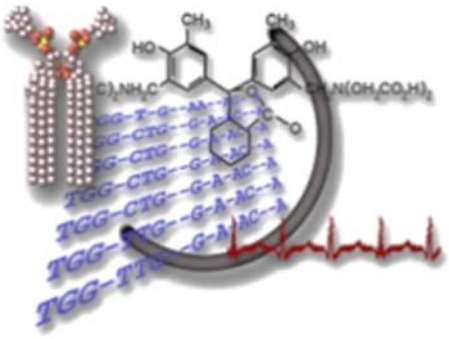
Pharmacological and Pharmaceutical Sciences Research Retreat

August 10, 2018

Kirkwood Regional Center

2301 Oakdale Blvd., Coralville, IA



The University of Iowa College of Pharmacy

Department of Pharmaceutical Sciences and Experimental Therapeutics

Hosted by:

The Pharmacological Sciences Training Program

Department of Pharmacology

Department of Pharmaceutical Sciences and Experimental Therapeutics

Research Retreat

Schedule

8:00 - 9:00 AM Continental Breakfast and Poster Set up

9:00 AM DEO's Welcome

9:05 - 10:20 AM **Session I: Presentations by trainees appointed to the TG**

9:05 - 9:20 Alicia Ortiz, Biochemistry
Mentor: Kris DeMali, PhD

9:20 - 9:35 Eden Maack, MNPC/PSET
Mentor: Robert Kerns, PhD

9:35 - 9:50 Jordan Kohlmeyer, Molecular Medicine
Mentor: Dawn Quelle, PhD

9:50 - 10:05 Blake Monroe, PTT/PSET
Mentor: Ethan Anderson, PhD

10:05 - 10:20 Grant Walters, Neuroscience
Mentor: Yuriy Usachev, PhD

10:20 - 10:40 AM Break

10:40 - 10:55 AM Group Photo

11:00 - 12:00 PM **Session II: Keynote Faculty Presentation**

Joey V. Barnett, PhD
Vice Chair, Professor of Pharmacology, Medicine, Pediatrics and Pathology, Microbiology & Immunology
Director of the Office of Medical Student Research
Assistant Dean of Physician-Researcher Training
Vanderbilt University School of Medicine
Nashville, TN

"Rethinking PhD Training"

12:00 - 12:30 PM Lunch Break

12:30 - 2:30 PM Poster Session - Viewing / Judging

12:30 - 1:30 Odd number posters

1:30 - 2:30 Even number posters

Research Retreat

Schedule

2:30 - 3:30 PM **Session III: Faculty Presentations**

2:30 - 3:00 PM Catherine Marcinkiewcz, PhD, Assistant Professor, Department of Pharmacology
"Serotonin Signaling in Anxiety and Reward"

3:00 - 3:30 PM Ryan Smith, PhD, Assistant Professor, Pharmaceutics & Translational Therapeutics
"Functional Genetics to Identify Anti-Addiction Drug Targets"

3:30 - 4:00 PM **Awards and Wrap-up**

Faculty Presentations are 25 minutes + 5 minutes for Q & A

Trainee Presentations are 12 minutes + 3 minutes for Q & A

Keynote Speaker

Joey V. Barnett, PhD, Professor

Vice Chair, Professor of Pharmacology, Medicine, Pediatrics and Pathology, Microbiology & Immunology
Director of the Office of Medical Student Research
Assistant Dean of Physician-Researcher Training
Vanderbilt University School of Medicine

Rethinking PhD Training

Discuss history of Ph.D. training and models of Ph.D. training that have developed, challenges to effective training (Rigor and Reproducibility, "student-centered" incentives, career development, generic or professional development) as well as opportunities these challenges offer. Recent innovations in training will be presented as examples to be considered.

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Abstracts

1 E3 Ubiquitin Ligase Mutations in X-linked Intellectual Disability

Jianing Song[1], Ronald Merrill, Ph.D.[1], Rikki Kephart[1], Yujia Liu[1], Marie Shaw, BSc Hons[2,3], Renée Carroll, BSc[2,3], Vera Kalscheuer, Ph.D.[4], Fiona McKenzie, Ph.D.[5], Lachlan Jolly, Ph.D.[2,3], Jozef Gécz, Ph.D. [2,3], and Stefan Strack, Ph.D.[1]

[1]Department of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA, USA. [2]Robinson Research Institute, The University of Adelaide, Adelaide, SA, Australia. [3]School of Paediatrics and Reproductive Health, The University of Adelaide, Adelaide, SA, Australia. [4]Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany. [5]Genetic Services of Western Australia, Subiaco, WA, Australia.

Intellectual disability (ID), which affects 1-2% of the general population, is a devastating neurodevelopmental disorder with the most lifetime costs of all diagnoses in the U.S. However, males are more susceptible to ID than females and are often found to have severe outcomes. Mutations in X-chromosomal genes are thought to account for this male-biased phenomenon. *KLHL15* was recently identified as a novel XLID gene. It encodes Kelch-like protein 15 (KLHL15), a substrate adaptor of a Cullin-3 (CUL3)-based E3 ubiquitin ligase complex that targets proteins, including the brain-enriched B′*β* regulatory subunit of protein phosphatase 2A (PP2A), for degradation by the ubiquitin/proteasome system (UPS). Several KLHL15 mutations have been found in the poorly characterized BACK domain, which is a "hotspot" for many deleterious variants of the other KLHL family members resulting in either Mendelian diseases or human cancers. We identified both loss-of-function (ΔFY241, ::ACOT9) and gain-of-function (R249H) alleles, and we ***hypothesize*** that small deletions and point mutations in KLHL15's BACK domain lead to structural rearrangement that change the alignment between bound substrates and the ubiquitin-transfer (E2/E1) complex to either slow or accelerate substrate ubiquitination and degradation, causing dysregulated protein turnover of $CUL3^{KLHL15}$-targeted substrate(s) and eventually pathogenesis of ID.

