-proteasome pathway. Although the previous study showed stabilization of the ATP13A2 isoform 1 proteins upon proteasome inhibition, the results indicated that the proteins are not ubiquitinated, which is a hallmark of this pathway. We questioned whether other ATP13A2 isoforms behaved in a similar manner. To test this idea, we focused on the third ATP13A2 isoform. We measured the degradation rates of wild type and two mutant ATP13A2 proteins in transfected HeLa cells following inhibition of protein synthesis with cycloheximide. Our results suggest that the wild type and mutant proteins turnover at different rates: one mutant is degraded rapidly, similar to the wild type, whereas the other is degraded substantially slower. Degradation of all three proteins is stabilized in cells treated with MG132, an inhibitor of the proteasome. Immunoprecipitation of ATP13A2 showed ubiquitination of the proteins. These results suggest degradation of the proteins via the ubiquitin-proteasome system. Also, characterization of the proteins by immunofluorescence staining indicated colocalization with ER markers. Further characterization of these proteins and comparison between the various isoforms will hopefully provide insight into the role of ATP13A2 in Parkinson's disease (PD).

30. SEARCH FOR THE DNA BINDING SEQUENCE OF THE CYTOPLASMIC HEME BINDING PROTEIN PHUS USING THE GENOMIC SELEX SYSTEM

Aaron Smith and Angela Wilks

Poster Presentation; Westminster Hall

The cytoplasmic heme binding protein PhuS, encoded within the Fur-regulated Pseudomonas heme utilization (phu) operon, has previously been shown to traffic heme to the iron regulated heme oxygenase (HO). Furthermore, recent data also suggests that PhuS plays a role in directly or indirectly sensing and maintaining the cells iron and heme homeostasis. In keeping with PhuS having a regulatory function is the observation that apo-PhuS, in contrast to the heme bound form, has DNA-binding properties. The PhuS DNA binding activity has been further investigated by gel-shift assays and fluorescence anisotropy to determine the binding affinity and by systematic evolution of ligands by

exponential enrichment (SELEX) to determine sequence specificity.

31. CONTROL OF SEQUENTIAL ACTIONS IN TYPICALLY DEVELOPING CHILDREN

Viswanathan, P. Whitall, J.

Poster Presentation; Westminster Hall

Introduction: The present study investigates the age-related changes in motor control in typically developing (TD) children, concerning control of sequential actions when reaching to grasp or lift an object. We hypothesized that younger TD children would not integrate their planning for sequential movements as early as children 10 years and older. Methods: We tested a developmental landscape of typically developing (TD) children in the age range of 6-10 years (n=25) plus 10 young adults. We used a motor screen (Movement Assessment Battery for Children) and used a cutoff of greater than 20% to assess that the motor development was age-appropriate. All subjects performed the following conditions, with each arm, in a randomized order: an isolated reach, a reach-to-grasp, and, reach-to-grasp and lift action in a modified discrete Fitts Law paradigm. Subjects were asked to move as fast and accurately as they could. We also calculated separate indices of difficulty for each subject to enable ease of comparison. Results: Scaling the experiment tasks to body proportion yielded similar indices of difficulty values across the subject groups. As compared to adults and older children, 6-year-old TD children were slower, showed increased delay and more variable onset of the lift phase, which suggests a sequential planning mode. Our results suggest that 6-year-old children showed inability to execute a movement sequence as a whole. Instead, they break down the movements into separate units suggesting a lesser degree of ballistic control in these children.

32. ROLE OF NUCLEAR FACTOR KAPPA B (NF-ºB) IN LUNG INFLAMMATION INDUCED BY FE AND SE

Potnis, P.A., Squibb, K.S., and Elnabawi, A.K.

