University of North Carolina at Chapel Hill
Department of Pharmacology Research Retreat

March 27, 2015 – William and Ida Friday Center

8:30–9:00am Continental Breakfast (Atrium)

9:00–9:15am State of the Department Address (Dogwood)
Gary L. Johnson, PhD, Kenan Distinguished Professor and Chair

Invited Speakers

9:15-9:45am **Juan Song, PhD,** Assistant Professor, Department of Pharmacology
"Location, Location, Location: Visualizing in vivo Dynamics of Migrating Neuroblasts in the Adult Hippocampus"
Introduced by Gary Johnson, PhD

9:50–10:20am **Jason Kralic, PhD**, Founder & Principal Advisor, Innervate BD Solutions, LLC
"Career Paths for the PhD Pharmacologist"
Introduced by Rob Nicholas, PhD

10:30a–12:00pm **Poster Session 1** (presenters 1-21)

12:00–1:00pm **Lunch** (Trillium Room)

Keynote Speaker

1:15–2:00pm **Christopher M. Counter, PhD**, Professor, Departments of Pharmacology and Cancer Biology, and Radiation Oncology, Duke University
"A bad penny: Copper, MEK1/2 and Cancer"
Introduced by Channing Der, PhD

Graduate Student and Postdoc Presentations (Session I)

Introduced by Samantha Miller and Patrick McCarter

2:00–2:15pm **Tim Stuhlmiller, PhD**, Postdoctoral Fellow, Johnson Lab
"Inhibition of BET Family Bromodomains Suppresses Lapatinib-induced Kinome Reprogramming in HER2-positive Breast Cancer"

2:15–2:30pm **Kate Lansu,** Graduate Student, Roth Lab
"Ligand Discovery and Functional Analysis of the Novel Opioid Orphan Receptor MRGPRX2"

2:30–2:45pm **Orrin Stone**, Graduate Student, Hahn Lab
"A Versatile System to Generate Biosensors or Optogenetic Tools from Autoinhibitory Proteins"

2:45–4:00pm **Poster Session 2** (presenters 22-42)

Graduate Student and Postdoc Presentations (Session 2)

Introduced by Marissa Cann and Carrie Rubel

4:00–4:15pm **Derek Franklin**, Graduate Student, Zhang Lab
"CROT is a Novel p53 Target Gene that links p53 Stress Response to Lipid Metabolism"

4:15–4:30pm **Nicole Baker**, Graduate Student, Der Lab
"PAK1 Kinase Regulates Cell Growth and Macropinocytotic Uptake in K-Ras Mutant Pancreatic Cancer"

4:30–4:45pm **Andrew Hardaway, PhD**, Postdoctoral Fellow, Kash Lab
"Nociceptin Signaling Modulates the Expression of Limited Access Binge Eating"

5:00–6:30pm **Social Mixer and Award Presentations (Magnolia Lounge)**

University of North Carolina at Chapel Hill
Department of Pharmacology Research Retreat

March 27, 2015 – William and Ida Friday Center

Contact Information for 2015 Pharmacology Retreat Speakers

Keynote Speaker

Christopher Counter, PhD,
Professor, Department of Pharmacology
Associate Professor of Radiation Oncology
Duke University School of Medicine
Phone: 919-684-9890
Email: chris.counter@duke.edu
Website: http://pharmacology.mc.duke.edu/faculty/counter.html

Juan Song, PhD
Assistant Professor of Pharmacology
University of North Carolina at Chapel Hill
Phone: 919-843-9363
Email: juansong@email.unc.edu
Website: www.med.unc.edu/pharm/people/primaryfaculty/juan-song-1

Jason Kralic, PhD
Founder & Principal Advisor
Innervate BD Solutions, LLC
Phone: 919-491-4995
Email: library@ncbiotech.org
Website: http://directory.ncbiotech.org/content/innervate-bd-solutions-llc

University of North Carolina at Chapel Hill
Department of Pharmacology Research Retreat

March 27, 2015 – William and Ida Friday Center

ORAL PRESENTATIONS

Session 1
(2:00 – 2:45)

1. **Tim Stuhlmiller, PhD, Postdoctoral Fellow, Johnson Lab**
   "Inhibition of BET Family Bromodomains Suppresses Lapatinib-induced Kinome Reprogramming in HER2-positige Breast Cancer"

2. **Kate Lansu, Graduate Student, Roth Lab**
   "Ligand Discovery and Functional Analysis of the Novel Opioid Orphan Receptor MRGPRX2"

3. **Orrin Stone, Graduate Student, Hahn Lab**
   "A Versatile System to Generate Biosensors or Optogenetic Tools from Autoinhibitory Proteins"

Session 2
(4:00 – 4:45)

1. **Derek Franklin, Graduate Student, Zhang Lab**
   "CROT is a Novel p53 Target Gene that Links p53 Stress Response to Lipid Metabolism"

2. **Nicole Baker, Graduate Student, Der Lab**
   "PAK2 Kinase Regulates Cell Growth and Macropinocytotic Uptake ikn K-Ras Mutant Pancreatic Cancer"

3. **Andrew Hardaway, PhD, Postdoctoral Fellow, Kash Lab**
   "Nociceptin Signaling Modulates the Expression of Limited Access Binge Eating"

Inhibition of BET family bromodomains suppresses Lapatinib-induced kinome reprogramming in HER2-positive breast cancer.

Timothy J. Stuhlmiller[1], Samantha M. Miller[1], Jon S. Zawistowski[1], Kazuhiro Nakamura[1], Adriana S. Beltran[1], James S. Duncan[1], Steven P. Angus[1], Kyla A. L. Collins[2], Deborah A. Granger[1], Rachel A. Reuther[1], Lee M. Graves[1], Shawn M. Gomez[2], Pei-Fen Kuan[3], Joel S. Parker[4], Xin Chen[1], Noah Sciaky[1], Lisa A. Carey[5], H. Shelton Earp[5], Jian Jin[6], Gary L. Johnson[1],* *Department of Pharmacology[1], Joint Department of Biomedical Engineering at UNC-Chapel Hill and NC State University[2], Department of Biostatistics[3], Department of Genetics[4], Department of Medicine[5], Eshelman School of Pharmacy[6], Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

The *ERBB2* oncogene is amplified or overexpressed in roughly 25% of breast cancers and serves as the primary driver of tumor cell growth in the majority of these cancers. Therapeutics such as lapatinib that target ERBB2 often provide initial clinical benefit but resistance frequently develops. Using a chemical proteomics method, we found the adaptive responses leading to lapatinib resistance involve reprogramming of the kinome through reactivation of ERBB2/ERBB3 signaling and transcriptional upregulation and activation of multiple tyrosine kinases. The heterogeneity of induced kinases prevents their targeting by a single kinase inhibitor, underscoring the challenge of predicting effective kinase inhibitor combination therapies. We hypothesized that to make the tumor response to single kinase inhibitors durable, the adaptive kinome response itself must be inhibited. Genetic and chemical inhibition of BET bromodomain chromatin readers suppresses transcription of many lapatinib-induced kinases involved in resistance including ERBB3, IGF1R, DDR1, MET, and FGFRs, preventing downstream SRC/FAK signaling and AKT reactivation. Combining inhibitors of kinases and chromatin readers prevents kinome adaptation at an epigenetic level by blocking transcription, generating a durable response to lapatinib and overcoming the dilemma of heterogeneity in the adaptive response.

Ligand discovery and functional analysis of the novel opioid orphan receptor MRGPRX2.

K. Lansu, W.K. Kroeze, and B.L. Roth. *Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA.*

Orphan G protein-coupled receptors have no known endogenous ligands and make up approximately 30% of the non-olfactory GPCRs. These understudied receptors have potential pharmacological and therapeutic relevance. Although orphan GPCRs are expressed throughout the body several subtypes are expressed exclusively in the brain and/or spinal cord and may be tractable targets for the treatment of pain. One such class of receptors is the Mas-Related G Protein-Coupled Receptor X (MRGPRX) family of orphan GPCRs that are expressed (or just express) in the dorsal root ganglion and mast cells. These receptors have few known ligands and have poorly characterized function in vivo. We used a ?-arrestin recruitment assay to screen approximately 6 800 compounds against the MRGPRX family of receptors and discovered that many opiate compounds (eg. morphinans benzomorphans) activate MRGPRX2 but not MRGPRX1 or MRGPRX4. We confirmed these activities using calcium flux and PI hydrolysis assays and created preliminary structure-activity relationship (SAR) for the MRGPRX2 receptor. Our data indicate that MRGPRX2 is activated by opiate drugs and may be a novel G?q-coupled opioid receptor with physiological relevance.

A versatile system to generate biosensors or optogenetic tools from autoinhibitory proteins.

O. J. Stone, O. Dagliyan, and K. M. Hahn. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

The relative timing and localization of protein activity is often critical in determining cell behavior. Recent advances in our understanding of these important aspects of cell signaling networks have been made possible by the development of tools that enable visualization or manipulation of protein activity in living cells with high spatio-temporal precision. Visualization of protein activity has been accomplished with fluorescent biosensors that report protein activation states by using Förster resonance energy transfer (FRET) readouts of a conformational change or protein interaction. Optogenetic probes based on light-dependent dimerization or conformational changes have enabled manipulation of protein activity with light. However widespread adoption of these approaches is limited by the significant difficulty associated with developing new biosensors or optogenetic probes. We have developed a general platform for the rapid development of both biosensors and optogenetic probes from autoinhibitory proteins. Autoinhibition is a common theme in protein regulation whereby an autoinhibitory domain negatively regulates protein activity via intramolecular interactions with other domains. Upstream regulators of autoinhibited proteins often exert their effects by modulating autoinhibition via phosphorylation or binding interactions. We have leveraged the high affinity (Kd < 22 nM) interaction of the E. coli protein SspB with the small peptide SsrA to generate orthogonal binding partners for autoinhibited proteins. Insertion of SsrA into a key C-terminal regulatory region of Src an autoinhibited kinase does not affect Src function or localization. However binding of SspB to SsrA-tagged Src results in disruption of autoinhibition and kinase activation. We have also successfully applied this strategy to control p21-activated kinase 1 (Pak1) indicating this method may be broadly applicable to other autoinhibited proteins. In addition we have shown that SspB binds selectively to activated Src and thus our approach may also be useful for biosensor development.

Carnitine octanoyl transferase (CROT) is a novel p53 target gene that links p53 stress response to lipid metabolism.

D. Franklin and Y Zhang. *Dept. Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA and Department of Radiation Oncology UNC Hospitals Chapel Hill, NC, USA.*

The canonical tumor suppressor functions of p53 include apoptosis cell cycle arrest and senescence. A recently developed knock-in mouse model expressing a p53 mutation that abolishes these recognized activities of p53 surprisingly exhibits decreased tumor formation and increased survival compared to p53 knockout mice. Furthermore despite this mutation resulting in disruption of p53-mediated apoptosis senescence and cell cycle arrest p53-dependent regulation of cellular metabolism remains intact implicating p53-dependent metabolic regulation as being critical for the tumor suppressor activity of p53. Multiple mRNA microarrays using small molecule activators of p53 have identified carnitine octanoyl transferase (CROT) as a potential p53 target gene. Further experimentation confirmed that CROT is a novel p53 target gene that is up regulated in response to p53 activation in a number of cell lines and stimuli. Moreover decreased expression of CROT facilitated increased cell proliferation of the MCF-7 breast cancer cell line under hypoxic conditions suggesting a possible tumor suppressive role for CROT. Future studies will focus on the potential tumor suppressive role of lipid catabolism and CROT expression specifically.

PAK1 kinase regulates cell growth and macropinocytotic uptake in K-Ras mutant pancreatic cancer.

Nicole Baker1, Janine LoBello2, Haiyong Han2, Channing J. Der1. *1University of North Carolina Dept. of Pharmacology Chapel Hill NC 2Translational Genomics Research Institute Phoenix AZ, USA.*