2 Regulation of Cardiac Remodeling by CDK8-Selective Kinase Inhibitor, Senexin A

Rachel A. Minerath[1], Allison M. Vaske[2], Duane D. Hall[2], and Chad E. Grueter[2]

[1]University of Iowa, Department of Pharmacology, Iowa City, IA
[2]University of Iowa, Department of Internal Medicine-Cardiology Division, Iowa City, IA

Pathological cardiac hypertrophy represents a major risk factor for heart failure (HF). The hypertrophic response is orchestrated in part through transcriptional alterations that ultimately modify cardiac function. Mediator is a multiprotein complex that coordinates signal dependent transcription factors with basal transcriptional machinery including RNA polymerase II and general transcription factors. Transcriptional regulation by Mediator complex can be modified by the association of the Mediator complex kinase submodule with the core complex. Cyclin-dependent kinase 8 (CDK8) is a kinase present in the submodule that has been demonstrated to have a complex role in transcriptional regulation through a number of mechanisms involving both transcriptional activation and inhibition. Our lab has demonstrated that CDK8 expression is upregulated in murine HF models as well as in failing human hearts. In addition, cardiac CDK8 over-expression promotes transcriptional remodeling that results in dilated cardiomyopathy suggesting that CDK8 inhibition may represent a novel pharmacological target. This study utilizes CDK8 kinase inhibitor, Senexin A, which is being evaluated as an anti-cancer chemotherapeutic agent. Here, we demonstrate that Senexin A inhibits kinase activity in an *in vitro* neonatal rat cardiomyocyte (NRCM) model resulting in reduced cardiomyocyte hypertrophy as well as a reduction in expression of specific cardiomyocyte hypertrophy markers. These studies demonstrate a role for CDK8 activity in transcriptional regulation of genes associated with pathological cardiac remodeling that drives cardiac hypertrophy and ultimately HF.

Abstracts

3 **mTORC1 signaling regulates vascular endothelial function via reactive oxygen species and NFκB signaling**

John J. Reho, Deng-Fu Guo, Andrew Olson, Kamal Rahmouni

Department of Pharmacology, University of Iowa, Iowa City, IA

The mechanistic target of rapamycin complex 1 (mTORC1) is an important intracellular energy sensor that regulates protein synthesis through its downstream signaling components the S6-kinase and the ribosomal S6 protein. Recently, our laboratory has demonstrated a critical role of the mTORC1 signaling pathway in the cardiovascular regulation with implications for obesity and hypertension. In this study, we tested the hypothesis that the mTORC1 signaling pathway in the vasculature is involved in the regulation of vascular endothelial function and that dysregulation of this pathway contributes to the cardiovascular disorders associated with obesity. We began by assessing the effects of activating mTORC1 signaling in cultured mouse lung endothelial cells (MLECs) using leucine (10mM; 16hrs) or a constitutively active S6-kinase adenoviral construct (Ad-S6KCA; 48hrs). Activated mTORC1 signaling was confirmed by the increased phosphorylated levels of the ribosomal S6 protein (2-4 fold; $p<0.05$). Increasing mTORC1 signaling elevated mRNA expression of oxidative stress markers (NOX1 and NOX2; 1.5-5 fold; $p<0.05$), decreased mRNA expression of superoxide dismutase 2 (0.5 fold; $p<0.05$) and increased reactive oxygen species (ROS) generation (via dihydroethidium staining; 1.5 fold; $p<0.05$) in MLECs demonstrating a pro-oxidant gene environment evoked by activation of mTORC1 signaling. Blockade of the IKKβ subunit of the NFκB transcriptional complex (BMS-345541; 300nM) prevented mTORC1 signaling induced ROS generation in MLECs demonstrating a critical role of NFκB signaling in the cellular response to mTORC1 activation. Next, we used leucine and Ad-S6KCA to test the consequence of enhancing mTORC1 signaling on endothelial function in aortic rings isolated from wildtype mice. Both leucine and an adenovirus expressing a constitutively active form of S6K (Ad-S6KCA) impaired endothelial dependent acetylcholine-induced relaxation (~10-15%; $p<0.05$) without changing endothelial-independent relaxation responses evoked by sodium nitroprusside indicating endothelial but not smooth muscle dysfunction in response to increased mTORC1 signaling. Blockade of mTORC1 signaling using a dominant negative S6K adenoviral construct (Ad-S6KDN) as well as inhibition of ROS signaling by Tempol (non-specific free radical scavenger) ameliorated mTORC1-induced endothelial dysfunction ($p<0.05$). To determine the involvement of mTORC1 signaling in the endothelial dysfunction associated with obesity, we utilized diet-induced obese mice that display vascular endothelial dysfunction as compared to lean controls. Obese mice exhibited increased mTORC1 signaling in the aorta and mesenteric artery indicated by the elevated phosphorylated levels of the ribosomal S6 protein (1.5-3 fold; $p<0.05$) and this was associated with increases in NOX2 expression (1.5 fold; $p<0.05$) further indicating ROS signaling in the vascular pathology associated with obesity. We conclude that mTORC1 signaling is a novel regulator of vascular endothelial function through its effects on the NFκB complex and ROS signaling. Our data also indicate that dysregulation of the mTORC1 signaling pathway may be involved in the endothelial dysfunction associated with obesity.

Abstracts

4 **Contrasting effects of high fat diet and DOCA-salt on primary neuronal cilia**

Jingwei Jiang, Kamal Rahmouni

Department of Pharmacology, University of Iowa Carver College of Medicine, Iowa City, IA

Virtually, every mammalian cell is equipped with an antenna like primary cilium, a cell surface protrusion that is thought to act as a sensory organelle. Many of the rare genetic disorders that cause shorter, absent or disrupted cilia are associated with obesity and cardiovascular dysfunction in humans and rodents, which suggest that cilia length contribute to energy balance and cardiovascular homeostasis. Here, we examined the length of the primary neuronal cilia in the brain nuclei that contribute to metabolic and cardiovascular regulation in high fat diet-induced obese (DIO) mice and DOCA-salt mice. Cilia length was examined by adenylate cyclase 3 (AC3) immunostaining, followed by confocal 3D reconstruction, and quantification by IMARIS imaging analysis software. Analysis of the cilia length and distribution showed reduced frequency of cilia that are over 10 μm in the brain of DIO mice compared to control mice fed normal diet fed mice (17.02±1.36% vs 23.78±1.15%, p=0.032). Interestingly, the most pronounced difference in cilia length was observed in the dorsomedial hypothalamus with the DIO mice displaying significantly shorter cilia (6.90±0.06 μm) relative to controls (7.32±0.14 μm in controls, n=5/group p<0.05). Conversely, we found that average neuronal cilia length was elongated in 3-week DOCA-salt treated mice compared to sham group. The number of primary neuronal cilia that are over 10 μm was significantly increased in DOCA-salt mice by 8% (p=0.0114). On the other hand, the number of cilia that are 4-5 μm in length was significantly decreased in DOCA-salt mice compared to sham controls (11.73±1.70% vs 18.73±2.02%, p=0.0385). The supraoptic nucleus was the only nucleus that displayed difference in the length of cilia that are 5-10 μm in length (7.46±0.24 μm vs 6.76±0.15 μm, n=5/group, p=0.0509). Our data demonstrate plasticity of neuronal cilia in response to high fat diet and DOCA-salt treatment in defined brain regions. Our results raise the possibility that primary neuronal cilia may function as part of environmental surveillance system in the brain that control energy homeostasis and cardiovascular function. Further analysis of the role of primary neuronal cilia in cardiovascular regulation is underway.

