

# **Twenty-Eighth Annual**

***Daniel T. Watts***

## **Research Poster Symposium**

**A Scientific Forum for the VCU Community**

**October 18-19, 2011**

**Hermes A. Kontos  
Medical Sciences Building  
Virginia Commonwealth University**



## *In Memory of*

### **Daniel T. Watts (1917-1994)**

The Daniel T. Watts Poster Research Symposium is named in honor of Daniel T. Watts, former Dean of the School of Basic Health Sciences and Graduate Studies who passed away in 1994 at the age of 77. Dean Watts was a nationally recognized pharmacologist. In 1946 and 1947, he worked on projects to determine human tolerance to the acceleration forces experienced in aviators' ejection seats. From 1947 to 1953, he taught pharmacology at the University of Virginia. He served as chair of Pharmacology at West Virginia University from 1953 to 1966 before coming to the Medical College of Virginia in 1966, continuing to serve as Dean as the institution was incorporated into Virginia Commonwealth University in 1968. Dean Watts held interests in intercollegiate athletics as well as biomedical research and graduate education and represented the University in that capacity. He retired as Dean, Basic Health Sciences in 1982.

During his tenure at this institution he established the foundation of the research enterprise in basic health sciences that continues today. His legacy continues both in the breadth of research and educational programs particularly the development of Ph.D. training. The growth of research and graduate training build on the pioneering efforts of John C. Forbes and C.C. Clayton, establishing the traditions which continue today. Shortly after his retirement, the Poster Symposium was initiated as a tribute to Dr. Watts and his effort, serving as an illustration of the research and development for continuing generations of life/health science researchers.





Daniel T. Watts



**Session I - Tuesday, October 18, 2011**

**Presentations: A-01 to A-48**

**Session II – Wednesday, October 19, 2011**

**Presentations: B-01 to B-49**



## Session I – Tuesday 18 October

### **A-01 Access and Barriers to Healthcare in Three Northern Honduran Communities Served by the Honduras Outreach Medical Brigada Relief Effort (HOMBRE)**

Catherine Pearson, Michael Stevens MD MPH, Kakotan Sanogo MS, Gonzalo Bearman MD MPH  
Medicine-Infectious Disease

### **A-02 The Stat5-Fyn Partnership in Mast Cell Activity**

Nicholas A. Pullen, Brian O. Barnstein, Yves T. Falanga, Tenchee Dhasela Lama Tamang\*, Bridget S. Wilson, John J. Ryan \*undergraduate researcher  
Biology

### **A-03 Amphetamine induces a persistent serotonin transporter leak current**

Ernesto Solis, Jr. and Louis J. DeFelice  
Physiology and Biophysics

### **A-04 An open channel blocker dislodges the allosteric coupling between ligand binding and channel opening**

Shengjun Wu, Changan Xie, Xinping Xu, Christina Vorvis, Farzana Marni, Weihua Gao, Qinglian Liu and Lei Zhou  
Physiology and Biophysics

### **A-05 Biochemical and biophysical studies of AIM2-dsDNA complex**

Govinda Remesh, S. and Escalante, C. R.  
Physiology and Biophysics

### **A-06 The role of ADAM10 in Germinal Center Formation and Antibody Production**

Natalia S. Chaimowitz, Rebecca K. Martin, Dae-Joong Kang, Hannah Zellner, David R. Gibb and Daniel H. Conrad  
Microbiology and Immunology

### **A-07 Capturing Biomolecules: Towards The Design Of High Affinity Small Molecules Containing Electrophilic Bait.**

Susan Daniela Selaya and Matthew C.T. Hartman, PhD  
Chemistry

### **A-08 CAVEOLAE AS REGULATORS OF G-PROTEIN COUPLED RECEPTOR SIGNALING IN GASTROINTESTINAL SMOOTH MUSCLE**

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Department of Physiology and Biophysics

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Jennifer A. Mietla, Dayanjan S. Wijesinghe, Eric K. Mayton, Nadia F. Lamour, and Charles E. Chalfant  
Biochemistry and Molecular Biology

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Annamarie Dalton and William Barton, PhD  
Biochemistry and Molecular Biology

**A-11 Compensatory roles of Krüppel-like transcription factors EKLF and KLF2 in mouse embryonic erythropoiesis**

Divya S. Vinjamur, Mohua Basu, Jerry B Lingrel, Jack L. Haar, Joyce A. Lloyd  
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Martin Michael Dcona; Deboleena Mitra; Matthew C.T. Hartman\*  
Chemistry

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Physiology and Biophysics

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Sami Dodhy  
Internal Medicine

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Human and Molecular Genetics

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Human and Molecular Genetics

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Department of Biostatistics

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Biostatistics

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Human and Molecular Genetics

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1Integrative Life Sci, 2Ctr for Study of Bio Complexity, 3Dept of Biochem and Mol Biol

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Biochemistry and Molecular Biology

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Niti Vanee, Stephen S. Fong  
Integrative Life Sciences

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Physiology and Biophysics

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Biostatistics

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Danielle Weaver<sup>1</sup>, William Budd<sup>2</sup>, Emmanuel Petricoin<sup>3</sup>, Joy Ware<sup>4</sup>, Zendra Zehner<sup>5</sup>  
1Ctr for Study of Bio Complexity, 2Integrative Life Sci, 3Center Applied Proteomics & Mol Medicine,  
George Mason Univ, 4Dept of Pathology, 5Dept of Biochem & Mol Biol.

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Dr. Jessica Ketchum, Dr. Juan Carlos Arango-Lasprilla, Jenna Czarnota  
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Yves T. Falanga, Naty Chaimowitz, Nicholas A. Pullen,  
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Physiology and Biophysics

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Human and Molecular Genetics

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Ruifeng Qi, Qinglian Liu  
Department of Physiology and Biophysics

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Biochemistry and Molecular Biology

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Kyle K Payne, Amir A Toor, Masoud H Manjili  
Microbiology and Immunology

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Amir A Toor, Kyle K Payne, Harold M Chung, Catherine H Roberts, Roy T Sabo, Maciej Kmiecik, William Clark, Christy McLaughlin, Angela Buskey, Jennifer Anderson, Rose H Manjili, Susan D. Roseff, John M McCarty, Masoud H Manjili  
Microbiology and Immunology

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aMegha A. Desai, bNinad M Walavalkar, cMerlin N. Gnanapragasam, dJ. Neel Scarsdale, eDavid C. Williams, fGordon D. Ginder aDepartment of Human and Molecular Genetics, bIntegrative Life Sciences program, cDepartment of Human and Molecular Genetics, dIns Human and Molecular Genetics

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Lane, BM. Lister, JA  
Human and Molecular Genetics

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Xinping Xu\*, Divya Prasanna Kumar\*, Christina Vorvis\*, and Evans Boateng Sarbeng\*, and Qinglian Liu  
Physiology and Biophysics

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Human and Molecular Genetics

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Prashant V. Thakkar, Shirley M. Taylor, Ph.D.  
Microbiology and Immunology

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Psychiatry

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Chun-Mei Xia, Sharon J. Yu, Jarren C. Kay, and Li-Ya Qiao  
Physiology and Biophysics



## **A-01 Access and Barriers to Healthcare in Three Northern Honduran Communities Served by the Honduras Outreach Medical Brigada Relief Effort (HOMBRE)**

Catherine Pearson, Michael Stevens MD MPH, Kakotan Sanogo MS, Gonzalo Bearman MD MPH

### Medicine-Infectious Disease

**Background:** Rural Honduras lacks adequate access to health services. Medical relief efforts aim to address these gaps but often lack knowledge of local healthcare access and barriers. This study is a descriptive and comparative analysis of perceived barriers to healthcare in three northern Honduran communities served by the VCU Honduras Outreach Medical Brigada Relief Effort (HOMBRE). One site (Coyoles) is suburban while Lomitas and La Hicaca are mountainous and rural.

**Methods:** An IRB approved, 25-item questionnaire was completed by structured individual interview by study personnel at the point of care. Eligible subjects included any persons 18 years or older receiving care at the June 2011 HOMBRE clinics. Study participation was voluntary and anonymous. A descriptive and comparative analysis of survey responses was performed.

**Results:** 220 random surveys were collected: 140 in Coyoles, 50 in Lomitas, and 30 in La Hicaca. 70% of respondents in Lomitas reported no contact with a healthcare provider in the last 12 months, compared to 43% in La Hicaca, and 28% in Coyoles ( $p < 0.0001$ ). The majority (59%) of respondents in Coyoles accessed their health provider in less than 30 minutes. A 1-3 hour trip to access healthcare was reported by 14% Coyoles, 36% Lomitas and 80% La Hicaca ( $p < 0.0001$ ). The majority (56%) of Lomitas respondents reported a 3-8 hour trip. Other major barriers were cost, 52% Coyoles, 88% Lomitas and 63% La Hicaca ( $p < 0.0001$ ), facility overcrowding, reported by 66% Coyoles, 94% Lomitas and 73% La Hicaca ( $p = 0.0006$ ) and transportation, reported by 37% Coyoles, 90% Lomitas and 67% La Hicaca ( $p < 0.0001$ ).

**Conclusion:** Honduran communities in the same region experience differences in healthcare access. Barriers include distance, cost, transportation, and facility overcrowding. These findings should guide future relief efforts.

## **A-02 The Stat5-Fyn Partnership in Mast Cell Activity**

Nicholas A. Pullen, Brian O. Barnstein, Yves T. Falanga, Tenchee Dhasela Lama Tamang\*, Bridget S. Wilson, John J. Ryan \*undergraduate researcher

### Biology

Stat5 expression is required for development, survival, and IgE-mediated mast cell (MC) activity. Tyrosine phosphorylation (pY) of Stat5 is generally accepted as the activation switch for this transcription factor; pY-Stat5 is swiftly, and transiently, induced by crosslinking of the high affinity IgE receptor (FcεRI) in MC. We sought to delineate the components of this transduction process, as understanding this mode of signaling is essential to understanding MC function – the chief mediators of allergic responses. We found that likely suspects such as Syk, PI3K, Akt, BTK, and Jak2 had no influences on early response pY-Stat5. Fyn kinase was found to be the essential positive regulator of this signal, with Lyn kinase in opposition. Additionally, a heretofore-unknown pre-association of Stat5 and Fyn was discovered. Further analysis of the adaptor protein Gab2 and phosphatase SHP-1 revealed their negative parts in this signaling apparatus, as deficiency in either enhances pY-Stat5 detection. The separable roles of Stat5A and Stat5B were finally demonstrated with late-phase mast cell activation studies where Stat5B was shown to be the specific regulator of cytokine production in response to FcεRI crosslinking.

## **A-03 Amphetamine induces a persistent serotonin transporter leak current**

Ernesto Solis, Jr. and Louis J. DeFelice

### Physiology and Biophysics

Amphetamine and related compounds increase serotonin (5HT) levels in the brain and cause profound behavioral effects. One target for these drugs is the serotonin transporter (SERT), which normally regulates synaptic 5HT levels. SERT agonists, such as 5HT and amphetamine, induce SERT-mediated currents coupled to Na<sup>+</sup>. We employed two-electrode voltage-clamp to measure SERT currents on *Xenopus laevis* oocytes voltage-clamped to -60 mV. We discovered a leak SERT current induced by exposure to amphetamine that persists long after its removal. In this work, we characterize the amphetamine-induced leak current in SERT and compare it to responses by several amphetamine derivatives including 3,4-methylenedioxymethamphetamine (MDMA, also known as ecstasy), para-chloroamphetamine (pCA), and methamphetamine. Understanding this novel effect of amphetamine-related drugs on SERT has implications in the understanding of human behavior.

**A-04 An open channel blocker dislodges the allosteric coupling between ligand binding and channel opening**

Shengjun Wu, Changan Xie, Xinping Xu, Christina Vorvis, Farzana Marni, Weihua Gao, Qinglian Liu and Lei Zhou

Physiology and Biophysics

For ligand-gated ion channels, it had been long proposed that ligands open the channel by specifically stabilizing the channels in the open state. Using fluorescently labeled ligands as marker, recent studies using the technique of patch-clamp fluorometry (PCF) directly demonstrated in cyclic-nucleotide gated (CNG) and hyperpolarization activated cyclic-nucleotide gated (HCN) channels that the channels in the open state have a higher binding affinity for ligand. However, within the full-length channel protein, it was unclear how discrete molecular elements, especially the regions far away from the ligand binding site, coordinate this dynamic ligand - channel interaction. Here we probed the ion conducting pore in HCN channel expressed in frog oocytes with classical pore blockers. Two ionic blockers, Cs<sup>+</sup> and Mg<sup>2+</sup>, effectively blocked the channel but had no effect on cAMP binding. However, an open channel blocker, ZD7288, significantly reduces the binding of cAMP to the functioning full-length channel. Independent biochemical assays on isolated protein segment excluded the direct interaction between ZD7288 and the cAMP binding domain (CNBD). Moreover, we identified residues near the inner activation gate in S6 that can remotely affect this dynamic interaction between ligand and channel. Thus, our results directly illustrated the allosteric coupling between inner activation gate and ligand binding site in HCN channel and provide novel insights into the implementation of protein allostery in ligand-gated ion channels.

## A-05 Biochemical and biophysical studies of AIM2-dsDNA complex

Govinda Remesh, S. and Escalante, C. R.

### Physiology and Biophysics

The successful recognition of pathogen associated molecular motifs (PAMPs) is achieved by the innate immune system with the aid of pathogen recognition receptors (PRRs). One such receptor, Absent in Melanona 2 (AIM2), is known to bind dsDNA inducing production of interleukin 1 $\beta$  (IL 1 $\beta$ ) and interleukin 18 (IL 18). AIM2 forms large multi-protein complexes, the inflammasome that initiates an inflammatory response leading to programmed cell death or pyroptosis. Thus, AIM2 binds and oligomerizes around cytoplasmic ds DNA and causes the recruitment of ASC (apoptosis associated speck like protein with a CARD domain) and ASC in turn interacts with pro-caspase 1. The inflammasome thus formed with dsDNA acting as the triggering molecule along with bound AIM2, ASC and pro-caspase 1 causes the release of IL 1 $\beta$  and IL 18. A step towards understanding the formation of AIM2 inflammasome is to delineate the interaction of AIM2 with DNA. In particular, the number of AIM2 molecules needed to induce recruitment of ASC as well as the binding mode of DNA to protein. Spectro-fluorometric binding studies of AIM2 with varying lengths of dsDNA under different salt conditions provided a gross understanding of the protein-DNA interactions. Furthermore, using analytical ultracentrifugation the oligomerization state of AIM2 around the varying lengths of DNA was analyzed. In addition, we generated a model of AIM2 bound to a 16bp DNA and selected several residues that seemingly interact with DNA. In order to confirm our model, residues were mutated to alanine at the proposed sites and the binding of the mutants with dsDNA was tested. We found that residues R214A and K245A had a significant effect on DNA binding. These studies will direct our future goal of the structure determination of the AIM2-dsDNA complex by X-ray crystallography.

## **A-06 The role of ADAM10 in Germinal Center Formation and Antibody Production**

Natalia S. Chaimowitz, Rebecca K. Martin, Dae-Joong Kang, Hannah Zellner, David R. Gibb  
and Daniel H. Conrad

### Microbiology and Immunology

Germinal centers (GCs) are the sites of B cell proliferation, somatic hypermutations and subsequent affinity maturation as well as isotype class switching. Furthermore, plasma cells and memory B cells are also generated in GCs. GCs are thus critical for a successful humoral response. Many aspects of germinal center formation and B cell differentiation, however, remain unclear. We have demonstrated that a member of the disintegrin and metalloprotease family, ADAM10, is critical for B cell responses to protein antigens. Immunohistochemistry and flow cytometry analysis demonstrated that B cell specific ADAM10B<sup>-/-</sup> mice fail to form proper GCs and have abnormal lymphoid structure after immunization. Furthermore, these mice have very low levels of circulating antigen specific IgG after immunization. Consistent with this finding, antigen specific IgG secreting cell numbers are decreased both in the spleen and bone marrow of knock out mice. Furthermore, follicular helper T cell numbers and IL-21 levels are also decreased in ADAM10B<sup>-/-</sup> mice. Interestingly, ADAM10-deficient B cells migrate better to CXCL13 than WT B cells. When ADAM10 was deleted subsequent to IgG1-class switching, ADAM10IgG1<sup>-/-</sup> displayed decreased levels of antigen specific IgG1 following immunization, although plasma cell numbers were comparable between wild type and ADAM10IgG1<sup>-/-</sup>. These results demonstrate that ADAM10 plays a role in maintenance of lymphoid architecture following immunization and suggest that ADAM10 also plays a role in antibody secretion.

**A-07 Capturing Biomolecules: Towards The Design Of High Affinity Small Molecules Containing Electrophilic Bait.**

Susan Daniela Selaya and Matthew C.T. Hartman, PhD

Chemistry

Development of an intra-cellularly active small molecule tag with high selectivity for biomolecules of interest is an ongoing challenge and would be a powerful tool in cellular biology. In particular, the study of protein-protein interactions is a key role in various human diseases but elucidation of protein interfaces is difficult and disrupted by large tags. Therefore, site selective attachment using small molecules to specific proteins is a primary goal. To this effect it has been demonstrated that electrophilic reagents tested for activity based protein profiling can discriminate between nucleophilic peptides in different protein microenvironments. Generation of nucleophilic peptides can be accomplished using mRNA display. Utilizing this powerful tool it is possible to generate a library containing  $10^{12}$ - $10^{14}$  diverse peptides. These peptides contain nucleophilic amino acids whose reactivity can be tested against various electrophiles. Sulfonate esters are a source of electrophiles that have been studied extensively. The electrophiles in this study contain three major components. The first is the electrophilic end using a sulfonate ester such as tolylsulfonate ester. The second component includes a polyethylene oxide linker that provides flexibility and spatial distance between the two ends of the small molecule. Finally, the third component is an affinity tag such as biotin or a fluorescent marker such as Texas red. Utilizing mRNA display to generate a diverse peptide library, these tagged electrophiles were designed as bait to covalently bind specific peptides. After multiple rounds of selection utilizing mRNA display, enrichment of the peptides that react site specifically are isolated using affinity chromatography and sequenced to observe trends between electrophiles and their corresponding peptides.

**A-08 CAVEOLAE AS REGULATORS OF G-PROTEIN COUPLED RECEPTOR SIGNALING IN GASTROINTESTINAL SMOOTH MUSCLE**

Sayak Bhattacharya, Sunila Mahavadi, Senthilkumar Rajagopal, Karnam S. Murthy

Department of Physiology and Biophysics

Caveolae are membrane invaginations that contain the scaffolding proteins caveolin-1, -2 and -3. Caveolins are known to regulate G-protein coupled receptor signaling. The role of caveolin-1, the main caveolar protein in the regulation of Gq/13-coupled m3 and Gi-coupled m2 muscarinic receptor signaling in smooth muscle has not been examined. **AIM:** To examine the role of caveolin-1 in the regulation of m3 and m2 receptor signaling in gastric smooth muscle. **METHODS:** Caveolar fractions are prepared using sucrose density gradient centrifugation and localization of m3 and m2 receptors examined by western blot. M3/Gq-coupled PI hydrolysis, m3/G13-coupled Rho kinase and ZIP kinase activity via RhoA, and M2/Gi-coupled inhibition of cAMP formation were measured using receptor-specific antagonists, and pharmacological (using methyl cyclodextrin; MCD) and molecular approaches (using siRNA or caveolin-1<sup>-/-</sup> mice) to suppress caveolin-1 functions. Muscle contraction was measured by scanning micrometry in isolated muscle cells. **RESULTS:** Carbachol-induced PI hydrolysis, Rho kinase and ZIP kinase activity, and muscle contraction via m3 receptors were attenuated with MCD or caveolin-1 siRNA, and in caveolin-1<sup>-/-</sup> mice. In contrast, carbachol-induced inhibition of cAMP formation via m2 receptors was unaffected by MCD or caveolin-1 siRNA. **CONCLUSION:** Caveolin-1 positively regulates m3, but not m2 receptor signaling in gastric smooth muscle.

## A-09 Characterization of eicosanoid synthesis in a genetic ablation model of ceramide kinase

Jennifer A. Mietla, Dayanjan S. Wijesinghe, Eric K. Mayton, Nadia F. Lamour, and Charles E. Chalfant

### Biochemistry and Molecular Biology

Eicosanoids are bioactive signaling lipids and major mediators of the inflammation process. Activation of the arachidonic acid liberating enzyme, group IV phospholipase A2 (cPLA2 $\beta$ ), is the initial rate limiting step in the production of eicosanoids induced by various inflammatory agonists. Multiple reports have demonstrated that ceramide kinase (CERK)-derived ceramide-1-phosphate (C1P) is an activator of cPLA2 $\beta$ , but recently, there has been controversy as to the effects of genetic ablation of CERK. To examine the effects of the genetic ablation of CERK on eicosanoid synthesis, mouse embryonic fibroblasts (MEFs) were isolated from CERK $^{-/-}$  and  $+/+$  mice, and the profiles of multiple eicosanoids were investigated utilizing a comprehensive lipidomics approach. In general, wild-type MEFs produced quantifiable levels of 8 out of 15 eicosanoids examined. Specifically arachidonic acid, PGE2, 6-keto PGF1 $\alpha$ , PGF2 $\alpha$ , 5 HETE, 11 HETE, 12 HETE, and 15 HETE were produced at appreciable levels. In baseline experiments, the levels of 7 out of 8 detectable eicosanoids were dramatically lower in the CERK $^{-/-}$  MEFs compared to wild type cells. For example, arachidonic acid levels were reduced approximately 80%, and 11 HETE levels were reduced approximately 70%. Importantly, induction of eicosanoids by calcium ionophore was dramatically reduced by approximately 61% in the CERK $^{-/-}$  MEFs as compared to wild type MEFs. Furthermore, the reported controversy in regards to CERK with eicosanoid synthesis was due to adaptations utilizing C1P from fetal bovine serum to compensate for the genetic ablation of CERK. Overall, we demonstrate that there are significant and dramatic differences in the eicosanoid levels in the CERK $^{-/-}$  MEFs compared to wild type counterparts validating the role of C1P in the biosynthesis of eicosanoids.

## A-10 Characterizing interactions between endothelial integrins and the Tie receptors

Annamarie Dalton and William Barton, PhD

Biochemistry and Molecular Biology

Angiogenesis is a complex cellular process involving a multitude of related growth factors and growth factor receptors including VEGF, VEGFR1/2, Angiopoietin, and Tie receptors. Unlike vasculogenesis, which primarily occurs early in development, angiogenesis extends from early development through adult life. In the adult, angiogenesis is primarily restricted to areas of active wound repair or solid tumor growth and development, suggesting that the tumor angiogenic switch could serve as an effective therapeutic target.