Poster Presentation; Westminster Hall

Epithelial cells lining the lung face a constant threat of sustaining damage caused by inhaled atmospheric pollutants. Metals present in ambient particulate matter have been strongly implicated in causing lung inflammatory diseases. We studied the effects of Fe or Se, at environmentally relevant concentrations, on induction of oxidative stress and generation of the chemokine MCP-1 in cultured human lung alveolar epithelial A549 cells. To characterize the underlying mechanisms of induction of the inflammatory response, we investigated the influence of these metals on the activation of the nuclear factor kappa B (NF-κB) intracellular signaling pathway. Exposure to Fe or Se increased cellular ROS levels measured by the fluorescent probe DCFH-DA as early as 30 min after exposure, with enhanced release of MCP-1 at 24h. Immunoblotting of cytosolic and nuclear extracts for p65 (NF-κB dimer) showed that both Fe and Se induced the nuclear translocation of NF-κB. MCP-1 levels in response to Fe were significantly suppressed in the presence of the antioxidant N-acetylcysteine (NAC); similar effects were obtained in response to Se. In the presence of BMS-345541, a specific inhibitor of NF-κB, Fe- and Se-induced release of MCP-1 was significantly decreased, indicating the involvement of NF-κB in the metal-induced chemokine release. Results indicate that both Fe and Se posses the potential for inducing lung inflammation via an oxidative stress pathway, and that the induction of NF-κB activity is, at least in part, responsible for up-regulating genes encoding for inflammatory chemokines. [Supported by: MD DHMH CH605CRT]

33. **THE IMPACT OF INCIDENT ATRIAL FIBRILLATION ON THE TERTIARY PREVENTION OF CHRONIC HEART FAILURE**

Xianghua Yin

Oral Presentation; SSW/Law Building 519

Heart failure and atrial fibrillation (AF) are very common, particularly in the elderly. Owing to common risk factors both disorders are often present in the same patient. In addition, there is increasing evidence of a complex, reciprocal relation between CHF and AF. The data regarding the impact of AF on prognosis of chronic heart failure (CHF) patients are inconclusive but are compatible with a deleterious effect. This presentation will report the results from secondary analyses on the Warfarin and Antiplatelet Therapy in Chronic Heart Failure (WATCH) Trial data from 1,587 patients recruited from 142 participating sites. Baseline data indicated that WATCH had enrolled moderately ill patients with an average left ventricular ejection fraction of 25%. 44% patients were in NYHA Class II and most of the others were in Class III. The mean age was 62 years and 85% were men. 80% patients had suffered prior MI; half had hypertension; one-third had diabetes. The mean follow-up period was 23 months. By the end of the trial, 60 patients developed AF. The mortality of this group of patients was substantially higher than that of patients without AF (patients with AF 35% vs. those without AF 16%, P=0.0002). The Cox regression shown incident atrial fibrillation was a marginally significant predictor of mortality among CHF patients after controlling for age, comorbidity, left ventricular ejection fraction (LVEF), biochemistry results, and medications. Those predictors that could be altered by external interventions justify the implications of current study on tertiary prevention of CHF.

34. **STRUCTURAL IMPACTS ON HEALTH OUTCOMES FOR ELDERLY WITH CHRONIC DISEASES**

Shannon O'Connor

Poster Presentation; Westminster Hall

Statement of the Problem: As current studies have noted a trend of lower health outcomes among ethnic minorities in the larger population, emphasis has been placed on uncovering the extent to which this trend persists into old age. Some researchers have hypothesized that lowered health outcomes may be causally related to socioeconomic factors, suggesting that ethnic minority status is only spuriously related to lowered health outcomes. Studies attempting to explore the mechanisms through which socioeconomic factors lead individuals to experience lowered health outcomes indicate that health care coverage may be a key factor. Although some research has been conducted to study the impact of both socioeconomic and health care coverage factors on health outcomes for the elderly, few studies examine this relationship among elderly living with chronic diseases. This study seeks to examine the impact of such factors on health outcomes for older individuals suffering from chronic, obesity-related ambulatory-care sensitive (ACS) diseases. Objectives: To determine the extent to which socioeconomic and health care coverage factors differentially affect health outcomes for elderly minority individuals with chronic disease. Method: This study analyzes cross-sectional data from the 2004 National Long Term Care Survey, a nationally representative longitudinal and cross-sectional survey of individuals aged 65 years and older with Medicare coverage. Individuals were divided by race into three categories including White, Black, and other. A separate indicator was used to determine respondents' ethnicity as either Hispanic or non-Hispanic. Data from 3,933 non-institutionalized respondents with chronic conditions were analyzed using SAS statistical analysis software version 9.1.3.