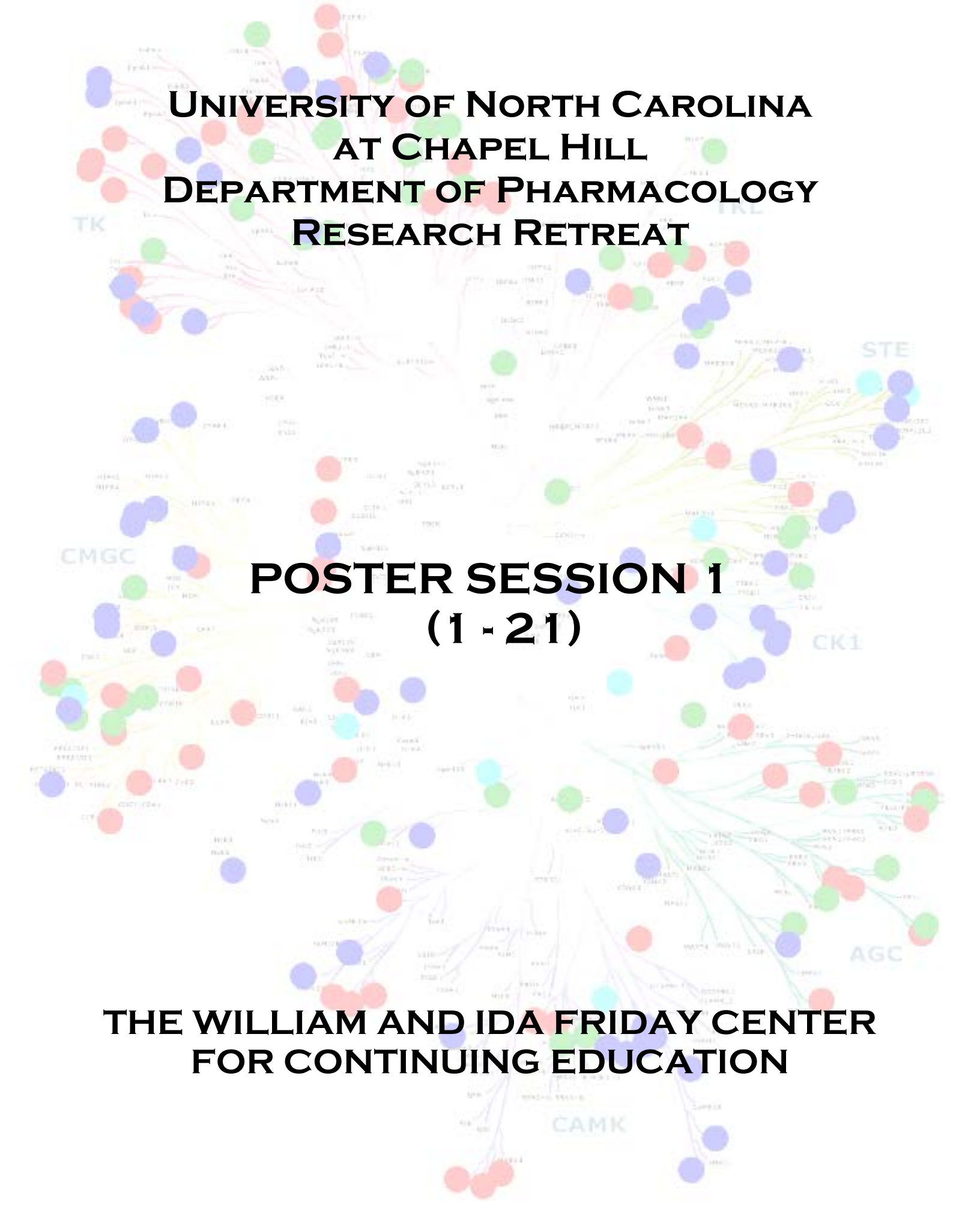
Pancreatic ductal adenocarcinoma (PDAC) is an extremely lethal cancer characterized by a high frequency of activating mutations in the *KRAS* oncogene (95%), which is a well-validated driver of PDAC growth. However, to date, no successful anti-K-Ras therapies have been developed. Inhibitors targeting components of KRAS downstream signaling pathways, when used as monotherapy or in combination, have been ineffectual for long-term treatment of *KRAS* mutant cancers. The lack of success of these inhibitors is due, in part, to the importance of other effectors in K-Ras-dependent cancer growth and the upregulation of compensatory signaling programs that overcome inhibitor activity. Consequently, in order to design effective combinatorial-targeted therapies, there is a pressing need to better understand the role of other effector signaling events that support mutant K-Ras- driven PDAC growth. We hypothesize that the PAK1 serine/threonine kinase is a key component downstream of mutant K-Ras in PDAC. In support of this, we found that PAK1 protein levels are overexpressed in pancreatic cancer cell lines and in patient tumor samples when compared to normal tissues. Additionally we determined that stable shRNA-mediated suppression of PAK1 protein expression inhibited PDAC anchorage-independent and –dependent growth in vitro. We also found that a pharmacologic inhibitor of PAK1 and other Group I PAK isoforms (PAK2 and PAK3) inhibited PDAC growth. Additionally, mutant K-Ras is known to upregulate processes in order to support the increased metabolic needs of cancer cell growth, such as macropinocytosis, a mechanism by which cells are able to uptake protein and carbohydrates from the extracellular environment in bulk. Inhibition or genetic ablation of PAK1 results in a marked decrease in macropinocytosis in PDAC cells. This data suggest inhibition of PAK1 in K-Ras mutant PDAC prevents cell growth and interferes with PDAC cell metabolism. Therefore PAK1 could represent a promising therapeutic target in pancreatic cancer.

Nociceptin signaling modulates the expression of limited access binge eating.

J.A. Hardaway, J.A. Sugam, M. Kim, C.M. Bulik, T.L. Kash. *Dept. Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.*

Binge eating disorder (BED) is the most common eating disorder that afflicts between 2-3% of both males and females over their lifetime. BED is characterized by persistent episodes of binge eating the consumption of an excessive amount of food paired with a sense of loss of control. Similar patterns of binge-like feeding can be generated in mice using pharmacological behavioral or circuit level strategies. For example the injection of nociceptin (NOC) peptide into the brain produces a significant increase in feeding but the functional requirement of this pathway during palatability driven binge eating is unknown. We used a behavioral model of BED where mice were provided with intermittent 1-hour daily access to a highly palatable and fat-rich food (HFD). Intermittently fed mice will eat significantly more in this session than animals provided with continuous 24-hour exposure demonstrate rapid onset consumption during the first 10 minutes and slowly escalate their binge intake over several weeks. Treatment with the NOC receptor antagonist SB 612111 significantly reduces both the maintenance and induction of intermittent HFD consumption. Using NOC reporter mice we determined that NOC neurons of the central amygdala (CeA) are activated following intermittent access binging. We targeted NOCCeA neurons using the inhibitory designer receptors exclusively access by designer drugs (DREADDs) hM4D. Pharmacogenetic inhibition of NOCCeA neurons significantly and selectively reduces HFD intake. We hypothesize that central amygdala NOC neurons and NOC receptor signaling may represent an important node for binge eating and an intriguing target to study for the treatment of BED respectively.

University of North Carolina at Chapel Hill
Department of Pharmacology
Research Retreat



POSTER SESSION 1
(1 - 21)

THE WILLIAM AND IDA FRIDAY CENTER FOR CONTINUING EDUCATION

Department of Pharmacology Research Retreat

POSTER SESSION 1 (1 - 21)

1. **Defining the Adaptive Kinome Response to BRAF and MEK Inhibition in Melanoma.** Steven P. Angus[1], Timothy J. Stuhlmiller[1], Deborah A. Granger[1], Rachel A. Reuther[1], Sara Hanna[1], David B. Darr[2], Jamie L. Jordan[2], William Y. Kim[2,3], Lee M. Graves[1], David Ollila[3], Stergios Moschos[3], Carrie Lee[3], Norman E. Sharpless[2,3], and Gary L. Johnson[1]. *Departments of Pharmacology[1], Genetics[2], and Medicine[3], Lineberger Comprehensive Cancer Center, University of North Carolina Chapel Hill, NC 27599, USA.*

2. **Molecular Dissection of Breast Luminal Cell Transcription Factor Networks.** F. G. Bargiacchi[1], H. S. Earp[1], and C. M. Perou[2,3]. *[1]Department of Pharmacology, [2]Department of Genetics; [3]Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, NC, USA.*

3. **Differential Regulation of Synaptic Versus Extrasynaptic α4 $GABA_A$-Receptors by Protein Kinase C and Protein Kinase A.** J. Peyton Bohnsack, Stephen L. Carlson, and A. Leslie Morrow. *Bowles Center for Alcohol Studies, Depts. of Pharmacology and Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

4. **The Use of Novel Peptide Probes to Study Proteasome Regulation by Src Family Kinases in Diffuse Large B-cell Lymphoma.** M. L. Cann, M. A. Priestman, Q. Wang, and D. S. Lawrence. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC USA and Division of Chemical Biology and Medicinal Chemistry, University of North Carolina School of Pharmacy, Chapel Hill, NC 27599, USA.*

5. **SCF and APC E3 Ubiquitin Ligases Define an Interacting Negative Feedback Loop that Control Cell-Cycle Progression.** Rajarshi Choudhury and Michael J. Emanuele, *Lineberger Comprehensive Cancer Center and Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

6. **Chronic Intermittent Ethanol Exposure (CIE) Modulates BNST GABA and Glutamate Transmission and Social Behavior in a kappa opioid receptor (KOR) Dependent Manner.** Nicole A. Crowley[1,2], Nora M. McCall[2], and Thomas L. Kash[2]. *Neurobiology Curriculum, University of North Carolina at Chapel Hill, Chapel, NC 27599, USA; Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.*

7. **Engineered Allosteric Control of Protein Activity in Living Cells.** O. Dagliyan, P. C. Hsu, A. Karginov, D. Shirvanyants, S. Yagishita,H. Kasai, N. V. Dokholyan, K. M. Hahn. *Department of Biochemistry and Biophysics, Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA; Center for Disease Biology and Integrative Medicine, The University of Tokyo, Bunko-ku, Tokyo, Japan.*

8. **Cellular Mechanisms of ?-Hemolysin-mediated Activation of the NLRP3 Inflammasome.** Ejiofor A. D. Ezekwe, Jr, Joseph A. Duncan. *Department of Pharmacology and Department of Medicine Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.*

9. **Patient-Derived Fibroblasts Support Dual Role of Stroma in Pancreatic Ductal Adenocarcinoma.** Elizabeth Flate[1], Richard A. Moffitt[1], S. Gabriela Herrera Loeza[1] and Jen Jen Yeh[1,2,3]. *[1]Lineberger Comprehensive Cancer, [2]Department of Surgery, [3]Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599, USA.*

10. **Divergent Roles of CAAX Motif Posttranslational Modifications in Ral GTPase Regulation.** L. R. Gentry, T. D. Martin, D. Tsygankov, C. J. Der. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

11. **Hippo Pathway Activation in Chemo-Sensitive and –Insensitive Breast Cancer.** Sara Hanna[1], Jerry Usary[2], Chuck Perou[2], and Gary Johnson[1]. *[1]Department of Pharmacology, [2]Genetics Department, School of Medicine, University of North Carolina, Chapel Hill, NC, 27599, USA.*

12. **Size Selection of cDNA Library for mRNA-Seq Using KAPA Stranded mRNA-Seq Kit.** K. Inoue and M. Calabrese. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

13. **Identification of Sugar-Responsive Protein Networks Associated with Regulator of G-protein Signaling 1 in Arabidopsis.** D. K. Jaiswal[1], M. Tunc-Ozdemir[1], D. Urano[1], E. G. Werth[2], W.O. Slade[2], L. M. Hicks[2], and A. M. Jones[1,3]. *Departments of Biology[1], Chemistry[2], and Pharmacology[3], University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

14. **High Throughput Screening Identifies Small Molecules that Enhance the Pharmacological Effects of Oligonucleotides.** B. Yang[1], X. Ming[1], C. Cao[1], B. Laing[1], A. Yuan[1], M. A. Porter[1], E. A. Hull-Ryde[1], J. Maddry[2], M. Suto[2], W. P. Janzen[1], and R. L. Juliano[1]. *[1]UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA; [2]Southern Research Institute, Birmingham, AL 30205, USA.*

15. **Testing the Theoretical Limit of Gradient Sensing in Yeast.** V. V. Lakhani[1,2,3], T. C. Elston[1,2,3]. *[1]Molecular and Cellular Biophysics Program, [2]Bioinformatics and Computational Biology Curriculum, [3]Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

16. **Identifying and Characterizing Protein Cofactors Involved in Long Noncoding RNA-mediated Gene Silencing.** D. M. Lee[1,2], C. Chu[3], H. Y. Chang[3], and J. M. Calabrese[1]. *[1]Department of Pharmacology and [2]Curriculum in Genetics and Molecular Biology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; [3]Stanford University School of Medicine, Stanford, CA USA.*

17. **Gene Expression and cis-eQTL Analyses of Peripheral Blood Cells from Elderly Patients with Obstructive Coronary Artery Disease.** (Lenhart, K. presenting) Robert N. Schuck[1], Eleanor Hilliard[2], Craig R. Lee[1], Xuming Dai[2,3], George A. Stouffer[2,3], Cam Patterson[4], and Jonathan C. Schisler[2,5]. *[1]Division of Pharmacotherapy and Experimental Therapeutics UNC Eshelman School of Pharmacy; [2]McAllister Heart Institute and, [3]Divison of Cardiology at the Univ. of North Carolina, Chapel Hill, NC 27599 USA; [4]Presbyterian Hospital/Weill-Cornell Medical Center, New York, NY 10065, USA; [5]Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

18. **microRNA Regulation of HK2 and its Pseudogene in Promoting Pancreatic Cancer Glycolysis.** M. B. Lipner, M. B. Nedrud, and J. J. Yeh. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

19. **Gaba Cells of the Dorsal Raphe Projecting to the Central Nucleus of the Amgydala Modulate Behavioral Reponses to a Fear-Associated Context.** E. G. Lowery-Gionta and T. L. Kash. *University of North Carolina School of Medicine Bowles Center for Alcohol Studies, Chapel Hill, NC 27599, USA.*

20. **Manipulation of vGlut3-expressing Cells of the Bed Nucleus of the Stria Terminalis Reduces Contextual Fear Learning.** Christopher Mazzone[1], Calvin Snyder[1], Thomas Kash[2]. *[1]Curriculum in Neurobiology, University of North Carolina at Chapel Hill; [2]Department of Pharmacology University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

21. **Ranking Proposed Yeast Feedback Networks through Approximate Bayesian Computation Model Estimation and Selection.** Patrick McCarter[1,3], Justin English[2], Henrik Dohlman[1,2,3], Tim Elston[1,2]. *Curriculum in Bioinformatics and Computational Biology[1], Department of Pharmacology[2], Department of Biochemistry and Biophysics[3], University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

Defining the Adaptive Kinome Response to BRAF and MEK Inhibition in Melanoma.

Steven P. Angus1, Timothy J. Stuhlmiller1, Deborah A. Granger1, Rachel A. Reuther1, Sara Hanna1, David B. Darr2, Jamie L. Jordan2, William Y. Kim2 3, Lee M. Graves1, David Ollila3, Stergios Moschos3, Carrie Lee3, Norman E. Sharpless2 3, and Gary L. Johnson1. *Departments of Pharmacology1 Genetics2 and Medicine3 Lineberger Comprehensive Cancer Center University of North Carolina Chapel Hill North Carolina 27599 USA.*

Early clinical studies have shown that concurrent administration of BRAF and MEK inhibitors Dabrafenib and Trametinib is more active in patients with BRAFV600E/K melanoma than either single agent alone. However progression to resistance ultimately occurs. Therefore therapeutic strategies to counter primary resistance and prevent the emergence of secondary resistance are needed. Following pharmacological or progressive genetic perturbations dynamic and system-wide adaptive changes (reprogramming) in the expression and activity of multiple kinases (collectively termed the kinome) clearly occur in tumor cells. Thus a comprehensive understanding of the kinome at baseline during the adaptive response to kinase inhibitor treatment and in the context of acquired resistance is critical. To this end we are analyzing tumor samples from patients in a UNC clinical trial (LCCC1128) administered the combination of Dabrafenib and Trametinib at baseline and at the time of progression on therapy. We use Multiplexed Inhibitor Beads (MIBs) mixtures of covalently immobilized linker-adapted kinase inhibitors coupled with mass spectrometry (MS) to assess the kinome activation state. Our enhanced MIB/MS technology allows us to study over 80% of the expressed kinome using lysates from cell lines genetically engineered mouse model (GEMM) tumors patient-derived xenografts or human melanoma samples. We have identified several kinases (e.g. DDR1 MAP3K1 AURKA) that are activated upon acute treatment with BRAFi and/or MEKi as well as in drug-resistant human melanoma cell lines and BrafV600E/Pten-/- GEMM-derived tumors and cell lines. Furthermore we have initiated studies to identify common genetic alterations observed in human melanoma samples that confer resistance to BRAFi/MEKi (e.g. MYC overexpression PTEN loss MEK2 mutation) and determine the consequent effects on the kinome. The integration of these studies will lead to the identification of novel and rationally predicted therapies for patients with advanced melanoma refractory to BRAFi/MEKi.