The Tie receptor tyrosine kinases are a major focus of therapeutic research. They act primarily downstream of VEGF and VEGFR1/2 and are predominantly responsible for vessel stability via regulation of endothelial cell survival, migration, and adhesion. Interestingly, the Tie2 ligands, Angiopoietin-1 and -2, function as agonist and antagonist, respectively. For example, Angiopoietin-2 can block Tie2 signaling initiated by Angiopoietin-1, and in the presence of VEGF, promote new sprouting and vessel formation while Angiopoietin-1 expression on its own causes vessel stability and quiescence. Many of the functions of the Tie receptors have been elucidated from studying their structures and interactions with other cell surface receptors. Unfortunately, the currently available models do not illuminate the role of macromolecular interactions in Tie signaling. We have identified  $\alpha 5\beta 1$  and  $\alpha v\beta 3$  integrin molecules as binding partners of both the Tie1 and Tie2 receptors through FRET microscopy and co-immunoprecipitation. Additionally, lentiviral mediated knockdowns of endothelial integrins are being used to study changes in innate morphological characteristics of this cell type as well as downstream signaling effects. A better understanding of these interactions will be key to developing effective anti-angiogenic agents.

## **A-11 Compensatory roles of Krüppel-like transcription factors EKLF and KLF2 in mouse embryonic erythropoiesis**

Divya S. Vinjamur, Mohua Basu, Jerry B Lingrel, Jack L. Haar, Joyce A. Lloyd

### Human and Molecular Genetics

The Krüppel-like factors EKLF (erythroid Krüppel-like factor or KLF1) and KLF2 are essential for embryonic erythropoiesis. They are highly homologous within their zinc finger DNA-binding domains and are expressed at similar levels in primitive erythroid cells. EKLF/KLF2 double knockout (KO) embryos die earlier than either single knockout and show a more severe phenotype with regard to erythroid cell morphology. EKLF/KLF2 double knockout (KO) embryos have a significantly greater reduction in *Ey*- and  $\beta$ h1-globin mRNA at embryonic day 10.5 (E10.5) than do either of the single knockouts, indicating that EKLF and KLF2 can partially compensate for each other. EKLF has an erythroid specific pattern of expression whereas KLF2 is expressed in many tissues including the lung, lymphocytes, erythroid and endothelial cells. We investigated the cell-specific functions of KLF2 using an erythroid-specific conditional knockout mouse model (ErGFP-Cre). We show here that KLF2 regulates mouse embryonic globin gene expression in an erythroid-cell autonomous manner. We further studied EKLF/KLF2 double conditional knockouts using Tie2-Cre mediated deletion of KLF2 in both erythroid and endothelial cells on an EKLF null background. EKLF/KLF2 double conditional knockouts recapitulate the pale and anemic phenotype of EKLF/KLF2 double knockouts. They display decreased expression of embryonic  $\beta$ h1- and *Ey*-globins. The anemic phenotype observed in EKLF/KLF2 double knockouts is dose dependent and can be rescued by the presence of a single copy of the KLF2 gene but not by a single copy of the EKLF gene. Further, EKLF-null erythroid cells display a 2.5-fold increase in KLF2 mRNA expression indicative of a compensatory mechanism.

## A-12 Developing Unnatural Cyclic Peptide Inhibitors

Zhong Ma, E. Railey White, David E. Hacker, Matthew C.T. Hartman

### Chemistry

The breast cancer associated protein 1 (BRCA1) was initially discovered because of its association with familial inherited breast and ovarian cancers. It has since been discovered that this large multifunctional protein is part of a complex network of DNA damage repair. BRCA1 is involved in DNA double strand break (DSB) repair via homologous recombination (HR) and is also known to interact with the Fanconi Anemia (FA) repair pathway of intrastrand crosslinks (ICLs). Inhibitors of BRCA1 have implications for chemotherapeutics and as tool for further study of BRCA1 and its many roles in cellular processes including DNA repair. The best characterized binding domain of BRCA1, the BRCT (BRCA1 C Terminal) domain, and is one of the most commonly mutated regions of BRCA1 in hereditary cancers. The BRCT domain is known to interact with phosphoserine containing proteins within the DNA repair pathway, but the functions of these interactions are not completely understood. We have used an mRNA display in vitro selection to find non-phosphorylated peptides containing non-genomic amino acids that bind to a BRCT-GSH fusion of BRCA1. The sequences resulting from the selection have common homologies and testing of binding affinities and cellular function is currently underway.

### **A-13 Development of in vivo quantification of angiogenesis and lymphangiogenesis**

Masayuki Nagahashi, Subramaniam Ramachandran, Eugene Y. Kim, Jeremy C. Allegood, Omar M. Rashid, Akimitsu Yamada, Sheldon Milstien, Sarah Spiegel and Kazuaki Takabe

#### Surgery/Biochemistry

Sphingosine-1-phosphate (S1P), a pleiotropic bioactive lipid mediator, regulates many cellular processes important for breast cancer progression. Previously, we clarified that orthotopic implantation of syngeneic cancer cells into immune intact mice models human breast cancer progression better than xenograft subcutaneous implantation. Utilizing this model, we discovered a critical role for S1P produced by sphingosine kinase 1 (SphK1) in tumor-induced angiogenesis and lymphangiogenesis. We found that S1P levels gradually increased in the tumor and in the circulation and that a specific SphK1 inhibitor, SK1-I, blocked these increases. Further, SK1-I reduced lymph node and lung metastases and overall tumor burden in vivo. S1P and Ang2 stimulated, and SK1-I inhibited, in vitro angiogenesis and lymphangiogenesis. We developed a new method to quantify both angiogenesis and lymphangiogenesis in the same sample by combining Directed In Vivo Angiogenesis Assays (DIVAA) with Fluorescence Activated Cell Sorting (DIVAA/FACS). With this method, we demonstrated that S1P and Ang2 enhanced directed angiogenesis and lymphangiogenesis in vivo whereas SK1-I suppressed it. Importantly, SK1-I also decreased angiogenesis and lymphangiogenesis not only in the primary tumor, but also in lymph nodes, the host tumor microenvironment. Taken together, our results demonstrate that S1P produced by SphK1 is an important factor in breast cancer-induced angiogenesis and lymphangiogenesis and that the SphK1/S1P axis deserves consideration as a target for breast cancer treatment. This work was supported by Sumitomo Life Social Welfare Services Foundation grant to MN, NIH (5K12HD055881) and Susan G. Komen for the Cure (KG090510) to KT, and NCI (R01CA61774) to SS.

## A-14 Drug delivery using photocaged cell impermeable drug conjugate

Martin Michael Dcona; Deboleena Mitra; Matthew C.T. Hartman\*

### Chemistry

Selectivity of pharmacological action is of central importance in drug therapy. The development of photoactivatable prodrugs may be particularly important in minimizing the adverse side effects associated with current cancer chemotherapeutics. In order to minimize off target effects of the anticancer agent, doxorubicin; we have designed a light-activated system to add control to its site specific delivery. Three components are incorporated into the design of this photoactivatable prodrug; (1) A photosensitive molecule that is chemically modified under UV light (2) An agent that alters cell permeability (3) doxorubicin. In this approach, we utilized the concept of photocaging that involves protection of drug molecule with light sensitive protecting group (nitroveratryl). We conceptualized that if a cell impermeable moiety is attached to the photocaged drug, we could make a prodrug that is cell impermeable in dark and when UV light is irradiated on this molecule, the drug gets released from the photocage and can permeate the cell membrane. We synthesized a photocaged doxorubicin molecule and attached it with hydrophilic moiety which is sulfated as a result of which we expect that Dox-nitroveratryl conjugate to be cell impermeable and to be activated only when irradiated with light. This drug delivery strategy works very well with the esophageal tumor cell line and could be used for internal tissue diseases that are accessible via endoscopic fiber optic technology.

## A-15 Dynamic oligomeric behavior of AAV-2 Rep68

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### Physiology and Biophysics

The Adeno-Associated Virus (AAV) Rep proteins (Rep40, Rep52, Rep68 and Rep78) belong to the SF3 family of helicases with a central AAA+ domain. The larger Rep68 and Rep78 have an additional N-terminal domain that binds DNA specifically and contain an endonuclease activity that is critical during DNA replication. The combination of the two domains gives these proteins extraordinary multifunctionality to work as initiators of DNA replication, regulators of transcription in addition to their essential role during site-specific integration into human chromosome 19. Structural characterization of Rep68 protein has proved difficult to study due to its tendency to aggregate even at moderate concentrations. We report here a comprehensive study using a multifaceted approach to understand the oligomeric nature of Rep68. We show that Rep68 aggregation originates from the oxidation of residue Cys151. Mutation of this residue produces a Rep68 protein that is amenable to structural studies. Sedimentation velocity studies show that Rep68 is in a complex dynamic equilibrium of multiple oligomers, dominated by two major species that sediment at ~4S and 13S. The 4S species corresponds to a monomer-dimer in rapid equilibrium. Electron microscopy studies show that the 13S specie is a heptameric ring. Surprisingly, the presence of ATP or ADP generates a larger species that sediments at ~27S. The role of any of these structures in any of the Rep68 activities remains to be investigated but illustrates the special character of AAV Rep68 needed to carry out the variety of DNA transactions during the AAV virus life cycle.

## A-16 Effect of an oxygen therapeutic agent on systemic parameters of transgenic sickle cell mice

Sami Dodhy

Internal Medicine

Transgenic mouse models have been developed to mimic human sickle cell disease (SCD) to investigate its complex pathophysiology. Oxygen therapeutic agents (OTAs) are solutions have been developed to allow hemoglobin to circulate in the plasma and enhance oxygen delivery. Because of this oxygen delivery capacity, OTAs may be used as adjunct treatments for sickle cell disease. MP4CO (Sangart, Inc., San Diego) is an OTA that releases carbon monoxide and then circulates as an oxygen carrier. Carbon monoxide might have beneficial effects in sickle cell disease. The purpose of this project is to study the effects of MP4CO on systemic parameters of transgenic SCD mice. Transgenic mice were anesthetized, tracheostomized, and their jugular vein was cannulated for fluid infusion. A rectal thermometer, pneumogram, and ECG leads were placed on the mice appropriately to acquire the core temperature, breath rate and heart rate, respectively. The mice underwent fifteen minutes of baseline measurements, one hour of hypoxia at 12% oxygen and one hour of reoxygenation at 21% oxygen. Two different substances were injected during hypoxia: Lactated Ringer's at 8 ml/kg or MP4CO at both 4 ml/kg and 8 ml/kg. Successful experiments required tight control of temperature (maintained at  $37.0 \pm 0.5$  C) and surgical technique. Preliminary data show that Tg SCD mice given a dose of MP4CO at 4 ml/kg had a lower heart rate during reoxygenation and an even lower heart rate with 8 ml/kg during reoxygenation when compared to mice infused with Lactated Ringer's. Breathing rates of Tg mice given MP4CO at 4 ml/kg and 8 ml/kg were respectively higher and deviated less from baseline during both after the infusion and during reoxygenation when compared to Lactated Ringer's. Survival and arterial oxygen saturation were unaffected by MP4CO. The results show that MP4CO affects heart rate and respiration rate but was well tolerated in Tg SCD mice. Further investigation is required.

## A-17 Evidence of Shared Polygenic Risk Among Smoking Behaviors and Body Composition

Roseann E. Peterson\*, Xiangning (Sam) Chen, Jingchun Chen, Bradley T. Webb, Hermine H. Maes

### Human and Molecular Genetics

Obesity and nicotine dependence (ND) represent complex heterogeneous diseases which pose serious public health problems, affecting 33 and 20 percent of Americans, respectively. While cross-sectional studies of ND are typically supportive of a negative relationship between smoking and body mass index (BMI), a positive association is supported by the observations that, within smoking cohorts, heavy smokers tend to be of increased bodyweight compared to light smokers. A growing body of literature demonstrates the utility of genome-wide association studies (GWAS) for identifying single nucleotide polymorphisms (SNP) that contribute to disease risk. The GWAS approach has been applied to BMI and smoking behaviors (SB) using sample sizes in the tens of thousands and yielded several putative risk variants of small effects on individual traits. Many traits show comorbidity but most studies do not examine common versus specific variants. The purpose of this study was to investigate whether variants affecting BMI or SB were common to multiple behaviors or were trait specific. 75 BMI and 54 SB associated SNPs were catalogued from large-scale GWAS meta-analyses. These variants were tested for association in  $n=2,802$  (41% African-American) older community-dwelling adults (68-80 yrs) from the HABC study. **Preliminary Results:** Current smokers had significantly lower BMI and abdominal visceral fat than never or former smokers in males and females ( $p < 0.0001$ ). There were three BMI SNPs associated with both body composition variables and smoking traits: rs1900273 in STK33, rs2145270 near BMP2 and rs12127438 in the 1q42.2 locus. Additionally, there were three SB SNPs associated with multiple traits: rs11072774 in CHRN4, rs2640732 in SCARA3 and rs6945244 in PDE1C. Results indicate shared genetic risk between smoking and body composition. Future research should confirm these associations and address mechanisms behind the common genetic architecture underlying these traits.

**A-18 EXAMINING THE ASSOCIATION BETWEEN GPSM1 AND HUMAN ALCOHOL DEPENDENCE (AD)  
USING ASSOCIATION AND EXPRESSION STUDIES**

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Human and Molecular Genetics

Relapse, a hallmark of addiction, may result from neuroadaptations in the brain during chronic drug use resulting in the brain's inability to function normally during periods of withdrawal. Model organism studies can mimic certain aspects of the process of human withdrawal, craving and relapse. G-protein signaling modulator 1 (GPSM1) expression in rat nucleus accumbens (NAc) increases following withdrawal from chronic ethanol exposure and results in an increased motivation to seek ethanol (Bowers et al., 2008). We investigated the role of GPSM1 in human AD and alcohol-related phenotypes using association data and expression studies. Tag SNPs for the LD block surrounding GPSM1 (chr9:138261561-138455700) were selected using HAPMAP and Haploview. The Irish Affected Sib Pair Study of Alcohol Dependence case-control sample (N=562 cases, N=569 controls) was genotyped and association analyses performed using Haploview and Plink. 2 of the 6 SNPs (rs10781510, rs3124994) were significantly associated with AD (P=0.01, P=0.003) and DSM-IV AD symptom 3 (P=0.009, P=0.004). Symptom 3 gauges if the subject has ever drunk more than intended. This phenotype suggests an increased, uncontrollable motivation to drink (craving) for alcohol that may relate to the observed phenotype in rats. Primers were designed to uniquely amplify all 4 Refseq GPSM1 transcripts and real-time PCR run to assess RNA levels in whole brain tissue. Human alcoholic (N=41) and control (N=41) brain samples from the TRC in Sydney, Australia will be examined for RNA expression of the 4 transcripts in the NAc.

## A-19 EXPRESSION AND FUNCTION OF BILE ACID RECEPTOR TGR5 IN GASTROINTESTINAL SMOOTH MUSCLE

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### Physiology and Biophysics

Bile acids, the main active constituents of bile, act, in addition to their role in nutrient absorption, as signaling molecules to exert genomic and non-genomic effects via nuclear receptors and the plasma membrane G protein-coupled receptors TGR5. TGR5 is expressed in epithelial cells and enteric neurons of the gastrointestinal tract and may mediate the effects of bile acids on motility and secretion. However, the mechanisms by which bile acids regulate smooth muscle function are not known. **AIM.** To determine the expression of TGR5 in gastrointestinal smooth muscle and identify their signaling pathways. **METHODS.** TGR5 expression was determined by RT-PCR and western blot. G protein activation in response to TGR5 selective ligand, oleanolic acid (OA) was measured as increase in Galpha binding to [35S]GTPgammaS. OA-induced increase in cAMP was measured by ELISA and muscle relaxation by scanning micrometry. **RESULTS.** TGR5 expression was detected by RT-PCR and western blot in rabbit gastric muscle and human intestinal muscle. OA activated Galphas, but not Galphaq, Galphai1, Galphai2, or Galphai3. Consistent with activation of Galphas, OA increased cAMP levels. OA did not elicit contraction, but caused relaxation of carbachol-induced muscle contraction. **CONCLUSION.** Smooth muscle cells express membrane receptors (TGR5) for bile acids preferentially coupled to Gs. The receptors mediate stimulation of adenylyl cyclase activity and muscle relaxation.

## A-20 Expressions of ATP-binding cassette transporters varies among subtypes in breast cancer

Akimitsu Yamada, Takashi Ishikawa, Masayuki Nagahashi, Kazuaki Takabe

### Surgery

**Background:** The ATP binding cassette (ABC) transporters are membrane bound proteins that efflux agents from cells and exert crucial roles to survive themselves. Thus, ABC transporters form different biological features of cells. Breast cancer consists of several subtypes which show different biological behaviors from each other. We therefore examined expressions of several important ABC transporters in light of subtypes of breast cancer.

**Patients & Methods:** Tissue microarray (TMA) was constructed from representative area in tumors collected from 289 patients with breast cancer. Based on the immunohistochemical expressions with estrogen- and progesterone-receptors (ER, PgR) and human epidermal growth factor receptor 2 (HER2), these tumors were subdivided into four subtypes. For triple negative subtype, cytokeratin 5/6 and epidermal growth factor receptor were also examined for further subdividing basal- or non-basal subtypes. The protein expressions of ABC transporter were examined in these tumors. The positive expression rates of each transporter were compared between subtypes. Disease-free survival (DFS) in the context of expressions of each transporter was analyzed

**Results:** TMA contained breast cancer with 190 luminal A (67.3%), 17 luminal B (6.0%), 26 HER2 (9.2%), and 50 triple negative (17.5%) subtypes. The expression of ABCB1 did not differ among subtypes. In light of the hormonal expression of tumors, the rate of ABCC1 and ABCG2 expression are significantly higher in hormone negative tumors than positive ones ( $p=0.003$  and  $0.003$ , respectively). The expressions of ABCC1 and C11 were associated with the worse DFS.

**Conclusion:** The expression profile of ABC transporters was different among breast cancer subtypes. The expression of some transporters may be associated with the prognosis of breast cancer. The further studies of ABC transporters in each subtype of breast cancer may provide important information for the different biology of breast cancer subtypes.

## A-21 Factor Analysis of the Multidimensional Attitude Scale in a Population of Ethiopian College Students

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Department of Biostatistics

**Introduction:** The purpose of this study was to examine the attitudes of Ethiopians college students towards people with persons with disabilities using a series of factor analyses. The primary goal of this study was to determine if three-factor solution on Jewish Israelis population (Findler et al., 2007) could be confirmed using an Ethiopian population, and if not to determine if a modified version of the three-factor solution or an entirely new factor solution better described this population.

**Methods:** The 34 item MAS were collected from a sample of 245 participants. The goodness of fit of CFA models were evaluated through statistical indices: the standardized root mean square ( $SRMR \leq 0.08$ ), the root mean square error of approximation ( $RMSEA \leq 0.06$ ), the comparative fit index ( $CFI \leq 0.9$ ), and the non-normed fit index ( $NNFI > 0.9$ ). All analyses were conducted by using SAS V.9.2.

**Results:** The three factor solution of MAS based on the Jewish Israeli population did not confirm well in an Ethiopian population ( $SRMR=0.0992$ ,  $RMSEA=0.0792$ ,  $CFI=0.711$ ,  $NNFI=0.6906$ ). However, the modified CFA model did provide a substantial improvement in fit ( $SRMR=0.0623$ ,  $RMSEA=0.0660$ ,  $CFI=0.8764$ ,  $NNFI=0.8630$ ). The EFA suggested that a five-factor solution was more appropriate in the Ethiopian population than the three-factor solution or modified three-factor solution, explaining over 93% of the total sample variance.

**Conclusions:** The factor structure of the MAS based on the Jewish Israeli population did not confirm well to the Ethiopian population. Differences in culture, education, social status, and environment could explain the improvements seen in modified CFA and in the EFA.

## A-22 FTY720 Suppressed Breast Cancer Lung Metastasis using a 4T1-Luc 2 Cell Implantation Model

Barbara Adams, Masayuki Nagahashi, Subramaniam Ramachandran, Nitai Hait, Sheldon Milstien, Sarah Spiegel and Kazuaki Takabe

### Surgery

**Objectives:** Sphingosine-1-phosphate (S1P) is a lipid mediator, which plays an important role in breast cancer progression including cell proliferation, invasion and angiogenesis. FTY720 inhibits S1P by binding to its specific receptor, S1P1, and is already approved by the FDA as an S1P receptor modulator for multiple sclerosis. The objective of this study is to investigate the effect of FTY720 in breast cancer progression.

**Methods:** We used a syngeneic model with orthotopically implanted 4T1-luc2 cells. Metastatic lesions and the overall tumor burden were quantified using bioluminescence technology. S1P in serum was measured using mass spectrometry and S1P1 gene expression was evaluated by qPCR. One mg/kg of FTY720 was given orally each day for treatment after implantation.

**Results:** Overall tumor burden increased exponentially after 4T1-luc2 cell implantation, and metastases to the lymph nodes and the lungs were apparent by days 4 and 14, respectively. Elevation of serum S1P levels were also observed at the time of metastatic lesion progression. S1P1 gene expression was highest in the lung metastasis, followed by the primary tumor and then the cells in vitro. One mg/kg FTY720 daily suppressed the overall tumor burden to half of the size of the control group at 10 days after treatment (n=6, p=0.015). Lastly, the growth of the metastatic lesions following mastectomy was also suppressed by daily administration of FTY720.

**Conclusion:** Increasing serum S1P levels during the course of breast cancer progression, and high S1P1 gene expression in metastases implicate that FTY720 may be effective against advanced breast cancer. The administration of FTY720 has the ability to suppress overall tumor burden growth, as well as the growth of metastatic disease.

## A-23 Genes Have Both Trait-Specific and General Association with Measures of Alcohol Use and Abuse

Jacquelyn L. Meyers, Emma Nyman, Anu Loukola, Richard J. Rose, Jaakko Kaprio & Danielle M. Dick

### Human and Molecular Genetics

**Background:** Finntwin12 provides a unique opportunity to both model the genetic architecture of measures of alcohol consumption and problems and to use the resulting latent genetic factors in gene finding analyses.

**Methods:** Here we present the best fitting twin model of 5 measures of alcohol consumption (frequency of alcohol use, quantity of alcohol use, frequency of intoxication, maximum drinks consumed in a 24 hr. period) and problems (DSM-4 Alcohol Dependence symptom count) as well as results from genome wide association analyses performed on each of the resulting latent genetic factors.

**Results:** There was some overlap in the genes that each GWAS produced however, some latent genetic factors returned unique genes. Different measures of alcohol consumption and alcohol problems may be influenced by different genes.

**Conclusions:** Prioritization of the association signals must be conducted to identify those genes and SNPs which contribute to one's regular alcohol use and risk for problematic alcohol use and distinguish real signals from false positives. Signals implicated in this sample must be replicated in independent samples.

## **A-24 Genome-wide association studies with stratifications**

Wenan Chen, Guimin Gao

### Biostatistics

Genome-wide association studies (GWAS) have identified hundreds of genetic variants with more than 100 disease and traits. In a typical GWAS, population stratification needs to be controlled or it may result in a number of false positives. In an association test, another important assumption is the mode of inheritance. If the underlying mode of inheritance of the genetic variant is consistent with the model assumption, we can have high power detecting the susceptible locus. However, if the model is misspecified, the power could be very low. In this study, we developed an association test accounting for population stratification, whose power is high when the mode of inheritance is additive or multiplicative, and maintains good power when the mode of inheritance is dominant or recessive comparing to other methods.