Models were constructed to examine the impact of socioeconomic and health insurance coverage variables on two dependent variables, any overnight hospitalization in the past year and any emergency room (ER) visits in the past month. Logistic regression analyses were employed to determine the impact of socioeconomic and health insurance coverage variables on health outcome variables. Measures of access and continuity of health care were also included in the study, as well as demographic factors and measures of activities of daily living (ADLs).
Results: Contrary to the proposed hypotheses, no significant differences were found between White and Black elderly respondents with chronic disease when examining impacts on hospitalizations in the past year, although significant differences were noted for respondents with other (non-Black) race as 38% less likely than Whites to be hospitalized, OR=0.62, p<.05. No significant differences were found when examining socioeconomic factors including income, education, and welfare enrollment, even after controlling for demographic factors and various chronic diseases. Individuals with a history of diabetes (OR=1.21, p<.05), heart attacks (OR=4.89, p<.001), or heart problems (OR=2.12, p<.001) were all significantly more likely to have had an overnight hospitalization. Respondents with Medicare or private insurance coverage were more likely to be hospitalized overnight (OR=2.13, p<.05 and OR=1.30, p<.01, respectively). In examining ER visits in the past month, no significant differences between respondents were found among race, ethnicity, socioeconomic, or insurance variables. Diabetes patients were less likely to have been hospitalized in the past 30 days (OR=0.74, p<.05), while those suffering from heart problems were 42% more likely (OR=1.42, p<.01).
Significance:
Findings of this study confirm that health coverage factors play a role in determining health outcomes for elderly individuals with chronic diseases, although only a small portion of the variance can be accounted for using these models. Final models in the present study did not reveal racial or ethnic differences across health outcomes for elderly individuals living with chronic ambulatory-care sensitive diseases. Results of this study indicate that hospitalizations may be a better measure of health outcome than visits to emergency facilities, or that a longer duration of time during which urgent medical services can be recorded may have more explanatory power for determining health outcomes. Further research is needed to clarify the findings of this study regarding the relative impact of insurance coverage variables on health outcomes among elderly with chronic diseases. Continued research is also needed to determine if the association of race and ethnicity on health outcomes in this study are representative of the larger population of elderly individuals with chronic diseases which are sensitive to ambulatory health care services, using various measures of health outcomes. Future studies should also oversample minority groups in the data collection stage in order that analyses will be more powerful in explaining the relationship of race and ethnicity to health outcomes.

35. REASONS FOR MINIMAL USAGE OF ORDERSETS IN COMPUTERIZED PROVIDER ORDER ENTRY (CPOE)

Rekha Mathew

Oral Presentation; SSW/Law Building 519

Setting of project: This project was conducted at the Johns Hopkins Medical Institute (JHMI) to evaluate the reasons for minimal usage of ordersets created for the providers at the hospital in the Computerized Provider Order Entry (CPOE) application. Starting and ending dates of project: October 2008-December 2008
Background and significance: Ordersets are defined as groups of orders that reflect the best practices for a given procedure or diagnosis. Several studies have demonstrated that the integration of