5 **High Throughput Compatible Assays and Resources at UIHTS Core**

Kuo-Kuang Wen, Ph.D.[1], **Meng Wu, Ph.D**[1,2,3]

[1]High Throughput Screening Core Facility, [2]Division of Medicinal & Natural Products Chemistry, Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, and [3]Department of Biochemistry, Carver College of Medicine, University of Iowa

In this poster, a set of novel high throughput compatible assays is presented. They include spheroid 3D cell HT System, arrayed Kinome CRISPR gRNAs and Kinase inhibitors, high content live cell time-course imaging of mitochondria Dynamics, FDA approved drugs/repurposing, and combinational (i.e. synergistic between drugs, synthetic lethal among genes) assays.

Abstracts

6 **RhoA-myosinII axis protects circulating tumor cells from fluid shear stress-induced damage**

Devon Moose[1,2], MS, Benjamin Krog[1,3], MS, Lei Zhao[1], PhD, Gretchen Burke[1], BS, Lillian Rhodes[1], and Michael Henry[1,2,4], PhD

[1]Department of Molecular Physiology and Biophysics, Carver College of Medicine, [2]Cancer Biology, Carver College of Medicine, [3]Department of Biomedical Engineering, College of Engineering, [4]Holden Comprehensive Cancer Center, Carver College of Medicine, University of Iowa

Circulating tumor cells (CTCs) are exposed to hemodynamic forces, which have long been thought to be mechanically destructive to CTCs. However, recent studies show that cancer cell lines from diverse histologies exhibit resistance to brief (millisecond) pulses of high-level (750-6400 dyn/cm^2)fluid shear stress (FSS), whereas non-transformed epithelial cells are sensitive to this mechanical insult (PMID: 23226552, 26447202). Moreover, exposure of cancer cells to FSS results in cortical stiffening (PMID: 25908902). Herein, we elucidate the mechanism of FSS resistance in cancer cells, and extend these findings to experimental models of CTCs. We show that although some cancer cell lines exhibit elevated levels of membrane repair relative to non-transformed counterparts, intrinsic resistance to plasma membrane damage is a more consistent feature distinguishing cancer cells. FSS resistance is detectable in cancer cells acutely isolated from primary mouse TP53/PTEN mutant prostate tumors, not just a feature of cultured cancer cell lines. Our findings indicate that cancer cells respond to FSS by activation of RhoA-myosinII contractility which protects them from nanometer-scale damage to the plasma membrane. Moreover, we present evidence that the RhoA myosinII axis protects CTCs from mechanical damage in animal models. Treatment of PC-3 cancer cells with a non-toxic dose of the myosin II inhibitor blebbistatin (20mM; 3h) reduced the number of intact cells arrested in the lung microvasculature immediately following tail vein injection. Additionally, treatment of mice bearing orthotopically implanted, metastatic PC-3 derived prostate tumors with blebbistatin (2.5mg/kg; 3h) acutely reduced steady-state CTC levels by approximately 10-fold. Taken together, our data indicate that viable CTCs actively resist destruction by hemodynamic forces and are likely to be more mechanically robust than is commonly thought.

7 **Targeting a Novel RABL6A-RB1 Pathway Suppresses MPNST Pathogenesis**

Jordan Kohlmeyer[1], Courtney Kaemmer[3], Allison Moreno Samayoa[2], Chandra Maharjan[3], Vickie Knepper-Adrian[4], Dave Gordon[5], Rebecca Dodd[4], Ben Darbro[5], Munir Tanas[6] and Dawn E. Quelle[1,3,6]

Molecular Medicine Graduate Program[1], Post Baccalaureate Research Education Program[2], and Departments of Pharmacology[3], Internal Medicine[4], Pediatrics[5] and Pathology[6] at the University of Iowa, Carver College of Medicine and Holden Comprehensive Cancer Center, Iowa City, IA

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive, deadly sarcomas that arise from the myelinating nerve sheath. These tumors can arise sporadically (50%) or in association with the cancer predisposition syndrome, neurofibromatosis type I (NF1). The only known cure for MPNSTs is complete surgical resection with wide margins; however, many tumors are non-resectable or cannot be fully resected due to their location and/or large size. In most MPNSTs, the retinoblastoma (RB1) tumor suppressor pathway is inactivated through hyper-activation of CDK4/6 kinases that disable RB1, often through loss of cell cycle inhibitors such as p16 and p27.

RABL6A is an oncogenic GTPase and newly recognized inhibitor of the RB1 pathway whose role in MPNST biology is not known. We examined RABL6A protein levels in human MPNST lines and found it is upregulated compared to non-transformed Schwann cells (NHSC), *supporting our hypothesis that RABL6A drives MPNST pathogenesis through inactivation of RB1, and this pathway represents an important, new therapeutic target.* Tissue microarray analyses showed marked upregulation of RABL6A coincident with p27 loss in human MPNSTs compared to patient-matched PNFs. *In vitro* assays demonstrated RABL6A is essential for MPNST proliferation and survival. Loss of RABL6A caused significant MPNST cell death and G1 phase arrest concurrent with p27 upregulation and accumulation of active, hypo-phosphorylated RB1. Conversely, RABL6A overexpression enhanced MPNST cell proliferation and RB1 phosphorylation. These data suggested MPNSTs will be inhibited by drugs targeting the RB1 pathway. Indeed, the selective CDK4/6 inhibitor, palbociclib (PD0332991), killed MPNST cells in an RABL6A-dependent manner *in vitro* and suppressed MPNST growth in mouse tumor orthotopic xenografts. *Our findings establish a critical role for RABL6A in MPNST pathogenesis and identify RABL6A-RB1 signaling as a novel, clinically relevant target for MPNST therapy using FDA-approved CDK4/6 inhibitors.*