## **A-25 KLF2 is Required for Normal Cardiac Development in Mice**

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### Human and Molecular Genetics

KLF2 is expressed in endothelial cells in the developing heart, particularly in areas of high shear stress, such as the atrioventricular (AV) canal. Ablation of KLF2 leads to high output cardiac failure in mouse embryos in a mixed genetic background, and they die by embryonic day 14.5 (E14.5). This work identifies an earlier and more fundamental role for KLF2 in the cardiac development of mice in the FVB/N genetic background. FVB/N KLF2<sup>-/-</sup> embryos die by E11.5. In E9.5 WT hearts, the cells lining the AV cushions, the primordia of the AV valves, form a single layer. In E9.5 FVB/N KLF2<sup>-/-</sup> hearts, these cells form multiple, disorganized layers and there are 2-fold more cells. In E10.5 FVB/N KLF2<sup>-/-</sup> embryos, the AV cushions are hypoplastic and the cells accumulating and lining the AV canal have endothelial characteristics, indicating a defect in endothelial to mesenchymal transformation (EMT). E10.5 FVB/N KLF2<sup>-/-</sup> hearts have a reduction in glycosaminoglycans in the cardiac jelly, which correlates with the lack of EMT. These AV phenotypes are genetic background dependent. At E10.5, cardiac function in FVB/N KLF2<sup>-/-</sup> embryos is abnormal. Echocardiography indicated that cardiac output and ejection fraction are significantly higher, but the velocity of blood in the descending aorta is significantly reduced compared to WT. Therefore, KLF2 is required for normal endocardial cushion development and heart function in FVB/N embryos. The mechanism of action of KLF2 in directing cardiac jelly synthesis and endocardial cushion remodeling may be through its regulation of the UDP- glucose dehydrogenase, GATA binding protein 4 and T-box 5 genes.

**A-26 MICRORNA DYSREGULATION IN PROSTATE CANCER: BIOLOGICAL NETWORK ANALYSIS REVEALS PREFERENTIAL REGULATION OF HIGHLY CONNECTED PROTEIN NODES**

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MiRNAs are small molecules that influence a large number of cellular processes including cell growth, differentiation, and apoptosis. miRNAs regulate gene expression post-transcriptionally by binding to mRNA targets causing translational inhibition or mRNA degradation. Dysregulation of miRNA expression can lead to the development of cancer. We have found over 100 miRNAs are dysregulated affecting 608 protein targets in prostate cancer. To date, considerable research has focused on the function and expression of single miRNAs. However, complex processes require the interaction, regulation and coordination of many molecules including miRNAs and proteins. Highly connected molecules often serve important roles in the cell. We propose that interactions among these molecules can be modeled as a network and topology can be described mathematically. A protein interaction network confirmed miRNA target proteins essential to cellular stability are highly connected. They affect pathway components known to be involved in cancer initiation and progression such as MAPK14, VEGF, and ErbB2. This phenomenon, not observed in a network of randomly chosen prostate proteins, demonstrated that miRNAs contribute to the overall health of the prostate and their aberrant expression could destabilize the homeostatic balance leading to cancer. As a protein becomes more highly connected, it is subject to regulation by multiple miRNAs and dysregulation of any of these miRNAs could lead to disease. This integrative, networks approach revealed important miRNAs and proteins in prostate cancer that will be useful to identify specific disease biomarkers, which may be used as targets for therapeutics or drugs in themselves.

**A-27 Molecular analysis of FhbB, the factor H binding protein of the periodontal pathogen  
*Treponema denticola***

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Microbiology and Immunology

Periodontal disease affects nearly 116 million people in the United States and is the most common infection of middle-aged adults. *Treponema denticola* is a periodontal pathogen that is highly abundant in periodontal pockets. In order to survive and thrive in the oral cavity, *T. denticola* must be able to evade human defense mechanisms including the complement system that is abundant and active in crevicular fluid. We have demonstrated that *T. denticola* and several other pathogenic spirochete species evade complement-mediated destruction by binding the human complement regulatory protein factor H (FH). The sole *T. denticola* protein involved in FH binding is FhbB. A *T. denticola* fhbB deficient mutant was found to be highly sensitive to complement mediated killing in human serum. Interestingly, bacterial FH binding proteins show little or no homology suggesting that their interaction with FH is not mediated by primary sequence but rather by common structural features. Since FhbB is the smallest known FH binding protein, analysis of its structure could result in the identification of the minimal determinants required for binding. In this study, X-ray crystallography was used to determine the molecular structure of FhbB. The structure, determined to a resolution of 1.8 angstroms, revealed unique folds within FhbB. To assess the molecular basis of the interaction between FhbB and FH, site-directed mutations were constructed and surface plasmon resonance analyses were performed. Specific residues critical for binding FH were identified. The data presented here represent a significant step forward in our understanding of the molecular basis of interactions between FH and microbially produced binding proteins.

**A-28 NOVEL ROLE OF THE  $\mu$ -OPIOID/NOCICEPTIN ORPHANIN Q RECEPTOR SYSTEM IN OLIGODENDROCYTE DEVELOPMENT AND BRAIN MYELINATION**

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Biochemistry and Molecular Biology

While the classical function of myelin is to facilitate saltatory conduction, this membrane and the myelin making oligodendrocytes are now recognized as regulators of plasticity and remodeling in the developing central nervous system (CNS). As such, oligodendrocyte maturation and myelination are among the most vulnerable processes along CNS development. We showed that rat brain myelination is altered by buprenorphine (BUP), an opioid receptor agonist currently in clinical trials for the treatment of pregnant opioid addicts. Perinatal exposure to low BUP doses induced accelerated and increased expression of all splicing variants of myelin basic proteins (MBPs), cellular and myelin components that are markers of mature oligodendrocytes. In contrast, supra-therapeutic drug doses delayed MBP brain expression and resulted in a decreased number of myelinated axons. Using cultured oligodendrocytes, we found that the in vivo effects on myelination can result from direct drug action on the oligodendrocytes. Furthermore, just like in vivo, BUP effects on cultured cells also exhibit a bell-shaped dose response. Low drug doses induce an up-regulation of all MBP isoforms, which is mediated by the  $\mu$ -opioid receptor (MOR). Interestingly, this stimulatory effect is lost at higher drug concentrations, but is unexpectedly restored by inhibition of the nociceptin/orphanin Q receptor (NOPR). Moreover, the endogenous NOPR ligand nociceptin counteracts the MOR-dependent stimulatory effects. This finding is particularly intriguing because the NOPR/nociceptin system has been linked to behavior and pain regulation but a role in CNS development has not been described. Our observations indicate that a delicate balance between MOR and NOPR signaling plays a crucial role in the timing of oligodendrocyte maturation and myelin formation. Exposure to BUP, and other opioids, may disrupt the normal interplay between these two receptors, altering the developmental pattern of brain myelination.

## A-29 Optimization Of Terpenoid Precursors in *Thermofibida fusca* Using in silico Modeling

Niti Vanee, Stephen S. Fong

### Integrative Life Sciences

Natural products play an important role in manufacturing of several active pharmaceutical ingredients (APIs). APIs or precursors of APIs can be produced in living organisms with the major challenge of designing and optimizing metabolic pathways to obtain the compounds of interest. In this capacity, living organisms can act as renewable catalysts with high product specificity to produce APIs with potential cost savings over purely synthetic chemistry synthesis routes.

Cellulolytic organisms are being heavily studied for the production of biofuels, given that lignocellulosic biomass would be a cheap, abundant, and renewable starting material for chemical production. A challenge with cellulolytic microorganisms is that they are typically poorly characterized and often difficult to genetically manipulate. Here we propose that the aerobic, cellulolytic actinobacterium, *Thermofibida fusca*, may be a good candidate for cellulolytic production of APIs.

In the current study, in silico characterization and analysis of the non-mevalonate pathway (or DXP pathway) will be performed using a genome-scale metabolic model of *T.fusca*. The DXP pathway leads to the production of terpenoid precursors that have applications in nutraceuticals and pharmaceuticals. Modeling analysis illustrates the presence of the genes required to form substrate for producing monoterpenoids, sesquiterpenoids, and diterpenoids precursors. The constructed metabolic model provides a basis for conducting in silico strain design predictions to optimize the production of terpenoid precursors through metabolic flux analysis to provide a rational basis for determining the distinct possible target gene sets for increased product synthesis. In parallel with these computational predictions, experimental work is being conducted to verify specific biochemical pathways and to implement novel pathways in *T. fusca*.

**A-30 Pituitary Adenylate Cyclase-Activating Peptide (PACAP) and Substance P (SP) induce the release of Brain-Derived Neurotrophic Factor (BDNF) from the longitudinal muscle**

Mohammad Alqudah, Sunila Mahavadi, Zachary L. Bradley, Jarren C. Kay, Karnam S. Murthy, and John R. Grider

Physiology and Biophysics

BDNF has been implicated in neuronal development and plasticity. While BDNF is expressed in neural and non-neuronal cells, the expression and secretion of BDNF in gastrointestinal smooth muscle is not well defined. **Aim:** To determine if BDNF is expressed and secreted from intestinal smooth muscle. **Methods:** BDNF expression in intestinal segments was evaluated immunohistochemically. Longitudinal muscle cells were cultured for 24 to 48 hours in the absence or presence of SP or PACAP (10 to 100 nM); BDNF content in cells and secretion into media was measured by western blot and ELISA. **Results:** Immunohistochemically, BDNF was identified primarily in the longitudinal rather than circular muscle layer of mouse and rabbit intestine. Western blot analysis of BDNF from longitudinal muscle cultures confirmed basal BDNF expression and demonstrated time- and concentration-dependent increase in BDNF content in response to both neuropeptides, with PACAP being more effective than SP. Similar results were obtained for release of BDNF into culture medium. **Conclusion:** In the basal state, intestinal smooth muscle cells from the longitudinal layer express and secrete BDNF. Contractile and relaxant neuropeptides modulate this expression and secretion suggesting crosstalk between enteric neurons and smooth muscle via interplay between neuropeptide transmitters and neurotrophins.

## A-31 Predictors of Headache Density after Traumatic Brain Injury

Amber R. Wilk, Dr. Jessica M. Ketchum, Dr. William C. Walker, Jenny H. Marwitz

### Biostatistics

**Objective:** To determine if a specific set of variables are predictive of headache (HA) density at 3, 6, and/or 12 month post traumatic brain injury (TBI). Setting: Traumatic Brain Injury Model System and HA Module Database. Participants: 450 adult participants admitted to inpatient rehabilitation (February 2008 to June 2009). Outcome Measure: HA Density Index, a function of frequency, duration, and pain intensity. Methods: Generalized linear mixed model theory was used to fit an ordinal logistic regression model to model the repeated measures of the 6 level ordinal outcome as a function of sex, pre-injury history of HA, limitations due to HA, type of TBI, evidence of a skull, spine, or face fractures, and time post-traumatic amnesia (PTA). **Results:** Three months post-TBI, history of pre-injury HA was the only significant predictor of HA density post-TBI. The odds of more severe HAs post-TBI were greater for those with a pre-injury history of migraine HAs (OR = 10.38, 95% CI = 1.99, 54.02) or non-migraine HA (OR = 4.12, 95% CI = 1.62, 10.46) versus with no history of HA. Six months post-TBI, history of pre-injury HA, sex, and type of TBI were significant predictors of HA density post-TBI. The odds of more severe HAs post-TBI were greater for females versus males (OR = 2.59, 95% CI = 1.31, 5.13), for those with a pre-injury history of migraine HA (OR = 10.11, 95% CI = 2.20, 46.54) or non-migraine HA (OR = 3.20, 95% CI = 1.25, 8.20) versus no history of HA, and for those with penetrating versus closed injuries (OR = 4.14, 95% CI = 1.13, 15.17). Twelve months post-TBI, the odds of a more severe HA post-TBI were greater for those with a pre-injury history of migraine HA versus no history of HA (OR = 8.66, 95% CI = 1.62, 45.96). **Conclusion:** Sex, pre-injury history of HAs, and TBI type were significant predictors of the severity of post-injury HA density. Females, those with pre-injury HAs, and penetrating injuries were at the greatest risk for more severe HAs after injury.

## A-32 Proteomics Suggests ErbB2/ErB3 as a New Target of miR17-3p Regulation

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miRNAs are small molecules that post-transcriptionally regulate expression of target mRNAs. Previously, data from miRNAarray screens coupled to gene arrays have been used to predict mRNA targets. This approach assumes that all miRNAs regulate gene expression by degrading their respective targets, which is not always the case. Since the end result of miRNA targeting is reduced protein synthesis, we propose that proteomics using RPMA, Reverse Phase Protein MicroArrays, is a better approach for deducing targets of miR regulation. To investigate this hypothesis, we used a progressive prostate cancer cell model consisting of the immortalized p69 human prostate cell line and its highly tumorigenic, metastatic variant, M12. RPMA data from the M12 subline showed a significant increase in ErbB2 [1.7X] and ErbB3 [2X] protein expression with a significant decrease [ $>2X$ ] in miR 17-3p expression via qRT-PCR compared to the parental p69 cell line. M12s stably transformed to increase miR17-3p expression 5-fold (M12+miR17-3p), resulted in a significant decrease in ErbB2 [1.9X] and ErbB3 [1.4X] protein expression via RPMA data. RNA Hybrid prediction software suggested that both the 3'UTRs of ErbB2 and ErbB3 possess possible miR17-3p binding sites with a favorable  $\Delta G$ . Using a dual luciferase reporter gene assay, inclusion of the ErbB2 3'UTR resulted in a decrease [2X] in luciferase activity compared to the parental vector in M12+miR17-3p cells. RPMA has suggested new targets for miR17-3p regulation, which were not evident from previous approaches.

### A-33 Risk Factors of Suicide Two Years Post Traumatic Brain Injury

Dr. Jessica Ketchum, Dr. Juan Carlos Arango-Lasprilla, Jenna Czarnota

#### Biostatistics

**Objective:** To determine which demographic, injury, rehabilitation, and 1 year post-injury characteristics are associated with suicide risk in individuals between 1 and 2 years post traumatic brain injury (TBI).  
**Design:** Retrospective study. **Setting:** Longitudinal data set of the TBI Model Systems National Database.  
**Participants:** 4575 individuals aged 18-89, hospitalized with TBI between 1997 and 2007. Participants were living and non-vegetative 1 year post injury, with available year 1 follow-up data, and discernible suicide risk at year 2 (attempted or successful). **Main Outcome Measure:** Suicide risk 2 years post-injury (yes/no). **Results:** A multiple logistic regression model indicated that employment status, level of education, level of alcohol consumption, arrests, total satisfaction with life, and attempted suicide in the year following injury were significant predictors of suicide risk 2 years post-injury, after controlling for the effects of each other (all p-values  $\leq 0.042$ ). Unemployment, less than a high school level of education, and occasional excessive alcohol use (as compared to never) were associated with increased odds of suicide risk 1-2 years post-injury. Arrests, lower satisfaction with life, and attempted suicide in the year following injury were also associated with increased odds of suicide risk. **Conclusions:** Surviving a TBI can be a life altering event with severe consequences (e.g. loss of support system, loss of job/income, increased psychological stress, decreases in function/independence). Increased risk of suicide has been found to occur more frequently in TBI patients than non TBI patients, thus identifying which factors are associated with an increased risk in this population is crucial. Based on the results of this study there are a variety of modifiable factors that increase risk for suicide post TBI. Interventions specific to the TBI population should be developed and implemented for individuals who are at an increased risk of suicide.

## A-34 Roles for Fyn and Lyn Kinases in FcγR Signaling and IgG-mediated Systemic Anaphylaxis

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### Biology

Mast cells (MCs) are key players in the innate immune system and atopic diseases. Once activated, they degranulate and produce cytokines, promoting the recruitment of other immune cells and the clearance of the infectious agent. In this study we focused on MC activation via the immunoglobulin G (IgG) receptors, FcγR. We found that crosslinkage of mouse mast cells with an antibody recognizing the low affinity receptors FcγRIIb and FcγRIII induced tyrosine phosphorylation of both Lyn and Fyn. We used Fyn- or Lyn-deficient (KO) mice to demonstrate that FcγR, like the IgE receptor, FcεRI, triggers mast cell degranulation and cytokine release via a Fyn- and Lyn-dependent manner. Lyn KO mast cells displayed increased FcγRIIb-mediated degranulation and cytokine release, while a decrease was observed using Fyn KO MC. We show that the role for Fyn and Lyn downstream of FcγR is not restricted to the MC lineage, as similar effects were found in basophils and macrophages. Furthermore, these effects were consistent in vivo, when measuring IgG-mediated passive systemic anaphylaxis (PSA). Collectively, these data demonstrate Fyn and Lyn activation during FcγR signaling and highlight their regulatory contribution to IgG-mediated inflammation.

**A-35 STRUCTURAL STUDIES OF REB1-TER3 COMPLEX & UNDERSTANDING REPLICATION TERMINATION USING SAXS & X-RAY CRYSTALLOGRAPHY**

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Physiology and Biophysics

DNA replication is the basis for biological inheritance and its proper completion is critical for cell viability. Progress of replication forks can be blocked by various endogenous events or exogenous factors. The blockage or collapse of replication forks could lead to pathological conditions such as cancer. However, physiologically programmed fork arrest occurs in cells at sites known Replication Fork Barriers (RFB) where replication termination proteins bind tightly to DNA and thus result in replisome arrest. RFBs ensure the proper directionality & prevent collisions of replisome with transcription machinery approaching from the opposite direction. Such polar fork arrest occurs mainly in nontranscribed spacers of ribosomal DNA (rDNA). Previous studies have identified three well-defined RFB (Ter1-Ter3) in fission yeast *Schizosaccharomyces pombe* that serves as binding site for replication terminator proteins, Reb1 and Sap1. Recent studies suggested Reb1 binds to both Ter2 & Ter3 sites.

In our studies, to understand the molecular mechanism of eukaryotic fork arrest, we focused on binding of Reb1 with Ter3 site. Biochemical analysis of protein-DNA complex helped us to define the minimum protein construct capable of binding to Ter3 sequence, exhibit replication termination and give a stable complex. Further, preliminary crystal screening helped us in designing the minimal base pairs requirement of Ter3 sequence. Moreover, these screening conditions gave us a base for optimizing the crystallization condition in order to get a high-resolution crystal. SAXS & AUC studies helps in understanding the biophysical characteristics of the Reb1 protein alone as well as in complex with the Ter3 DNA. Further recently obtained crystal diffraction patterns confirms the presence of DNA in complex with protein and provided us a little information about the lattice structure of crystal. The crystal diffracted to a resolution  $\sim 5\frac{1}{2}$ .

**A-36 Study of a common deletion in ERBB4 in the Irish high-density schizophrenia families and case/controls**

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Human and Molecular Genetics

Rare copy number variants (CNVs) and recurrent, low frequency CNVs are associated with schizophrenia. Common CNVs, by contrast, have not been looked at in detail, although there is clear potential for such CNVs to act as common, small effect-size variants in the etiology of common complex traits. In CNV calls from a genome-wide association study (GWAS) of Irish multiplex pedigrees, we observed an apparent excess of a common deletion in the first intron of ERBB4 when in affected family members compared to unaffected ones. We directly compared the frequency of this common CNV in 1) genetically independent members of the Irish multiplex pedigrees (n=277) and Irish population controls (n=448) and 2) Irish singleton cases (n=462) compared to Irish population controls. Family CNVs were called from Illumina 610 Quad array probe intensity data using PennCNV. A study of N=1320 North American population samples of European descent estimated the ERBB4 CNV frequency at 4.5%. We directly assessed Irish family, Irish singleton, and Irish control copy number status by real-time PCR. We called CNV genotypes using TaqMan copy number assays from ABI and COPY CALLER software. ERBB4 deletion frequency of 9.8% was seen in Irish family compared with 4.5% in the North American population sample (chi-sq=12.2, p=0.0005). Deletion frequency of 6.6% was seen in Irish singleton cases (N=462) compared to 7.7% Irish population controls (N=448) (chi-sq=0.8, p=0.36), and 9.8% Irish genetically independent family members (N=111) compared to Irish population controls (chi-sq=1.0, p=0.31). Although we detect an apparent excess of a common ERBB4 deletion in our multiplex family members compared to a North American population sample, the observed difference was shown to be due to ethnic differences. The difference in ERBB4 deletion frequencies is not statistically significant between Irish family compared to Irish population controls, nor between singleton cases compared to Irish population controls.

### **A-37 The complex structure of DnaK with ATP**

Ruifeng Qi, Qinglian Liu

Department of Physiology and Biophysics

The structures of DnaK itself and complexed with ADP have already previously resolved, but its complex structure with ATP has not been obtained so far. In this research, we successfully obtained the complex crystal and resolve its structure at 1.8 Å, by trying make new construct and all kinds of mutations. Compared with the structures of DnaK itself or complexed with ADP, there is a great difference in the complex one with ATP. Thereby, the whole process of DnaK binding and hydrolyzing ATP is shown clearly. In the complex structure, some new important sites of DnaK interacting with ATP are observed and some mutations about them and relevant biochemical assay are under conducting in our lab.

### **A-38 THE CRITICAL ROLE OF SPHINGOSINE KINASE 1 AND SPHINGOSINE-1-PHOSPHATE IN CD40-MEDIATED IMMUNE RESPONSE**

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Biochemistry and Molecular Biology

The tumor necrosis factor (TNF) receptor family member CD40 plays an essential role in the activation of antigen presenting cells, B cell maturation, and isotype antibody class switching critical for adaptive immunity and autoimmune diseases. Binding of CD154 to CD40 recruits a signaling complex containing TNF receptor associated factor 2 (TRAF2), TRAF3, and TRAF6 to its cytoplasmic C-terminal tail, prompting further recruitment of cellular inhibitor of apoptosis 1 and 2 (cIAP1/2). Within this complex, TRAF2 is K63-ubiquitinated, and cIAPs, which require TRAF2 for their K63-linked ubiquitination, promote K48-linked ubiquitination of TRAF3 and proteasomal degradation. This is necessary for subsequent release of the complex and propagation of downstream signaling pathways. Recently, our lab discovered that sphingosine-1-phosphate (S1P) produced by sphingosine kinase 1 (SphK1) is an essential cofactor for TRAF2-catalyzed K63-linked polyubiquitination of RIP1, a critical event in activation of NF- $\kappa$ B. We have now found that ligation of CD40 activates SphK1 and that SphK1 activity was required for downstream activation of NF- $\kappa$ B and JNK pathways. Moreover, SphK1 was important for efficient recruitment of cIAP1/2 and TRAF3 to CD40 and degradation of TRAF3. Furthermore, S1P not only enhanced autoubiquitination of TRAF6, cIAP1, and cIAP2 in vitro, but also enabled cIAP1-mediated K48 ubiquitination of TRAF3. Importantly, CD40-mediated antibody production, isotype switching, and plasma cell differentiation required SphK1 activity. Taken together, our results suggest that SphK1 is a key regulator of CD40-mediated complex formation by generating S1P which is required for the K63 ubiquitination of TRAF2, recruitment of cIAP1/2, and in turn, K48 ubiquitination and degradation of TRAF3, necessary for propagation of further downstream signaling. Therefore, targeting SphK1 might be a useful therapeutic approach for autoimmune diseases.