evidenced-based decision support, practice guidelines, and disease-specific ordersets into CPOE can result in reduction of medical errors, shorter-order turn-around times, and greater overall efficiency of care. Development of standardized ordersets involves a lot of time, effort and resources. At the Johns Hopkins Medical Institute, each department has an orderset department coordinator who acts as a liaison between the department and Clinical Information Systems (CIS) department. All ordersets require sponsors and clinicians working closely with the CIS team in creating the ordersets. Ordersets are released to providers only after careful scrutiny and approval from a multidisciplinary Provider Order Entry Review Committee. During the period of August 2007 to August 2008, there were 1039 ordersets available for JHMI providers. Specific aims: The main aim of this study was to assess the reasons for minimal usage of ordersets in CPOE. Methods: A report was run to identify the usage of ordersets from August 2007 to August 2008. The report showed that 361 order sets were used less than 10 times and out of them 148 were not used at all. The least used ordersets from each department were identified. Web surveys with choices for reasons for minimal usage were sent to providers of these departments by the orderset coordinators. Results of the survey were analyzed after 2 months. Providers included fellows, residents and nurse practitioners. Outcomes: A total of 85 providers from 11 departments participated in the survey. Findings revealed the following reasons for minimal usage of ordersets. Cannot find in browse (3%), did not know about it (69%), too long to use (2%), rare case (9%), POE functionality issues (1%), and content not appropriate (5%). The main reason for minimal usage of the identified order set was the providers not knowing about it. Lessons learned: One of the most important factors for efficient use of ordersets is providers' awareness of them. Prior to implementation, specific communication strategies must be set to inform providers of their existence.

36. **COMPARISON OF CARDIOVASCULAR EVENTS OF THIAZOLIDINEDIONES USE WITH OTHER ORAL ANTI-DIABETIC DRUGS USE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS: AN OBSERVATIONAL STUDY OF A MEDICAID POPULATION**

Fadia T. Shaya, PhD, MPH,Zhiqiang Lu, BSPharm, PhD Candidate, Kyongsei Sohn, Ph.D, MBA, Matthew R. Weir, MD

Oral Presentation; SSW/Law Building 519

Context: Recent studies have suggested that thiazolidinedione (TZD) drug treatment of patients with Type 2 diabetes mellitus (DM) is associated with an increase in the risk of myocardial infarction compared to placebo or other anti-diabetic (OAD) drug regimens. Objective: Our objective was to investigate the evidence of difference in risks of acute myocardial infarction (MI) and stroke (hemorrhagic and non-hemorrhagic) systematically between TZD and other OAD medication users in a largely under-represented, high risk Medicaid population. Design, Setting, and Patients: We analyzed patient encounter data, using propensity scoring methods and logistic regression to compare the risk of cardiovascular (CV) events in patients with Type 2 DM in a high risk, largely minority Medicaid population. Main Outcome Measures: Outcomes were identified through ICD9 codes 410-411 for acute myocardial infarction (MI), strokes, 430-438, and in combination with revenue codes (emergency codes: 450-459) in case of MI. Results: Using retrospective medical encounter and prescription data analyses, we did not find evidence that the TZD drug class as a whole increased the risk of cardiovascular events, such as acute myocardial infraction and strokes combined, compared to other OAD medication treatments in a high risk cohort of patients with diabetes. Conclusions: We found no conclusive evidence that TZD drug treatments increased the risk of cardiovascular events (MI and stroke) among high risk patients with Type 2 DM.

Further research capturing risk factors that are unobserved in encounter data is recommended.

37. RELATIONSHIP BETWEEN SERUM LEVELS OF INFLAMMATORY CYTOKINES AND BONE TURNOVER MARKERS IN THE YEAR FOLLOWING HIP FRACTURE

J. A. Chan, R. R. Miller, J. A. Yu-Yahiro, W. G. Hawkes, J. R. Hebel, M. D. Shardell, G. E. Hicks, M. C. Hochberg, E. A. Streeten, J. Magaziner, D. Orwig