Abstracts

8 **Characterization of *de novo* mutations in PP2A/B'δ (PPP2R5D) that cause intellectual disability reveals altered substrate specificity and activity regulation**

Chian Ju Jong, PhD, Ronald A. Merrill, PhD, Yufang Kong, BS, Stefan Strack, PhD

Department of Pharmacology, University of Iowa, Carver College of Medicine, Iowa City, IA-52242

De novo germline mutations in protein phosphatase 2A (PP2A) subunits have been identified in intellectual disability (ID) and human overgrowth. The most commonly mutated subunit is B'd (*PPP2R5D*) and most of these mutations switch a negatively charged to a positively charged residue (commonly glutamate to lysine) in the highly conserved acidic loop that extends towards the active site of the catalytic subunit. We hypothesized that *PPP2R5D* mutations change PP2A substrate specificity (e.g. from positively to negatively charged phospho-peptides), which would enhance cellular signaling cascades that promote growth and proliferation. To identify cellular and biochemical phenotypes of *PPP2R5D* mutations causing ID, we generated stable HEK293 cell lines inducibly expressing either wild-type or mutant B'δ using the PP2A reduction model. We characterized the most common E198K, as well as the less severe E420K mutation in *PPP2R5D*. The PP2A reduction cell lines were treated with doxycycline or vehicle for at least three days followed by assessment of PP2A subunit expression, cellular growth, and substrate dephosphorylation by unbiased phospho-proteomics. Wild-type and mutant B'δ were overexpressed by about 3-fold, concomitant with a 2 to 3-fold loss of endogenous PP2A regulatory subunits, indicating successful PP2A reduction. Wild-type B'δ expression caused growth arrest after four days of doxycycline treatment. On the other hand, the E198K and to a lesser extend the E420K mutations attenuated the growth inhibitory effect of B'δ. The proliferative effects of wild-type and mutant B'δ are likely associated with a change in substrates specificity. Our global phosphoproteomic analyses of SILAC-labeled lysates suggests that wild-type B'δ preferentially dephosphorylates substrates containing positively charged residues, while E198K-mutant B'δ favors substrates containing negatively charged residues adjacent to the phosphorylation site. Our data also suggest that the E198K mutation confers high basal activity to the PP2A holoenzyme, while wild-type PP2A/B'δ requires phosphorylation by PKA for full activity. Using the PP2A reduction model, we provide preliminary evidence that *de novo* mutations in *PPP2R5D* blunt the growth inhibitory effect of B'δ likely by a change in PP2A substrates specificity. We speculate that altered PP2A activity deregulates signaling pathways mediating cell proliferation, differentiation, and morphogenesis, which in turn leads to abnormal brain development.

Abstracts

9 **Loss of the NIAM tumor suppressor cooperates with Myc activation in B-cell malignancies**

Chandra K. Maharjan[1], MS; Angela M. Schab[1], BS; Ryan M. Sheehy[1,2], PhD; Amy L. Whillock[3], BS; Michael Pisano[3], BS; Chandini Reddi[1], BS; Jacqueline E. Reilly[1], PhD; Jackson Nteeba[1], PhD; Maureen C. Lamb[4], BS; Timothy Ginader[5], MS; Brian J. Smith[5], PhD; Nicholas Borcherding[6], MS; Xuefang Jing[6], PhD; Van Tompkins[6], PhD; Fenghuang Zhan[7], PhD; Gail Bishop[3], PhD; David K. Meyerholz[6], DVM, PhD; Carol J. Holman[6], MD; Siegfried Janz[6], MD; and Dawn E. Quelle[1,2,4,6,8], PhD

[1]Pharmacology, [2]Free Radiation and Radiation Biology, [3]Immunology Program, [4]Molecular and Cell Biology Program, [5]Biostatistics, [6]Pathology, [7]Internal Medicine and [8]Medical Science Training Program in the University of Iowa College of Medicine and Holden Comprehensive Cancer Center

Background and Rationale: Diffuse large B-cell lymphoma (DLBCL) is the most common and an aggressive subtype of B-cell malignancies. A better understanding of molecular mechanisms underlying its development will identify novel chemotherapeutic targets and treatment strategies. Myc oncogene activation is a primary initiating event in B-cell lymphoma development. Subsequent alterations in the ARF-Mdm2-p53 tumor suppressor pathway, including inactivation of the ARF and p53 tumor suppressors or overexpression of the Mdm2 oncoprotein, cooperate with Myc activation to drive mature B-cell tumor progression and connote worse outcome. NIAM (Nuclear Interactor of ARF and Mdm2) is a novel regulator of ARF-Mdm2-p53 signaling whose loss causes B-cell lymphoma at an incidence of ~20-30% in older animals. NIAM-deficient mice were crossed with B-cell specific Myc transgenic mice (which develop a spectrum of B-cell lymphomas) to test if NIAM loss promotes Myc-driven B-cell lymphomagenesis.

Key Findings: Dual mutant mice (Myc transgenic; NIAM-deficient) showed reduced survival, increased tumor incidence, and accelerated spontaneous lymphoma development compared to Myc transgenic or NIAM-deficient animals. NIAM deficiency on a Myc transgenic background shifted tumor type from follicular lymphoma (FL) to the more aggressive DLBCL. Molecular studies revealed that NIAM is a new binding partner of c-Myc whose loss increases endogenous c-Myc expression. Analyses of endogenous c-Myc mRNA levels indicated that its regulation by NIAM is post-transcriptional. Functionally, NIAM loss enhances the proliferation of splenic B-cells without altering their apoptosis. This work uncovers NIAM as a new negative regulator of Myc that suppresses Myc-driven B-lineage malignancies.