Molecular Dissection of Breast Luminal Cell Transcription Factor Networks.

F.G. Bargiacchi1, H.S. Earp1, and C.M. Perou 2 3. *1Dept. Pharmacology; 2Dept. Genetics; 3Dept. Pathology, University of North Carolina School of Medicine, Chapel Hill, NC USA.*

During mammary development, cellular differentiation and lineage commitment to various epithelial and mesenchymal cell types are driven by hormonal and paracrine signaling mechanisms. Understanding mechanisms that govern differentiation into distinct cell populations is critically important for a complete understanding of development and breast tumorigenesis. Studies have shown that retroviral transduction of fibroblasts with four transcription factors can initiate the conversion of a somatic cell into an embryonic stem cell-like state with capabilities of differentiating into cell types of all three germ layers. Based on these data, our goal is to determine whether mammary specific transcription factors (TFs) can directly induce transdifferentiation to an ER+/luminal cell phenotype in mouse embryonic fibroblasts via an iPS type approach. To address this we have developed a model in which CDKN2A KO Balb/c mouse embryonic fibroblasts (MEFS) are transduced utilizing a lentiviral vector system which allows the simultaneous expression of multiple candidate genes. After screening our nine candidate TFs for their abilities to induce various epithelial- and breast-specific attributes, we are focusing efforts on ESR1, FOXA1, PGR, and AR. Based on earlier data from human mammary epithelial cells (HMECs) ectopic overexpression of these TFs have both unique and overlapping contributions towards inducing the expression of genes responsible for luminal differentiation. Combining these TFs has created combinations that produce a luminal progenitor phenotype as defined by gene expression histological markers for epithelial/breast development as well as functionality. Examining the expression patterns of these combos using a classifier of differentiation status (Differentiation Score, Prat et al. 2010) identified several genes that drive a transition towards the luminal subtype. Since there are presently no cell lines or mouse models of Luminal A/ER+ breast cancers, the creation of such of a line would be of tremendous value.

Differential regulation of synaptic versus extrasynaptic α4 $GABA_A$-receptors by protein kinase C and protein kinase A.

J. Peyton Bohnsack, Stephen L. Carlson, and A. Leslie Morrow, *Bowles Center for Alcohol Studies Departments of Pharmacology, and Psychiatry University of North Carolina School of Medicine, Chapel Hill, NC 27599 USA.*

Ionotropic gamma aminobutyric acid receptors ($GABA_A$-Rs) are responsible for the majority of inhibitory neurotransmission in the mammalian brain. $GABA_A$-Rs neurotransmission can be divided into two subtypes: phasic inhibition regulated by synaptic $GABA_A$-Rs, and tonic inhibition regulated by extrasynaptic $GABA_A$-Rs. $GABA_A$-Rs containing the α4 subunit are found in both synaptic and extrasynaptic populations. Previous reports have demonstrated regulation of $GABA_A$-Rs by protein kinase C (PKC) and protein kinase A (PKA). To assess changes in expression for the different populations of α4 GABAA-Rs we validated a biochemical strategy for the separation of synaptic and extrasynaptic membrane fractions in cultured cortical rat neurons based on a technique used for the separation of synaptic and extrasynaptic glutamate receptors. Validation demonstrated that the synaptic markers postsynaptic density protein 95 and neuroligin 2 were highly enriched in the synaptic fraction while $GABA_A$-α4 subunit was highly enriched in the extrasynaptic fraction. Western blot analysis was utilized to determine the relative change in GABAA-R subunits. Pharmacological activation of PKC using phorbol 12 13-dibutyrate (100 nM) increased synaptic $GABA_A$-α4 subunit expression by 233.9 ± 56.4% ($p = 0.034$). In contrast pharmacological activation of PKA using Sp-cAMP (50 μM) decreased synaptic $GABA_A$-α4 expression by 77.95 ± 6.29% ($p = 0.018$). In the extrasynaptic fraction activation of PKC did not significantly alter the expression of the $GABA_A$-α4 or $GABA_A$-δ subunit expression. In contrast PKA activation increased extrasynaptic $GABA_A$-α4 expression by 152.4 ± 14.27% ($p = 0.017$). These data suggest that PKA activation opposes the effects of PKC on synaptic $GABA_A$-α4 receptors. The differential regulation of these distinct populations of receptors could have importance for both homeostatic and disease-state regulation of inhibitory neurotransmission in the brain.

Proteasome Regulation by Src Family Kinases in Diffuse Large B-cell Lymphoma.

M.L. Cann, M.A. Priestman, Q. Wang, and D.S. Lawrence. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA and Division of Chemical Biology and Medicinal Chemistry University of North Carolina School of Pharmacy Chapel Hill NC USA.*

Diffuse large B-cell lymphoma (DLBCL) patients can be categorized into two clinically relevant subtypes: activated B-cell (ABC) and germinal center B-cell (GCB). Since patients with ABC DLBCL tend to have a much worse prognosis than their GCB DLBCL counterparts there is great interest in identifying effective drug targets for the treatment of this subtype. Overexpression or hyperactivity of the Src family kinases and the proteasome has been implicated in a variety of different cancers and we are using novel peptide probes to explore the functional relationships between these proteins in DLBCL. We have developed biochemically- and photophysically-distinct green red and far-red real-time peptide sensors to monitor the proteasome s chymotrypsin-like trypsin-like and caspase-like activities respectively. Using these sensors we demonstrate that DLBCL cell lines have a very high chymotrypsin-like activity relative to healthy primary B-cells. Furthermore treatment of DLBCL cell lines with the Src family kinase inhibitor dasatinib results in a marked decrease in chymotrypsin-like proteasome activity for the ABC DLBCL cell lines but minimal change in the GCB DLBCL cell lines. Western blot analysis demonstrates a modest reduction in expression of the ?5 proteasome subunit which confers the Ch-L activity of the proteasome. Rather protein expression of the proteasome 19S regulatory particle decreases significantly with dasatinib treatment. We have also developed a fluorescent peptide sensor to monitor activity of Src family kinase members and we will use this sensor in subsequent studies to identify which Src family member is responsible for the decrease in proteasome activity in response to dasatinib.

SCF and APC E3 Ubiquitin ligases define an interacting negative feedback loop that control cell-cycle progression.

Rajarshi Choudhury and Michael J Emanuele, *Lineberger Comprehensive Cancer Center and Department of Pharmacology UNC School of Medicine Chapel Hill NC-27599, USA.*

Ubiquitination is a post-translational modification where ubiquitin is deposited to a substrate protein. Recent studies predict that a significant number of proteins in human proteome are post translationally modified by ubiquitin machinery. Proper cell-cycle transitions are driven by waves of ubiquitin-dependent degradation of key regulators by the anaphase-promoting complex (APC) and Skp1-Cullin1-F-box (SCF) E3 ubiquitin ligase complexes. But precisely how APC and SCF activities are coordinated to regulate cell-cycle progression remains largely unclear. We have demonstrated that APC/Cdh1 earmarks the SCF component Cyclin F for degradation and demonstrated that Cyclin F reciprocally controls APC/Cdh1 activity by governing Cdh1 degradation most probably by ubiquitination. Our work reveals a negative repression mechanism for SCF to control APC thereby illustrating an elegant dual repression system between these two E3 ligase complexes to create the ordered cascade of APC and SCF activities governing timely cell-cycle transitions.

Chronic Intermittent Ethanol Exposure (CIE) modulates BNST GABA and Glutamate Transmission and social behavior in a kappa opioid receptor (KOR) dependent manner.

Nicole A. Crowley[1,2], Nora M. McCall[2] , & Thomas L. Kash[2.] [1]Neurobiology Curriculum, *University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA [2]Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA.*

Kappa opioid receptors (KORs) have been shown to be involved in alcohol consumption and withdrawal both in humans and rodents. Recently a greater emphasis has been placed on the role of KORs in ethanol withdrawal as a potential therapeutic target. DBA/2J mice were exposed to chronic intermittent ethanol vapor for 16hrs/day for 5 days and then 24 hours following the final exposure mice underwent a social approach test as a proxy for ethanol-induced anxiety and social deficits. We found that alcohol exposed mice show decreased social preference for a novel mouse as compared to air-exposed controls. This behavior is normalized by administration of the KOR antagonist JDTic (10mg/kg). In order to understand the neurocircuitry underlying this KOR-mediated social behavior we conducted slice electrophysiology experiments in the bed nucleus of the stria terminalis (BNST) a region implicated in alcohol withdrawal induced anxiety. We found that CIE exposed mice have decreased KOR modulation of evoked excitatory post-synaptic currents (eEPSCs) as compared to air-exposed controls. In contrast CIE exposed mice have greater KOR modulation of evoked inhibitory post-synaptic currents (eIPSCs) as compared to air-exposed controls. In addition in CIE-exposed mice eIPSCs show a potentiation by the KOR antagonist norBNI indicating there may be tonic KOR activation at these synapses following ethanol exposure. Taken together this work supports KORs as a therapeutic target for alcohol addiction and related behaviors.

Engineered allosteric control of protein activity in living cells.

O. Dagliyan, P.C. Hsu, A. Karginov, D. Shirvanyants, S. Yagishita, H. Kasai, N.V. Dokholyan, K. M. Hahn. *Dept. Biochemistry and Biophysics, Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC USA; Center for Disease Biology and Integrative Medicine, The University of Tokyo, Bunko-ku, Tokyo, Japan.*

For many cellular behaviors, it is essential that signaling is precisely coordinated in space and time. Such spatiotemporal dynamics can only be fully understood using tools that examine and manipulate protein behavior in living cells. While it is very valuable to visualize signaling dynamics, testing hypotheses about spatio-temporal regulation requires that we actually control such activity by manipulating protein activity at precise times during cell behaviors. Previously, we developed an approach to control kinases, in which we inserted a ligand-controlled, engineered domain (uniRapR) into kinase loops that are allosterically coupled to the active site. The engineered domain rendered the kinases catalytically inactive. Addition of the small molecule rapamycin rescued catalytic activity by binding to the inserted uniRapR domain. This was not dimerization induced by rapamycin, but rather a rapamycin-induced change in protein dynamics. Here we describe development of another uniRapR kinase, PAK1 and extension of the method to a new protein family, guanine nucleotide exchange factors (GEFs). GEFs activate monomeric GTPases by exchanging GDP for GTP. We targeted Vav2, a GEF of Rac1, RhoA and Cdc42. The site identified for uniRapR insertion could also be used to control activity with light. We demonstrate this for Src kinase through insertion of a LOV domain for allosteric control of kinase activity. These switchable constructs were used to modulate the morphodynamics of living cells. The approaches were also applied to other Rho GEFs, validated biochemically. Switchable GEFs are allowing us to manipulate the kinetics of GEF-GTPase circuits in live, migrating cells.

Cellular Mechanisms of ?-Hemolysin-mediated Activation of the NLRP3 Inflammasome.

Ejiofor A.D. Ezekwe Jr, Joseph A. Duncan. *Department of Pharmacology and Department of Medicine Division of Infectious Diseases University of North Carolina at Chapel Hill Chapel Hill NC USA*.

Staphylococcus aureus toxin ?-hemolysin is an important and well-studied virulence factor in staphylococcal infection. It is a soluble monomeric protein that once secreted by the bacterium forms a heptameric pore in the membrane of a broad range of host cell types. Hemolysin was recently discovered to bind and activate a disintergin and metalloprotease 10 (ADAM10). In epithelial and endothelial cells ADAM10 activation is required for the toxin s activity against these cells. In host monocytic cells ?-hemolysin activates the nucleotide-binding domain and leucine-rich repeat containing gene family pyrin domain containing 3 (NLRP3) inflammasome leading to production of pro-inflammatory cytokines and cell death. We now show that ADAM10 is critical for ?-hemolysin-mediated activation of the NLRP3 inflammasome as siRNA knockdown or chemical blockade of ADAM10-?-hemolysin interaction leads to diminished inflammasome activation and cell death. Unlike epithelial cell and endothelial cell damage which requires ?-hemolysin induced ADAM10 activation ADAM10 protease activity was not required for NLRP3 inflammasome activation. This work confirms the importance of ADAM10 in immune activation by ?-hemolysin but indicates that host cell signal induction by the toxin is different between host cell types.

Patient-Derived Fibroblasts Support Dual Role of Stroma in Pancreatic Ductal Adenocarcinoma.

Elizabeth Flate[1], Richard A. Moffitt[1], S. Gabriela Herrera Loeza[1] and Jen Jen Yeh[1,2,3]
[1]Lineberger Comprehensive Cancer Center, [2]Department of Surgery, [3]Department of Pharmacology, University of North Carolina, Chapel Hill, USA.

Studies have increasingly shown the importance of the abundant desmoplastic stroma in pancreatic ductal adenocarcinoma (PDAC). While the tumor-associated stroma was long regarded as tumor-promoting, recent studies have suggested that the stroma can be protective. Such differential findings illustrate the complexity of tumor stroma and emphasize the need to understand the influence of the pancreatic tumor microenvironment. Methods: We used non-negative matrix factorization of gene expression data of 381 primary and metastatic samples to identify two novel stroma subtypes. Because the major cellular component of tumor stroma is a heterogenous population of cancer-associated fibroblasts (CAFs) we isolated primary CAFs and normal adjacent fibroblasts (NAFs) from tumors of ten PDAC patients. Immunofluorescence was used to verify cells as CAFs defined by the presence of SMAα and vimentin as well as the absence of EpCAM. We assessed the ability of CAF and NAF-conditioned media (CM) to affect the migration of a PDAC tumor cell line using transwell assays. Results: The two stroma subtypes are associated with patient outcomes. Patients with samples associated with activated stroma had worse survival (10.5mo) when compared to samples associated with normal stroma (26.2mo p=0.0097). Media conditioned by primary NAFs did not affect the migration of PDAC tumor cells as compared to control non-conditioned media. Media conditioned by CAFs isolated from six different samples differentially affected tumor cell migration. Three out of six CAF cell lines increased the migration of tumor cells and likely represent the activated stroma subtype. The other three CAF cell lines did not affect migration and may be representative of the normal subtype which is not tumor-promoting. Conclusion: We have identified two stroma subtypes which are associated with prognosis. Our results using CAF-CM indicate that not all CAFs isolated from PDAC patients are tumor-promoting suggesting that there is inherent CAF heterogeneity in PDAC patients.