## A-39 Cancer Testis Antigens as prognostic biomarkers for breast cancer patients

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### Microbiology and Immunology

We have previously reported that the presence of a distinct immune function gene signature network at the tumor lesions of patients with early stage breast cancer could predict relapse-free survival following conventional therapies. We hypothesized that expression of cancer testis antigens (CTA) may be responsible for converting weakly immunogenic breast tumors into highly immunogenic tumors, and result in relapse-free survival. To test this hypothesis, we performed qRT-PCR analysis of RNA extracted from tumor lesions of patients with breast cancer from which we compared CTA expression levels of those who relapsed within 1-3 years with those who remained relapse-free during 5-7 years follow-up. We detected an increased expression of a number of CTA in tumor lesions of patients who remained relapse-free but not in those with tumor relapse. We also showed that treatment of human breast tumor cell lines with a demethylating agent, Decitabine, induced expression of CTA in the tumors. Altogether, these data suggest that lack of CTA expression in tumor lesions of breast cancer patients at the time of diagnosis may predict high risk of tumor relapse, and that using Decitabine in a neoadjuvant setting may convert patients with high risk into those with low risk of tumor relapse.

**A-40 Adaptive Immunotherapy in Multiple Myeloma: Epigenetic Modification and Immunomodulation by Sequential Azacitidine and Lenalidomide to Generate Cancer Testis Antigen Specific Cellular Immune Response**

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Microbiology and Immunology

Malignant plasma cells in patients with multiple myeloma (MM) occasionally express highly immunogenic cancer testis antigens (CTA). Since CTA expression is regulated by DNA methylation, we hypothesized that CTA may be induced by 5-azacitidine, and facilitate CTA-specific immune responses against MM; the addition of lenalidomide (L) may then augment any ensuing adoptive cell therapy (ACT). We initiated a phase II clinical trial of Aza administered sequentially with L in patients with residual disease following initial therapy. Three cycles of Aza (75 mg/m<sup>2</sup> day 1-5 SQ) and L (10 mg PO daily, day 6-21) were administered every 4 weeks; autologous lymphocytes were collected around day 21 of the 2nd and 3rd cycles of Aza-L and cryopreserved for ACT. Subsequent stem cell mobilization was followed by melphalan 200 mg/m<sup>2</sup> and stem cell transplantation (SCT). GM-CSF was used post-transplant for hematopoietic engraftment. ACT was performed between day +30 to +60. To date 6 patients have undergone SCT and no unexpected post transplant toxicities were observed. Four patients received ACT at a median 42 days following SCT with no immediate or remote infusional toxicities. With a median follow up of 9 months post-transplant, all four ACT recipients are progression free with either complete remission (n=1) or VGPR (n=3). qRT-PCR evaluating a panel of 10 CTA in unfractionated bone marrow specimens collected before and after Aza-L from five patients demonstrated 6-8 discrete CTA induced in each patient. This expression was mainly detected in CD138+ plasma cells. NY-ESO 1-specific T cell response was detected following Aza-L treatment, and lasted for several months post-transplant. CD3+ T cell counts before and after ACT demonstrated a marked increase in T cell counts at two (mean 959/μl; n=4) and eight (1277/μl) weeks, compared with baseline (414/μl; P=0.05). This study was supported by celgene corporation.

## **A-41 The role of MBD2-NuRD corepressor complex in the developmental regulation of fetal gamma-globin gene**

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### Human and Molecular Genetics

Epigenetic mechanisms such as DNA methylation and histone modifications play a pivotal role in the developmental regulation of embryonic/fetal  $\beta$ -type globin genes, wherein the MBD2/NuRD (Nucleosome Remodeling and Deacetylase) complex is an important player. The MBD2/NuRD co-repressor complex binds methylated DNA through MBD2 and represses transcription of embryonic/fetal globin genes and aberrantly methylated tumor suppressor genes in human cancers. The complex also includes the components RbAp46/48, HDAC1/2, MTA1/2, p66 $\alpha/\beta$  and Mi-2 $\alpha/\beta$ . Previous studies in the lab showed that MBD2 contributes to the silencing effect on the human  $\gamma$ -globin and  $\epsilon$ -globin genes in adult erythroid cells of  $\beta$ YAC transgenic mice. However, it does not exert this effect by binding near the  $\gamma$ -globin gene promoter.

In an attempt to identify factors through which MBD2 might be exerting its repressive effect, we conducted microarray expression analyses to identify protein coding gene(s) and/or microRNAs regulated by MBD2. TZFP (testis zinc-finger protein) and miR-210 were identified as candidate downstream effectors of MBD2. MBD2<sup>-/-</sup> mice show increased expression of TZFP and miR-210 by 4-fold and 3-fold, respectively. MBD2 knockdown CD34<sup>+</sup> hematopoietic progenitor cells also show increased expression of TZFP and miR-210. We are investigating the functional effects of TZFP and miR-210 by their over-expression in CD34<sup>+</sup> HPCs. We are also investigating the mode of interaction of MBD2 with other components of the NuRD-co-repressor complex. Preliminary studies have identified a central region of MBD2 i.e. p55BR to be responsible for interaction with the NuRD components RbAp46/48, HDAC1/2 and MTA1/2. Through both structural and functional studies aimed at understanding the mechanism of gene silencing by the MBD2-NuRD corepressor complex, new molecular targeted therapy for beta globin disorders and cancer may be facilitated.

## A-42 The Role of Mitf and Otx Transcription Factors in Zebrafish RPE Development

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### Human and Molecular Genetics

The retinal pigment epithelium (RPE) is a layer of pigmented cells between the retina and choroid that is essential to the proper development and function of the vertebrate eye. In murine models of eye development, Mitf and Otx transcription factors have been identified as essential to RPE development with similar functions in activating pigment-related genes but the relationship between these factors and their specific role in the RPE developmental pathway have not yet been clearly defined. Using morpholino knockdown as well as mutant fish lines, the role of the two Mitf transcription factors (Mitfa and Mitfb) and two Otx transcription factors (Otx1a and Otx2) in zebrafish RPE development was explored. The loss of Mitf activity in mitfa, mitfb, or double mitf null mutant fish lines had no effect on RPE pigmentation or development. The loss of Otx2 activity through morpholino knockdown can produce a RPE deficient phenotype in a small percentage of embryos, while the additional knockdown of Otx1a leads to widespread and severe RPE developmental abnormalities. Analysis of ocular sections and immunohistochemistry revealed that the retinal layers including the rod and cone photoreceptors remain relatively unaffected in mitf mutants as well as in RPE-deficient Otx morphants, except in cases of severe ocular degeneration. Expression analysis studies have revealed that Otx transcription factors and in particular Otx1a are necessary for the proper expression of mitfa and mitfb. Combined Mitf and Otx loss of function experiments suggest that Mitfa and Mitfb both have a function in zebrafish RPE development but the presence of Otx transcription factors can compensate for the loss of Mitf activity due to their at least partially redundant functions. Current experiments are exploring the pigment gene activation potential of each of these transcription factors to further clarify the regulatory network controlling zebrafish RPE development.

**A-43 The unique peptide substrate binding properties of Hsp110 molecular chaperone determines its distinct chaperone activity**

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Physiology and Biophysics

Hsp70 molecular chaperones play essential roles in maintaining protein homeostasis. Hsp110, an Hsp70 homolog, is highly efficient in preventing protein aggregation, but lacks the hallmark folding activity seen in Hsp70s. To understand the mechanistic differences between these two chaperones, we first characterized the distinct peptide substrate binding properties of Hsp110s. In contrast to Hsp70s, Hsp110s prefer aromatic residues in their substrates, and the substrate binding and release exhibit remarkably fast kinetics. Sequence and structure comparison revealed significant differences in the two peptide-binding loops: the length and properties are switched. When we swapped these two loops in an Hsp70, the peptide binding properties of this mutant Hsp70 are converted to Hsp110-like, and more impressively, it functionally behaves like an Hsp110. Thus, the peptide substrate binding properties implemented in the peptide-binding loops determine the chaperone activity differences between Hsp70s and Hsp110s.

**A-44 Transcription Factors KLF1 and KLF2 Positively Regulate Embryonic and Fetal  $\beta$ -Globin Genes through Direct Promoter Binding**

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Human and Molecular Genetics

Krüppel-like factors (KLFs) control cell differentiation and embryonic development. KLF1 (erythroid Krüppel-like factor) plays essential roles in embryonic and adult erythropoiesis. KLF2 is a positive regulator of the mouse and human embryonic  $\beta$ -globin genes. KLF1 and KLF2 have highly homologous zinc finger DNA-binding domains. They have overlapping roles in embryonic erythropoiesis, as demonstrated using single and double KO mouse models. Ablation of the KLF1 or KLF2 gene causes embryonic lethality, but double KO embryos are more anemic and die sooner than either single KO. In this work, a dual human  $\beta$ -globin locus transgenic and KLF knockout mouse model was used. The results demonstrate that the human  $\epsilon$ - (embryonic) and  $\gamma$ -globin (fetal) genes are positively regulated by KLF1 and KLF2 in embryos. Conditional KO mouse experiments indicate that the effect of KLF2 on embryonic globin gene regulation is at least partly erythroid cell-autonomous. KLF1 and KLF2 bind directly to the promoters of the human  $\epsilon$ - and  $\gamma$ -globin genes, the mouse embryonic  $E\gamma$ - and  $\beta$ h1-globin genes, and also to the  $\beta$ -globin locus control region, as demonstrated by ChIP assays with mouse embryonic blood cells. H3K9Ac and H3K4me3 marks indicate open chromatin and active transcription, respectively. These marks are diminished at the  $E\gamma$ -,  $\beta$ h1-,  $\epsilon$ - and  $\gamma$ -globin genes and locus control region in KLF1 and KLF2 embryos, correlating with reduced gene expression. Therefore, KLF1 and KLF2 positively regulate the embryonic and fetal  $\beta$ -globin genes through direct promoter binding. KLF1 is required for normal histone modifications in the  $\beta$ -globin locus in mouse embryos.

**A-45 Understanding mechanisms generating 5-hydroxymethylcytosine in mammalian mitochondrial DNA**

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Microbiology and Immunology

DNA methylation plays a pivotal role in governing the normal function of many cellular processes including genomic imprinting, regulation of gene expression and development. Recent identification of 5-hydroxymethylcytosine(5hmC) in the mammalian nuclear DNA has added another element to epigenetic regulation of gene expression. 5hmC is generated by Tet family of methylcytosine dioxygenases(Tet1, Tet2 and Tet3), by hydroxylation of 5-methylcytosine(5mC) in a 2-oxoglutarate and Fe<sup>2+</sup> dependent manner. This conversion of 5mC to 5hmC enables active demethylation in the adult brain through a process involving base excision repair pathway, thus converting 5mC back to cytosine.

We have recently reported significant levels of 5mC and 5hmC modification in immunoprecipitates of mammalian mitochondrial DNA(mtDNA). We also provide first evidence that an isoform of DNA Methyltransferase 1(DNMT1) is translocated to the mitochondria, which suggests that DNMT1 is responsible for generation and maintenance of 5mC in mtDNA. However, mechanism generating 5hmC in the mtDNA remains unexplored. Our goal is to determine the enzyme responsible for generation of 5hmC and elucidate its functional significance in the mitochondria. Mitoprot analysis on Tet family predicts that Tet1 may be translocated to the mitochondria. Preliminary results indicate that a band of expected size(235kDa) is present on immunoblots of mitochondrial fraction from mouse embryonic stem cells with an antibody directed against Tet1. This band, however, is not protected from trypsin treatment of mitochondria indicating that Tet1 may not be transported to the mitochondrial matrix. Stable knockdown of Tet1 in cells does not alter 5hmC signal in hydroxyMeDIP assays. We now seek to determine if Tet2/Tet3 may be involved in 5hmC generation. Understanding these mechanisms will give us insights about the epigenetic regulation of normal mitochondrial function and changes that occur in diseases involving mitochondrial dysfunction

## A-46 Variable selection using LASSO in Bayesian D-Optimal Supersaturated Design

Jiayi Hou, Dr.Charlie Kish

### Biostatistics

Experimental design, first proposed by Ronald A. Fisher in the famous 'The Lady Testing Tea' experiment, has demonstrated broad and prevalent applications in the pharmaceutical industry over a long period of time. In formulation development, the traditional fractional factorial design screening methodology often constrained the discovery of important effects to a limited number of active pharmaceutical ingredient, excipients, and several selected combinations of both. A Bayesian D-Optimal Supersaturated Design modifies the original screening experimental design by expanding the predictor space into a higher-dimensional space with consideration of all combinations of pairs of ingredients while preserving the prior property of individual effects by distinguishing terms of primary and potential interest under Bayesian framework. Compared to the traditional design, this aggressive expansion does not require additional budget, time, or effort to accomplish. Statistical literature has proposed several approaches for analyzing data from supersaturated designs, but no consensus approach has emerged. We applied the data-mining technique LASSO to data from a Bayesian D-Optimal supersaturated design. By using this variable selection process, important individual as well as combination effects can be identified under the Bayesian D-Optimal aliasing structure. Thus, this approach has demonstrated an efficient, robust, solution to the supersaturated situation while still retaining the parsimonious property of a good statistical model.

**A-47 Variants in the 15q25 gene cluster are associated with risk for schizophrenia and bipolar disorder**

Kia J. Jackson, Ayman H. Fanous, Jingchun Chen, Kenneth S. Kendler, Xiangning Chen

Psychiatry

Rates of tobacco smoking are significantly higher in patients with schizophrenia ( $\approx$  80-90%) compared to the general population ( $\approx$  20-30%). The underlying mechanism for this comorbidity is unclear. One hypothesis is that there are common genetic factors that predispose to both nicotine dependence (ND) and schizophrenia. To explore this hypothesis, we examined the association of the most significant candidate region to date implicated in ND and smoking behavior, the 15q25 gene cluster, with schizophrenia and bipolar disorder. Five variants in the 15q25 gene cluster (rs951266, rs16969968, rs1051730, rs8040868, rs17477223) were selected for analysis in 7 schizophrenia and 3 bipolar datasets ( $n=15\ 780$ ). A meta-analysis revealed four markers significant for risk for schizophrenia and bipolar disorder (rs951266, rs16969968, rs8040868, rs17477223). All markers were associated with the presence of negative symptoms of schizophrenia. Gene expression analysis using publically available post-mortem brain expression data indicated an association between genotypes of the rs1051730 variant and CHRNA5 expression in brain and peripheral blood mononuclear cells, and with the rs16969968 and rs17477223 variants in brain. Overall, these results suggest that variants in the 15q25 gene cluster are associated with risk for schizophrenia/bipolar illness, negative symptoms of schizophrenia, and influence CHRNA5 expression in the brain and peripheral blood mononuclear cells. Because the direction of the association is the same as that found for ND, these results support the notion that there are genetic mechanisms common to schizophrenia, ND, and bipolar disorder.

**A-48 Visceral inflammation increased serotonergic innervation of lumbar dorsal root ganglia:  
implications in viscerosomatic cross-sensitization**

Chun-Mei Xia, Sharon J. Yu, Jarren C. Kay, and Li-Ya Qiao

Physiology and Biophysics

One of the physiological functions of 5-hydroxytryptamine (5-HT) is its modulatory role in pain perception. Release of 5-HT by serotonergic neurons causes either pro- or anti-nociceptive action by binding to different subtypes of 5-HT receptors. Here we examined the serotonergic innervation of L5 DRG in two models with visceral inflammation, namely trinitrobenzenesulfonic acid (TNBS)-induced colitis and cyclophosphamide (CYP)-induced cystitis; both of them are accompanied by somatic hypersensitivity. We found that cystitis at 8h and 48h post CYP injection caused 4 to 5-fold increases, while colitis at 7 days and 21 days caused 1.5 to 2-fold increases in axonal outgrowth in L5 DRG visualized by sucrose-potassium phosphate-glyoxylic acid (SPG) staining which was associated with sympathetic sprouting and serotonergic innervation. 5-HT immunostaining revealed "beaded" fibers presumably small and regularly spaced varicosities. The number and density of the 5-HT fibers were time-dependently increased in DRG of both models. The excitatory effects of 5-HT was examined by immunostaining of 5-HT<sub>4</sub>, a G<sub>s</sub>-protein coupled serotonin receptor, and showed marked increases in the number of 5-HT<sub>4</sub> cells in colitis by 1.5 to 2.3-fold, and in cystitis by 2-fold. These results suggest that serotonin/5-HT<sub>4</sub>-mediated sensory hypersensitivity may have a role in visceral inflammation-induced somatic cross-sensitization.

## Session II - Wednesday 19 October

### **B-01 Relationship between radiation-induced autophagy and senescence in MCF7 breast tumor cells**

Khushboo Sharma, Rachel W. Goehe, Dr. David Gewirtz  
Pharmacology and Toxicology

### **B-02 A variable target comparison of whole breast and partial breast irradiation fractionation regimens. Do the current margins make sense?**

E. Al Sulaimani, D. Arthur, D. Todor  
Radiation Oncology

### **B-03 Access and Sanitation of Drinking Water and Diarrheal Disease in the Department of Yoro, Honduras**

1Gabriela E. Halder, MPH, 2Gonzalo Bearman, MD, MPH, 2Michael P. Stevens, MD, MPH  
1Department of Medical Education, 2Department of Internal Medicine, Division of Infectious Diseases

### **B-04 OSTEOPONTIN: AN ACUTE INFLAMMATORY MEDIATOR OF SUCCESSFUL SYNAPTIC RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY**

Julie L. Chan and Linda L. Phillips  
Anatomy and Neurobiology

### **B-05 Agonist-Induced Rho Kinase and ZIP kinase Activity Levels in Different Regions of the Stomach**

Otman Al-Shboul, Sayak Bhattacharya, Sunila Mahavadi, and Karnam S. Murthy  
Physiology and Biophysics

### **B-06 Analysis of Human polynucleotide phosphorylase (hPNPaseold-35) function**

Upneet K. Sokhi<sup>1, 2, 3</sup>, Devanand Sarkar<sup>1,2,3</sup> and Paul B. Fisher<sup>1, 2, 3</sup>  
Human and Molecular Genetics

### **B-07 Characterization of Mutations affecting SaPI1 Capsid Size Determination**

Erin A. Wall, P. K. Damle, Altaira Dearborn, M. Spilman, G. E. Christie, T. Dokland  
Microbiology and Immunology - Molecular Biology and Genetics

### **B-08 Characterizing how estrogen-related receptor (ERR) regulates metabolic changes under hypoxia using Drosophila melanogaster**

Yan Li, Keith D. Baker  
Biochemistry and Molecular Biology

### **B-09 Chinese culture, perceived social support and consistent condom use among young drug users: A Path-analytic model analysis**

Jennifer Nield, Jian Li, Hongjie Liu, Jianhua Li, Jian Luo  
Epidemiology

### **B-10 Chronic Morphine Treatment Influences Mu- Opioid Receptor Agonist Effects on Intracranial Self-Stimulation**

Ahmad Altarifi and S. Stevens Negus  
Pharmacology and Toxicology

**B-11 Confidence Interval Adjustment for Variance Components in Twin Studies**

Hao Wu and Michael C. Neale

Psychiatry

**B-12 DEFORMABLE REGISTRATION AND ANALYSIS OF SMALL ANIMAL 18F-FLT AND 18F-FDG PET/CT IMAGES**

C Bass, J He, M Axente, S Gobalkrishnan, J Hirsch, G Hugo, J Zweit, A Pugachev

Medical Physics

**B-13 Differential Development of Tolerance and Withdrawal in  $\beta$ -Arrestin2 Knockout Mice Ileum and Colon Circular Muscle Following Repeated Exposure to  $\mu$ -Opioid Receptor Agonists.**

Maguma, H.T., Dewey W., Akbarali H.I.

Pharmacology and Toxicology

**B-14 Electrophysiological Characterization of Mouse Enteric Neurons**

TH Smith, WL Dewey and HI Akbarali

Pharmacology and Toxicology

**B-15 ETHANOL-INDUCED CELL FATE ALTERATIONS IN C. ELEGANS**

Lindsay Kondo, Richard Raabe, Kalyann Kauv, Mia Bolling, Andrew Davies, and Jill Bettinger

Pharmacology and Toxicology

**B-16 Genetic analysis of acute ethanol responsive behaviors in C. elegans**

Joseph Alaimo, Keith Shelton, Andrew Davies, and Jill Bettinger

Pharmacology and Toxicology

**B-17 HIV-1 Neuropathogenesis: Effects of Morphine and gp120 on Glia**

Samano, K.L., Knapp, P.L., & Hauser, K.F.

Pharmacology and Toxicology

**B-18 Identification of critical inter-subunit contacts in protein allostery guided by collective molecular motions**

Farzana Marni, Shengjun Wu, Gaureve Shah, Changan Xie, Xin-ping Xu, Qinglian Liu and Lei Zhou

Physiology and Biophysics

**B-19 IDENTIFICATION OF GENES THAT MEDIATE ETHANOL-INDUCED ACUTE FUNCTIONAL TOLERANCE IN C. ELEGANS**

Ka-Po Leung, Mia Bolling, Gina Blackwell, Jennifer Gardner, Andrew Davies, and Jill Bettinger

Pharmacology and Toxicology

**B-20 IFI16 REQUIRES COOPERATIVE ACTION OF TWO HIN DOMAINS IN SENSING THE INTRACELLULAR MICROBIAL DNA**

Jaiswal, R., and Escalante, C.R.

Physiology and Biophysics

**B-21 Intracranial Self Stimulation: Examining the Rewarding Properties of Abused Inhalants**

Matthew Tracy

Pharmacology and Toxicology

**B-22 Intramitochondrial injury during Ischemia-Reperfusion**

Hema S Aluri, Edward J. Lesnefsky, Jr., MD

Physiology and Biophysics

**B-23 Knowledge-driven analysis identifies novel developmental growth factors involved in Hepatitis C- induced Hepatocellular Carcinoma**

Martha K Behnke, Mark Reimers, Robert A Fisher

Integrative Life Sciences

**B-24 MAGL Inhibition: Potential Treatment for Nicotine Dependence**

Pretal Muldoon, Aron Lichtman, Ph.D., Imad Damaj, Ph.D.

Pharmacology and Toxicology

**B-25 Molecular Biomarkers of Human Kidney Transplantation in Ischemia Reperfusion Injury**

Mba, MU; Maluf, DG; Dumur, CI; Scian, M; Posner, MP; King, AL; Gehr, TWB; Sharma, A;Cotterell, AU; Ren, Q & Mas, VR

Physiology and Biophysics

**B-26 Multi-spectral fluorescence imaging of adoptive immune cell therapy using a cell membrane probe.**

Fatma Youniss<sup>1</sup>, Gopalakrishnan Sundaresan<sup>1</sup>, Laura Graham<sup>2</sup>, Collin Berry<sup>1</sup>, Harry Bear<sup>2</sup>, Jamal Zweit<sup>1</sup>.