Abstracts

10 The roles of estrogen-related receptor alpha in cardiometabolic control

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Department of Pharmacology, The University of Iowa

The estrogen-related receptor alpha (Esrra) is an orphan nuclear receptor with strong homology to estrogen receptor alpha (ERα), whereas exhibits estrogen-independent constitutive transcriptional activity to regulate genes in cellular energy metabolism. Esrra is highly expressed in metabolically active tissues such as skeletal muscles, heart, brown adipose tissues, kidney, and brain. While previous reports show that Esrra knockout (KO) mice are hypotensive and resistant to high fat diet-induced obesity (DIO), systemic understanding of the roles of ESRRA in cardiometabolic control is limited. We therefore performed a variety of metabolic and cardiovascular measures to evaluate the cardiometabolic consequences of mice lacking Esrra globally. Our results revealed that Esrra KO mice were hypoactive and were resistant to DIO mainly due to decreased food intake. Despite of lower body weight and plasma leptin level, female Esrra KO mice tend to have elevated blood glucose level (p=0.08) without notable changes of insulin and glucagon levels. Non-invasive blood pressure measurement by tail-cuff sphygmomanometer showed that male Esrra KO mice had significantly lower blood pressure when it was measured in light period. On the other hand, the blood pressure measured during dark period was significantly lower in female, but not male, Esrra KO mice compared to their WT littermates. Pulse wave velocity test showed that vascular stiffness was comparable between genotypes in both genders. Echocardiographic measurements revealed that the ejection fraction of male, but not female, KO mice was significantly higher than that of WT littermates, while female KO mice did not show any obvious changes. These results indicate a multifaceted role of Esrra in the regulation of metabolic and cardiovascular functions likely in a gender- and circadian cycle-dependent manner. Future studies with conditional deletion approach are necessary to tease apart complex roles of Esrra in distinct cardiometabolic processes.

11 A novel RABL6A-PP2A-AKT pathway drives pancreatic neuroendocrine tumor growth

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The Department of Pharmacology[1], Molecular Medicine Graduate Program[2], Free Radical & Radiation Biology Training Program[3] and the Departments of Internal Medicine[4], Surgery[5], Pathology[6], and Pediatrics[8] in the College of Medicine, Holden Comprehensive Cancer Center, University of Iowa, Iowa City, Iowa. Case Western Reserve University[7], Cleveland, Ohio.

Hyperactivated AKT/mTOR signaling is a hallmark of pancreatic neuroendocrine tumors (PNETs). Drugs targeting the pathway are used clinically but tumor resistance invariably develops. A better understanding of factors controlling AKT/mTOR signaling and PNET pathogenesis is needed to improve current therapies. We discovered that RABL6A, a new oncogenic driver of PNET proliferation, is required for AKT activity. Silencing *RABL6A* caused PNET cell cycle arrest that coincided with selective loss of AKT-S473 (not T308) phosphorylation and AKT/mTOR inactivation. Restoration of AKT phosphorylation rescued the G1 phase block triggered by *RABL6A* silencing. Mechanistically, loss of AKT-S473 phosphorylation in RABL6A depleted cells resulted from increased protein phosphatase 2A (PP2A) activity. Inhibition of PP2A restored phosphorylation of AKT-S473 in RABL6A depleted cells whereas PP2A reactivation in control cells using a specific small molecule activator of PP2A (SMAP) abolished that phosphorylation. Importantly, SMAP treatment effectively killed PNET cells in a RABL6A-dependent manner and suppressed PNET growth *in vivo*. This work identifies RABL6A as a new inhibitor of the PP2A tumor suppressor whose expression is required for AKT signaling in PNET cells. Our findings offer novel targets, PP2A and RABL6A, for PNET therapy.

Abstracts

12 **Gi/o-GPCR signaling promotes cancer stem cell tumorigenicity and HER2-driven breast cancer progression**

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Cancer stem cells (CSCs) are a subpopulation of stem-like cells that contribute to tumor initiation and progression, therapy resistance and metastatic dissemination of $HER2^+$ breast cancer. In this study, we showed a subset of G protein-coupled receptors that signal through the Gi/o proteins (Gi/o-GPCRs) were overexpressed in human HER2-amplified breast cancer cell lines and tissues as well as mouse CSCs from HER2-driven mammary tumors. They were required for proliferation of CSCs *in vitro* and *in vivo*. Blocking Gi/o-GPCR signaling via mammary gland-specific expression of an inducible inhibitor, pertussis toxin, suppressed HER2-driven spontaneous formation of mammary tumors and lung metastasis. Further studies revealed aberrant HER2 signaling upregulated Gi/o-GPCR expression in breast cancer cells, which in turn activated EGFR/HER2 via Src and PI3K pathways. Targeting Gi/o-GPCR signaling sensitizes $HER2^+$ breast cancer to HER2-targeted therapies. Together, our data demonstrate that targeting Gi/o-GPCR signaling may represent a new approach to eliminate CSCs to augment HER2 therapeutics.

13 **Synthesis of Quinazoline-2,4-Dione Dimers**

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Bacterial type II topoisomerases are targets for broad spectrum antibiotics. There are typically two different type II topoisomerases in bacteria, DNA gyrase and topoisomerase IV, and these topoisomerases are responsible for relaxing positive supercoiling of DNA. Fluoroquinolones and other quinolone-class antibiotics bind to DNA and type II topoisomerase to form a drug-DNA-topoisomerase ternary complex. Formation of ternary complex blocks religation of nicked DNA ultimately leading to double strand DNA breaks and cell death. Target-mediated resistance to fluoroquinolones, that being amino acid substitution(s) in gyrase or topoisomerase IV that impedes formation of ternary complex, is a significant clinical problem. Quinazoline-2,4-diones (diones) bind bacterial type II topoisomerases and form a ternary complex similar to fluoroquinolones. Many diones are equipotent with wild-type and fluoroquinolone-resistant topoisomerases, and thus overcome target-mediated fluoroquinolone resistance. However, diones have lower absolute antibacterial potency than fluoroquinolones because diones are excellent substrates for bacterial efflux pumps. Past work in the Kerns lab has shown that C7-linked fluoroquinolone dimers overcome efflux-based fluoroquinolone resistance in Gram-positive bacteria while maintaining or having improved antibacterial activity with different Gram-positive organisms. Fluoroquinolone dimers also overcome target-mediated resistance to fluoroquinolones based on mutations in topoisomerase IV by inhibiting gyrase more potently. Currently, I am exploring strategies for synthesizing diones that can overcome or evade efflux-based resistance. Guided by the previous work showing fluoroquinolone dimers evade bacterial efflux pumps, I am synthesizing C7-linked dione dimers for similar evaluation. The different routes and methods for synthesis of dione dimers will be presented.