Poster Presentation; Westminster Hall

The purpose of this study was to examine the relationship between serum levels of inflammatory cytokines and markers of bone turnover in the year following hip fracture. Women who sustain a hip fracture have increased rates of bone loss (decline in BMD) as well as continually elevated serum levels of inflammatory cytokines up to one year post-fracture. Several proinflammatory cytokines have been associated with bone remodeling; however with inconsistent results. Furthermore, little is known about a potential relationship after hip fracture. The Baltimore Hip Studies (BHS4) cohort was a randomized clinical trial in which participants were assigned to either a home-based exercise program or standard of care group. Women age 65 years and older (mean 82 years ± 6.8) were enrolled within 15 days after fracture (n=180). Serum samples were analyzed for sTNF-αR1, IL-6, bone alkaline phosphatase (BAP), C-terminal telopeptide of type I collagen (CTX), and C-terminal propeptides of type I procollagen (CICP) at baseline and 2-, 6-, and 12-months after fracture. Generalized estimating equations were used to model the association of sTNF-αR1 and IL-6 levels with markers of bone turnover over time adjusting for age, BMI, type of fracture and intervention group. The analysis included 88, 95, 107, and 91 participants at baseline, 2-, 6-, and 12-months after fracture respectively. Higher levels of sTNF-αR1 were associated with higher levels of CTX during the year after fracture ($p<0.0001$). For each ng/ml increase in sTNF-αR1, CTX was 0.16 (95% CI: 0.10, 0.22), 0.13 (95% CI: 0.05, 0.22), 0.12 (95% CI: 0.04, 0.20), and 0.09 (95% CI: 0.02, 0.16) ng/ml higher at baseline, 2-, 6-, and 12-months. Serum IL-6 levels were also positively associated with CTX levels during the year post-fracture ($p<0.0001$). There was no evidence of association between sTNF-αR1 and IL-6 with either BAP or CICP ($p>0.10$). These data demonstrate that serum levels of sTNF-αR1 and IL-6 are positively associated with increased bone turnover. The results suggest that inflammation leads to increased bone resorption, contributing to the excess decline in bone mineral density in the year following hip fracture.

38. MONTE CARLO SIMULATION TO PROJECT DOXYCYCLINE EFFECT ON REDUCING NUMBER OF ELECTIVE ABDOMINAL AORTIC ANEURYSM INVASIVE REPAIR PROCEDURES IN THE OLDER U.S. POPULATION, WITHIN A 20-YEAR TIME FRAME

Xinggang Liu M.D., Michael Terrin M.D., C.M., M.P.H., Richard Hebel Ph.D.

Poster Presentation; Westminster Hall

BACKGROUND: Abdominal Aortic Aneurysm (AAA) is found in 4%-8% of older men and 0.5%-1.5% of older women and presents a public health challenge to the older U.S. population. Expanding AAA progresses to lethal complications such as rupture. Doxycycline is a potentially effective agent to slow AAA expansion and postpone expensive endovascular repair procedures. Slowing AAA expansion could translate into reduced numbers of elective invasive repair procedures in the older population, which has both a substantial AAA prevalence and mortality from other causes. METHODS: We conducted an epidemiologic/economic study using Monte Carlo simulation (SAS 9.1.3, SAS Institute, Inc) to project the number of elective Abdominal Aortic Aneurysm invasive repairs that could be prevented by doxycycline treatment in the older U.S. population, within a 20-year time frame. The number of older AAA patients (>=55 years old) in U.S.

in 2010 was projected from our own AAA prevalence assessments and U.S. Census Bureau's 2008 National Population Projections. From year 2011 on, we estimated new eligible AAA cases each year (3% of each year's new 55-year-old population), adding them to the eligible baseline 2010 prevalence cohort (60% of all 2010 AAA patients as conservative adjustment for inadequate screening). Patients of different age-groups were simulated to have different AAA diameter means and standard deviations, which were obtained from our preliminary survey data. Patient follow-up was simulated every 6 months, assuming a reduced AAA diameter growth rate in the doxycycline treatment group and invasive repair procedures ordered at AAA diameter of 5.5 cm. Patients were censored by age-specific natural mortality abstracted from the CDC 2005 Mortality report. Sensitivity simulations were performed to test how results change under different parameter settings. RESULTS: Assuming doxycycline treatment reduced the average aneurysm expansion rate from 0.25 cm per year to 0.15 cm per year, and patients censored for randomly occurring mortality by age-specific rates set to 1.25 times the U.S. general population rates (on account of expected co-morbidity), doxycycline could prevent 508,400 of 2,509,370 elective invasive repair procedures in the U.S. older population within a 20-year span (25,420 per year). If average elective procedures cost $30,000, the total number of dollars saved on elective invasive repairs could amount to 15 billion dollars ($762,600,000 per year). Increased treatment effect and higher natural mortality (2.0 or 1.5 fold of U.S. age-specific mortality) were both associated with greater reduction in number of elective invasive repair procedures and cost savings. CONCLUSION: Doxycycline could be an effective medicine to postpone or prevent elective invasive repair procedures in the older AAA population, and subsequently have significant public health impact. Monte Carlo simulation allows us to project treatment effect on health care utilization and costs.