Center for Molecular Imaging

**B-27 Activation of the Receptor for Advanced Glycation End Products (RAGE) and Oxidative Stress Mediate Up-Regulation of RhoA/Rho Kinase Pathway and Smooth Muscle Contraction in Diabetes**

Sunila Mahavadi, Olivia Manion, Shobha Ghosh, and Karnam S. Murthy

Physiology and Biophysics

**B-28 O-linked  $\beta$ -N-acetylglucosamine modification of MYPT1 reciprocally regulates its phosphorylation at Ser695 and Thr696 by PKG and Rho kinase, respectively: A novel mechanism that contributes to increased smooth muscle tone in diabetes.**

Sunila Mahavadi, John R. Grider and Karnam S. Murthy

Physiology and Biophysics

**B-29 Opioids and HIV-1 Associated Neurodegeneration: Possible Regulation by P2X4 Purinergic Receptors in Primary Mouse Striatal Cells**

Sorrell, M.E. (1), Zou, S. (2), Knapp, P.E. (1, 2), and Hauser, K.F. (1); (1) Dept. Pharmacol. & Toxicol., (2) Dept. Anat. & Neurobiol., Virginia Commonwealth Univ. Coll. of Medicine, Richmond, VA, USA.

Pharmacology and Toxicology

**B-30 Pharmacogenomic study of side effects for antidepressant treatment options in STAR\*D**

Shaunna L. Clark, Daniel E. Adkins, Karolina Aberg, John M. Hetteema, Joseph L. McClay, Renan P. Souza, Edwin J.C.G. van den Oord

Center for Biomarker Research and Personalized Medicine

**B-31 Photo-controlled Drug Release From A Caged Drug Conjugate**

Deboleena Mitra, Martin Michael Dcona, Matthew C.T.Hartman

Department of Chemistry, Massey Cancer Center

**B-32 PROBING STRUCTURE-ACTIVITY REALTIONSHPIS OF SIMOCYCLINONE D8'S COUMARIN BINDING SITE**

Lauren M Gaskell and Keith C Ellis

Medicinal Chemistry

**B-33 PROCESSING OF BLOCKED OXIDATIVELY MODIFIED DNA DOUBLE-STRAND BREAK ENDS BY METNASE**

Susovan Mohapatra, Suk-Hee Lee, Vijay Menon, Robert A. Hromas, Lawrence F. Povirk

Pharmacology and Toxicology

**B-34 REGULATION OF PRO-INFLAMMATORY TH17 RESPONSES DURING T. CRUZI INFECTION BY IL-12-FAMILY CYTOKINES**

Drew Cobb, and Ronald B. Smeltz

Microbiology and Immunology

**B-35 Role of Interdomain linker on AAV-2 Rep68 oligomerization**

Zarate-Perez, F., Villamil-Jarauta, M., Burgner, J., Das, K., Kekilli, D., Linden, M., and Escalante, C.R.

Physiology and Biophysics

**B-36 Role of the HPA Axis in Behavioral Responses to Ethanol**

Blair N. Costin and Michael F. Miles

Pharmacology and Toxicology

**B-37 Sphingosine kinase 1 inhibition attenuates mast cell-dependent allergic asthma in mice**

Megan M. Price<sup>1</sup>, Carole A. Oskeritzian<sup>1</sup>, Yves T. Falanga<sup>2</sup>, Kuzhuvelil B. Harikumar<sup>1</sup>, Jeremy C. Allegood<sup>1</sup>, Sergio E. Alvarez<sup>1</sup>, Daniel Conrad<sup>3</sup>, John J. Ryan<sup>2</sup>, Sheldon Milstien<sup>1</sup>, and Sarah Spiegel<sup>1</sup>

Biochemistry and Molecular Biology  
Departments of <sup>1</sup>Biochemistry and Molecular Biology and <sup>3</sup>Microbiology and Immunology;  
<sup>2</sup>Department of Biology

**B-38 Sphingosine-1-Phosphate: a missing cofactor for lysine 63-linked poly-ubiquitinations in pro-inflammatory cytokines mediated signaling**

Kuzhuvelil B. Harikumar, Nitai C. Hait, Jeremy Allegood, Eugene Y. Kim, Michael Maceyka, Sheldon Milstien, Sarah Spiegel, and Tomasz Kordula

Biochemistry and Molecular Biology, Massey Cancer Center

**B-39 Structure-Guided design of bivalent simocyclinone D8 analogs that display an improved antibacterial activity by binding to DNA gyrase**

Jenson Verghese\* and Keith C. Ellis

Medicinal Chemistry

**B-40 The Discriminative Stimulus Effects of Nitrous Oxide**

Kellianne Richardson and Keith L. Shelton

Pharmacology and Toxicology

**B-41 THE IMPACT OF ADOLESCENT NICOTINE EXPOSURE ON DRUG DEPENDENCE IN ADULTHOOD**

Mai Alajaji, M.S. and M. Imad Damaj, Ph.D

Pharmacology and Toxicology

**B-42 The interaction between the cannabinoid agonist Win55,212-2 and radiation in breast cancer**

Emery, S., Sumner, E., Lichtman, A., & Gewirtz, D.

Pharmacology and Toxicology

**B-43 The role of Alpha5\* Nicotinic Acetylcholine Receptors in the acute and chronic effects in mice**

Dawson, AJ; Miles MF; Damaj IM

Pharmacology and Toxicology

**B-44 The role of CCR5 in morphine and HIV-1 Tat mediated neuropathogenesis**

Elizabeth M. Podhaizer, Pamela E. Knapp, and Kurt F. Hauser

Pharmacology and Toxicology

**B-45 Transcriptional transitions in response to hypoxia and energy expenditure**

Divya Padmanabha

Biochemistry and Molecular Biology

**B-46 TriplatinNC; Heparan Sulfate Mediated Cell Entry and Nucleolar Localization.**

Erica Peterson, Heveline Silva, Vijay Menon, Brad Benedetti, Ralph Kipping, Nicholas Farrell

Chemistry

**B-47 TRIUMPH OVER ADVERSITY: A QUALITATIVE STUDY OF NARRATIVE, COPING AND EXPERIENCE IN INDIVIDUALS DIAGNOSED WITH CANCER**

Utkarsh Subnis

Social and Behavioral Health

**B-48 Using Genetic Information from Genome Wide Association Studies in Risk Prediction for Alcohol Dependence in the COGA and SAGE GWAS Samples**

Jia Yan, Fazil Aliev<sup>1</sup>, Kenneth S. Kendler<sup>1</sup>, Bradley T. Webb<sup>1</sup>, Mark A. Schuckit<sup>2</sup>, John I. Nurnberger Jr.<sup>3</sup>, Howard J. Edenberg<sup>4</sup>, John R. Kramer<sup>5</sup>, Alison M. Goate<sup>6</sup>, Jay A. Tischfield<sup>7</sup>, Danielle M. Dick<sup>1</sup>

Human and Molecular Genetics

**B-49 Decision Analysis Model Evaluating the Cost-Effectiveness of Fidaxomicin and Vancomycin in the Treatment of Clostridium Difficile Infection (CDI) from a Hospital Perspective**

Maryam Alowayesh, David Holdford, Spencer Harpe

School of Pharmacy - Department of Pharmacotherapy and Outcomes Science



**B-01 Relationship between radiation-induced autophagy and senescence in MCF7 breast tumor cells**

Khushboo Sharma, Rachel W. Goehe, Dr. David Gewirtz

Pharmacology and Toxicology

Our current studies involve efforts to understand the relationship between autophagy and senescence. Senescence is a phenomenon where cells undergo a form of cellular arrest but are still metabolically active. Autophagy is a catabolic process involving the degradation of the cell's own components such as mitochondria and endoplasmic reticulum to generate energy under conditions of stress. A recent study suggested the existence of a functional relationship between these two processes in the context of oncogene-induced senescence. We therefore sought to understand the nature of this relationship in MCF-7 breast tumor cells responding to ionizing radiation. MCF-7 cells treated with radiation (a dose of 5Gy) demonstrated a transient growth arrest followed by proliferative recovery. Acridine orange staining for autophagic vesicles and beta-galactosidase staining for senescence demonstrated that the two processes appeared to be coincident responses. This relationship was confirmed by quantification of various proteins associated with autophagy (p62 and LC3) and senescence (p21 and p53). In addition, we wanted to determine the potential role of H2AX, an indicator of DNA double strand breakage, in regards to autophagy and senescence. Confocal imaging of H2AX punctuate formation showed induction of DNA damage at early time points after radiation. The results of these studies indicate a possible linkage between autophagy and senescence after radiation; however further studies are necessary in order to determine whether senescence is dependent on prior autophagy.

**B-02 A variable target comparison of whole breast and partial breast irradiation fractionation regimens. Do the current margins make sense?**

E. Al Sulaimani, D. Arthur, D. Todor

Radiation Oncology

**Purpose:** A novel, variable target approach is used in this study to establish equivalence, in a radiobiological sense, between whole breast and partial breast irradiation schemes. We are questioning the meaning of margins, as they are currently used to create planning target volumes. Ultra-short fractionation regimens have been recently proposed for APBI, reducing the number of fractions from 10 to as few as two. This work addresses the concept of "equivalence" between the currently accepted regimens (both WBI and APBI) and the newly proposed ones.

**Methods:** Four early stage breast cancer patients were included. Two patients were treated with interstitial BT and two patients with balloon BT. Three regimens for WBI were simulated by creating uniform doses for all targets. EUBED and gBEUD were computed and the parameter  $a$  identified based on equivalence of standard WBI with interstitial APBI. The equivalence of new proposed ultra-short regimens in balloon BT namely (7Gy x 4fx), (8.25Gy x 3fx) and (10.25Gy x 2 fx) was then compared with the "traditional" APBI.

**Results:**  $a=-1$  was identified from WBI and APBI equivalence. At GTV+1.0 cm margin, we find gBEUD for ultra-short regimens to be 20% or higher than current standard fractionation scheme.

**Conclusions:** Somehow unexpected is that despite a larger margin (15mm), the interstitial implants seem to be equivalent, within the validity of the radiobiological model considered, for significantly smaller targets than the one the treatment was planned for. For the balloon BT, the planned margin of 1cm around the balloon, seemed a lot more consistent with the "true" target for which the WBI and APBI would be equivalent. When the new, ultra-short regimens were compared with the traditional (3.4Gy x 10fx), we find that their equivalent targets are larger than the 1cm planning margin, even when compared with the standard WBI regimen.

**B-03 Access and Sanitation of Drinking Water and Diarrheal Disease in the  
Department of Yoro, Honduras**

1Gabriela E. Halder, MPH, 2Gonzalo Bearman, MD, MPH, 2Michael P. Stevens, MD, MPH  
1Department of Medical Education, 2Department of Internal Medicine, Division of Infectious Diseases

Honduras has recently focused on increasing the availability of clean water in order to lower the incidence of diarrheal diseases, which are currently among the top three leading causes of disease in children up to five years of age. We examine and compare drinking water access and sanitation, as well as self-reported diarrheal disease incidence among the Lomitas, La Hicaca, and Coyoles communities of the Department of Yoro area of Honduras. In June 2011, 263 randomly selected participants receiving care from a medical brigade completed a 20-item language-specific, interviewer-administered, anonymous water sanitation questionnaire. We documented and analyzed data on drinking water sources and sanitation methods, in addition to diarrheal disease rates, at both the individual and community level. Over half of the participants obtained their drinking water from a private faucet, 20.9% purchased bottled water, and 12.5% obtained water from a river. Nearly one-third identified using filters to sanitize their water, 19% purchased bottled water and 19% used no sanitation method. Only 14.8% identified using chlorine. In the community of Lomitas, 60% of participants used no sanitation method. In this community over a quarter of its participants had experienced diarrhea within the last 30 days, which was the highest incidence rate reported among all three communities ( $p=0.03$ ). The surveyed communities of Yoro vary in terms of water access and sanitation and self-reported diarrheal incidence, reflecting the need for area-specific aid.

**B-04 OSTEOPONTIN: AN ACUTE INFLAMMATORY MEDIATOR OF SUCCESSFUL SYNAPTIC RECOVERY FOLLOWING TRAUMATIC BRAIN INJURY**

Julie L. Chan and Linda L. Phillips

Anatomy and Neurobiology

Osteopontin (OPN) is an inflammatory cytokine documented to modulate CNS growth and plasticity. Following traumatic brain injury (TBI), it is likely a player during reactive synaptogenesis as a substrate of acutely activated matrix metalloproteinases, and a recruiter of microglia for debris clearance. Our previous studies following unilateral entorhinal cortex lesion (UEC) showed elevated OPN transcript and protein at 2-7d post-injury. Questions regarding OPN's role during TBI inflammation and its contribution to successful synaptogenesis are addressed here at more acute (1d) post-injury intervals. Further, we tested OPN response following maladaptive synaptic plasticity in combined midline fluid percussion injury and bilateral entorhinal cortex lesion (TBI+BEC). By contrasting OPN profile during acute response, we can identify alterations in expression which might correlate with effective synaptic recovery. Rats subjected to UEC, TBI+BEC, or sham injury were evaluated for OPN at 1 or 2d post-injury via Western blot or confocal IHC utilizing antibodies to OPN and either microglia or astroglia. At 1d post-UEC, we observed a robust OPN increase matching the response at 2d, suggesting rapid and intense OPN activation during the acute inflammatory response. Following maladaptive TBI+BEC, OPN increased at 1 and 2d, however expression was significantly reduced relative to adaptive recovery. Finally, confocal imaging at 1d showed OPN localization in reactive microglia and astrocytes, and microglia diffusely distributed within the deafferented zone relative to distinct laminar organization at 2d. Together, these results support a significant OPN role in acute inflammatory response to TBI. Further, differences in OPN between adaptive and maladaptive recovery suggest immune-related regulation of successful synaptic repair. This process may involve OPN effects on microglial activation and migration, facilitating efficient debris clearance to promote axonal sprouting.

## B-05 Agonist-Induced Rho Kinase and ZIP kinase Activity Levels in Different Regions of the Stomach

Othman Al-Shboul, Sayak Bhattacharya, Sunila Mahavadi, and Karnam S. Murthy

### Physiology and Biophysics

The molecular events that regulate the tonic and phasic phenotypes of smooth muscle are attributed to differential expression of contractile proteins and signaling pathway that regulate MLC kinase and MLC phosphatase activity, and thus MLC20 phosphorylation. The activity of MLC phosphatase is inhibited by Rho kinase/ZIP kinase-dependent pathways. **AIM.** To determine whether Rho kinase and ZIP kinase activity levels correlate with the contractile phenotypes in proximal (tonic) versus distal (phasic) stomach. **METHODS.** Muscle cells were isolated separately from proximal and distal regions of rabbit stomach. Acetylcholine (ACh)-induced stimulation of Rho kinase and ZIP kinase activity was determined by immunokinase assay. **RESULTS.** ACh (0.1  $\mu$ M) caused stimulation of Rho kinase and ZIP kinase activity in muscle cells of both proximal and distal stomach. However, stimulation of both kinases was significantly higher in muscle cells from proximal stomach compared to distal stomach. Y27632, a selective blocker of Rho kinase, inhibited stimulation of ZIP kinase activity suggesting that stimulation of ZIP kinase activity is dependent on Rho kinase activity. **CONCLUSION.** Higher activity levels of Rho kinase and ZIP kinase, which normally inhibit MLC phosphatase and increase MLC20 phosphorylation, in proximal stomach correlate with its tonic phenotype compared to the distal stomach that exhibits phasic phenotype

## **B-06 Analysis of Human polynucleotide phosphorylase (hPNPaseold-35) function**

Upneet K. Sokhi<sup>1</sup>, Devanand Sarkar<sup>1,2,3</sup> and Paul B. Fisher<sup>1, 2, 3</sup>

### Human and Molecular Genetics

hPNPaseold-35 is an evolutionarily conserved 3', 5' exoribonuclease predominantly involved in mRNA degradation. It is a type I IFN-inducible early response gene that was identified as a gene upregulated in terminally differentiated and senescent fibroblasts. Overexpression of hPNPaseold-35 via a replication incompetent adenovirus (Ad.hPNPaseold-35) arrests multiple cancer cell types in the G1 phase of the cell cycle, with cells eventually undergoing apoptosis. A potential mechanism of this growth inhibition and eventual apoptosis induction is the ability of hPNPaseold-35 to selectively degrade c-myc mRNA. This was further proven by the overexpression of c-myc that partially but significantly protects human melanoma cells from Ad.hPNPaseold-35-mediated growth inhibition, indicating that c-myc down-regulation might directly mediate the growth-inhibitory properties of Ad.hPNPaseold-35. Since the protection offered by c-myc overexpression was incomplete, additional targets of hPNPaseold-35 are likely to exist that may play a role in mediating its growth-inhibitory effects. The goal of the present study was to identify these additional candidate genes that may be directly or indirectly regulated by hPNPaseold-35, which would help us better understand the physiological implications of this interesting enzymatic protein. In order to identify the target genes a whole genome cDNA microarray was performed to assess gene expression changes that occur when hPNPaseold-35 was silenced in HO-1 melanoma cells. The genes that are upregulated in the hPNPaseold-35 knockdown cell line might be potential direct degradation targets of hPNPaseold-35 according to our current hypothesis. Finally, validation of these targets may provide us a unique opportunity for defining specific molecules and pathways that may represent new targets of hPNPaseold-35 contributing to its growth-inhibitory effects, which may be further used for developing approaches to enhance its therapeutic efficacy.

## **B-07 Characterization of Mutations affecting SaPI1 Capsid Size Determination**

Erin A. Wall, P. K. Damle, Altaira Dearborn, M. Spilman, G. E. Christie, T. Dokland

Microbiology and Immunology - Molecular Biology and Genetics

SaPIs are mobile genetic elements in staphylococci that parasitize helper phages for their own high frequency horizontal transmission within a phage-encoded virion. One striking feature of this interaction is the redirection of capsid assembly to favor the creation of a small T=4 capsid rather than the normal T=7 capsid of helper phage 80alpha; this allows packaging of the smaller SaPI DNA while excluding a complete helper phage genome. It has been shown that two proteins encoded by prototype island SaPI1, gp6 and gp7, are involved in this process. These proteins were found in SaPI1 procapsids but not in mature SaPI1 transducing particles, suggesting that they play a transient role in capsid assembly. In the course of investigating these proteins, strains were constructed that carried SaPI1 ORF 6 and ORF 7 inserted individually or as a pair into the capsid gene cluster of helper phage 80β. The insertion of 6 alone or 7 alone did not affect capsid size or phage growth. However, insertion of 6 and 7 together led to the formation of predominantly small capsids and severely impaired plaque formation, consistent with the inability of the mutant phage to package its complete genome. Plaques obtained at a low frequency from phage carrying the insertion were presumed to be compensatory mutants that had recovered the ability to form large capsids. Some of these viable mutants had deleted much or all of the insertion, while others carried point mutations ½ all of which occurred in ORF 6. Missense mutations mapped to residues either involved in structural integrity or the C-terminal motif shared between gp6 and the 80alpha scaffold protein, consistent with the proposed function of gp6 as an alternative scaffold in small capsid formation.

**B-08 Characterizing how estrogen-related receptor (ERR) regulates metabolic changes under hypoxia using *Drosophila melanogaster***

Yan Li, Keith D. Baker

Biochemistry and Molecular Biology

Estrogen-related receptors (ERRs) are orphan nuclear receptors that do not have known endogenous ligands. Mammalian ERRs ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) have been found to transcriptionally regulate enzymes involved in fatty acid oxidation and mitochondrial oxidative phosphorylation. More studies showed that ERRs are involved in more aspects of energy metabolism, such as glycolysis and pento phosphate pathway. Recently, dERR has been found to directly interact with *Drosophila* hypoxia inducible factor 1 $\alpha$  (dHIF-1 $\alpha$ ). HIF-1 $\alpha$  is the main regulatory factor that responds to changes under hypoxia. To study the regulatory role of the ERR/HIF-1 $\alpha$  complex in hypoxia, microarray analysis was carried out on partial clear gut third instar larvae of *err* mutant, *dhif-1 $\alpha$*  mutant and *err*, *dhif-1 $\alpha$*  double mutant flies both under hypoxia and normoxia. 1127 genes were identified as hypoxic genes, and 48 genes were found significantly changed in all three mutant genotypes under hypoxia. Our result showed that ERR clearly has an impact on Sima hypoxic pathway in *Drosophila*. Further studies will be performed to identify binding element of both Sima and ERR from the promoter region of representative genes.

**B-09 Chinese culture, perceived social support and consistent condom use among young drug users: A Path-analytic model analysis**

Jennifer Nield, Jian Li, Hongjie Liu, Jianhua Li, Jian Luo

Epidemiology

**Background/ Objectives:** The purpose of this study was to examine the interrelationships among Chinese culture, drug-use stigma, HIV stigma, perceived social support and consistent condom use among young drug users.

**Methods:** Respondent-driven sampling (RDS) was used to recruit 426 young drug users in Yunnan, China. The Individualism-Collectivism Interpersonal Assessment Inventory (ICIAI) was used to measure cultural norms and values in the context of three social groups: family members, friends, and colleagues/neighbors. Path-analytic modeling was used to analyze the interrelationships.

**Results:** The results of path analytic modeling illustrated several key statistically significant associations: (1) higher levels of family ICIAI (more collectivism) were associated with lower levels of perceived social support from drug use alters ( $\beta$ (standardized coefficient) = -0.12); (2) friend ICIAI was positively associated with perceived social support from drug use alters ( $\beta$ = 0.22); (3) neighbor/colleague ICIAI were negatively associated with drug use stigma and perceived social support from drug user and sex partner alters ( $\beta$ =-0.14/ -0.29/-0.24 respectively); (4) drug use stigma and perceived social support from drug use alters were positively associated with consistent condom use ( $\beta$  = 0.20/ 0.18respectively) but perceived social support from sex partner alters was negatively associated with consistent condom use ( $\beta$  = -0.21).

**Conclusions:** This study provides insight on how collectivistic aspects of Chinese culture may influence drug use, HIV stigma and perceived social support, which in turn may affect the choice to practice safe sex. Behavioral interventions targeting safer sex among young drug users should take the characteristics of Chinese culture into consideration.

## **B-10 Chronic Morphine Treatment Influences Mu- Opioid Receptor Agonist Effects on Intracranial Self-Stimulation**

Ahmad Altarifi and S. Stevens Negus

Pharmacology and Toxicology

ICSS is a behavioral assay used to evaluate abuse-related effects of drugs. Facilitation of ICSS is often interpreted as an abuse-related drug effect. Many factors can influence mu agonist effects on ICSS, including drug efficacy at mu receptors, dose and pretreatment time. This study tested the hypothesis that mu opioid agonist effects on ICSS are determined in part by the degree of opioid dependence and tolerance. Adult male rats were equipped with electrodes targeting the medial forebrain bundle. Stimulus intensities were individually determined, and during daily behavioral sessions, 10 stimulus frequencies were available under an FR1 schedule. The primary dependent measure was rate of reinforcement. Once ICSS stabilized, the mu agonist fentanyl was tested under before initiation of morphine treatment (the "no treatment" condition), and during treatment with low-dose morphine (3.2 mg/kg/day) and high-dose morphine (18 mg/kg/day). Behavioral sessions were conducted 23 hr after each daily morphine injection, at a time after dissipation of any direct morphine effects. Consistent with previous findings, chronic morphine produced a dose-dependent rightward shift in baseline ICSS frequency-rate curves determined 23 hr after daily morphine administration. During the "no treatment" condition, fentanyl produced only a dose-dependent decrease in ICSS. Chronic morphine produced a dose-dependent tolerance to fentanyl-induced rate-decreasing effects and an increase in expression of fentanyl-induced facilitation of ICSS. In general, fentanyl reversed withdrawal-associated decreases in ICSS, but it did not increase ICSS above pre-morphine baseline levels. These findings suggest that chronic morphine produced (1) dependence as indicated by withdrawal-associated decreases in baseline ICSS 23 hr after daily morphine treatments, (2) tolerance to fentanyl-induced rate-decreasing effects, (3) an increase in expression of abuse-related facilitation of ICSS produced by fentanyl.