Divergent roles of CAAX motif posttranslational modifications in Ral GTPase regulation.

L. R. Gentry, T. D. Martin, D. Tsygankov, C. J. Der. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

The Ras-like (RalA and RalB) small GTPases function as critical effectors of RAS oncogene-driven human cancer. Intriguingly although RalA and RalB share strong amino acid sequence identity (82%) and structural/biochemical properties they often have divergent roles in cancer and the hypervariable region at the C-terminus is thought to modulate these differences. Subcellular localization and function of many small GTPases is regulated in part by processing of the C-terminal CAAX motif (C = cysteine A = aliphatic amino acid X = terminal residue). However the role of posttranslational modifications of the CAAX motif in Ral isoform-selective functions has not been addressed. Here we find that CAAX-signaled modifications by Ras-converting endopeptidase (RCE1) and isoprenylcysteine carboxymethyltransferase (ICMT) have differential consequences on RalA and RalB subcellular localization activity and protein stability. We found that loss of RCE1 caused mislocalization as well as sustained activation of RalA and RalB. In contrast ICMT is required for RalB but not RalA localization at the plasma membrane and that this requirement is dictated by the hypervariable region. Instead RalA depended on ICMT for efficient endosomal localization. We further found that the absence of ICMT caused increased RalB but not RalA protein stability. Finally we determined that dual prenylation of the CAAX motif by palmitoylation and geranylgeranylation was critical for RalB but not RalA subcellular localization. In summary we have identified striking isoform-specific consequences of CAAX-signaled posttranslational modifications that contribute to the divergent regulation of RalA and RalB.

Hippo pathway activation in chemo-sensitive and -insensitive breast cancer.

Sara Hanna[1], Jerry Usary[2], Chuck Perou[2], and Gary Johnson[1]. *[1] Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA; [2] Genetics Department, School of Medicine, University of North Carolina, Chapel Hill, NC, USA.*

Triple negative breast cancers (TNBC) lack the receptors used in targeted therapy for other breast cancer subtypes, making chemotherapy the main option for treatment. However, only about 22% of TNBC patients respond to chemotherapy treatment. It is therefore important to understand the difference in tumor response between chemo-sensitive and chemo-insensitive TNBC. We have the opportunity of studying tumor samples from TNBC patients (WHIM Washington-Human-In-Mouse) who have achieved pCR (pathologic complete response) from chemotherapy, and also from those that were insensitive to chemotherapy treatment and had disease progression. These tumors are passaged in mice as patient derived xenografts (PDXs) and have been shown to faithfully mimic the patients' original clinical chemo-response. We have performed MIB/MS analysis comparing the response to Carboplatin and Paclitaxel treatment in either a chemo-sensitive (WHIM30) or chemo-insensitive (WHIM2) TNBC PDX. We see many differences in kinase response, including multiple survival and apoptotic regulators such as NFkB, JNK, and the Hippo pathways. The Hippo pathway is an evolutionarily conserved regulator of tissue growth and cell fate. It is regulated through a signaling cascade of core kinases (MST1, MST2, LATS1, and LATS2) resulting in the phosphorylation and deactivation of YAP1 (Yes-associated protein). While this pathway is commonly disregulated in cancers, and often associated with poor prognosis, few germline or somatic mutations have been discovered within the pathway, suggesting that the disregualtion could be a result of irregular kinase activity. MIB/MS analysis shows increased MST1/MST2 activity in chemo-sensitive PDXs in response to Carboplatin and Paclitaxel treatment. Immunoblotting also shows a subsequent increase in YAP1 phosphorylation and deactivation in the chemo-sensitive PDXs. Work in TNBC cell lines corroborates the data seen in PDXs. We plan to continue these studies in both TNBC PDXs and cell lines in order to determine the role of the Hippo pathway in chemo-sensitivity.

Size selection of cDNA library for mRNA-Seq using KAPA Stranded mRNA-Seq Kit.

K. Inoue and M. Calabrese. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

KAPA Stranded mRNA-Seq Kit is widely used for Illumina sequencing. cDNA libraries prepared by this product are applicable to a diverse range of RNA-seq applications such as single nucleotide variation discovery and splice junctions. We prepared cDNA libraries from 30 Coriell b-lymphoblastoid cell lines from people corresponding to a family tree (CEPH) in order to investigate the mechanism of X-chromosome inactivation through analysis of single nucleotide polymorphisms (SNP) of each library. For this purpose we attempted to prepare cDNA libraries with an average size of 500bp (450~550) because we need to see the SNP in both strands to confirm that mutation data is certain. However libraries prepared by following their manual generated bands ranging 250~550 bp on an agarose gel. In order to obtain a cDNA library suitable for our purposes we developed a size selection protocol for the KAPA Kit using a published protocol (Quail MA Swerdlow H Turner DJ. Curr Protoc Hum Genet. 2009 Jul Chapter 18:Unit 18.2.) with some modifications. We changed the ratio of Agencourt AMPure XP reagent and reaction mixture after 2nd Strand Synthesis from 1.8:1.0 to 0.6~0.8:1.0 and washed beads with 80% ethanol while breaking beads with pipetting. The libraries prepared with ratios of 1.8 0.8 and 0.6 AMPure XP reagent to reaction mixture demonstrated 250~550 450~600 and 500~750 bp bands on a gel respectively. We further narrow down the conditions to 0.75 0.7 and 0.65 and they yielded 483 506 and 613 bp on average on a tape station. Based on these results we suggest general conditions to modulate length of cDNA fragments for next-generation sequence analysis of allele-specific gene expression and alternative splicing.

Identification of sugar-responsive protein networks associated with regulator of G-protein signaling 1 in Arabidopsis.

D.K. Jaiswal1, M. Tunc-Ozdemir1, D. Urano1, E.G. Werth2, W.O. Slade2, L.M. Hicks2, and A.M. Jones1,3. *Department of Biology1 Chemistry2 and Pharmacology3 University of North Carolina Chapel Hill NC 27599 USA.*

Sugar-induced signal transduction pathways play significant roles in many physiological processes in plants such as photosynthetic efficiency and output cell wall sugar economy and pathogen defense. It is now well established that G-proteins mediate sugar signaling however the co-ordinated function of regulator of G-protein signaling (AtRGS1) a putative cell surface receptor for D-Glucose in context with other proteins interacting in different physical environments is still unclear. AtRGS1 consists of an N-terminal seven transmembrane domain a distinctive feature of G-protein-coupled receptors and a C-terminal RGS domain which negatively regulate receptor signaling. Sugar causes physical uncoupling of AtRGS1 and the self-activating G protein AtGPA1 by endocytosis of the AtRGS1 protein. To understand the molecular mechanism associated with activation and trafficking of AtRGS1 we used AtRGS1-TAP tag in conjunction with mass spectrometry and identified 478 putative interactors of AtRGS1. Among the identified proteins we found many previously known interactors as well several new and functionally relevant proteins. Gene Ontology analysis showed the high representation of pathways associated with sugar metabolism signal transduction transport and responsiveness to various stresses.

High Throughput Screening Identifies Small Molecules that Enhance the Pharmacological Effects of Oligonucleotides.

B.Yang[1#], X. Ming[1#], C. Cao[1#], B. Laing[1], A. Yuan[1], M. A. Porter[1], E.A. Hull-Ryde[1], J. Maddry, [2] M. Suto[2], W.P. Janzen[1*] and R.L. Juliano[1, *]. 1. *UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, 27599, USA; 2. Southern Research Institute, Birmingham, AL, 35205, USA.*

The therapeutic use of antisense siRNA and splice-switching oligonucleotides has been constrained by the limited ability of these membrane-impermeable molecules to reach their intracellular sites of action. We sought to address this problem using small organic molecules to enhance the effects of oligonucleotides by modulating their intracellular trafficking and release from endosomes. A high throughput screen of >150 000 compounds yielded several hits that markedly potentiated the actions of splice switching oligonucleotides in cell culture. These compounds also enhanced the effects of antisense and siRNA oligonucleotides. The hit compounds preferentially caused release of fluorescent oligonucleotides from late endosomes rather than other intracellular compartments. Studies in a transgenic mouse model indicated that these compounds could enhance the in vivo effects of a splice-switching oligonucleotide without causing significant toxicity. These observations suggest that selected small molecule enhancers may eventually be of value in oligonucleotide-based therapeutics.

Testing the Theoretical Limit of Gradient Sensing in Yeast.

V. V. Lakhani [1,2,3], T. C. Elston [1,2,3]. *[1] Molecular and Cellular Biophysics Program; [2] Bioinformatics and Computational Biology Curriculum; [3] Dept. Pharmacology, University of North Carolina, USA.*

During mating yeast cells must measure the direction of highest pheromone concentration and grow in that direction towards a mate a clear evolutionary advantage lies in rapid and accurate measurement of the pheromone gradient. Yeast use a spatial detection mechanism. In a gradient more receptors will be bound on one side (the front) than another (the back). In cases of shallow gradients (0.5 nM/um) centered at the Kd of the receptor this difference may be 100 +/- 50 bound receptors the error arises from stochastic binding and unbinding. Since the noise is on the same scale as the signal a non-trivial mechanism for measuring is needed: time-averaging. Fast binding and unbinding rates allow for frequent sampling and hence a rapid & accurate measure of the gradient. Contradictory to this expectation however the yeast pheromone receptor is suspected to have poor binding (1.6*10^5 1/M*s) and unbinding (10^-3 1/s) rates. With these rates resampling the environment just once takes over 15 minutes. We aim to demystify this paradoxical model of gradient sensing. We have created a computer program to simulate individual molecules to stochastically solve the reaction-diffusion equation (discretely in time and continuously in space). To simulate second-order reactions we independently customize the binding radius and the time step of the simulation. For spatial accuracy our binding radius is equal to the size of the receptor and the diffusion step size is on the same scale (by choosing a small time step). Experimentally measured reaction rates and diffusion constants are also used. Calculations of reactions and diffusion are computed on a GPGPU.

Identifying and characterizing protein cofactors involved in long noncoding RNA-mediated gene silencing.

D.M. Lee1 2, C. Chu3, H.Y. Chang3, and J.M. Calabrese1. *(1)Dept. Pharmacology and (2)Curriculum in Genetics and Molecular Biology University of North Carolina Chapel Hill NC USA. (3)School of Medicine Stanford University Stanford CA USA.*

Epigenetic control of gene expression is crucial in both development and normal cell function and a wide variety of health problems can be attributed to its dysregulation. Investigating the functional mechanisms of gene silencing is important in understanding both basic biological function and dysfunction which leads to disease. One mode of gene repression is mediated by long noncoding RNAs (lncRNAs) expressed transcripts longer than 200 nucleotides which do not code for proteins. A key example is the action of the Xist lncRNA which is responsible for silencing of the inactive X chromosome in female mammalian cells. The wide-scale silencing abilities of Xist make it a paradigmatic model for studying lncRNA-mediated gene silencing but the specific mechanisms by which silencing is achieved remain unclear. In addition Xist action provides the opportunity to learn more about RNA-protein interactions. Starting from a list of around 100 proteins shown to physically interact with Xist in the cell we are working to identify and characterize Xist cofactors which contribute to its silencing ability. We are using an RNAi knockdown approach to screen through the list looking for reduction in silencing ability upon knockdown. We hypothesize that Xist functions as a recruitment scaffold to bring together effector proteins increasing local concentration to allow aggregation and formation of a silencing complex. We have shown that the Xist associated proteins display a highly significant enrichment of long low-complexity sequences relative to the entire mouse proteome and we believe that these facilitate polymerization. We are employing computational and experimental methods to understand how these low complexity sequences contribute to RNA-mediated gene silencing and RNA-protein interactions in general.

Gene expression and cis-eQTL analyses of peripheral blood cells from elderly patients with obstructive coronary artery disease.

(Kaitlin Lenhart presenting)

Robert N. Schuck1, Eleanor Hilliard2, Craig R. Lee1, Xuming Dai2 3, George A. Stouffer2 3, Cam Patterson4, and Jonathan C. Schisler2 5. *1Division of Pharmacotherapy and Experimental Therapeutics Eshelman School of Pharmacy 2McAllister Heart Institute and 3Division of Cardiology at The University of North Carolina at Chapel Hill Chapel Hill NC 27599 USA 4Presbyterian Hospital/Weill-Cornell Medical Center New York NY 10065 USA 5Department of Pharmacology The University of North Carolina at Chapel Hill Chapel Hill NC 27599 USA.*

Obstructive coronary artery disease (CAD) risk increases with age moreover elderly patients particularly those with multiple CAD-associated risk factors will often exhibit poorer prognoses following acute coronary events in comparison to the general population. Although it has been well-established that inflammation plays a key role in CAD pathogenesis studies in elderly patients have not consistently shown elevated inflammatory biomarkers in association with poorer outcomes indicating the possibility of distinct pathophysiological mechanisms underlying CAD in the aging population. Indeed we found no correlation between CAD severity and expression of the circulating inflammatory biomarkers MCP-1 hs-CRP and CAMs (E-selectin P-selectin ICAM-1 and VCAM-1) from elderly (>65 years of age) patients enrolled in the Supporting a Multi-disciplinary Approach to Researching Atherosclerosis (SAMARA) study. Nevertheless global gene expression analysis of peripheral blood mononuclear cells (PBMC) from these patients indicated a significant enrichment of immune system development and inflammatory genes which positively correlated with CAD severity. Additionally an evaluation of cis-expression quantitative trait loci (eQTLs) identified 66 SNPs associated with altered expression of 30 CAD-linked genes throughout the genome. This study provides novel insight into new molecular phenotypes that may play a role in the development and progression of CAD in the elderly. Moreover we demonstrate the importance of treating elderly patients as a discrete population who are frequently under-represented in clinical trials. Further work is required to tease apart key biological processes underlying CAD in this high-risk population which may facilitate the development of novel therapeutic strategies to improve prognosis.

microRNA regulation of HK2 and its pseudogene in promoting pancreatic cancer glycolysis.