Abstracts

14 Carnosine is a potent scavenger of biogenic aldehyde metabolites of neurotransmitters

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Parkinson's disease (PD) is a neurodegenerative disease that originates from insults such as oxidative stress within the neuron. Within these dopaminergic cells, the neurotransmitter dopamine (DA) is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) via monoamine oxidase before oxidation to an acid metabolite. DOPAL is a highly protein-reactive aldehyde and toxic to neurons. Increased levels of this toxic aldehyde are hypothesized as chemical triggers for diseases such as PD. Therefore, a scavenger of these electrophiles is predicted to prevent or mitigate cell injury. Carnosine is a dipeptide comprised of the amino acids beta-alanine and histidine. Historically carnosine has been shown to act as an antioxidant, scavenger of reactive oxygen species (ROS), as well as scavenger of alpha-beta unsaturated aldehydes. The hypothesis presented in this study is that carnosine acts as a selective aldehyde scavenger towards toxic neurotransmitter metabolite DOPAL and the aldehyde metabolite of norepinephrine but not towards the primary lipid peroxidation product 4-hydroxy-2-nonenal(4-HNE) or other alpa,beta-unsaturated aldehydes. This reactive behavior is in direct contrast to another well-known ROS and aldehyde scavenger glutathione (GSH).

15 Metabolic Influences of Bisphenol F Exposure in Population-based Heterogeneous Stock Rats

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Bisphenol F (BPF) is marketed as a 'safe' substitute for bisphenol A (BPA), an endocrine disruptor associated with obesity and heart disease (i.e. cardiometabolic disease), in manufacturing polycarbonates and in common consumer products. Characterizing the health effects of BPF in an animal model under controlled exposure conditions is critical; health risks associated with human BPF exposure are vastly unknown. The hypothesis of this pilot project is that Heterogenous Stock (HS) rats are a model for identifying genes contributing to BPF-induced cardiometabolic disease based on genetically regulated xenobiotic metabolism and underlying genetic susceptibility.

Littermate pairs of male HS rats were randomly assigned to control and treated groups at wean and exposed to vehicle or 50 µg BPF/kg body weight/day for five weeks in drinking water. Cardiometabolic measures, tissues, urine, and feces were taken to determine if BPF exposure interacts with genome variation to influence metabolic outcomes and genetic regulation of BPF metabolism and excretion. Preliminary data suggest that BPF treatment increases gain in body weight since wean ([#]8 weeks: Vehicle = 156.7±4.9 g; BPF = 164.4±4.0 g, @n=20/group, *p=0.0447). BPF treatment alters body composition determined using nuclear magnetic resonance (NMR) at seven weeks by increasing Fat% (Vehicle = 8.0±0.3%,; BPF = 9.0±0.4%, n=23/group, *p=0.0913) and decreasing Lean% (Vehicle = 65.1±0.3%; BPF = 64.3±0.3%, n=22/group, *p=0.016), mirroring the significant increase in body-weight-adjusted gonadal and peritoneal white adipose tissue (WAT) mass in eight-week-old BPF males (GWAT: Vehicle = 7.9±0.4 mg/g; BPF = 9.1±0.5 mg/g, n=21/group, *p=0.0354; PWAT: Vehicle = 10.0±0.5 mg/g; BPF = 12.7±0.6 mg/g, n=20/group, *p=0.0015). Preliminary data indicate that BPF treatment increases body weight and adiposity, which are risk factors for cardiometabolic disease. Future studies will determine if the response to BPF is genetically regulated.

[#]Measures reported as Mean±SEM, [@]n = number of litters, *two-tailed paired t-test

Abstracts

16 **CRISPR-Cas9 Gene Editing Yields a Novel Rat Model of Cardiometabolic Disease**

Karen C. Clark, Valerie A. Wagner, Jacob A. Malek, Alyssa E. Arroyo, Nicole A. Pearson, Justin L. Grobe. PhD, Anne E. Kwitek, PhD

Despite strong evidence that the Metabolic Syndrome (MetS) and its defining features (central obesity, dyslipidemia, hypertension, hyperglycemia) are highly heritable, the genetic etiology is complex and relatively few causative genes are known. For this reason, we employ the Lyon Hypertensive (LH) rat, a well-characterized genetic model of MetS. Using several genetic and computational approaches, we identified a novel gene (*C17h6orf52*) on rat chromosome 17 as a putative master regulator of gene expression that broadly affects several features of cardiometabolic disease. Currently, we are evaluating *C17h6orf52* knockout rats to determine this gene's effects *in vivo*. In this study, frameshift mutations by CRISPR-Cas9 in exon 2 of *C17h6orf52* are shown to change a variety of MetS phenotypes, including body composition, body weight, blood pressure and serum cholesterol in LH-derived female rats.

C17h6orf52$^{-/-}$ (*C17*$^{m2/m2}$) females have significantly more body fat mass than wild-type control at 8 weeks of age, measured by nuclear magnetic resonance (NMR) as a percentage of total body weight ($^{\#}$WT=5.18±0.137, n=3; *C17*$^{m2/m2}$=6.74±0.387, n=5; *p<.05), and this difference increases by 11 weeks of age (WT=5.57±0.094, n=3; *C17*$^{m2/m2}$=8.08±0.413, n=5, *p<.01). In addition, *C17*$^{m2/m2}$ females have increased body weight (18 weeks: WT =228.6g±9.101, n=4; *C17*$^{m2/m2}$=247.7g±3.414, n=6; $^{\$}$p=.052), and increased diastolic blood pressure after a 4% dietary salt challenge (WT=98.97mmHg±.623, n=4; *C17*$^{m2/m2}$=104.9mmHg±1.549, n=6, *p=.08). Finally, female mutants develop higher serum cholesterol (WT=189.6mg/dl±11.64, n=5; *C17*$^{m2/m2}$=220.9mg/dl±12.63, n=5; $^{\$}$p=.1), as well as significantly higher LDL cholesterol (WT=16.16mg/dl±3.472, n=5; *C17*$^{m2/m2}$=25.18mg/dl±1.633, n=5; $^{\$}$p=.0466)

These data suggest that *C17h6orf52* is *protective* against cardiometabolic risk factors common to MetS, given that mutation confers a greater burden of MetS features on an already obese and hypertensive rat. The continued study of this rat model of Metabolic Syndrome has the potential to functionally validate an uncharacterized regulatory gene, and provide novel targets for pharmacological intervention in the treatment of obesity.