## **B-11 Confidence Interval Adjustment for Variance Components in Twin Studies**

Hao Wu and Michael C. Neale

Psychiatry

Variance component models (e.g. ACE and ADE models) have been widely used in twin studies to determine different sources of contribution (e.g. genetic, environmental, etc.) to a target phenotype. The traditional likelihood ratio test (LRT) for a variance component parameter has been found flawed as it fails to account for the fact that a variance component is non negative, and has been subsequently corrected. However, the confidence interval for such a parameter, which is closely related to the test, has yet been adjusted and may give rise to conflicting results with the corrected LRT. In this work, we present two different ways to adjust the CI of a variance component which may give consistent results with the LRT.

## B-12 DEFORMABLE REGISTRATION AND ANALYSIS OF SMALL ANIMAL 18F-FLT AND 18F-FDG PET/CT IMAGES

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Medical Physics

### **Purpose:**

To demonstrate utility of a novel PET tracer for multi-tracer studies it is necessary not only to confirm co-localization of the tracer with its intended target, but also to prove that the tracer provides complimentary information to FDG PET. Here we report on the methodology used to deformably co-register and compare intratumoral distributions of FDG and FLT as imaged in the same animal with PET/CT on two consecutive days.

### **Methods and Materials:**

Nude mice bearing FaDu (human H&N) tumor xenografts were imaged with 18F-FDG and 18F-FLT on two consecutive days using a small animal PET/CT scanner (Siemens Inveon). PET data was reconstructed and loaded into Pinnacle 9.1. Despite careful repositioning of the animal using an animal-specific pad, misalignment of FDG and FLT PET images hindered voxel-by-voxel analysis. To perform objective co-registration of the PET images, we relied on associated CT images. A DVF was generated using Pinnacle 9.1's demons deformable registration algorithm by deforming the second day's PET/CT images to the first day CT. The CT-based DVF was then applied to the second day PET image. After registration, voxel-by-voxel analysis of co-registered FLT and FDG PET images was carried out in Matlab. To ensure accuracy a brief study was conducted to test the error in the algorithm.

### **Results:**

Deformable image registration tools available in Pinnacle are adequate for co-registration of the animal PET/CT. Voxel-by-voxel analysis of co-registered FLT and FDG PET/CT images produced an average correlation coefficient of .57 ( $p < 10^{-5}$ ). All errors in the registration algorithm were smaller than .4 mm.

**Conclusions:** It is feasible to co-register PET images using deformable registration of the associated CT images using Pinnacle 9.1.

**B-13 Differential Development of Tolerance and Withdrawal in  $\beta$ -Arrestin2 Knockout Mice Ileum and Colon Circular Muscle Following Repeated Exposure to  $\mu$ -Opioid Receptor Agonists.**

Maguma, H.T., Dewey W., Akbarali H.I.

Pharmacology and Toxicology

Major drawbacks to morphine's excellent pain relieving effects include the development of analgesic tolerance and persistent constipation. We previously demonstrated that tolerance develops with repeated morphine exposure in the ileum but not the colon; however, the cellular mechanisms utilized vary among agonists targeting the mu-opioid receptor. Since genetic deletion of  $\beta$ -arrestin2 ( $\beta$ -arr2) has been reported to abrogate development of tolerance to the analgesic effect of morphine, we assessed the development of tolerance and withdrawal to different opioid agonists in the ileum and colon of C57BL/6 wild type (WT) and  $\beta$ -arr2 knockout (KO) mice. Tolerance was determined by assessing the ability of repeated in vitro opioid exposure to induce contraction of the circular muscle. Significant desensitization to morphine, DAMGO and fentanyl was observed in the ileum of WT and  $\beta$ -arr2 KO. Compared to the 1st exposure, morphine-induced contraction was decreased to 42.1% in the WT and 66.7% in  $\beta$ -arr2 KO during the 4th exposure whereas DAMGO contractions decreased to 70.0 % and 43.5% respectively; similarly, fentanyl contractions were reduced to 28.1% and 27.0 %. In contrast, the colon of WT did not develop desensitization to either morphine or DAMGO, however slight tolerance was observed to fentanyl with the 4th contraction reduced to 61.6 %. Unlike the WT, tolerance was either induced (morphine, 46.6%; DAMGO, 52.2%) or acutely magnified (fentanyl, 20.2%) in the colon of  $\beta$ -arr2 KO. The development of withdrawal was observed with all agonists in the ileum but not colon for both groups. Our findings suggest attenuation of  $\beta$ -arr2 to be permissive for the development of opioid tolerance without affecting development of withdrawal.  $\beta$ -arr2 potentially represents an important target for treatment of opioid-induced bowel dysfunction. Exploration of  $\beta$ -arr2 signaling pathways would be critical to assess mechanisms involved in reversal of opioid effect in the colon of  $\beta$ -arr2 KO mice.

## B-14 Electrophysiological Characterization of Mouse Enteric Neurons

TH Smith, WL Dewey and HI Akbarali

### Pharmacology and Toxicology

The enteric nervous system functionally controls digestion. Previous whole cell voltage-clamp electrophysiological characterization of enteric neurons has been performed in guinea pigs and other larger animals. Development of a murine model would allow the use of genetically altered mice to study the electrophysiological effects of specific proteins. We have successfully isolated cells of the mouse enteric nervous system from longitudinal muscle/myenteric plexus preparations (LMMP) of the ileum. Immunocytochemistry revealed most cells stained positive for the neuronal markers  $\beta$ III-tubulin and p75NTR. A few stained for the glial marker, GFAP. Positive staining was also seen for choline acetyltransferase, neuronal nitric oxide synthase, the  $\mu$  opioid receptor and  $\beta$ -arrestin 2. Neurons displayed action potentials, and were sorted based on the presence or absence of an after hyperpolarization (AHP; average  $7.5 \pm 0.7$  mV and  $111.5 \pm 12.6$  ms). The 33 neurons had an average size of  $20.7 \pm 1.6$  pF, and a rheobase of  $32.9 \pm 4.5$  pA. Neurons lacking an AHP were more polarized than those without an AHP (resting membrane potential [RMP] =  $-51.8 \pm 2.2$  mV vs.  $-49.6 \pm 1.6$  mV, respectively) and had a greater AP height ( $49.9 \pm 7.4$  vs.  $38.4 \pm 2.9$ , respectively). Clotrimazole (10  $\mu$ M), a putative IK(Ca<sup>2+</sup>) blocker, blocked the AHP. Neurons with an AHP had significantly larger Na<sup>+</sup> and K<sup>+</sup> currents as indicated by current-voltage curves. In neurons with an AHP, morphine (3  $\mu$ M) decreased the excitability of these neurons by reducing the ability of the cell to fire multiple action potentials, increasing the rheobase of the cell (10.0  $\pm$  0.1 pA without drug to 26.6  $\pm$  4.9 pA with morphine), and reducing the height of the action potential (52.8  $\pm$  6.7 mV without drug to 42.2  $\pm$  6.5 mV with morphine). Morphine did not alter the RMP of the neurons. In conclusion, we have successfully electrophysiologically characterized mouse neurons via whole cell patch clamp technique.

## B-15 ETHANOL-INDUCED CELL FATE ALTERATIONS IN C. ELEGANS

Lindsay Kondo, Richard Raabe, Kalyann Kauv, Mia Bolling, Andrew Davies, and Jill Bettinger

### Pharmacology and Toxicology

Fetal alcohol syndrome (FAS) is the leading preventable cause of mental retardation, but the molecular mechanisms underlying FAS are not well understood. We have taken a genetic approach to studying the effect of ethanol on a discreet cell fate decision occurring during embryogenesis in the nematode, *C. elegans*. AWC cells are a pair of olfactory neurons that allows *C. elegans* to discriminate between volatile attractive odorants in odor chemotaxis and odor discrimination behavioral assays. Early in development, AWC neurons make an activity dependent cell fate decision, and subsequently particular groups of G protein-coupled receptors are asymmetrically expressed in the two AWCs. A GFP tagged STR-2 allows us to monitor cell fate decisions between AWC neurons. SLO-1, a voltage-gated potassium channel is also expressed in these neurons, and activation of this channel can modify the AWC cell fate decision so that both AWCs adopt the same cell fate (which we identify as both cells expressing the GFP marker, or a 2 AWCON cell fate). Previous studies from our lab have shown that SLO-1 is a major molecular target of ethanol and mediates ethanol sensitivity. We tested if ethanol exposure during embryogenesis could cause defects in the AWC cell fate decision and found that ethanol exposure could alter the AWC cell fate, which requires the SLO-1 channel. Furthermore, by altering the lipid composition of the cell membrane, we can render this cell fate decision resistant to the effects of ethanol. To determine if this change in AWC cell fate has functional consequences, we are currently testing the ability of animals that had been exposed to ethanol during embryogenesis to perform in chemotaxis and odorant discrimination assays, which requires proper function of the AWCs. We predict that exposing embryonic worms to ethanol will cause functional behavioral changes in the animal due to altered AWC cell fate decisions.

## B-16 Genetic analysis of acute ethanol responsive behaviors in *C. elegans*

Joseph Alaimo, Keith Shelton, Andrew Davies, and Jill Bettinger

### Pharmacology and Toxicology

Alcohol abuse is a complex disorder with a poorly understood etiology that includes both genetic and environmental influences. One factor found to influence drinking behavior and subsequent liability for dependence is variation in genes encoding ethanol metabolism machinery. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are enzymes involved in ethanol metabolism. We directly tested the effects of altering the function of these enzymes on ethanol responsive behaviors in worms. We tested two ADH enzymes encoded by the genes *sodh-1* and *H24K24.3*. We have found that at 400 mM ethanol *sodh-1(ok2799)* animals are hypersensitive to ethanol's depression of locomotion relative to wild type, but *H24K24.3(RNAi)* animals are not. We tested internal ethanol tissue concentrations and found that tissue concentration is increased in *sodh-1(ok2799)*, but not in *H24K24.3(RNAi)*. Importantly, we found that both strains develop robust acute functional tolerance to ethanol, indicating that these enzymes are dispensable for this process. The nematode genome encodes 13 ALDH enzymes that are highly conserved compared with human ALDHs. Knockdown by RNAi of nine of these genes did not alter ethanol's depression of locomotion, but knockdown of *alh-6(RNAi)* and *alh-13(RNAi)* resulted in hypersensitivity to this effect. Interestingly, internal tissue ethanol concentrations in these two strains appear to be slightly higher than in wild type, suggesting that the lack of ALDH function may cause a buildup of acetaldehyde, which would be converted by ADH into ethanol. Collectively, these data suggest that altered ethanol metabolism in worms results in a mild but detectable effect on ethanol response behaviors.

## B-17 HIV-1 Neuropathogenesis: Effects of Morphine and gp120 on Glia

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### Pharmacology and Toxicology

The mechanisms by which opioids perturb HIV neuropathogenesis remain unresolved; however, HIV+ individuals who use heroin show an increase in the frequency and extent of pathological hallmarks of HIV-encephalitis. The HIV coat glycoprotein, gp120, is neurotoxic and it is suggested that the presence of glia, especially microglia, are instrumental to this damage. While our lab has shown co-morbidity of opioids with HIV Tat through effects on glia, fewer studies have addressed the interactive effects of morphine and HIV gp120. We focus on the striatum since it is a preferential target of HIV and it also contains high levels of the mu-opioid receptor (MOR). We hypothesize that morphine will potentiate reactive gliosis induced by gp120 and that this will be manifested by microglial and astrocyte activation that will culminate in neuron injury and death. To assess this in vivo, mice were stereotaxically injected in the striatum with gp120 (100 ng) or vehicle (0.9% saline) and treated with subcutaneous time-release morphine (5 mg/day) and ½ naltrexone (12 mg/day) pellets. Immunohistochemical studies co-localizing Iba-1 (a microglial marker) with 3-nitrotyrosine (a marker of nitrosative stress) as well as GFAP (an astrocyte marker) with MOR (mu-opioid receptor) were performed. Of importance, morphine facilitated microglial activation by gp120, as gp120 + morphine treated animals displayed a significant increase in Iba-1 positive microglia expressing 3-nitrotyrosine compared to vehicle + morphine treated animals. The proportion of astrocytes (GFAP immunopositive) in which MOR was co-detected increased significantly after gp120 alone or in combination with morphine compared to morphine + vehicle treated animals. Interestingly, this data suggests that opioids may alter the CNS response to HIV gp120 at the level of glia, and future in vitro studies will investigate the molecular mechanisms that influence opioid-gp120 interactive neuropathogenesis.

**B-18 Identification of critical inter-subunit contacts in protein allostery guided by collective molecular motions**

Farzana Marni, Shengjun Wu, Gaureve Shah, Changan Xie, Xin-ping Xu, Qinglian Liu and Lei Zhou

Physiology and Biophysics

Given the much advanced understanding of the atomic detail of various ligand  $\gamma$  receptor systems, the nature of the allosteric coupling between ligand binding and protein function remains largely unclear. Here we used the tetrameric HCN channel as a model system to study the crucial inter-subunit contacts in cAMP-dependent allostery. Based on the computational analysis of collective molecular motions, we first systematically surveyed the intersubunit contacts with a focus on the stable contacts. Lead candidates were further tested by mutagenesis and functional assays. Because the inter-subunit contacts critical for allosteric coupling are located far from the ligand binding site, we focused on the dynamic interaction between the ligand and the whole protein, using the technique of patch-clamp fluorometry. We pinpointed several inter-subunit contacts that are critically involved in the coupling between ligand binding and channel function. Thus, following the theme of protein structure, dynamics, and function, our study not only provides new understanding of the implementation of allosteric coupling in HCN channels but also illustrates a research strategy that can be applied to other ligand - receptor systems.

## **B-19 IDENTIFICATION OF GENES THAT MEDIATE ETHANOL-INDUCED ACUTE FUNCTIONAL TOLERANCE IN C. ELEGANS**

Ka-Po Leung, Mia Bolling, Gina Blackwell, Jennifer Gardner, Andrew Davies, and Jill Bettinger

### Pharmacology and Toxicology

Alcohol abuse and alcoholism are prevalent diseases in our society that are physically and socially damaging. There are few adequate treatments available today largely, in part because the molecular mechanisms behind the development of alcoholism are still unclear. We know from human genetics studies that there is a significant genetic component that influences disease susceptibility, and that an individual's initial sensitivity and development of acute functional tolerance (AFT) after alcohol consumption are strong predictors of lifetime development of addiction. We have taken a genetic approach to study ethanol sensitivity and the development of tolerance in the nematode, *C. elegans*. We performed a forward genetic screen for mutations in genes that are required for the development of AFT to ethanol. We identified a mutation, *bet11*, that causes mutant animals to be defective in the development of AFT in response to ethanol treatment. We have used genetic mapping to localize the gene that is disrupted by the *bet11* mutation to an approximately 80-gene interval on Chromosome I. We are currently using transformation rescue and whole genome sequencing to identify the gene in this interval that is required for the development of AFT to ethanol. Additionally, a special feature of the *bet11* allele is that we have strong genetic evidence that allelic variation in the gene that it disrupts can act in combination with natural alleles in another gene to influence ethanol response in wild populations. We are currently mapping to identify the allelic variation in the other gene involved in this synthetic interaction. We have localized the interacting allele to a region of Chromosome III and are currently refining our genetic map of this interval. Identifying and characterizing the genes that are responsible for alcohol-induced development of AFT will give us a better understanding of the neurobiological mechanisms that lead to alcohol abuse and alcoholism.

## **B-20 IFI16 REQUIRES COOPERATIVE ACTION OF TWO HIN DOMAINS IN SENSING THE INTRACELLULAR MICROBIAL DNA**

Jaiswal, R., and Escalante, C.R.

### Physiology and Biophysics

Detection of intracellular microbial DNA is one critical factor in inducing the innate immune response. Immune response to viral RNA has been well characterized; however, knowledge about how microbial DNA is sensed is limited. IFN inducible gene of HIN-200 family encodes a class of homologous proteins that share 200 amino acids domain (HIN) contains a conserved MPHATVAT motif. Structurally, most of HIN-200 family proteins possess: an N-terminal conserved  $\alpha$ -helical PYRIN domain and at C-terminal 1 or 2 conserved HIN domain that binds to DNA. Recently, AIM2 & IFI16 were shown to be involved essentially in sensing the intracellular microbial dsDNA through HIN Domain and hence results in activation of inflammatory response. Unlike other members of Human HIN-200 family, IFI16 possesses two conserved HIN domains. The structural similarity to mouse homolog, p202a as well as the ability to sense intracellular microbial DNA and binding to various transcription factors makes IFI16 an important target for medicinal research and drug design.

As a first step towards understanding the mechanism how IFI16 senses and interacts with microbial DNA and the importance of two HIN domains, we focus on biochemical characterization and X-ray structure of IFI16-DNA complex. Binding assays suggested that individual HIN domains of IFI16 are incapable of binding to DNA alone. Further, the synergistic action of two HIN domains along with the linker region is critical in sensing and binding of dsDNA. SAXS studies of the protein alone and in complex with DNA help in understanding how the two HIN domains arrange themselves along DNA upon binding. We are screening the minimum length of linker between two domains that can still bind to DNA as well as the appropriate length of DNA in order to facilitate the crystallization of the complex. Further, we are testing a number of approaches to understand the biophysical and biochemical nature of the interaction of IFI16 with dsDNA.

## **B-21 Intracranial Self Stimulation: Examining the Rewarding Properties of Abused Inhalants**

Matthew Tracy

Pharmacology and Toxicology

Inhalants are a diverse group of abused substances classified only by their route of administration. One challenge to assessing the abuse liability of inhalants is the development of a predictive animal model. In the current study our goal was to quantify the reward altering effects of the commonly abused solvent, toluene, using an intracranial self-stimulation (ICSS) model in mice. In ICSS, an electrode is permanently implanted into the medial forebrain bundle which is innervated by mesolimbocortical reward pathway projections of the brain. Stimulation of the electrode will directly activate the reward pathway. Seven days following implantation C57BL6/J mice were then given the opportunity to respond on a lever for self-stimulation across ten frequencies from 158-56 Hz in descending, one minute increments. Each ten minute component was run three times within a single session over the course of 70 minutes, with a twenty minute timeout between components. Treatment conditions were applied twenty minutes prior to the third component and responding was compared to the second baseline component. Once a baseline level of responding was established, we examined the ability of 1000-6000 parts per million (PPM) toluene vapor as well as 3, 10, and 17 mg/kg cocaine injected intraperitoneally to alter the rewarding effects of self-stimulation. As expected, cocaine enhanced responding for the rewarding effects of ICSS. Exposure to toluene at 1000 and 2000 PPM significantly enhanced ICSS responding. At 3000 and 4000 PPM, responding was biphasic; increased responding occurred at lower frequencies and decreased responding at higher frequencies. Exposure to 6000 PPM produced decreased levels of responding at all frequencies. This data suggests that toluene has reward enhancing pharmacological actions mediated via interaction with the mesolimbocortical pathway, and that ICSS can be adapted to study the rewarding properties of inhaled substances.

## B-22 Intramitochondrial injury during Ischemia-Reperfusion

Hema S Aluri, Edward J. Lesnefsky, Jr., MD

### Physiology and Biophysics

Mitochondria are the effector organelles that are damaged during ischemia (ISC) when there is no blood flow. Resumption of metabolism by damaged mitochondria during reperfusion (REP) results in increased cell injury. Current therapeutic interventions to pre-condition and post-condition the heart during ischemia rely on cyto-protective signaling pathways. However, these therapeutic interventions are ineffective during certain conditions like aging and diabetes due to defects in the signaling cascades. Hence direct therapeutic targeting of dysfunctional mitochondria will provide the potential to bypass the upstream signaling defects and intervene directly with the effector organelle.

This project addresses the role of cyt c in oxidizing cardiolipin during ischemia resulting in cardiac injury. Cardiolipin (CL) is an oxidatively sensitive phospholipid that is unique to mitochondria and is modified during ischemia-reperfusion (ISC-REP) in the heart. We hypothesize that during ISC oxidants from complex III modify cyt c, forming a peroxidase which causes oxidative damage to cardiolipin. Oxidation of cardiolipin causes loss of fusion proteins which maintain long, elongated mitochondria. In turn, it also recruits fission proteins that cause mitochondria to fragment. Fragmented mitochondria are removed by lysosomes.

Modification and depletion of CL disrupts normal physiology, impairs electron transport, disrupts membrane integrity and augments cell death via the release of cyt c from mitochondria. Identification of the innovative pathobiology during ISC-REP recognizes a novel therapeutic target, CL peroxidase, which will be a focal point for new therapeutic interventions to decrease cardiac injury.

**B-23 Knowledge-driven analysis identifies novel developmental growth factors involved in Hepatitis C- induced Hepatocellular Carcinoma**

Martha K Behnke, Mark Reimers, Robert A Fisher

Integrative Life Sciences

**Background:** Hepatocellular Carcinoma (HCC) ranks fifth among all cancers. Hepatitis C (HCV) is the major cause of HCC in North America. Knowledge-driven analysis may help elucidate which molecules, receptors, and effectors are acting coordinately and which are aberrant in HCV-induced HCC.

**Methods:** Patients with cirrhosis and HCC were treated at VCUHS and tissue samples were obtained. Controls were obtained from donor livers. Microarray images were assessed for technical quality and chips with severe visual patterns and neighboring probe correlation  $>0.5$  were excluded. Data from 15 normal, 36 cirrhotic, and 38 T1/T2 HCC tissues were analyzed using Principal Components Analysis (PCA), heat maps and moderated t-test ( $FDR < 0.05$ ). After screening several growth factor families, we identified the Fibroblast Growth Factor (FGF) family for further investigation. Along with their regulators (Bone Morphogenic Proteins (BMPs) and BMP antagonists Gremlin (GREM1/2) and Follistatin), FGFs contribute to Endothelial-to-Mesenchymal Transition in other types of cancer. However, their roles are not well-characterized in HCC.

**Results:** PCA identified GREM1 and Follistatin as the dominant components separating HCC from cirrhosis and normal tissue. GREM1 was strongly expressed in HCC tissues, while Follistatin was often up-regulated in the HCC samples without GREM1 over-expression. FGF7 and FGF13 were also over-expressed in a subset of HCC samples. These patterns were confirmed in an independent dataset obtained from the Gene Expression Omnibus (GEO).

**Conclusion:** The FGF-BMP circuit is well-studied in embryological development but poorly understood in cancer. This approach has demonstrated that Gremlin and Follistatin are over-expressed in early HCV-induced HCC. These proteins directly bind BMPs to inhibit their ability to regulate FGFs. These results suggest Gremlin/Follistatin over-expression as a possible novel mechanism in the development of HCV-induced HCC.