M.B. Lipner, M.B. Nedrud, and J.J. Yeh. *Dept. Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA*

Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer death in the United States. Resistance to chemotherapeutics and failure of proposed targeted therapies contribute to a dismal 5% five-year survival. Metabolic pathways like anaerobic glycolysis critical to PDAC initiation and progression are emerging as therapeutic targets. The glycolytic enzyme hexokinase 2 (HK2) is preferentially upregulated in PDAC and HK2 expression is independently associated with patient survival. However, there are no specific HK2 inhibitors and HK2 dysregulation is incompletely understood. Downregulation of microRNAs (miRs), a class of endogenous gene repressors, has been shown to contribute to cancer progression and may represent a novel therapeutic modality. miR-143 targets HK2 and is downregulated by critical PDAC oncogenes, indicating that dysregulation of the HK2-miR network is an important component of PDAC oncogenesis. HK2 shares almost all predicted miR binding sites with HK2P1, an expressed pseudogene that is overexpressed in PDAC cell lines. HK2 and HK2P1 may be coregulated by competing for a pool of shared miRs, as has been hypothesized by the competing endogenous RNA theory. This project will evaluate novel miR repressors of HK2 and HK2P1 and assess the ability of HK2P1 to potentiate the effects of HK2 by competing for shared miRs. miR prediction software and PDAC expression data was used to identify four candidate miR repressors of HK2 and/or HK2P1. Transient and stable miR modulations followed by a dual luciferase reporter assay, qPCR, and immunoblotting will assess miR-mediated HK2 and HK2P1 repression in PDAC cell lines. To assess HK2P1 as an HK2 ceRNA, similar assays will be performed following siRNA-mediated HK2P1 knockdown and lentiviral-induced HK2P1 3'UTR overexpression. Finally, tumor suppressing functions of candidate miRs will be assessed with in vitro growth and invasion assays and mouse xenografts of PDAC cell lines with inducible miR expression.

Gaba Cells of the Dorsal Raphe Projecting to the Central Nucleus of the Amgydala Modulate Behavioral Responses to a Fear-associated Context.

E. G. Lowery-Gionta & T. L. Kash. *University of North Carolina at Chapel Hill Bowles Center for Alcohol Studies Chapel Hill NC 27599, USA.*

The dorsal raphe is a midbrain structure that has been implicated in anxiety and stress responses. This structure is comprised mainly of serotonin cells, which innervate the forebrain, and GABA cells, which are thought to function primarily as interneurons. Here, we investigated the role of dorsal raphe GABA neurons in anxiety and fear using Vgat-Cre mice and intra-dorsal raphe expression of DREADDs. Using GFP-tagged channelrhodopsin (ChR2) expression in Vgat-Cre mice to map the projections of dorsal raphe GABA neurons, we identified a novel GABAergic pathway from the dorsal raphe to the central nucleus of the amygdala (CeA). We then examined the contribution of this specific pathway to anxiety- and fear-related behaviors by site-directly expressing the inhibitory opsin archaerhodopsin (Arch) or the excitatory opsin ChR2 in the dorsal raphe and implanting optical fibers over the CeA. We found that inhibition of dorsal raphe GABA cells by the Gi-coupled hm4di DREADD enhanced anxiety-like behavior in the elevated plus maze (EPM), light/dark box, and open field chamber. Optical inhibition of the GABAergic projection from the dorsal raphe to the CeA by Arch enhanced anxiety-like behavior in the open field chamber but did not alter behavior in other assays. Optical inhibition of this pathway during Pavlovian fear learning reduced freezing only in the footshock-paired context. Conversely, activation of this pathway by ChR2 reduced anxiety-like behavior in the elevated plus maze without effects in other anxiety assays. Unexpectedly, activation of this pathway also altered freezing only in the footshock-paired context. Together, these results reveal a novel GABAergic pathway from the dorsal raphe to the CeA that is involved in anxiety- and fear-related behaviors. Ongoing experiments are characterizing this pathway using slice electrophysiology. (NIH-NIAAA R01-AA019454 to TLK, NIA-NIAAA F32-AA022549 to EGLG).

Manipulation of vGlut3-expressing cells of the bed nucleus of the stria terminalis reduces contextual fear learning.

Christopher Mazzone, Calvin Snyder, Thomas Kash. *Curriculum in Neurobiology University of North Carolina at Chapel Hill. Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

Anxiety and fear-related disorders are debilitating conditions that affect nearly 20% of adults in the United States. Identifying neural substrates capable of altering these affective states is critical for the development of future pharmacological therapies. The bed nucleus of the stria terminalis (BNST) is a component of the extended amygdala that can directly alter anxiety states and is directly impacted by stress. Of particular interest the BNST is composed of heterogeneous cell populations with respect to neurotransmitter content. However the BNST also contains a recently discovered population of neurons expressing vesicular glutamate transporter 3 (vGlut3) whose transmitter content and functional connectivity is unknown. To selectively target and characterize BNST vGlut3 neurons we used a vGlut3-ires-cre mouse and locally infused cre-inducible optogenetic and pharmacogenetic viral constructs to assess both functional anatomical circuitry and behavioral responses to BNST vGlut3 neuron stimulation and inhibition. Using whole cell patch clamp electrophysiology we show that BNST vGlut3-expressing neurons form local GABAergic synapses thus demonstrating a novel source of local inhibition within the BNST. Further using a combination of in vivo pharmacogenetic and optogenetic approaches we find that manipulation of BNST vGlut3 neurons impairs contextual fear learning without altering cued fear. In contrast acute modulation of these neurons did not impact anxiety-like behavior. Together these findings provide a novel population of GABAergic cells within the extended amygdala selectively involved in the development of learned contextual fear thus providing a new target for anxiety-related disorders such as post-traumatic stress disorder.

Ranking proposed yeast feedback networks through Approximate Bayesian Computation model estimation and selection.

Patrick McCarter[1,3], Justin English[2], Henrik Dohlman[1,2,3], Tim Elston[1,2]. *Curriculum in Bioinformatics and Computational Biology*[1], *Department of Pharmacology*[2], *Department of* Biochemistry and Biophysics[3], *University of North Carolina School of Medicine, Chapel Hill, NC, USA*.

In humans Mitogen-Activated Protein Kinases (MAPK) protect cells against ischemia hyper-osmolarity uv-irradiation and other stressors. Dysregulated MAPK pathways are also found in Alzheimer's Disease Amyotrophic Lateral Sclerosis and cancer. In this work we detail a multi-disciplinary research plan that combines mathematical modeling biochemical and genetic experimentation to better understand how MAPKs coordinate cellular responses to environmental stress. Using an innovative biochemical technique we have recently accumulated experimental evidence that strongly suggests that MAPK activation in the yeast High-Osmolarity Glycerol (HOG) Pathway is encoded via positive feedback and that MAPK deactivation is encoded via negative feedback. The MAPK in the HOG pathway Hog1 is homologous to the mammalian p38 and JNK MAPKs. The HOG pathway transmits hyper-osmotic stress to the nucleus of a cell through the Hog1 MAPK, which is activated by two distinct signaling branches (Sho1, Sln1). We use mathematical modeling to identify the most likely HOG pathway feedback network that dynamically regulates the MAPK in response to sustained hyper-osmotic stress. We demonstrate that our modeling approach has thus far been successful by identifying models that agree with our preliminary experimental data. We now extend our modeling process to objectively rank the proposed and fitted models using Approximate Bayesian Computation (ABC) method to perform model selection. Many signaling mechanisms initially discovered in yeast have proven to be conserved in human cells. This work is significant because it will establish signaling mechanisms that allow multiple pathways to contribute to stress adaptation that are likely conserved across human cells.

University of North Carolina
At Chapel Hill
Department of Pharmacology
Research Retreat

POSTER SESSION 2
(22 - 42)

THE WILLIAM AND IDA FRIDAY CENTER FOR CONTINUING EDUCATION

Department of Pharmacology Research Retreat

POSTER SESSION 2 (22 - 43)

22. **Elucidating the Role of Cellular Kinases and Direct Phosphorylation in Regulation the NLRP3 Inflammasome.** J. H. Melehani, T. J. Stuhlmiller, G. L. Johnson, J. A. Duncan. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

23. **Epigenetic Suppression of Kinome Adaptation to Trametinib by Inhibition of BET Bromodomains.** S. Miller, J. Zawistowski, G. L. Johnson. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

24. **Elucidating the Role of NUSAP1 in Mitosis.** C. A. Mills and M. J. Emanuele. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

25. **Virtual Microdissection of Pancreatic Ductal Adenocarcinoma Reveals Tumor Subtypes.** R. A. Moffitt, R. Marayati, K. E. Volmar, K. A. Hoadley, J. M. Anderson, D. J. Bentrem, M. S. Talamonti, C. A. Iacobuzio-Donahue, M. A. Hollingsworth, and J. J. Yeh. *Department of Pharmacology, Lineberger Comprehensive Cancer Center, Dept. of Genetics, and Dept. of Surgery, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; Eppley Cancer Institute, University of Nebraska, Omaha, NE USA; Dept. of Surgery and Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL USA; Dept. of Surgery, North Shore University Health System, Chicago, IL USA; Dept. of Pathology, Johns Hopkins University, Baltimore, MD USA.*

26. **Investigating Regulation of BIRC6 in Drug Resistant Leukemia.** D. O. Okumu, M. Levine, L. Jones, R. Zhang, and L. M. Graves. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

27. **Modeling Cerebral Cavernous Malformations with Patient-Specific Induced Pluripotent Stem Cells.** J. F. Olivares, A. S. Beltrán, and G. L. Johnson. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA and NIDDKD NIH, Bethesda, MD USA.*

28. **Regulation of Adult Neurogenesis by the Hippocampal Cholecystokinin Network.** Olsen, R. H., Song, J. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

29. **Effects of Chronic Alcohol Consumption on Neuronal Function in the Primate Extended Amygdala.** K. E. Pleil, C. M. Helms, J. Sobus, J. B. Daunais, K. A. Grant, and T. L. Kash. *Department of Pharmacology and Bowles Center for Alcohol Studies, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; Division of Neuroscience Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR USA; Human Exposure and Atmospheric Sciences Division, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC USA; Dept. of Physiology & Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC USA.*

30. **The Unfolding Tail of CHIP Mutation in Gordon Holmes Syndrome.** C. Rubel, S. Soss, H. McDonough, W. Chazin, C. Patterson, J. Schisler. *McAllister Heart Institute and Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA and Center for Structural Biology, Vanderbilt University Nashville, TN USA.*

31. **Mechanisms of ERK-driven Expression and Function of the RacGEF PREX1 in Melanoma.** Meagan B. Ryan, Katherine H. Pedone, Alexander J. Finn, Nancy E. Thomas, Channing J. Der, Adrienne D. Cox. *Departments of Pharmacology, Dermatology and Radiation Oncology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

32. **Assessing Allele Specific Epigenetic Patterns in Long Non-coding RNA-Mediated Imprinted Regions.** Megan Schertzer and Mauro Calabrese. *Department of Pharmacology, Curriculum in Genetics and Molecular Biology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

33. **Second Messengers of Osmotic Stress that Regulate G Protein Signaling.** J. P. Shellhammer and H. G. Dohlman. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

34. **Differential Roles of Medial Prefrontal Cortical Projections to the Basolateral Amygdala and Nucleus Accumbens in a Model of Fear Extinction.** J. A. Sugam and T. L. Kash. *Department of Pharmacology, Bowles Center for Alcohol Studies, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

35. **Identifying Kinases that Regulate the Transcription Factor NRF2.** T. Y. Tamir[1], D. Goldfarb[2], M. P. Walker[3,4], and M. B. Major[1,2,3,4]. *[1]Department of Pharmacology, [2]Department of Computer Science, [3]Department of Cell Biology and Physiology, [4]Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

36. **Transcriptome-wide Identification and Study of Cancer-Specific Splicing Events Across Multiple Tumors.** Yihsuan S. Tsai[1], Daniel Dominquez[2], Shawn M. Gomez[1,2,3,4], Zefeng Wang[1,2]. *[1]Curriculum in Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill, NC 27599; [2]Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599; [3]Computer Science, University of North Carolina, Chapel Hill, NC 27599; [4]Joint Department of Biomedical Engineering at UNC-Chapel Hill and NC State University, Chapel Hill, NC 27599, USA.*

37. **Receptor Kinase Activation of Heterotrimeric G. Protein Signaling in MAMP-Induced Cell Death.** M. Tunc-Ozdemir[1], D. Urano[1], D. Jaiswal[1], S. D. Clouse[3], A. M. Jones[1,2]. *Departments of [1]Biology and [2]Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599; [3]Department of Horticultural Sciences, North Carolina State University, Raleigh, NC 27695, USA.*

38. **A Polar Expedition: Investigating Regulation of Polarity in the Pheromone Response Pathway.** L. Vered, M. Peña, B. Errede, T. Elston. *Departments of Chemistry, Pharmacology and Biochemistry & Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

39. **Exploring Mutations in the E3 Ubiquitin Ligase CHIP for Impacts on Activity and Protein Interactions.** C. Virus[1], C. Rubel[1], L. Carrier[2], J. Robbins[3], R. Page[4], D. Cyr[5], and J. C. Schisler[1,6]. *[1]McAllister Heart Institute, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; [2]Department of Experimental Pharmacology and Toxicology Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf, Hamburg Germany; [3]Cincinnati Children's Hospital Medical Center, Cincinnati, OH USA; [4]Dept. of Chemistry and Biochemistry, Miami University, Oxford, OH USA; [5]Dept. of Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; and [6]Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 29599 USA.*

40. **Binge Alcohol-Induced Microglial Priming.** T. Jordan Walter, Ryan Vetreno, Liya Qin, Fulton Crews. *Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.*

41. **Resonator Motifs in Mechano-Chemical Signaling Pathways Revealed by a New Optogenetic Method for Precise Oscillation of Signaling Circuits.** H. Wang[1], M. Vilela[2], R. Liu[3], G. Danuser[2], K. M. Hahn[1]. *[1]University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; [2]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA; [3]UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA.*

42. **Identification of Epigenetic Inhibitors of MEK Inhibitor-Induced Kinome Adaptation in Triple Negative Breast Cancer.** J. S. Zawistowski[1], B. T. Golitz[1], J. Jin[2], and G. L. Johnson[1]. *[1]Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA; [2]Depatment of Structural and Chemical Biology Icahn School of Medicine at Mt. Sinai, New York, NY USA.*

43. **Does the beta-lactam-insensitive L,D-transpeptidase NGO1484 play a role in resistance to beta-lactam antibiotics in Neisseria gonorrhoeae?** K. Daniels, R. A. Nicholas. *Department of Chemical Biology and Medicinal Chemistry, University of North Carolina Eshelman School of Pharmacy, and Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27599 USA.*

Elucidating the role of cellular kinases and direct phosphorylation in regulating the NLRP3 inflammasome.