$^{\#}$all measures are presented as mean±SEM, *RM 2-WAY ANOVA, Sidak's correction, $^{\$}$Students two-tailed t-test

Abstracts

17 **Sexually Dimorphic Metabolic or Lethal Consequences of Disrupting Angiotensinogen in Agouti Related Peptide-Expressing Cells**

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Genetic disruption of angiotensin (ANG) type 1A receptors (AT1A) in cells which express Agouti-related peptide (AgRP) abolishes thermogenic sympathetic nerve activity (SNA) and resting metabolic rate (RMR) responses to leptin and high fat diet (HFD). Although it is established that glia secrete angiotensinogen (AGT), *in silico* reanalysis of published single-cell RNAseq datasets describing transcriptomes of cells from mouse hypothalamus (GSE74672) and RNAscope fluorescent *in situ* hybridization independently confirm that AgRP neurons of the hypothalamic arcuate nucleus (ARC) in adult wildtype mice express AGT mRNA. We therefore sought to explore the functional significance of this expression pattern. Why, if AgRP neurons are bathed in AGT in the interstitial space, would they synthesize AGT? Mice were generated which lack AGT specifically in AgRP cells ($AGT^{AgRP\text{-}KO}$ mice) using the Cre-Lox approach (sire: AgRP-Cre^{+}, $AGT^{F/wt}$ x dam: AgRP-$Cre^{-/-}$, $AGT^{F/F}$). Of the first 116 offspring, this resulted in expected overall distributions across sex (53% female), Cre (47% Cre^{+}) and AGT (46% $AGT^{F/F}$) genotypes, but an underrepresentation of targeted $AGT^{AgRP\text{-}KO}$ mice at weaning (n=5 females + 1 male; X^2=52.0, df=3, p<0.001), indicating pre-weaning or *in utero* lethality of the genotype. Before and at 8 weeks of age, female $AGT^{AgRP\text{-}KO}$ mice exhibited normal body mass (n=5, 17.3±0.4 vs n=12 littermates 17.3±0.3 g); 5 weeks of 45% HFD caused significant weight gain in all mice (p<0.05), but $AGT^{AgRP\text{-}KO}$ mice gained less (body mass: 18.3±0.4 vs 19.7±0.3 g, p<0.05, and fat mass by NMR: 1.6±0.4 vs 2.3±0.2 g, p<0.05), which was not due to suppression of food intake (p=NS) or digestive efficiency as assessed by bomb calorimetry (p=NS). Furthermore, preliminary data suggest reduced total Aldosterone (n=5, 3576±1680 pg vs n=9 littermates 6921±1089 pg/day) and Corticosterone (n=6, 430.6±147.5 ng/day vs n=9 littermates 923.5±226.5 ng/day) levels in the urine of $AGT^{AgRP\text{-}KO}$ mice compared to control littermates. Finally, male $AGT^{AgRP\text{-}KO}$ mice exhibit reduced average kidney mass and altered gross histology compared to control littermates (n=1, 0.09 g vs n=14 littermate, 0.16±0 g). We conclude that (i) AGT is expressed by AgRP cells of the ARC, (ii) disruption of AGT in AgRP cells causes a developmental lethality that is more penetrant in males, and (iii) in surviving females, the disruption of AGT in AgRP cells causes resistance to weight gain through increased energy expenditure.

18 **A Modular Avidin-Based Nanoparticle System for Targeted Gene Delivery to the Liver**

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Non-viral gene delivery to the liver faces many challenges including maintaining DNA stability in circulation, avoiding macrophage uptake, and selectively inducing hepatocyte gene expression. Previously we developed a plasmid DNA carrier system utilizing a PEGylated polylysine/acridine peptide capable of condensing DNA into nanoparticles. These nanoparticles exhibit circulatory stability and hepatocyte gene expression in mice under hydrodynamic dosing, but lack intrinsic liver targeting. This study reports on the use of tetrazine click-chemistry to append NeutrAvidin to the surface of nanoparticles as a modular system for attachment of biotinylated targeting proteins. To furnish the reactive handle, a heterobifunctional 5-kDa PEG was synthesized containing the tetrazine moiety on one end and a maleimide on the other for attachment to the DNA-condensing peptide. The counterpart, *trans*-cyclooctene (TCO) labeled NeutrAvidin, was constructed using NHS ester coupling. Gel filtration chromatography and dynamic light scattering verified the covalent linkage of NeutrAvidin-TCO to the DNA nanoparticles. The sizes of NeutrAvidin-labeled nanoparticles were found to be dependent on the mole percentage of tetrazine in the nanoparticles. Furthermore, NeutrAvidin nanoparticles were functionalized with the biotinylated targeting proteins apolipoprotein E (LDL receptor-specific), *Sambucus nigra* lectin (sialic acid specific), and *Erythrina cristagalli* lectin (galactose specific). A HepG2-based binding assay with fluorescently labeled DNA revealed that the targeting proteins promoted cellular uptake over controls, with the sialic acid-binding lectin displaying the best internalization. Further work involves co-labeling the nanoparticles with biotinylated endosomal escape agents and elucidating the *in vitro* luciferase gene expression of these targeted systems. This novel strategy thus provides a modular platform for rapidly testing targeting ligands and optimizing targeted non-viral gene delivery systems.

Abstracts

19 **Investigating the Metabolic Changes in Response to Force on E-cadherin**

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Response to mechanical force is a major determinant of the form and function of cells. These forces are sensed by cell surface adhesion receptors and trigger robust actin cytoskeletal rearrangements and growth of the associated adhesion complex to counter the applied forces. This process is known as cell stiffening or reinforcement. The actin re-arrangements necessary for stiffening are energetically costly suggesting that mechanisms coupling force transduction and energy production might exist. Previous work demonstrated that, in response to force, AMPK is recruited and activated at the E-cadherin adhesion complexes, thereby stimulating actomyosin contractility, glucose uptake, and ATP production. This increase in glucose uptake and ATP is suggested to provide the energy necessary for reinforcement of adhesion complexes and the actin cytoskeleton, however how mechanical force modulates glucose uptake and glucose metabolism is not fully understood. One aspect of this study is to determine how glucose transporter-1 (GLUT1) affects force-induced metabolic changes and cell stiffening. Here we suggest that GLUT1 is the force-sensitive glucose transporter responsible for the force-induced glucose uptake necessary for the growth of adhesion complexes and reinforcement of the actin cytoskeleton. In further support of this notion, we show that GLUT1 is recruited to the cell-cell junctions and forms a complex with E-cadherin in response to force. Furthermore, we present evidence that inhibition of GLUT1 blocks force-induced cell stiffening. This study proposes a novel connection between glucose metabolism and the energy-intensive process of force-induced cell stiffening.