39. **THE 2004 NATIONAL NURSING ASSISTANT SURVEY: RELIABILITY AND VALIDITY OF SUPERVISORY SUPPORT, WORKPLACE ENVIRONMENT, AND JOB SATISFACTION MEASURES**

JiSun Choi; Meg Johantgen;Gary M. Lang

Poster Presentation; Westminster Hall

Objectives: In 2004, the first National Nursing Assistant Survey (NNAS) (HHS/ASPE, 2004) was conducted to examine recruitment, retention, and satisfaction among nursing assistants in nursing homes. Considering that this is a new survey instrument and the items are likely to be used in other research and evaluation, it is important to establish the measurement properties. This study examined 3 latent constructs: supervisory support, workplace environment, and job satisfaction. Hypothesized relationships among supervisory support, workplace environment and job satisfaction of nursing assistants are also examined. Methods: Using the publicly available 2004 National Nursing Assistant Survey (n = 2897), measurement and structural models were tested using LISREL 8.8. Results: A three-factor measurement model was developed and tested: supervisory support (10 items), workplace environment (7 items), and job satisfaction (5 items). The structural model demonstrated that nursing assistants' positive relationship with their supervisors directly influenced positive perception of workplace environment, which in turn predicted higher job satisfaction. Discussion: The study provides some of the first evidence for reliability and validity of supervision, workplace environment, and job satisfaction measures in the National Nursing Assistant Survey. The importance of workplace environment and supervisory support as contributors to satisfaction was demonstrated.

40. RELATION BETWEEN RETENTION AND TURNOVER AMONG SOCIAL WOKRES

Michin Hong

Poster Presentation; Westminster Hall

Although prior research has suggested the necessity of investigating retention and turnover separately, retention and turnover are usually treated as one construct, and numerous studies have only focused on turnover. This study examines the relation between retention and turnover intention among social workers based on Herzberg's motivation theory. A principal components analysis and a correlation test were conducted; the findings indicate that intention to stay and intention to leave are highly correlated each other and are likely to be two ends of one construct. Given the findings of the study, implications for research, theory and practice are discussed.

41. COMMUNITY FACTORS AND MATERNAL SMOKING: CONTRIBUTING TO THE PROBLEM OR HELPING WITH THE SOLUTION?

Shauna P. Acquavita

Poster Presentation; Westminster Hall

Maternal smoking is a significant issue as it not only affects the health of the mother, but also the baby. Pregnancy presents a window of opportunity to help women stop smoking as women are more likely to attempt to quit smoking during pregnancy then at any other point in their lifetime. In order to help pregnant women stop smoking, research must be done to examine what influences pregnant women to smoke. Examining both individual and community factors provides richer detail of the factors that impact smoking. While there has been studies conducted examining individual factors in relation to maternal smoking, research is lacking on community factors. This case control study examined what individual and community factors predicted maternal smoking in Baltimore city. A total of 1000 Birth certificates from the year 2000 of singleton births was randomly selected, 500 reporting tobacco use during pregnancy and 500 without, matched on education and marital status. Other individual factors included race, age, and medical assistance status. Community factors consisted of crime rate by census block group, education rate and poverty rate by census tract. Results found provide valuable information in designing maternal smoking interventions for urban areas and African American women.