J.H. Melehani, T.J. Stuhlmiller, G.L. Johnson, J.A. Duncan. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

The NLRP3 inflammasome pathway is a pro-inflammatory pathway important to a growing list of infectious and non-infectious diseases. The NLRP3 inflammasome is an innate immune molecular sensor made up of three proteins: NLRP3 and ASC an adaptor protein recruit Caspase 1 to form a polymeric structure in response to massive potassium efflux. The NLRP3 inflammasome drives two separate inflammatory processes: 1) activation of Caspase 1 and subsequent processing and release of the inflammatory cytokines IL-1? and IL-18 and 2) a Caspase 1-independent programmed necrotic cell death termed pyroptosis and release of endogenous pro-inflammatory factors. Activation of the NLRP3 inflammasome is rapid and is thought to be entirely regulated by post-translational mechanisms. In this study I will use a potent inflammasome-activating S. aureus pore-forming leukotoxin LukAB to investigate the role of cellular kinases and direct phosphorylation in the post-translational regulation of the NLRP3 inflammasome pathway. Kinases act as rapid on-off switches in the cell by phosphorylating target proteins. Electrostatic interactions are critical in inflammasome formation and NLRP3 ASC and Caspase 1 all have multiple predicted surface-exposed phosphorylation sites suggesting phosphorylation may be an effective means of regulating these protein-protein interactions. Expression of phospho-mimetic and non-phosphorylatable inflammasome components in THP1 monocytic cells will help tease out the role of these modifications. To identify critical kinases I have used multiplex-inhibitor bead mass spectrometry (MIB-MS) to evaluate changes in total kinase activity from baseline compared to a time course of LukAB intoxication. Two biological replicates of this analysis identified 180 kinases in common 28 of which were up- or down-regulated by more than 20% at any time point. I aim to validate select changes by immunoblot and characterize the role of these kinases in the NLRP3 inflammasome pathway. Together these studies will significantly advance our understanding of NLRP3 inflammasome regulation.

Epigenetic Suppression of Kinome Adaptation to Trametinib by Inhibition of BET Bromodomains.

S. Miller, J. Zawistowski, G. Johnson. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

Triple Negative Breast Cancer (TNBC) is an aggressive subtype of breast cancer with no FDA-approved targeted treatments. In preclinical models of TNBC tumor cells are responsive to the MEK inhibitor trametinib however resistance rapidly develops. Disruption of negative feedback loops allows tumor cells to bypass targeted inhibition of MEK through increased expression and activation of receptor tyrosine kinases leading to reactivation of ERK. These adaptive kinases are heterogeneous across patient samples and are dynamically activated allowing bypass of strategic combinations of kinase inhibitors. We proposed to overcome this global resiliency of the kinome by blocking the transcriptional response which upregulates adaptive kinase expression by using specific epigenetic inhibitors. Our work demonstrates that BET bromodomain inhibitors (JQ1 and iBET151) which target epigenetic histone readers synergize with trametinib to block TNBC growth in short term (4 days) and long term (4 weeks) growth assays. Combination BET bromodomain inhibitor treatment also blocks upregulation of adaptive kinases at the mRNA and protein levels. MIB/MS analysis shows decreased binding of adaptive kinases to inhibitor beads during combination treatment indicating a loss of adaptive kinase activity. Furthermore RNAseq analysis demonstrates that BET bromodomain inhibitors synergize with trametinib to preferentially block expression of adaptive response genes. I recently established a technique in our lab Translating Ribosome Affinity Purification (riboTRAP) which captures mRNA transcripts actively bound to ribosomes. Preliminary studies show that riboTRAP will provide a high-resolution view into dynamic regulation of mRNA transcripts with trametinib and BET bromodomain inhibitor treatment in cell line studies. RiboTRAP is also being optimized to enrich for mRNA transcripts specifically expressed in tumor cells of orthotopic xenograft models of TNBC. Ongoing studies will expand our understanding of TNBC bypass mechanisms to MEK inhibition and will test the ability of BET bromodomain inhibitors to block adaptive resistance leading to durable treatment of TNBC.

Elucidating the role of NUSAP1 in mitosis.

C. A. Mills and M. J. Emanuele. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

Improper chromosomal segregation is common in many cancer types and has even been shown contribute to its development however it is unclear how proper chromosome segregation and spindle stability is controlled. In an effort to better understand the mechanisms that regulate the processes our lab is seeking to identify the functions of Nucleolar and Spindle Associated Protein 1 (NUSAP1). NUSAP1 is a microtubule binding protein important for proper spindle assembly and chromosome segregation that has been shown to have increased expression in proliferating cell populations including tumors. NUSAP1 has been shown to play a role in stabilizing mitotic spindles but the specific function of NUSAP1 is unknown as are NUSAP1 interacting proteins. We utilized a multi-faceted approach including cell biology and biochemistry to better clarify the functions of NUSAP1 during mitosis. We began by identifying interaction partners with NUSAP1 during mitosis using an endogenous IP-MS approach. We have identified and confirmed Ran Binding Protein 2 (RBP2) as well as other members of the nuclear pore complex such as Ran GTPase Activating Protein 1 (RanGAP1) and Ubiquitin Carrier Protein 9 (Ubc9) as NUSAP1 interactors. During mitosis the RBP2/RanGAP1/Ubc9 (RRU) complex functions as a SUMO E3 ligase that attaches a small ubiquitin-like modifier or SUMO to target proteins. The SUMO post-translational modification has been shown to regulate many protein characteristics including localization function and even degradation. Here we hypothesize that NUSAP1 is could either be functioning to: 1.) localize the RRU to microtubules or 2.) regulate the activity of this SUMO E3 ligase during in turn regulating its targets such as Topoisomerase II and Borealin.

Virtual Microdissection of Pancreatic Ductal Adenocarcinoma Reveals Tumor Subtypes.

R. A. Moffitt, R. Marayati, K. E. Volmar, K. A. Hoadley, J. M. Anderson, D. J. Bentrem, M. S. Talamonti, C. A. Iacobuzio-Donahue, M. A. Hollingsworth and J. Yeh. *Dept. of Pharmacology, Lineberger Comprehensive Cancer Center, Dept. of Genetics, and Dept. of Surgery, University of North Carolina School of Medicine, Chapel Hill, NC, USA; Eppley Cancer Institute, University of Nebraska, Omaha, NE, USA; Dept. of Surgery and Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; Dept. of Surgery, NorthShore University HealthSystem, Chicago, IL, USA; Dept. of Pathology, Johns Hopkins University, Baltimore, MD, USA.*

Pancreatic Ductal Adenocarcinoma (PDAC) is a highly fatal disease, lacking effective targeted therapies, clinically useful biomarkers, or consensus subtypes. Understanding molecular mechanisms of disease in PDAC has the potential to yield rationally designed therapies. However, previous work using gene expression arrays have been hampered by the low cellularity of malignant epithelium in PDAC patient samples. We sought to avoid this pitfall by applying blind source separation to the analysis of a large set of PDAC gene expression microarray data, consisting of primary tumors (n=149), metastatic tumors (n=64), cell lines (n=17), normal pancreas (n=47), and distant site adjacent normal samples (n=89). We identified two novel tumor-specific subtypes, (“classical” and “basal-like”), as well as specific expression from normal, immune, and stromal compartments. By using our tumor-, normal- and stroma-specific gene expression signatures, we were able to estimate relative compositions of our samples that agreed with histopathology estimates of tumor cellularity ($p<0.001$). “Classical” and “basal-like” subtypes had differential prognostic value ($p=0.006$, hazard ratio of 1.93) that validated in recently published International Cancer Genome Consortium array data. Furthermore, our “basal-like” subtype was consistent with basal subtypes in both breast and bladder cancer data. We found that normal gene expression from liver, lung, lymph, or muscle tissue describe superficial differences between our metastatic samples, but that tumor-specific subtype expression is conserved within samples from the same patient, suggesting cell autonomous similarity among tumor sites within a patient. By applying a computational approach to a large cohort of data, we have overcome the low cellularity problem and generated new insights into the complex molecular composition of PDAC. These results and their prognostic value may provide decision support in a clinical setting where the choice and timing of therapies is critical.

Investigating regulation of BIRC6 in drug resistant leukemia.

D. O. Okumu, M. Levine, L. Jones, R. Zhang, and L. M. Graves. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

Baculoviral IAP repeat containing 6 (BIRC6) is a member of the inhibitors of apoptosis proteins (IAPs) a family of functionally and structurally related proteins known to inhibit apoptosis. BIRC6 has been implicated in drug resistance in several different human cancers but very little is known about the mechanisms of its regulation. In this study we investigated the role of BIRC6 in drug resistance and the mechanisms of BIRC6 regulation in human leukemia cell lines. BIRC6 mRNA and protein were highly expressed in several human leukemia cell lines including MYL K562 and RAJI cells. BIRC6 was substantially enriched in a drug resistant derivative of the MYL cell line (MYL-R) suggesting that BIRC6 contributes to drug resistance in leukemia. Knockdown of BIRC6 increased sensitivity to Imatinib suggesting that BIRC6 expression is a major contributor to drug resistance in MYL-R cells. Thus we next sought to elucidate the mechanisms regulating cellular levels of BIRC6. MYL-R cells are characterized by increased expression and activity of Lyn kinase. Inhibition of Lyn with Dasatinib significantly reduced BIRC6 mRNA and protein levels. Knockdown or pharmacological inhibition of a component of the P-TEFb complex CDK9 also markedly reduced BIRC6 mRNA levels. Thus BIRC6 expression is transcriptionally regulated. Proteomics analysis of MYL-R extracts revealed elevated levels of a BIRC6-unique hyper-phosphorylated peptide in MYL-R cells. The peptide was hyper-phosphorylated on four serine residues proximal to acidic residues consistent with the consensus sequence for CK2 kinase. Inhibition of CK2 using the small molecule inhibitor CX-4945 significantly reduced BIRC6 protein levels in Myl-R cells. Thus phosphorylation by CK2 may play an important role in the post-translational regulation of the protein. Accordingly our data suggest that BIRC6 plays an important role in mediating drug resistance and may be a novel and promising target in drug-resistant leukemia and other cancers.

Modeling Cerebral Cavernous Malformations with patient-specific induced pluripotent stem cells.

J.F. Olivares, A.S. Beltrán, and G.L. Johnson. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA and NIDDKD NIH Bethesda MD USA.*

Cerebral cavernous malformation (CCM) is associated with a wide range of neurovascular disorders for which complicated and risky surgery is the only treatment option. CCM stems from the bi-allelic loss of ccm1 -2 or -3 in brain endothelial cells (ECs) but the subsequent mechanism leading to lesion development is unclear. We have demonstrated the CCM phenotype being caused by an increase in cytoskeletal stability stemming from dysregulation of the small GTPase RhoA. Loss of CCM1 CCM2 and CCM3 in ECs increases the activity of RhoA and ROC kinase (ROCK) resulting in functional defects including in vitro loss of EC tube formation invasion of extracellular matrix and increased barrier permeability. Pharmacological inhibition of ROCK partially rescues the tube formation defect and decreases lesion burden in animal models. The aggressiveness of the CCM phenotype and the partial effect of ROCK implies that CCM proteins supports additional important EC functions which have hitherto been undiscovered in existing models of CCM deficiency. To address this we have developed a human CCM cell model based on induced pluripotent stem cells (iPSCs) derived from patients with CCM1 CCM2 and CCM3 mutations. We applied the CRISPR/cas9 system to create CCM null cells and CCM-YPET fusion proteins in iPSCs. We used these cells to study signaling network control by CCM proteins that regulate the formation and stability of vessel-like structures. The molecular characterization of this process will lead to new mechanistic insight and dissection of novel targets for CCM for which no cure is available.

Regulation of adult neurogenesis by the hippocampal cholecystokinin network.

Olsen, R.H., J. Song, Juan. *Dept. Pharmacology, Chapel Hill, NC, USA.*

Adult neurogenesis is a unique and poorly understood form of neuroplasticity that in humans is essentially restricted to the dentate gyrus (DG) of the hippocampus. Unlike developmental neurogenesis, this enigmatic process is tightly regulated by the activity of local neuronal circuits and afferent projecting systems. The identity of specific cell types, neurotransmitters, and receptors that facilitate this regulation remains critically understudied. Studies utilizing animal and cell culture models suggest that the neuropeptide cholecystokinin (CCK) serves as a and survival signals for neural stem cells in the adult brain We therefore will test the hypothesis that CCK may regulate adult neurogenesis by promoting neural stem cell proliferation and asymmetric neuronal fate specification, neuronal progenitor survival, and integration of immature neurons into the surrounding circuitry. We have found that neural stem cells express mRNA for the G_q-coupled CCK_2 receptor, and exhibit Ca^{2+} transients following stimulation with the CCK_2-receptor selective form of CCK (CCK8) which can be blocked by the CCK_2R antagonist YM022. Intravenous administration of CCK8 produces an increase in proliferating cells in the DG, and we have found that *in vivo* chemogenetic stimulation of CCK-releasing neurons in the DG produces an increase in stem cell proliferation. Because CCK-cells are a heterogeneous population and can release other signaling molecules, moving forward we will identify whether this effect can be blocked by knocking down CCK synthesis and what subset of CCK-cells may mediate this effect. To further clarify the role of the CCK-network in activity-dependent adult neurogenesis, we will utilize retrograde transynaptic tracing to identify interacting local and distant and neuronal populations.

Effects of chronic alcohol consumption on neuronal function in the primate extended amygdala.