## **B-24 MAGL Inhibition: Potential Treatment for Nicotine Dependence**

Pretal Muldoon, Aron Lichtman, Ph.D., Imad Damaj, Ph.D.

### Pharmacology and Toxicology

Nicotine is the main addictive component of tobacco that plays a major role in dependence. Emerging evidence suggest that the endogenous endocannabinoid system may modulate these effects. Our lab has previously reported that increase in AEA, enhanced nicotine withdrawal and reward of nicotine and was CB1 mediated (Merritt et. al. 2008). However, 2-AG has yet to be studied in nicotine's effect. 2-AG is the most abundant endocannabinoid in the brain, required for cannabinoids synaptic transmission and exerts its action via CB1 receptors. To assess 2-AG's role in Nicotine Dependence we enhanced 2-AG levels via MAGL inhibition by JZL184. Nicotine reward in the mouse was evaluated in an unbiased conditioned place preference paradigm (CPP). Our results showed that degradation of MAGL dose-dependently decreased nicotine preference compared to nicotine control in our CPP paradigm. This blockade of Nicotine CPP is not CB1 mediated. Finally, we wanted assess JZL184's effect on another important aspect of ND, nicotine withdrawal. We wanted to assess the role 2-AG's neurotransmission in both physical and affective measures of nicotine withdrawal. JZL184 did not alter Nicotine withdrawal induced anxiety-like behavior. However, JZL184 was able to dose-dependently block somatic signs and this effect was CB1 mediated. We then assessed JZL184's role in another affective measure the Nicotine Withdrawal Conditioned-Place Preference (CPA) model. Here, we took the lowest active dose of JZL184 (8mg/kg) and was able to completely block mecamylamine induced aversion. These results suggest that AEA and 2-AG have different roles in nicotine withdrawal and reward. MAGL inhibition is able to block both nicotine reward and physical somatic withdrawal signs. Interestingly, JZL184's effect in Nicotine CPP is not CB1 mediated whereas in physical somatic withdrawal signs they are.

## **B-25 Molecular Biomarkers of Human Kidney Transplantation in Ischemia Reperfusion Injury**

Mba, MU; Maluf, DG; Dumur, CI; Scian, M; Posner, MP; King, AL; Gehr, TWB; Sharma, A;Cotterell, AU; Ren, Q & Mas, VR

### Physiology and Biophysics

Ischemia reperfusion injury (IRI) is a multi-factorial process leading to delayed graft function (DGF). Identification of at-risk grafts would alter post-transplant (Tx) management and eventually improve post transplantation outcomes. Gene expression microarray analysis was done on 118 samples from zero-hour (K1) and 1 hour post-perfusion (K2) biopsies in 59 patients placed into DGF (17) and non-DGF (42) groups. Differentially expressed genes dealing with inflammation, metabolism and apoptosis were found within relevant comparisons of patient groups and seem to play a role in the development of IRI and DGF.

**B-26 Multi-spectral fluorescence imaging of adoptive immune cell therapy using a cell membrane probe.**

Fatma Youniss<sup>1</sup>, Gopalakrishnan Sundaresan<sup>1</sup>, Laura Graham<sup>2</sup>, Collin Berry<sup>1</sup>, Harry Bear<sup>2</sup>, Jamal Zweit<sup>1</sup>.

Center for Molecular Imaging

**Objectives:** The overall objective of this study is to non-invasively assess in-vivo targeting and retention of adoptively transferred T-lymphocytes at the tumor site.

**Methods:** 4T1 specific T-lymphocytes obtained from draining lymph nodes of 4T1 sensitized BALB/C mice were pulsed in-vitro with Bryostatin/ Ionomycin for 18 hours, and then divided into two populations that were grown in either Interleukin-2 (IL2) or a combination of interleukins 7 & 15 (IL7/15) for 10 to 13 days. T-lymphocytes were then directly labeled with a lipophilic NIR fluorescent probe (Xenolight DiR). In experiment one, recipient mice having a 4T1 tumor were injected with labeled cells. In experiment two, 4T1 tumor cells were implanted 1-week post-injection of labeled T-lymphocytes. Multi-spectral fluorescence imaging was done at various time points up to 24 days. The viability of lymphocytes and their functions were assessed by ATP based cell viability assay, flowcytometry and interferon- gamma (IFN $\gamma$ ) ELISA.

**Results:** IL7/15 is superior to IL2 for ex vivo expansion, but the in-vivo imaging data showed higher tumor retention of labeled T-lymphocytes for IL2 than IL7/15 and the signal persisted at the tumor site for weeks with a peak on day 6 post-injection of IL2 grown cells. In experiment two, IL2 grown cells moved out of lymphoid compartments to the site of 4T1 inoculation within two hours and peaked on day 3. Good visualization of labeled lymphocytes in tumor, liver, spleen, gut, lymph nodes, and in bone/ bone marrow was noted ex vivo. Flowcytometry, cell viability assay and IFN $\gamma$  ELISA showed that, the proliferation, viability and function of stained 4T1 specific T-lymphocytes was not affected.

**Conclusion:** Direct labeling of 4T1 specific T-lymphocytes by a fluorescent dye yielded relatively stable labeling and provided in vivo data on trafficking of these cells over an extended period of time.

**B-27 Activation of the Receptor for Advanced Glycation End Products (RAGE) and Oxidative Stress Mediate Up-Regulation of RhoA/Rho Kinase Pathway and Smooth Muscle Contraction in Diabetes**

Sunila Mahavadi, Olivia Manion, Shobha Ghosh, and Karnam S. Murthy

Physiology and Biophysics

Accumulation of advanced glycation end products (AGEs) and activation of the receptor for AGEs (RAGE) have been implicated in the oxidative stress and vascular dysfunction in the diabetic animal models. In smooth muscle MLC20 phosphorylation and contraction are dependent on inhibition of MLC phosphatase via RhoA/Rho kinase pathway. Studies have shown that up-regulation of Rho kinase activity contributes to the increased gastric smooth muscle contraction in diabetic ob/ob mice. However, the mechanisms that lead to up-regulation of RhoA/Rho kinase pathway in diabetes are not known. **Aim.** To determine whether activation of RAGE and oxidative stress are involved in the up-regulation of RhoA/Rho kinase pathway in gastric smooth muscle from ob/ob mice. **Methods.** Expression of RhoA, RAGE and SOD-1 in gastric smooth muscle from control and ob/ob mice was examined by western blot and qRT-PCR. Rho kinase activity in response to acetylcholine was measured by immunokinase assay. **Results.** Treatment of cells with glucose or S100B (RAGE ligand) significantly increased the expression of RhoA, RAGE and SOD-1, and H<sub>2</sub>O<sub>2</sub> levels compared to control cells. ACh-induced Rho kinase activity was augmented by glucose or S100B. The anti-oxidant NAC blocked the effect of glucose or S100B on RhoA, RAGE and SOD-1 expression, H<sub>2</sub>O<sub>2</sub> formation and Rho kinase activity. The results imply that hyperglycemia or activation of RAGE induces oxidative stress leading to increase in the expression of RhoA and Rho kinase activity. Expression of RhoA, RAGE and SOD-1, H<sub>2</sub>O<sub>2</sub> levels and ACh-induced Rho kinase activity were also significantly higher in smooth muscle from ob/ob mice compared to control mice. **Conclusion.** These studies demonstrate for the first time, in gastric smooth muscle, that activation of RAGE and RAGE-dependent increase in oxidative stress play a role in the up-regulation of RhoA/Rho kinase pathway, a key event associated with increased smooth muscle tone in diabetes.

**B-28 O-linked  $\beta$ -N-acetylglucosamine modification of MYPT1 reciprocally regulates its phosphorylation at Ser695 and Thr696 by PKG and Rho kinase, respectively: A novel mechanism that contributes to increased smooth muscle tone in diabetes.**

Sunila Mahavadi, John R. Grider and Karnam S. Murthy

Physiology and Biophysics

Altered gastric motility, a common diabetic complication, is associated with a decrease in nNOS activity and interstitial cells of Cajal, and an increase in smooth muscle tone. Regulation of the latter is dependent on inhibition of MLC phosphatase via inhibitory phosphorylation of its regulatory subunit MYPT1 at Thr696 by Rho kinase and stimulatory phosphorylation at Ser695 by PKG. O-linked  $\beta$ -N-acetylglucosamine (O-GlcNAcylation) modification of serine or threonine residues regulates protein phosphorylation and function. O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively, catalyze the addition and removal of O-GlcNAcylation. Aim. To determine the role of O-GlcNAcylation in the regulation of MYPT1 phosphorylation at Ser 695 by Rho kinase and Thr696 by PKG. Methods. O-GlcNAcylation of MYPT1 and PKG- $\alpha$ , and phosphorylation of MYPT1 at Thr696 and Ser695 were examined in MYPT1 or PKG- $\alpha$  immunoprecipitates in muscle cells from control and ob/ob mice and in cells treated for 48 h with OGA inhibitor, PugNAc (100  $\mu$ M), or 30 mM glucose in the presence or absence of OGT inhibitor, STO45849 (10  $\mu$ M). Results. Both MYPT1 and PKG- $\beta$  were O-GlcNAcylated in gastric muscle from ob/ob mice, and in muscle cells treated with glucose or PugNAc. Phosphorylation of MYPT1 at Thr696 was increased and decreased at Ser695 in gastric muscle from ob/ob mice, and in muscle cells treated with PugNAc or glucose. The effect of glucose on O-GlcNAcylation of MYPT1 and PKG- $\alpha$ , and phosphorylation of MYPT1 at Thr696 were attenuated by STO45849, suggesting that changes in MYPT1 phosphorylation at Thr696 and Ser695 could be due to O-GlcNAcylation of MYPT1 or PKG- $\alpha$ . Conclusion. O-GlcNAcylation of MYPT1 reciprocally regulates its phosphorylation at Thr696 by Rho kinase and at Ser695 by PKG. Both increase in Thr696 phosphorylation and decrease in Ser695 phosphorylation of MYPT1 could contribute to decreased MLC phosphatase activity and increased smooth muscle tone in diabetes.

**B-29 Opioids and HIV-1 Associated Neurodegeneration: Possible Regulation by P2X4 Purinergic Receptors in Primary Mouse Striatal Cells**

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Pharmacology and Toxicology

HIV-1-associated neurocognitive disorders (HAND) are seen in about 40% of AIDS patients and individuals who abuse opiates can have an increased incidence of HAND with more severe symptoms. Microglia play a role in HIV neuropathogenesis since activation produces inflammatory molecules that can lead to neuronal injury and death. Extracellular purines (ATP and UTP) can markedly increase microglial activation and/or neuronal injury. Morphine has been reported to increase microglial motility by modifying P2X4 signaling (Horvath and DeLeo, 2009). This is important because mu opioid receptor agonists can increase HIV-1 replication, potentiate the release of oxyradicals and glutamate, and transiently increase cytokine production in HIV-1 Tat-exposed microglia. To examine whether HIV-1 and/or opioid-induced neurotoxicity are mediated via purinergic signaling, co-cultures of neurons and mixed glia from mouse striatum were treated for 72 hours with vehicle, Tat and/or morphine  $\pm$  1/2 purinergic antagonists, PPADS or TNP-ATP, which block P2X1-3, 5-7 and P2X1-7 receptors respectively. Treatment with Tat and morphine, in combination or alone, caused neuronal death. Since treatment with PPADS alone was found to be toxic, this experiment did not show that the P2X4 receptor mediates the toxicity of Tat or morphine. Immunocytochemistry was performed on co-cultures of neurons with mixed glial cells. The proportion of P2X4 positive cells was decreased by 72 hour Tat + morphine treatment. ATP levels were investigated in response to treatment with Tat + morphine, and increased levels were seen at both 30 minutes and 1 hour after treatment. Finally, we wanted to see if our model was representative of what is occurring in the human brain. To do this we obtained human P2X microarray data sets taken from HIV infected and non-infected individuals (courtesy of NNTC). Data suggests that P2X4 cells may be preferentially targeted in the disease. Support: NIH DA18633, DA19398, and DA27374.

## **B-30 Pharmacogenomic study of side effects for antidepressant treatment options in STAR\*D**

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Center for Biomarker Research and Personalized Medicine

Understanding individual differences in susceptibility to antidepressant therapy side effects is essential to optimize the treatment of depression. Clinical trials have suggested that only 50% of patients with uncomplicated major depressive disorder respond to any single antidepressant with inability to tolerate side effects being the most common reason for discontinuing antidepressant therapy. Studies have consistently indicated that antidepressant response is substantially heritable, suggesting that pharmacogenomic approaches represent a promising avenue toward individualizing antidepressant treatment. Here we perform genome-wide association studies (GWAS) to search for genetic variation affecting the susceptibility to side effects. The analysis sample consisted of 1,675 depression patients, successfully genotyped for 421K SNPs, from the Sequenced Treatment Alternatives to Relieve Depression (STAR\*D) study. Outcomes included four indicators of side effects: general side effect burden, sexual side effects, dizziness and vision/hearing related side effects. Our criterion for genome-wide significance was a pre-specified threshold ensuring that, on average, only 10% of the significant findings are false discoveries. 35 SNPs satisfied this criterion. The top finding indicated 10 SNPs in SACM1L whose minor allele effects mediate the effects of bupropion on sexual side effects. SACM1L potentially alter cellular trafficking which has been shown to be associated with sexual dysfunction. EMID2 significantly mediates the effects of citalopram on vision/hearing side effects and has been associated with ocular functioning and pharmacogenetic side effect moderation in aspirin-induced asthma. Genome-wide significant findings were also found for SNPs in DTWD1, MAGI2, and CHL. Although our findings require replication and functional validation, this study demonstrates the potential of GWAS to discover genes and pathways that potentially mediate adverse effects of antidepressants.

### B-31 Photo-controlled Drug Release From A Caged Drug Conjugate

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The concept of targeted therapy has developed to overcome the less specificity and side effects associated with the chemotherapeutic treatment of cancer. We have developed a targeted drug delivery system using a photocaged molecule. In photocaging a biologically active molecule is made inactive by the attachment of a photocleavable group. On exposure to radiation the photocleavable entity is removed and the biologically active molecule is released. Using this concept we have designed a prodrug that consists of a cell impermeable hydrophilic molecule attached to a photocaged doxorubicin. The hydrophilic moiety makes the entire prodrug cell impermeable in dark. Upon irradiation with UV light the photosensitive group is removed and cytotoxic doxorubicin is released at the tumor site. This method will be further modified for the delivery of other cytotoxic agents in the cancer cells.

**Keywords:** targeted therapy, photocaged doxorubicin, dark, impermeable, light, permeable

### B-32 PROBING STRUCTURE-ACTIVITY REALTIONSHPIS OF SIMOCYCLINONE D8'S COUMARIN BINDING SITE

Lauren M Gaskell and Keith C Ellis

Medicinal Chemistry

Type II topoisomerases are a family of enzymes that control the winding and unwinding of bacterial DNA during replication. This family of enzymes is a validated target for antibacterial therapy, as the clinically used fluoroquinolone class targets type II topoisomerases. Fluoroquinolones act by trapping cut DNA on the enzyme, which eventually signals for cellular apoptosis. Recently, fluoroquinolone resistant strains of bacteria have been seen in the clinic. These strains are mutants able to maintain their regular catalysis while blocking the binding of the fluoroquinolone antibiotics. Simocyclinone D8 (SD8) is a natural product that has shown antibiotic activity against type II topoisomerases. SD8 has a novel mechanism of action as well as a novel binding site on type II topoisomerases.

Our goal is to develop more potent analogs of SD8. Various synthetic routes have been attempted to synthesize SD8's 8-fluoro-4,7-hydroxy-3-aminocoumarin without success. Analogs of the coumarin are being developed to explore the structure-activity relationships of type II topoisomerases' coumarin binding site. Data gathered from biological studies conducted with the coumarin analogs will guide development of more potent, drug-like analogs.

**B-33 PROCESSING OF BLOCKED OXIDATIVELY MODIFIED DNA DOUBLE-STRAND BREAK ENDS  
BY METNASE**

Susovan Mohapatra, Suk-Hee Lee, Vijay Menon, Robert A. Hromas, Lawrence F. Povirk

Pharmacology and Toxicology

Metnase, originally identified as a SET transposase, was found to increase radiosurvival following exposure to ionizing radiation. However, the role of Metnase nuclease domain in DNA double-strand break repair by non-homologous end joining needs to be further investigated. To assess the possibility that Metnase may act on oxidatively modified DNA end termini, its activity towards model DNA substrates, bearing a 3'-hydroxyl or a 3'-phosphoglycolate moiety was examined. A 3'-phosphoglycolate moiety on longer overhangs (4 and 6 bases) altered specificity and stimulated Metnase mediated cleavage of the terminal 3 nucleotides. However, an 8-oxoguanine residue at the single strand/double strand border did not affect specificity or extent of cleavage. A point mutation (D483A) in the DDE like motif of Metnase completely abrogated Metnase cleavage activity towards DNA ends. Total Metnase mediated cleavage also increased proportionally with the length of 3'-overhangs. These results suggest a possible role for Metnase endonuclease activity in processing of terminally blocked 3'-phosphoglycolate lesions following exposure to ionizing radiation or radiomimetic chemotherapeutics.

**B-34 REGULATION OF PRO-INFLAMMATORY TH17 RESPONSES DURING T. CRUZI INFECTION  
BY IL-12-FAMILY CYTOKINES**

Drew Cobb, and Ronald B. Smeltz

Microbiology and Immunology

The intracellular protozoan parasite *Trypanosoma cruzi* causes American trypanosomiasis, or Chagas disease. Protective immunity to *T. cruzi* infection requires CD4<sup>+</sup> IFN- $\gamma$ -producing Th1 cells and CD8<sup>+</sup> cytotoxic T cells. While the necessity for IFN- $\gamma$  production during *T. cruzi* infection has been well established, the role of the pro-inflammatory cytokine IL-17 has not been fully resolved. IL-17 has been shown to contribute to host resistance following infection with *T. cruzi*. However, exacerbated IL-17 production can have detrimental effects on host immunity to *T. cruzi*. Therefore, it's important to have a clear understanding of the signals involved in the regulation of Th17 development. We have previously determined that the Th1 transcription factor T-bet is necessary for inhibiting Th17 development during experimental *T. cruzi* infection. In this study, we sought to characterize the role of the IL-12-family cytokines IL-12 and IL-27 and their ability to regulate the development of Th17 cells following infection with *T. cruzi*. We found that IL-12 was important for limiting Th17 generation, as IL-12-deficient mice infected with *T. cruzi* failed to up-regulate T-bet, and developed parasite-specific Th17 responses with accompanying neutrophilia. In contrast, IL-27-deficient mice did not exhibit an increase in parasite-specific Th17 development, demonstrating that IL-27 does not play a significant role in regulating Th17 development during *T. cruzi* infection. We then examined the ability of IL-12 or IL-27 to affect the stability of Th17 cells from *T. cruzi*-infected mice *ex vivo*. IL-12 was capable of suppressing *T. cruzi*-specific IL-17 production in an IFN- $\gamma$ -independent manner. These results provide insight into the cytokine-dependent signaling that regulates Th17 responses during infection with the intracellular parasite *T. cruzi*.

## **B-35 Role of Interdomain linker on AAV-2 Rep68 oligomerization**

Zarate-Perez, F., Villamil-Jarauta, M., Burgner, J., Das, K., Kekilli, D., Linden, M., and Escalante, C.R.

### Physiology and Biophysics

Adeno-Associated virus (AAV) is the only eukaryotic virus with the ability to integrate site-specifically into a human chromosome. A set of non-structural proteins from the Adeno-Associated virus (AAV-2) the Rep proteins, perform multifunctional activities. These include the initiation of DNA replication, transcriptional regulation, and assembly of infectious viral particles. The AAV Rep proteins carry out these functions: Rep78, Rep68, Rep52 and Rep40. These proteins share a central SF3 helicase domain represented by the smallest protein of this group, the Rep40. However, unlike similar domains from other SF3 helicases, Rep40 does not oligomerize by itself. The longest proteins of this group Rep68 and Rep78 contain an additional domain named origin binding domain (OBD) at their N-terminus, and a linker of 20 residues connecting the two domains. We performed a detailed structural comparison of Rep40 with the helicase domains of SV40-Tag and Papilloma Virus E1 protein, and we found that the oligomerization interface is mediated by both the N-terminal oligomerization domain that forms a helical bundle of four or more  $\alpha$ -helices and the c-terminal AAA+ domain whose interface is stabilized by the presence of nucleotides. The Rep40 structure shows a significantly smaller oligomerization domain for this protein. We hypothesize that this difference is fundamental for the inability of this protein to form oligomers. We have constructed a series of Rep40 proteins with different lengths of the interdomain linker that produces an oligomerization in this protein as the extension of the same increases in length. Also we found an induced oligomerization of Rep40 using ATP or ADP. In addition, we show that linker residue Tyr224, plays a critical for oligomerization in the context of Rep68. These findings support the view that AAV Rep proteins are a special class of SF3 helicases that require the cooperative interaction of the OBD, linker and helicase domains to oligomerize.

## B-36 Role of the HPA Axis in Behavioral Responses to Ethanol

Blair N. Costin and Michael F. Miles

### Pharmacology and Toxicology

Glucocorticoid hormones modulate acute and chronic behavioral and molecular responses to drugs of abuse including psychostimulants and opioids. There is growing evidence that glucocorticoids might also modulate behavioral responses to ethanol. Acute ethanol activates the HPA axis, causing release of adrenal glucocorticoid hormones. Our prior genomic studies suggest glucocorticoids play a role in regulating gene expression in the prefrontal cortex (PFC) of DBA2/J (D2) mice following acute ethanol administration. However, few studies have analyzed the role of glucocorticoid signaling in behavioral responses to acute ethanol. Such work could be significant, given the predictive value for level of response to acute ethanol in the risk for alcoholism. We studied whether the glucocorticoid receptor (GR) antagonist, RU-486, or adrenalectomy (ADX) altered male D2 mouse behavioral responses to acute (locomotor activation, anxiolysis or loss-of-righting reflex (LORR)) or repeated (sensitization) ethanol treatment. Whole genome microarray analysis and bioinformatics approaches were used to identify PFC candidate genes possibly responsible for altered behavioral responses to ethanol following ADX. ADX and RU-486 both impaired acute ethanol (2 g/kg) induced locomotor activation in D2 mice without affecting basal locomotor activity. ADX mice showed microarray gene expression changes in PFC that significantly overlapped with acute ethanol-responsive gene sets derived by our prior microarray studies. Q-rtPCR analysis verified that ADX decreased PFC expression of *Fkbp5* while significantly increasing *Gpr6* expression. Our studies suggest that ethanol's activation of adrenal glucocorticoid release and subsequent GR activation may partially mediate ethanol's acute locomotor activating properties in male D2 mice. In addition, adrenal glucocorticoid basal tone can regulate PFC gene expression.