42. TRENDS IN THE RACIAL DISPARITY IN HEALTHCARE PROFESSIONAL RECOMMENDATIONS FOR COLORECTAL CANCER SCREENING

Shayna E. Rich, MA, MS, Fatmatta Kuyateh, MD, MS, Min Zhan, PhD, Diane M. Dwyer, MD, Carmela Groves, RN, MS, Mary-Claire Roghmann, MD, MS, Eileen K. Steinberger, MD, MS

Poster Presentation; Westminster Hall

Introduction: Colorectal cancer screening rates have increased over time, but screening rates remain lower for Blacks than Whites. It is not clear whether this difference is due to a disparity in patient-health care professional (HCP) interactions. This study examined whether there is a racial difference in reporting a recommendation for colorectal cancer screening from an HCP, and how this difference has changed over time. Methods: Secondary analyses of data from 8,412 White and 1,785 Black Maryland residents age 50 years or older in the samples of the 2002, 2004, and 2006 Maryland Cancer Surveys, cross-sectional population-based random digit dial surveys on cancer screening and risk behaviors. Respondents were asked whether they had ever received an HCP recommendation for a sigmoidoscopy/colonoscopy. Results: Overall, 71% of Whites and 61% of Blacks reported ever receiving an HCP recommendation for sigmoidoscopy/colonoscopy ($p<0.001$) For each race, reported recommendations for

sigmoidoscopy/colonoscopy increased significantly over time (66%-75% for Whites and 56%-66% for Blacks; p<0.001 for both), but the race disparity remained ~10% at each survey year. In multivariable analyses, the odds of reporting a recommendation among Whites were 1.5 times that of Blacks (95% CI 1.3-1.7). The effect of race on the odds of reporting an HCP recommendation for sigmoidoscopy/colonoscopy did not vary significantly across time (p=0.52). Conclusion: Whites were more likely than Blacks to report ever having received a recommendation for sigmoidoscopy/colonoscopy from an HCP. Although the proportion of patients receiving recommendations for sigmoidoscopy/colonoscopy is increasing over time, the gap between races has not changed significantly.

43. USE OF FREQUENT MANUAL REPOSITIONING FOR PRESSURE ULCER PREVENTION AMONG BEDBOUND HIP FRACTURE PATIENTS

Shayna E. Rich, MA, MS, David Margolis, MD, PhD, Michelle Shardell, PhD, William G. Hawkes, PhD, Ram R. Miller, MD, CM, Sania Amr, MD, MS, Mona Baumgarten, PhD

Poster Presentation; Westminster Hall

Setting of project: Hospital, Starting and ending dates of project: 2004-2007 Background and significance: National clinical practice guidelines for pressure ulcer prevention recommend frequent manual repositioning of bedbound patients, including those using pressure-redistributing support surfaces (PRSS), i.e., mattresses and overlays intended for pressure ulcer prevention. The extent to which these recommendations are being implemented is not known. Specific aims: Examine adherence to repositioning guidelines among bedbound hospitalized hip fracture patients Methods: Eligible patients were age≥65 years who underwent surgery for hip fracture and were bedbound at the time of a study visit in the first five days of hospitalization (n=238). Study nurses assessed the use of PRSS, and information on repositioning frequency for the same day was collected by medical chart review. Outcomes: Only 184 bedbound patients (59%) were repositioned frequently (at least every 2 hours). The proportion receiving frequent repositioning was similar for those using PRSS and those not using PRSS, 62% (78/125) and 56% (106/188), respectively. After accounting for within-patient correlation and adjusting for hospital, and pressure ulcer status and risk factors, the odds of frequent repositioning were significantly higher for bedbound patients using PRSS than for those not using PRSS (OR 2.7, 95% CI 1.2-6.1). Lessons learned: More than 40% of bedbound patients were not being repositioned at the recommended frequency, and frequent repositioning of patients was more common among those using PRSS than those not using PRSS. These results suggest that adherence to guideline recommendations for frequent repositioning is incomplete and improvements in implementation are needed.