K.E. Pleil, C.M. Helms, J. Sobus, J.B. Daunais, K.A. Grant, and T.L. Kash. *Dept. Pharmacology and Bowles Center for Alcohol Studies UNC School of Medicine Chapel Hill NC USA Div. Neuroscience Oregon National Primate Research Center Oregon Health & Science University Beaverton OR USA Human Exposure and Atmospheric Sciences Division National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park NC USA Dept. Physiology & Pharmacology Wake Forest School of Medicine Winston-Salem NC, USA.*

Alterations in hypothalamic-pituitary-adrenal (HPA) axis function contribute to many of the adverse behavioral effects of chronic voluntary alcohol drinking including alcohol dependence and mood disorders limbic brain structures such as the bed nucleus of the stria terminalis (BNST) may be key sites for these effects. Here we measured circulating levels of several steroid hormones and performed whole-cell electrophysiological recordings from acutely-prepared BNST slices of male rhesus monkeys allowed to self-administer alcohol for 12 months or a control solution. Initial comparisons revealed that BNST neurons in alcohol-drinking monkeys had decreased membrane resistance increased frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) with no change in spontaneous excitatory postsynaptic currents (sEPSCs). We then used a combined variable cluster analysis and linear mixed model statistical approach to determine whether specific factors including stress and sex hormones age and measures of alcohol consumption and intoxication are related to these BNST measures. Modeling results showed that specific measures of alcohol consumption and stress-related hormone levels predicted differences in membrane conductance in BNST neurons. Distinct groups of adrenal stress hormones were associated with the frequency of sIPSCs and sEPSCs and alcohol drinking measures and basal neuronal membrane properties were additional predictors of inhibitory but not excitatory PSCs. The amplitude of sEPSCs was highly correlated with age independent of other variables. Together these results suggest that chronic voluntary alcohol consumption strongly influences limbic function in non-human primates potentially via interactions with or modulation by other physiological variables including stress steroid hormones and age.

The Unfolding Tail of CHIP Mutation in Gordon Holmes Syndrome.

C. Rubel, S. Soss, H. McDonough, W. Chazin, C. Patterson, J. Schisler. *McAllister Heart Institute and Dept. of Pharmacology, The University of North Carolina Chapel Hill School of Medicine, Chapel Hill NC USA, and Center for Structural Biology, Vanderbilt University Nashville TN, USA.*

Gordon Holmes Syndrome (GHS) is a rare neurodegenerative disorder characterized by cerebellar ataxia with hypogonadism. The pathophysiological mechanisms and genetic causes of GHS are largely unknown and therapies limited. We identified a homozygous mutation (c.737C?T p.Thr246Met) in STIP1 homology and U-box containing protein 1 (STUB1) the gene that encodes for C-terminus of Hsp70 Interacting Protein (CHIP) in siblings that presented with GHS. CHIP plays a central role in regulating protein quality control as an E3 ligase and molecular chaperone. Human mutations of CHIP are increasingly linked to ataxias but the molecular mechanisms underlying this disease pathology remain undefined. Biophysical and cell culture studies reveal T246M CHIP has a disorganized Ubox domain and exists mostly as aggregates. Consequently T246M CHIP exhibits loss of E3 ligase activity but unexpectedly the amount of T246M CHIP interacting with substrates E2 enzymes and molecular chaperones is higher than wild-type CHIP. We hypothesize that the sequestering of CHIP interacting proteins drives disease pathology. Interestingly T246M CHIP maintains some chaperone activity as measured by its potentiation of AMPK activity in vitro and translocation and activation of HSF1 in cells. Thus the T246M mutation disrupts CHIP structure and function resulting in a dominant negative protein impairing ubiquitin-dependent protein degradation. We developed a CRISPR/Cas9 T246M CHIP mouse model of GHS and present preliminary data consistent with our hypothesis that T246M CHIP functions as a dominant negative protein causing GHS.

Mechanisms of ERK-driven expression and function of the RacGEF PREX1 in melanoma.

Meagan B. Ryan, Katherine H. Pedone, Alexander J. Finn, Nancy E. Thomas, Channing J. Der, Adrienne D. Cox. *Depts. Pharmacology, Dermatology and Radiation Oncology, University of North Carolina School of Medicine, Chapel Hill, NC, USA.*

In a gene array study to identify ERK MAPK-dependent genes in melanoma, we identified upregulated expression of PREX1, which encodes a guanine nucleotide exchange factor and activator of the small GTPase Rac1. Rac1 is a member of the Rho family of small GTPases. Aberrant Rho GTPase function can contribute to cancer cell proliferation, motility, invasion and metastasis. We recently determined that *Prex1*-deficient mice are viable but showed impaired metastatic but not tumorigenic growth in an *Nras/Ink4a*-driven mouse model of melanoma. We now extend these studies to assess aberrant expression of PREX1 protein in melanoma patients, to determine the mechanisms of increased PREX1 protein expression in human melanomas, and to characterize its contribution to melanoma biology. First, our immunohistochemical analyses of patient melanoma tissues revealed that PREX1 protein expression increased with more advanced disease and that increased PREX1 membrane association correlated with activation of ERK. Second, in a panel of melanoma cell lines, western blot and qRT-PCR analyses showed that increased levels of P-Rex1 protein correlated with increased PREX1 transcription. We also observed that pharmacological inhibition of the ERK MAPK pathway reduced PREX1 transcription. Interestingly, ERK1/2 also regulate PREX1 protein stability, likely through a proteasomal degradation mechanism. Using siRNA directed against PREX1, we have observed that loss of PREX1 leads to a decrease in melanoma cell Boyden chamber invasion and in collagen spheroid invasion as well as F-actin reorganization. PREX1 regulates Rac1 but not the related Rho GTPase Cdc42 in melanoma cell lines. Our ongoing studies will address the role of PREX1 in regulating additional Rho GTPases such as RhoG and TC10, as well as investigating the transcriptional mechanism whereby ERK regulates PREX1 expression. We propose that ERK1/2 regulation of PREX1 and Rac1 may be another contributor to ERK/MAPK therapeutic efficacy in melanoma.

Assessing Allele Specific Epigenetic Patterns in Long Non-coding RNA-Mediated Imprinted Regions.

Megan Schertzer and Mauro Calabrese. *Department of Pharmacology, Curriculum in Genetics and Molecular Biology*, *University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.*

Several long non-coding RNAs (lncRNAs) namely Kcnq1ot1 and Air are known to cause allele-specific gene silencing in cis within imprinted regions of the genome. Misregulation of gene expression in these distinct regions can lead to debilitating childhood developmental disorders such as Beckwith Wiedemann Syndrome and Silver Russell Syndrome and/or cancer. However the mechanisms of lncRNA-mediated silencing specifically their effect on the surrounding chromatin environment and what determines which genes are silenced versus genes that escape silencing are poorly understood. Using a unique F1 hybrid mouse trophoblast stem (TS) cell model that allows for quantitative allele-specific analysis in sequencing protocols we are profiling the allelic differences of the chromatin state in the imprinted regions surrounding the Kcnq1ot1 and Air lncRNAs. Preliminary ChIP-seq data for the repressive histone modification H3K27me3 reveals a pattern at each locus whereby H3K27me3 has the strongest allelic bias at the lncRNA TSS with this bias extending outward 1Mb and 400Kbs respectively. To gain further insight into the role of lncRNAs in these regions I have separately truncated each lncRNA in F1 hybrid TS cells using CRISPR-Cas9 to insert a triple poly-A site early in each lncRNA gene. This CRISPR-Cas9 approach will be used to assess whether deposition of H3K27me3 and recruitment of certain chromatin modifying complexes are dependent on the lncRNA. Further ChIP-seq data in WT and knockout cells will define allele-specific epigenetic profiles for these imprinted regions at a previously unattainable resolution. Given that patients with a mutation in these imprinted regions also have a silenced WT copy of the gene understanding the mechanism behind imprinted gene expression could lead to a treatment option that reverses silencing of the WT copy allowing for normal gene expression and thus alleviation of symptoms.

Second Messengers of Osmotic Stress that Regulate G Protein Signaling.

J. P. Shellhammer and H. G. Dohlman. *Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

All cells are subject to osmotic stress and will implement molecular signaling events that help to protect the organism. A failure to properly adapt can lead to pathologies including ischemia-reperfusion injury and diabetic neuropathy. Although the signaling processes involved in stress adaptation are incompletely understood we do know that multiple signaling nodes including mitogen-activated protein kinases (MAPKs) and the nutrient-sensing kinase AMPK are activated in response to cell stress. For proper adaptation these kinases must be coordinated with signals acting through G protein-coupled receptors responding to homeostatic cues such as hormones and neurotransmitters. In our recent metabolomics analysis of the model yeast S. cerevisiae we identified specific branched-chain amino acid (BCAA) metabolites that increase dramatically in response to osmotic stress. We are now working to determine the ability of these BCAA derivatives to act as second messengers that coordinate adaptation. We hypothesize that signals activating the stress-responsive MAPK (Hog1) lead to the production of BCAA-derivative second messengers that then bind to the G protein (Gpa1) and AMPK (Snf1) resulting in a conformational change promoting their phosphorylation leading to stress adaptation. Thus far we have found that ectopic addition of BCAA derivatives promotes Gpa1 phosphorylation in the absence of osmotic stress and that genetic deletion of a key BCAA regulatory enzyme reduces Gpa1 phosphorylation upon osmotic stress. Additionally we are working to assess conformational changes induced by binding of BCAA derivatives to Gpa1 and Snf1. We are further working to define the mechanism by which Hog1 promotes production of BCAA derivatives upon osmotic stress. Ultimately this work will define new mechanisms for adaptation to cell stress via a novel class of second messengers. These new mechanisms may lead to new targets for therapeutic intervention in the treatment of stress-related cell damage such as occurs during ischemia-reperfusion injury and other diseases.

Differential roles of medial prefrontal cortical projections to the basolateral amygdala and nucleus accumbens in a model of fear extinction.

J.A. Sugam, T.L. Kash. *Department of Pharmacology Bowles Center for Alcohol Studies University of North Carolina School of Medicine Chapel Hill NC USA.*

Stress and anxiety disorders pose a major public health concern with the overall economic costs in billions of dollars. Despite this massive economic cost there is relatively little known about the underlying circuit mechanisms that underlie stress and anxiety disorders. Post-traumatic stress disorder (PTSD) is one type of anxiety disorder that can also be influenced and exacerbated by other environmental stimuli including prior alcohol abuse. Fear extinction learning a preclinical model in which an animal learns to inhibit behavioral responses to fearful stimuli has been previously used to model PTSD symptoms. Glutamatergic projections from the prefrontal cortex (PFC) to the basolateral amygdala (BLA) are necessary for learning fear extinction however it is unknown how extinction induces plasticity in this circuit in comparison to other output structures of the PFC. To test this we used patch clamp electrophysiology coupled with retrograde tracing to record cellular activity in two different PFC projections in animals that underwent fear extinction. Specifically animals were injected with retrograde tracer in either the BLA or nucleus accumbens (NAc) prior to behavioral training. Following recovery animals were split into three groups fear naïve fear learning and fear extinction for recording. Interestingly Fear extinction resulted in a both a reduction in resting membrane potential and an increase in excitability of neurons that project from the infralimbic cortex to BLA compared to naïve animals. However we did not see changes in excitability in neurons that project from the prelimbic cortex to the BLA or in projections from the PFC to the NAc. This data suggests that the projections from the infralimbic cortex to the BLA are critical for mediating appropriate fear extinction behaviors. Future studies will use in vivo methods to further classify these pathways and evaluate the necessary role of these projections in mediating normal versus abnormal fear extinction.

Identifying kinases that regulate the transcription factor NRF2

T. Y. Tamir[1], D. Goldfarb[2], M. P. Walker[3, 4], and M. B. Major[1, 2, 3, 4], *[1]Dept. Pharmacology, [2]Dept. Computer Science, Dept. [3]Cell Biology and Physiology, [4]Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA.*

The cell's ability to sense, interpret, and respond to oxidative or electrophilic stress is primarily governed by the transcription factor Nuclear Factor Erythroid 2-Related Factor 2 (NRF2). When active, NRF2 provides strong cytoprotective functions by upregulating expression of: 1) xenobiotic metabolism enzymes, 2) phase II detoxification enzymes, 3) ATP-dependent drug efflux pumps, and 4) the proteasome. Under homeostatic conditions (low stress) NRF2 is ubiquitylated and targeted for degradation by the E3 ubiquitin ligase adaptor Kelch Like ECH-Associated Protein 1 (KEAP1). Electrophilic attack of reactive cysteines within KEAP1, during high oxidative stress, results in NRF2 stabilization, nuclear translocation, and transcriptional activation of genes containing Antioxidant Response Elements (AREs). NRF2 is a central gatekeeper of cellular fitness, and decline in NRF2 activity is observed with age as well in several neurodegenerative diseases. Conversely, somatic mutations within NRF2 or KEAP1 result in constitutive NRF2 activity in upper aerodigestive tumors including ~30% of lung cancer cases. NRF2-active tumors predict poor patient survival and resistance to chemotherapy. Although we know the basic mechanics of NRF2 degradation by KEAP1, relatively little is known of how other cellular signaling pathways impact NRF2. Establishing novel regulatory inputs by which the cell controls NRF2 promises to reveal new homeostatic mechanisms of NRF2 regulation, new pathways contributing to NRF2 increase or decrease in diseases, and new prognostic as well as therapeutic opportunities. To identify regulators of NRF2 and to maximize translational potential we employed an arrayed gain-of-function screen of 387 kinases using NRF2-responsive luciferase reporter. We specifically chose to study kinases because they function downstream of oncogenic drivers and are tractable drug targets. In our primary screen we identified over 20 kinases that regulate NRF2-mediated transcription. Low throughput validation screens revealed 12 high confidence NRF2 regulators. These results suggest potential novel regulators of NRF2 signaling in addition to KEAP1.

Transcriptome-wide identification and study of cancer-specific splicing events across multiple tumors.