### **B-37 Sphingosine kinase 1 inhibition attenuates mast cell-dependent allergic asthma in mice**

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Biochemistry and Molecular Biology

Departments of <sup>1</sup>Biochemistry and Molecular Biology and <sup>3</sup>Microbiology and Immunology;  
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Sphingosine-1-phosphate (S1P) produced by two sphingosine kinase isoenzymes, SphK1 and SphK2, has been implicated in IgE-mediated mast cell responses. However, studies of allergic inflammation in isotype-specific SphK knockout mice or in vivo siRNA knockdown have yielded conflicting results. Moreover, the role that S1P plays in vivo in a mast cell and IgE-dependent mouse model of allergic asthma has not yet been examined. We used an isoenzyme-specific SphK1 inhibitor, SK1-I, to investigate the contributions of S1P and SphK1 to mast cell dependent airway hyperresponsiveness (AHR) and airway inflammation. SK1-I inhibited antigen-dependent activation of human and murine mast cells and suppressed activation of NF- $\kappa$ B, a master transcription factor that regulates expression of pro-inflammatory cytokines. SK1-I treatment of mice sensitized to OVA in the absence of adjuvant, which develop mast cell-dependent allergic inflammation, significantly reduced OVA-induced AHR to methacholine; decreased numbers of eosinophils and levels of the cytokines IL-4, 5, 6, 13, IFN- $\gamma$ , and TNF- $\alpha$  and the chemokines eotaxin, and CCL2 in bronchoalveolar lavage fluid; and decreased pulmonary inflammation as well as activation of NF- $\kappa$ B in the lungs. Taken together, our findings demonstrate that SphK1 and S1P play key roles in mast cell-dependent, OVA-induced AHR and allergic inflammation and support the therapeutic potential of SphK1 inhibitors for the treatment of allergic airway inflammation. Supported by NIH Grants RO1AI50094 and U19AI077435 to S.S.

**B-38 Sphingosine-1-Phosphate: a missing cofactor for lysine 63-linked poly-ubiquitinations in pro-inflammatory cytokines mediated signaling**

Kuzhuvelil B. Harikumar, Nitai C. Hait, Jeremy Allegood, Eugene Y. Kim, Michael Maceyka, Sheldon Milstien, Sarah Spiegel, and Tomasz Kordula

Biochemistry and Molecular Biology, Massey Cancer Center

Sphingosine kinase 1, one of the isoenzymes that produce the potent bioactive lipid mediator sphingosine-1-phosphate (S1P), has long been implicated in the actions of pro-inflammatory cytokines. Yet its molecular mechanisms of action have largely remained unclear. We have recently shown that SphK1 and production of S1P is necessary for TNF- $\alpha$ -induced Lys 63-linked polyubiquitination of RIP1, phosphorylation of IKK and I $\kappa$ B $\alpha$ , and I $\kappa$ B $\alpha$  degradation, leading to NF- $\kappa$ B activation (Nature 465:1084, 2010). Surprisingly, these responses were mediated by intracellular S1P independently of its cell surface G protein-coupled receptors. S1P specifically binds to and stimulates the E3 ligase activity of TNF receptor-associated factor 2 (TRAF2) a key component in NF- $\kappa$ B signaling triggered by TNF $\alpha$ . S1P, but not dihydro-S1P, dramatically increased recombinant TRAF2-catalyzed Lys 63- but not Lys 48-linked polyubiquitination of RIP1 in vitro. Our data reveal that TRAF2 is a novel intracellular target of S1P, and that S1P is the missing co-factor for TRAF2 E3 ubiquitin ligase activity, providing a mechanistic explanation for the key role of SphK1 and its product S1P in TNF- $\alpha$  signaling and the canonical NF- $\kappa$ B activation pathway important in inflammatory, anti-apoptotic, and immune processes. We have found that S1P is also a cofactor for some other ubiquitin ligases that are critical for signaling by the pro-inflammatory cytokine IL-1. Taken together, our results suggest a new paradigm for regulation of Lys 63-linked polyubiquitination signaling by S1P.

**B-39 Structure-Guided design of bivalent simocyclinone D8 analogs that display an improved antibacterial activity by binding to DNA gyrase**

Jenson Verghese\* and Keith C. Ellis

Medicinal Chemistry

**Purpose.** To elucidate the minimal pharmacophore required for binding to the angucyclinone binding pocket of DNA gyrase by structure-guided design.

**Methods.** The crystal structure of SD8 bound to DNA gyrase identifies two distinct regions where SD8 binds to gyrase: the coumarin and the polyketide pockets. In-silico docking and scoring identified a surrogate to the coumarin. The chemically complex angucyclinone binds to the polyketide pocket by a multitude of contacts. Using chemical synthesis, guided by the crystal structure and chemical intuition we are dissecting the minimal structural requirements required for binding to the polyketide pocket of DNA gyrase.

**Results.** Using in-silico docking and scoring we have identified a poly-hydroxylated flavone - quercetin as a synthetically accessible substitute for the chloro-coumarin. The flavone was prepared in two steps from commercially available rutin. Selective esterification of the 3-hydroxyl over the 5-hydroxyl has been successfully carried out in good yields with mono-protected sebacic acid that would act as the bridge between the two pockets. Synthetic conditions have also been identified to unveil the free acid after the previous esterification followed by amidation with various substituted anilines with an increasing repertoire of functional groups designed to probe contacts that the angucyclinone makes with the polyketide pocket.

**Conclusions.** The synthetic route has been identified and worked out for preparing compounds which would serve to identify the minimal pharmacophore required for binding to the polyketide pocket of DNA gyrase. Assaying the inhibition of these compounds against DNA-gyrase would be the next step in identifying the minimal pharmacophore required for optimal binding and thereby anti-bacterial activity.

## **B-40 The Discriminative Stimulus Effects of Nitrous Oxide**

Kellianne Richardson and Keith L. Shelton

### Pharmacology and Toxicology

Despite the high prevalence of clinical use as well as illicit abuse, there is a limited understanding of the CNS actions of nitrous oxide (N<sub>2</sub>O). In vitro studies have shown that N<sub>2</sub>O oxide alters the function of NMDA, GABAA, and nicotinic acetylcholine receptors, amongst others. However, the receptor system or systems responsible for the intoxicating, subjective stimulus effects of N<sub>2</sub>O are uncertain. The drug discrimination procedure is a highly selective means of examining the neurotransmitter systems underlying a drug's in vivo pharmacological effects. Our overarching goal is to use drug discrimination in mice to assess the neurotransmitter systems responsible for producing the discriminative stimulus effects of N<sub>2</sub>O. We trained sixteen male B6SJLF1/J mice to discriminate 10 min of exposure to 60% inhaled N<sub>2</sub>O/40% oxygen versus 100% oxygen in daily 5-min operant sessions. Subsequently we began substitution tests with other compounds. Positive substitution between nitrous oxide and drugs with well characterized properties suggest similar mechanisms of action. We hypothesize that if the discriminative stimulus effects of N<sub>2</sub>O are mediated by NMDA antagonism and/or GABAA positive modulation, drugs with these mechanisms of action will fully substitute for N<sub>2</sub>O. Thus far the NMDA channel blockers ketamine and MK-801 have shown partial substitution for N<sub>2</sub>O. Of the 7 mice tested 3 fully substituted at 7 or 10 mg/kg ketamine. Of the 5 animals tested with MK-801, 3 fully substituted at 0.1–0.3 mg/kg. The same mice that fully substituted with ketamine also fully substituted with MK-801. Substitution of NMDA receptor competitive antagonist CGS19755 is currently being conducted. Once complete, the results of these studies will provide important information regarding the receptor systems underlying the abuse-related subjective stimulus properties of N<sub>2</sub>O.

## **B-41 THE IMPACT OF ADOLESCENT NICOTINE EXPOSURE ON DRUG DEPENDENCE IN ADULTHOOD**

Mai Alajaji, M.S. and M. Imad Damaj, Ph.D

### Pharmacology and Toxicology

Smoking among adolescents is a strong predictor of future drug abuse and dependence in adulthood. A number of studies has suggests that adolescents pre-exposed to nicotine may suffer permanent disruption of the brain's reward systems through changes in dopamine receptor function. We hypothesize that nicotine exposure during adolescence causes long lasting neurobiological alterations that increase the likelihood of cocaine use in adulthood. Conditioned-place-preference data showed that a 7-day exposure to 0.5 mg/kg of nicotine altered cocaine-induced responses. In contrast, neither 1 day exposure nor a low dose of nicotine (0.1 mg/kg) elicited this effect. A follow-up study was undertaken to determine if this enhancement generally applies to other drugs of abuse. Pre-exposure to 0.5mg/kg nicotine during early adolescence demonstrated significant enhancement to d-amphetamine and morphine preference in a CPP model. Similar to the effects seen with reward, exposure of early adolescent mice to nicotine also enhanced acute locomotor activity and locomotor sensitization to cocaine in adulthood. Our data strongly suggest that nicotine intake during adolescence may act to cross-sensitize the brain to cocaine's long-term changes in the brain. Further research will be required in order to more fully examine the mechanisms of action for the observed changes in cocaine rewards.

## **B-42 The interaction between the cannabinoid agonist Win55,212-2 and radiation in breast cancer**

Emery, S., Sumner, E., Lichtman, A., & Gewirtz, D.

### Pharmacology and Toxicology

Win55,212-2 (Win2) is a full efficacy agonist for the endocannabinoid system, which is the target of the psychoactive compound in marijuana,  $\Delta^9$ -tetrahydrocannabinol (THC). Pre-clinical work has shown that cannabinoids have antiproliferative actions in many cancers. Our studies were performed in the breast cancer cell lines: MCF-7, MDA-MB-231, or 4T1. Win2 at concentrations of 4, 8 and 12 $\mu$ M enhanced antiproliferative activity of ionizing radiation when combined with 1, 2 or 4 2Gy doses in a cell count assay with MCF-7 cells. This interaction was also confirmed in vivo using 5mg/kg Win2 twice weekly and 10Gy radiation. Win2 (8 $\mu$ M) for 24 hrs and 1 x 2Gy radiation was used for all subsequent experiments (Win/IR combo). This Win/IR combo was also shown to be effective in MDA-MB-231 and 4T1 cells. Interestingly neither THC nor the non-psychoactive cannabinoid Cannabidiol enhanced the response to radiation in MCF-7 cells, suggesting a possible drug-class specific effect. Additional studies using the Win/IR combo in MCF-7 cells suggested Win2 and IR caused growth arrest while the combination caused cell death between 24 and 48 hrs. At 24 hrs radiation alone had no effect on cell cycle distribution; yet Win2 either alone or in combination with radiation resulted in a robust G1 accumulation. At 48 hrs, Win2 alone or in combination with radiation caused a significant increase in the subG1 population implicating necrosis or apoptosis, but Annexin V/ PI staining via flow cytometry confirmed the presence of neither. The potential involvement of autophagy was investigated using the late autophagy inhibitor Chloroquine (CQ). CQ sensitized the cells to radiation and Win2, suggesting that autophagy is acting to protect the cells against radiation. Unexpectedly, CQ conferring protection on cells treated with Win2 and IR, suggesting a cytotoxic function of autophagy. Additional studies are required to understand the mode(s) of cell death and the role(s) of autophagy.

## **B-43 The role of Alpha5\* Nicotinic Acetylcholine Receptors in the acute and chronic effects in mice**

Dawson, AJ; Miles MF; Damaj IM

### Pharmacology and Toxicology

The high co-morbidity between alcohol and nicotine abuse suggests that nicotinic acetylcholine receptors (nAChRs), thought to underlie nicotine dependence, may also be involved in alcohol dependence. Genetic studies in rodents and humans suggest that the alpha5 nAChR-coding gene is significantly associated in both nicotine and alcohol dependence phenotypes. However, virtually no studies have investigated this receptor subtype with regard to alcohol's effects in vivo. We tested the hypothesis that alpha5 nAChRs mediate some acute and chronic effects of alcohol through experiments testing various behaviors in alcohol preferring C57BL/6J mice bred to lack alpha5 gene expression (alpha5 KO mice). We tested their response to alcohol's acute effects including initial sensitivity and functional tolerance through the loss of righting reflex assay, anxiolysis using elevated plus maze, hypothermia, and locomotor depression. For chronic effects, we chose to study alcohol intake and preference using two-bottle choice and aspects of alcohol reward using conditioned place preference. Our results showed that alpha5 KO mice had an enhanced response to alcohol-induced anxiolysis and hypothermia compared to wild type mice, but showed similar response to loss of righting reflex and locomotor depression. Conversely, in chronic experiments, alpha5 KO mice displayed significantly reduced ethanol intake and preference expression compared to wild type. These data suggest that alpha5 nAChRs may exert differential effects on the acute and chronic effects of alcohol. This is especially interesting given current literature in the field suggesting involvement of alpha5 nAChRs in nicotine intake regulation as well. Thus, this receptor subtype may represent a potential therapeutic target for the treatment of alcohol and nicotine co-abuse in the future.

## **B-44 The role of CCR5 in morphine and HIV-1 Tat mediated neuropathogenesis**

Elizabeth M. Podhaizer, Pamela E. Knapp, and Kurt F. Hauser

### Pharmacology and Toxicology

Neurocognitive impairments remain prevalent among HIV-1 infected individuals in the age of combined antiretroviral therapy. Additionally, substance abuse (such as heroin use), which occurs in approximately 1/3 of infected individuals, enhances the pathogenesis of the viral infection. CCR5 is a protein which reportedly interfaces with MOR, through both direct interaction, in the form of heterodimers, and/or indirectly through shared signaling pathways. CCR5 is also involved in the glial inflammatory response and may mediate opioid-HIV-1 interactions. We hypothesized that interference with CCR5 in glia would protect against HIV-1 Tat and morphine-mediated neurotoxicity. To assess the importance of CCR5, neuron-mixed glia co-cultures were subjected to HIV-1 Tat, morphine, or the combination in the presence or absence of the CCR5 antagonist, maraviroc. In this assay, the interactive neurotoxicity between Tat + morphine was specifically blocked by CCR5 inhibition. To determine the role of glia in this effect, CCR5 KO mixed glia were cultured with WT neurons. In this paradigm, CCR5 deletion in glia not only reduced the overall neurotoxic effect of morphine + Tat treatment, but also delayed its onset. Next we performed experiments aimed to assess the impact of CCR5 on glial inflammation/activation. Astroglial NF- $\kappa$ B p65 activation and chemokine release, processes which lead to elevations in CCR5 ligand secretion, were quantitated through ICC and ELISA, respectively. Maraviroc produced modest, but significant suppressions in nuclear translocation of p65, as well as RANTES and MCP-1 release. Additionally, microglial area was measured as a correlate of activation. HIV-1 had a time-dependent effect on microglial activation, and maraviroc altered specific stages of this process. These studies suggest that MOR-CCR5 may converge at the level of the glia to potentiate morphine and HIV-1 Tat-mediated neuropathogenesis. Support: P01 DA019398 and T32 DA007027.

## **B-45    Transcriptional transitions in response to hypoxia and energy expenditure**

Divya Padmanabha

Biochemistry and Molecular Biology

The interface of metabolism and hypoxia in disease progression has been the subject of renewed scrutiny. Several transcriptional activators are markedly induced by nutrient and oxygen deprivation. Cellular responses to hypoxia have been extensively studied in the context of the transcription factor HIF-1, a critical regulator of angiogenic and glycolytic genes in hypoxia. Interestingly, there exists clear evidence of HIF-independent pathways critical for hypoxia adaptation. However, relatively little is known about the oxygen sensors and regulatory pathways that mediate transcriptional responses independent of HIF. The nematode *Caenorhabditis elegans* has proven to be a powerful model system to study evolutionarily conserved signaling pathways regulating hypoxia responses. We are conducting a forward genetic screen to identify genes involved in adaptation responses to hypoxia but in a HIF-independent manner. We have generated reporter constructs in which GFP expression is controlled by regulatory sequences of a previously reported hypoxia-dependent HIF-independent gene F453.4. We also seek to understand temporal-specific transcript regulation by the nuclear hormone receptor estrogen-related receptor (ERR) with respect to energy expenditure. We have recently found that dERR facilitates the initiation of a pro-growth glycolytic program in the larval stages and the transition toward OXPHOS at adulthood, thereby suggesting that dERR maintains a striking level of functional conservation with its mammalian counterparts. We will use ChIP-Seq technology to define the full complement of ERR-bound elements in the *Drosophila* genome during the 2nd larval instar L2 and at the onset of adulthood. We expect to find ERR response elements that are common to both early and late time points as well as temporal-specific ERREs that are indicative of ERR function. We predict the temporal-specific ERREs will reveal the signature of ERR involvement in metabolic transition.

## **B-46 TriplatinNC; Heparan Sulfate Mediated Cell Entry and Nucleolar Localization.**

Erica Peterson, Heveline Silva, Vijay Menon, Brad Benedetti, Ralph Kipping, Nicholas Farrell

### Chemistry

TriplatinNC, a non-covalent derivative of the phase II clinical drug BBR3464, exhibits a distinct mode of DNA binding mediated through phosphate clamps, which display structural analogy to the polyarginine (arginine fork) DNA/RNA recognition motif. Furthering this analogy, we show that cellular accumulation of TriplatinNC, unlike cisplatin or oxaliplatin, is dependent upon the presence of charged cell surface glycosaminoglycans. Furthering the polyarginine analogy, the cellular accumulation of TriplatinNC, unlike cisplatin or oxaliplatin, is dependent upon the presence of cell surface glycosaminoglycans (GAGs), shown through comparison of uptake in wild-type Chinese Hamster Ovary (CHO) cell lines to those lacking heparan sulfate. The cellular localization of TriplatinNC and cisplatin were visualized in confocal microscopy experiments utilizing the fluorescently-tagged derivatives, TriplatinNC-NBD or cisplatin-NBD. TriplatinNC, but not cisplatin, shows distinct localization to the nucleolar region of human colon and ovarian carcinoma cell lines. It was hypothesized that this pattern of localization, combined with nucleic acid affinity, would allow TriplatinNC to disrupt rRNA synthesis, the primary function within the nucleolus. Using <sup>32</sup>P-metabolic labeling to monitor the rate of pre-rRNA transcript formation after cellular treatment, it was observed that both 47S pre-rRNA and processed rRNAs, 32S, 28S, and 18S levels decreased dramatically compared to the untreated control in a dose-dependent manner. While the localization and abundance of nucleolar proteins, RNA pol I and UBTF, were unaffected by treatments with TriplatinNC at early timepoints, levels of the clinical relevant proliferative marker Ki-67, decreased markedly within one hour of drug treatment.

**B-47 TRIUMPH OVER ADVERSITY: A QUALITATIVE STUDY OF NARRATIVE, COPING AND EXPERIENCE  
IN INDIVIDUALS DIAGNOSED WITH CANCER**

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Social and Behavioral Health

The increased occurrence of chronic illness has created a need to study the experience of illness from the patient perspective. Current medical training and education does not typically incorporate the patient experience of illness. Learning about patient perspectives is even more important in a disease such as cancer which has a tremendous psychosocial impact on patients. Patient narratives are an appropriate means of studying patient experience of cancer. More than ever, people are writing accounts of their experience of illness and treatment and patients are turning to these narratives for anecdotal information about particular illnesses and their treatments, conventional and alternative (Hawkins, 1999). This study takes a qualitative approach to studying the narratives of 21 patients who were diagnosed with cancer two years prior to the study to understand the patient experience of disease as well as examine the role that narratives play in the patient experience of disease. Distinct features of the patient experience of cancer were identified by the qualitative analysis. Features of the cancer experience included the impact of diagnosis, treatment, and prognosis on the cancer experience, perceptions of the etiology of cancer, coping mechanisms such as social support and spirituality and reactions to the health care system. Participants also used nuanced and unique metaphors for describing their cancer experience, for example a participant described the experience of parathyroid cancer was like having a "Rottweiler on a short leash". Results of this study suggest that personal cancer narratives -- written, read, told, and listened to -- can be highly useful for patients coping with a cancer diagnosis. The results also suggest that narratives might be an important public health tool for the dissemination of cancer related information.

**B-48 Using Genetic Information from Genome Wide Association Studies in Risk Prediction for Alcohol Dependence in the COGA and SAGE GWAS Samples**

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Human and Molecular Genetics

Genetic studies of alcohol dependence (AD) have reported numerous associated variants; however, the clinical validity of these variants has not been assessed. The Collaborative Study on the Genetics of Alcoholism (COGA) and the Study of Addiction: Genes and Environment (SAGE) genome wide association study (GWAS) samples were used to examine the aggregate impact of multiple genetic variants on clinical risk prediction for AD. Subsets of the COGA and SAGE samples were used as gene discovery and validation samples. Genetic sum scores were created by adding risk alleles of associated single nucleotide polymorphisms (SNPs) in discovery samples and then assessed for their ability to classify individuals as cases or controls in independent validation samples using receiver operating characteristic (ROC) curve analysis. We performed 3 analyses sets, which differed by method of SNP selection from GWAS results: (1) SNPs resulting from initial GWAS analysis; (2) SNPs from GWAS that are also associated in a second sample; (3) SNPs from GWAS analyses using varying "significance" criteria. ROC curve analysis using the first 2 methods did not result in significant discriminative ability for the sum scores. The 3rd method using less stringent p-value thresholds of 0.01 to 0.50 for SNP selection did yield significant area under the ROC curve estimates, ranging from 0.535 for sum scores created based on SNPs with  $p < 0.01$  to 0.573 for SNPs with  $p < 0.50$ . This study shows that these SNPs have limited clinical utility and supports a polygenic model involving hundreds of variants of small effect contributing to risk for AD.

**B-49 Decision Analysis Model Evaluating the Cost-Effectiveness of Fidaxomicin and Vancomycin in the Treatment of Clostridium Difficile Infection (CDI) from a Hospital Perspective**

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**Purpose.** This study compared the costs and effectiveness of fidaxomicin and vancomycin for the treatment of Clostridium difficile infection (CDI). This analysis was conducted from a hospital perspective to assist health-care providers in deciding whether to add fidaxomicin to their formularies. **Methods.** A decision tree model was built to examine whether fidaxomicin or vancomycin is more cost-effective for treating CDI. Outcomes data for this analysis were obtained from OPT-80-003 Clinical Study Group (fidaxomicin versus vancomycin) clinical trial. The main outcomes were: clinical cure, recurrence, and global cure. Cost data were per CDI patient: hospitalization costs (\$17,196), fidaxomicin cost (\$2,800), and vancomycin cost (\$26.8). **Results.** The baseline cost effectiveness analysis found that fidaxomicin was slightly more effective but more costly than vancomycin. That means that a hospital would pay an extra \$31,539 if fidaxomicin were chosen to treat each episode of CDI. The model is most sensitive to hospitalization costs, which is coherent with the literature. A one-way sensitivity analysis on drug cost found that fidaxomicin did not dominate whatever the cost was. A two-way sensitivity analysis on hospitalization cost and the clinical cure rate of fidaxomicin found that vancomycin was only dominated when fidaxomicin cure rates reached 97%, and this is highly unlikely. **Conclusion.** Based upon this analysis, vancomycin is the recommended drug of choice for CDI treatment. The hospital would pay an extra \$31,539 to treat each episode of CDI using fidaxomicin. Fidaxomicin would only be preferred if it had a higher clinical cure rate and a cheaper price according to the sensitivity analysis. As a conclusion, hospitals could consider fidaxomicin as second line treatment for cases in which patients have failed a course of treatment with vancomycin.