45. EFFECT OF OBSTRUCTIVE SLEEP APNEA ON SICKLE CELL DISEASE SEVERITY IN CHILDREN

Valerie E. Rogers

Poster Presentation; Westminster Hall

Statement of the Problem: Children with sickle cell disease (SCD) are at increased risk of developing obstructive sleep apnea (OSA) due to physiologic and SCD-disease-related adenotonsillar hypertrophy. In turn, OSA may increase vaso-occlusive episodes (VOE) through hypoxemia and inflammation, resulting in more severe SCD and increased need for medical care. Purpose: To test the hypotheses that SCD severity is increased by the presence and severity of OSA, and to determine whether treatment of OSA with adenotonsillectomy decreases SCD severity. Methods: Data were collected from medical records of 45 consecutive children with SCD aged 2-18

years referred to a sleep laboratory for evaluation of OSA. Severity of SCD was measured by medical care for VOE (# medical visits, # days of medical care), and a Sickle Cell Disease Severity Index (DSI) score. Analyses were descriptive and nonparametric, with exploratory regression modeling. Results: Seventy-three percent of the sample was diagnosed with OSA. Overall, as OSA severity increased, number of medical visits decreased ($H(2)=7.85$, $p=.017$). Only 11-17 year olds sought more medical care (NS) as OSA severity increased. Of the sleep study variables, medical visits were negatively associated with obstructive apnea-hypopnea index ($\hat{o}= -.341$, $p<.01$), peak end-tidal CO_2 ($\hat{o}= -.321$, $p<.05$) and respiratory arousals ($\hat{o}= -.341$, $p<.01$). DSI scores were negatively associated with mean SpO_2 ($\hat{o}= -.296$, $p<.01$) and sleep efficiency ($\hat{o}= -.232$, $p<.05$). Adenotonsillectomy was associated with a nonsignificant trend toward greater help-seeking, but with a medium-sized effect (# medical visits, $r = -.32$; # days of care, $r = -.35$). DSI scores decreased nonsignificantly in the post-adenotonsillectomy group but not in the group not having surgery. Conclusion: Hypotheses were not supported. The relationship between OSA and SCD severity is complex, with significant effect found only in certain subgroups (e.g. adolescents). Adenotonsillectomy shows some promise in decreasing SCD severity in certain children with OSA. Larger sample size and prospective studies are needed to enlighten these issues.

46. TRANSITION PATTERNS IN RESIDENTIAL LIVING SETTINGS AMONG ELDERLY MEDICARE BENEFICIARIES DURING 2000-2005

Masayo Sato, Alicia Arbaje, Thomas Shaffer, Myra Schneider, Ilene Zuckerman

Poster Presentation; Westminster Hall

Transitions between different residential living settings or across different health care settings represent vulnerable periods for older adults. An overall assessment of transition patterns has yet to be described in the literature. The objective of this study is to describe transition patterns across residential and healthcare settings over time and to determine whether annual transition patterns are consistent. This retrospective cohort study used the Medicare Current Beneficiary Survey (2000-2005). Beneficiaries who were aged 65 and over and who had complete residential records were included. Annual transition patterns were expressed with a string created by combining four types of settings: C (community), F (facility), S (skilled nursing facility, or SNF), and H (hospital). Patterns of annual transitions were consistent across all years of the study. Approximately 20% of beneficiaries who did not die during the year experienced transitions, and about 65% of beneficiaries who died had at least one transition. The most frequent transition patterns were Community-Hospital-Community (11.5%) among community-dwelling beneficiaries, and Facility-Hospital-Facility among institutionalized beneficiaries (7.9%). Institutionalized beneficiaries experienced more transitions than community-dwelling beneficiaries. Multiple transitions are mainly attributable to the use of SNFs and hospitals. These results show stable and consistent patterns in the Medicare population. These findings can help identify where issues related to transitions are most likely to occur. The consistency of these patterns can allow policy makers to plan interventions to improve quality of care and health-related quality of life in older adults that are more reliable and sustainable.

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