Yihsuan S. Tsai[1], Daniel Dominguez[2], Shawn M. Gomez[1,2.3,4], Zefeng Wang[1,2] *1Curriculum in Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill, NC 27599; 2Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599; 3Computer Science, University of North Carolina, Chapel Hill, NC 27599; 4Joint Department of Biomedical Engineering at UNC-Chapel Hill and NC State University, Chapel Hill, NC 27599, USA.*

Dysregulation of alternative splicing (AS) is one of molecular hallmarks of cancer with splicing alteration of numerous genes in cancer patients. However studying splicing mis-regulation in cancer is complicated by large noise of tissues-specific splicing. To obtain a global picture of cancer-specific splicing we analyzed transcriptome sequencing data from 1149 patients in TCGA project producing a core set of AS events significantly altered across multiple cancer types. These cancer-specific AS events are highly conserved more likely to maintain protein reading frame and mainly function in cell cycle cell adhesion/migration and insulin signaling pathway. Furthermore these events can serve as new molecular biomarkers to distinguish cancer from normal tissues to separate cancer subtypes and to predict patient survival. We also found that most genes whose expression is closely associated with cancer-specific splicing are key regulators of the cell cycle. This study uncovers a common set of cancer-specific AS events altered across multiple cancers providing mechanistic insight into how splicing is mis-regulated in cancers.

Receptor Kinase Regulation of Heterotrimeric G protein Signaling in MAMP-Induced Cell Death.

M. Tunc-Ozdemir[1], D. Urano[1], D. Jaiswal[1], S. D. Clouse[3], A. M. Jones[1, 2] *Departments of [1]Biology, and [2]Pharmacology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280 [3]Department of Horticultural Sciences, North Carolina State University, Raleigh, NC 27695-7609, USA.*

In Arabidopsis, there is essentially a single heterotrimeric G protein complex that is central for transduction of a myriad of signals. Paradoxically, plants lack conical G protein coupled receptors so it is unclear how signal discrimination is achieved. We proposed that serine/threonine receptor-like kinases (RLKs) provide the missing signal discrimination in the G protein pathway and elaborate here on this mechanism using microbe-associated molecular pattern (MAMP)-induced cell death. The MAMP designated FLG22 triggers G protein activation through its cognate RLK, FLS2 which is in complex with other RLKs, BAK1 and BIR1 and the G protein complex at the plasma membrane. BAK1 directly phosphorylates the Arabidopsis 7-transmenbrane receptor-like Regulator of G Signaling protein designated, AtRGS1, leading to trafficking to the endosome and spontaneous activation of the G protein complex. This process involves recruitment of BAK1 from the BAK1/BIR1 dimer which correlates with the phase containing the burst of reactive oxygen species occurring in the first 10-min.

A Polar Expedition: Investigating Regulation of Polarity in the Pheromone Response Pathway.

L. Vered, M. Peña, B. Errede, T. Elston. *Dept. Chemistry Dept. Pharmacology and Dept. Biochemistry and Biophysics. University of North Carolina Chapel Hill NC USA.*

Polarity establishment and maintenance is important to many cellular processes including morphogenesis differentiation and migration. Cell polarity is required for gradient tracking in which cells polarize in response to an external chemical gradient that may change in intensity duration or directionality. Models of polarity establishment primarily feature a strong positive feedback loop that amplifies an internal or external cue into a spatially polarized response. However the external cellular environment is dynamic and constantly changing. Therefore cells require regulatory mechanisms that allow them to counterbalance positive feedback and reorient their direction of polarity in response to changing environmental conditions. Here we reveal novel characteristics of polarity establishment in response to mating pheromone in the budding yeast Saccharomyces cerevisia. We show that the rate of polarity establishment is dependent on pheromone dosage. This result is surprising since polarity is assumed to be an all or nothing response where cells are either polarized or unpolarized. Our results indicate polarity establishment as a dosage-dependent nuanced response. Additionally we reveal a previously unknown negative feedback motif regulating loss of polarity in response to pheromone withdrawal. Lastly we show that cells retain memory of previous polarity sites. This memory mechanism might enable rapid response to an unstable or fluctuating cue. We also show that this memory mechanism is independent of Rsr1 the protein marking polarity site during mitosis. Our results demonstrate that polarity establishment in response to a dynamic external cue is likely regulated by unique previously unknown regulatory motifs. These motifs enable cells to quickly adapt to a constantly-changing cue. We anticipate our studies to lead to the discovery and characterization of these motifs in budding yeast. Furthermore these results will motivate similar investigations in other organisms in order to reveal common features of polarity networks.

Exploring mutations in the E3 ubiquitin ligase CHIP for impacts on activity and protein interactions.

C. Virus1, C. Rubel1, L. Carrier2, J. Robbins3, R. Page4, D. Cyr5, and J.C. Schisler1 6. *1McAllister Heart Institute University of North Carolina School of Medicine Chapel Hill NC USA; 2Dept. of Experimental Pharmacology and Toxicology Cardiovascular Research Center University Medical Center Hamburg-Eppendorf Hamburg Germany; 3Cincinnati Children s Hospital Medical Center Cincinnati OH USA; 4Dept. of Chemistry and Biochemistry Miami University Oxford OH USA; 5Dept. of Cell Biology and Physiology University of North Carolina School of Medicine Chapel Hill NC USA; and 6Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA.*

CHIP (C-terminus of HSC70 Interacting Protein) is a dual-function protein. It promotes protein folding by acting as co-chaperone for HSP70/90 but is also involved in protein degradation by tagging proteins with ubiquitin (E3 ubiquitin ligase function) and marking it thereby for proteasome processing. The protein clientele of CHIP is largely unknown. Known substrates are ErbB2 and NF-?B/p65 (breast/gastric cancer) and phospho tau ?-synuclein Parkin Huntingtin which links CHIP to Alzheimer s and Parkinson disease and ataxias. Several mutated CHIP variants have been found in patients suffering from cerebellar ataxia. Mutation T246M has been confirmed as cause of ataxia and hypogonadism in mouse models. The underlying mechanism of how mutated CHIP evokes dysfunctions is not yet understood. We plan to examine CHIP mutations located in the TPR domain responsible for HSP70/90 binding and U-box responsible for E2 interaction in a cell line based screening and by biophysical methods using purified protein. The cell line (HEK-293) stably express a dual reporter vector that reflects the ubiquitin-proteasome system (UPS) and autophagy status of the cell and is then subjected to genome-scale gene knockout by viral RNAi. The goal of this screen is to identify genes and/or pathways that are involved in the pathogenesis and/or the rescue of the deleterious protein due to the point mutation.

Binge alcohol-induced microglial priming.

T. Jordan Walter, Ryan Vetreno, Liya Qin, Fulton Crews. *Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.*

Binge drinking is a common form of alcohol abuse and has many detrimental effects. These effects include changes in the central immune system. The central immune system consists primarily of microglia, the resident macrophages of the brain, but also includes the many immune molecules expressed in the brain. We investigated the long-term effects of binge alcohol treatment on the central immune system using an intermittent adolescent ethanol (AIE) rat model. Rats were gavaged with ethanol (5 g/kg, 20-30%, i.g.) 2 days on and 2 days off from P25-P55 and sacrificed at P95. Immunohistochemical stains were performed for markers of microglial activation. Markers such as CD11b and Iba1 were increased following AIE, but markers of robust activation such as MHCII, CD68 and iNOS were not expressed. Using a mouse binge model, we further investigated the effects of binge alcohol on microglia. Mice gavaged once per day for ten days with ethanol (5 g/kg, 25%, i.g.) showed an increased inflammatory cytokine response to the inflammogen, poly I:C. Mice were also gavaged once with ethanol (6 g/kg, 25%, i.g.) and sacrificed 24 hours later. We found a decrease in expression of CD200, a molecule that inhibits microglia activation. These findings suggest binge alcohol causes microglial priming. Microglial dysfunction has been implicated in many neuropsychiatric diseases. Therefore, our results may give insight into therapeutic strategies for the detrimental effects of binge drinking.

Resonator motifs in mechano-chemical signaling pathways revealed by a new optogenetic method for precise oscillation of signaling circuits.

H. Wang[1], M. Vilela[2], R. Liu[3], G. Danuser[2], K.M. Hahn[1]. *1. University of North Carolina School of Medicine, Chapel Hill, NC, USA; 2. Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA; 3. Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA.*

We have developed an optogenetic approach to control protein activity that promises to be broadly applicable and is simple to apply (LOVTRAP for LOV trapping and release of proteins). Using mRNA display we developed Zdk a small protein that binds only to the dark state of the LOV2 domain which undergoes a large reversible conformational change induced by light between 400 and 500 nm. Zdark binds to the dark state of LOV2 with a Kd of ~27nM but shows no detectable binding to the lit state. In LOVTRAP, the LOV domain was anchored at the mitochondria and proteins of interest were fused to Zdark. In the dark the Zdark-protein fusion was sequestered at the mitochondria but released upon irradiation. LOVTRAP was used to regulate RhoA Rac1 Cdc42 Vav2 and a RhoA inhibitory peptide. By oscillating the activating light LOVTRAP could be used to generate oscillations in the activity of signaling proteins with precise frequency. We used this approach to study the cell edge dynamics induce by Vav2 a GEF that activates Rac1 RhoA and Cdc42. Simple activation of VAV2 or its downstream target Rac1 but not RhoA or Cdc42 increased the frequency of cell edge oscillations. Strong reinforcement of 3.3 mHz oscillations were observed when Vav2 activity was oscillated at frequencies that were multiples of 3.3 mHz, but not other frequencies. This showed that Vav2 was a component of a resonator circuit that controlled cell edge oscillations. The oscillations induced by VAV2 were dependent on PI3K suggesting a model for a resonator based on a positive feedback loop that includes mechanical and biochemical interactions. Because of the generalizable nature of our approach we hope that LOVTRAP can open the door to application of increasingly sophisticated signal processing tools to study not only cell migration but also other physiological oscillatory processes.

Identification of epigenetic inhibitors of MEK inhibitor-induced kinome adaptation in triple negative breast cancer.

J.S. Zawistowski1, B.T. Golitz1, J. Jin2, and G.L. Johnson1. *1) Dept. Pharmacology University of North Carolina School of Medicine Chapel Hill NC USA 2) Dept. Structural and Chemical Biology Icahn School of Medicine at Mt. Sinai New York NY USA*.

Kinase-targeted therapies are increasingly prevalent in cancer treatment strategies but ultimately fail to achieve a prolonged response as tumors develop resistance. A predominant mechanism of acquired resistance is the engagement of bypass signaling pathways that serves to promote cell proliferation and reactivate the inhibited driver kinase. Accordingly in triple negative breast cancer (TNBC) subtype-specific cohorts of receptor tyrosine kinases are both activated and transcriptionally upregulated in response to MEK inhibition. We have demonstrated that this upregulation can be attenuated by chemical inhibition of the BET family bromodomain chromatin reader BRD4 a protein that associates with acetylated histones and regulates P-TEFb control of transcription although mechanisms underlying this regulation and TNBC subtype specificity remain unclear. To gain insight into BRD4 control of the kinome adaptive response and to additionally identify new regulators of the response we screened a library comprised of 70 small molecule epigenetic inhibitors for their ability to attenuate MEK inhibitor-induced kinase upregulation in TNBC cell lines of the claudin-low (SUM159PT) or basal-like (HCC1806) molecular subtype. Epigenetic enzyme classes and proteins targeted by the inhibitor library included histone methyltransferases demethylases acetyltransferases deacetylases DNA methyltransferases topoisomerases 2OG oxygenases chromatin readers PARP and regulators of splicing. In SUM159PT cells we identified JIB-04 an inhibitor of JumonjiC domain-containing lysine demethylases to block upregulation of protein levels of adaptive response kinases PDGFRB and DDR1. SGC-CBP30 targeting the bromodomain of the histone acetyltransferases CBP/p300 inhibited adaptive response kinase upregulation in both SUM159PT and HCC1806 cells. The JumonjiC demethylase JMJD6 is known to associate with BRD4 and regulate P-TEFb transcriptional activation complexes while CBP/p300 acetylates lysines on histone H3 which serve as chromatin docking sites for BRD4 at promoters and enhancers. Thus both primary screen hits may target BRD4-associated complexes and may represent novel approaches for inhibiting the adaptive kinase response to MEK inhibitor.

Does the beta-lactam-insensitive L,D-transpeptidase NGO1484 play a role in resistance to beta-lactam antibiotics in Neisseria gonorrhoeae?

K. Daniels, R.A. Nicholas. *Dept. of Chemical Biology and Medicinal Chemistry, University of North Carolina Eshelman School of Pharmacy, and, Dept. of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA.*

Neisseria gonorrhoeae is the second-most prevalent sexually transmitted infection worldwide, causing an estimated 106 million infections in 2012. Over the past decade, the steady and inexorable increase of resistance toward multiple classes of antibiotics has severely limited treatment options for gonorrhea infections. Most alarmingly, infections caused by strains with high-level resistance to the extended-spectrum cephalosporin ceftriaxone, which is the last recommended beta-lactam antibiotic for treating gonococcal infections, have now been reported. These strains contain "mosaic" penA alleles encoding Penicillin-Binding Protein 2 (PBP2) variants with over 60 amino acid mutations that display markedly lower acylation rates with ceftriaxone. PBP2 is an essential transpeptidase that cross-links peptidoglycan and is the lethal target for ceftriaxone and other beta-lactam antibiotics. In other bacteria, increased L,D-transpeptidase activity can lead to beta-lactam antibiotic resistance due to the insensitivity of these enzymes to beta-lactams and their capacity to replace PBPs as essential peptidoglycan transpeptidases. BLAST searches of the gonococcal genome reveal one putative L,D-transpeptidase, NGO1484, but its role in resistance to beta-lactam antibiotics in this organism is unknown. Therefore, we investigated the role of this enzyme in beta-lactam resistance by knocking out the gene encoding NGO1484 in three antibiotic resistant N. gonorrhoeae strains: FA6140 (penicillin-resistant), 35/02 (cephalosporin-intermediate resistant), and H041 (cephalosporin-resistant). The 5' and 3' regions of ngo1484 separated by a kanamycin-resistance cassette were cloned into a pUC18us vector, and the resulting construct was used to transform the three resistant strains to kanamycin resistance. PCR amplification of genomic DNA confirmed the knock-out, and the MICs of penicillin and cefixime for these transformants and the parental strains were determined. The NGO1484 knock-out was shown to confer a 2-fold decrease in the MIC of cefixime (an expanded-spectrum cephalosporin) for strain 35/02, but showed no substantial difference in the MICs of cefixime for strain H041 or penicillin for strain FA6140. These data suggest that the L,D-transpeptidase NGO1484 does not play a significant role in the development of beta-lactam resistance, although its role in peptidoglycan synthesis requires further investigation.