20 **Development of PP2A reduction system to examine B'δ regulatory subunit-specific PP2A activity**

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BACKGROUND: Protein phosphatase 2A (PP2A) activity is essential for eukaryotic cells and is controlled through its many regulatory subunits. Mutations in regulatory subunits have been associated with a wide variety of human diseases from cancer to intellectual disability (ID). With such a milieu of PP2A complexes, we sought to reduce the complexity of regulatory subunits to examine the specific activity of B'd (PPP2R5D). Hypothesis – Engineered mutations in both the Aa and the B'δ will allow for interaction of the specifically engineered PP2A complexes to tease out specific B'δ regulatory function.

METHODS: The crystal structures of PP2A with its regulatory families have been determined and highlight key interacting residues between the A and B subunits. By swapping charged amino acids from the A and B subunits, we sought to generate engineered PP2A complexes that can only interact with each other and not the endogenous subunits. The inclusion of a hairpin to knockdown the endogenous A, and incorporation of the hairpin resistance into the engineered Aa, would reduce the overall complexity of the PP2A. To further increase expression, we integrated in one plasmid the bicistronic expression of the engineered Aa and the B'δ, along with the Aa hairpin, to provide stoichiometric ratios of the desired PP2A complex.

RESULTS: By swapping charges from the Aa and the B'δ, we see specific interaction of the engineered subunits. The engineered B'δ was not able to interact with the endogenous A subunits as expected. For the engineered Aa, all the endogenous subunits no longer bound except for the B'δ wildtype. The additional heat repeat at the c-terminus of B'δ appeared to stabilize this interaction as a deletion of the region eliminates binding. The incorporation of the Aa hairpin and the bicistronic mRNA expression of both the engineered Aa and the B'δ greatly reduced the overall PP2A complexity. We then used HEK293 cells to generate stable cell lines with an inducible PP2A reduction to determine the B'δ specific consensus sequence. This charge reversal with may allow for the development of PP2A reduction models for other B subunits.

CONCLUSION: The PP2A reduction model allows for reducing the complexity of PP2A and the determination of specific regulatory subunit directed activity.

Abstracts

21 **Determining how Pesticides alter Dopamine Metabolism**

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Pharmaceutical Sciences and Experimental Therapeutics

Pesticide exposure has been linked to Parkinson's disease (PD) and other neurodegenerative disorders. The "catechol-aldehyde" hypothesis that a buildup of intermediate aldehydes lead to neurotoxicity may underlie the neurotoxicity of pesticides. Dopamine (DA) is metabolized to a toxic catechol-aldehyde - 3,4-dihydroxyphenylacetaldehyde (DOPAL) - by monoamine oxidase (MAO) and then detoxified by aldehyde dehydrogenase (ALDH). Pesticides such as dieldrin and rotenone have been shown to affect ALDH activity and lead to an increase in DOPAL leading to toxicity. DOPAL toxicity can occur through protein modification and the formation of protein adducts. This work shows that treatment of dopaminergic N27 cells with DOPAL at non-toxic concentrations decreases the expression of the dopamine transporter (DAT). Dat modification could cause irregularities in DA cell trafficking. In addition, this study examined how DA metabolism is altered by organophosphate and pyrethroid pesticides, specifically chlorpyrifos and cypermethrin. These are insecticides that are neurotoxic to insects as well as humans. Cypermethrin has been shown to cause nigrostriatal degeneration with long-term exposure and may act synergistically with chlorpyrifos. We found that cypermethrin is taken up into dopaminergic N27 cells showing that it has potential to affect dopaminergic cells. Neither cypermethrin nor chlorpyrifos were found to be toxic at low micromolar concentrations using dopaminergic N27 cells. However, they could be acting together to cause increased toxicity. Understanding the mechanism of these insecticides and how they affect dopamine metabolism will further our understanding of PD and neurodegenerative diseases and be helpful in developing therapeutic strategies.

22

Determining the molecular identity of the mitochondrial Na^+/Ca^{2+} exchanger in neurons

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In neurons, mitochondria efficiently buffer Ca^{2+} influx during excitation, and then release Ca^{2+} back into the cytosol, which helps shape $[Ca^{2+}]_i$ transients and regulates many Ca^{2+}-dependent neuronal functions. The putative identity of the uniporter required for mitochondrial Ca^{2+} efflux was previously reported as the Na+/Ca^{2+}/Li+ exchanger known as NCLX (SLC8B1). However, our preliminary data in neurons showed that NCLX did not localize to mitochondria and that NCLX KO did not affect mitochondrial Ca^{2+} extrusion. As prior studies examined the role of NCLX in HeLa cells rather than neurons, we next used simultaneous imaging of cytosolic and mitochondrial $[Ca^{2+}]_i$ in HeLa cells and found that there was no effect of shRNA knockdown of NCLX in either Ca^{2+} amplitude or response duration to histamine, arguing against the role of NCLX in this process. To identify other genes involved in mitochondrial Ca^{2+} regulation we compared gene arrays from mice deficient in mitochondrial Ca^{2+} influx (MCU KO) to WT and identified the $Na^+/Ca^{2+}/K^+$ exchanger 2 (NCKX2/SLC24a2) as a putative target. In neurons, we found that NCKX2 was primarily localized to the mitochondria, suggesting a possible role of NCKX2 in mitochondrial Ca^{2+} extrusion. In Hela cells, we found that NCLX localized primarily to the ER whereas NCKX2 was localized to the mitochondria or Golgi. Overall, these data suggest that NCLX is not the main regulator of mitochondrial Ca^{2+} extrusion and indicate a potential role instead for NCKX2. Future studies will evaluate Ca^{2+} signaling in NCKX2 KO mice as well as NCLX conditional knockout mice.

Abstracts

23 **Exposure to Environmental Stress, High Salt Diet, and Angiotensin II in Mice Lacking the Brain-specific Alternative Renin Isoform Renin-b**

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Previous experience to various hypertensive conditions including psychosocial stress, high salt diet and exposure to Angiotensin II (Ang II) elevation enhances the response to subsequent hypertensive challenges. Importantly, the activation of the brain renin angiotensin system (RAS) is required for the sensitization to these hypertensive challenges. We previously reported that the brain-specific renin isoform (Ren-b) tonically inhibits the activation of the brain RAS and the ablation of Ren-b results in brain RAS disinhibition leading to blood pressure (BP) elevation. Interestingly, we observed a high degree of variability in the BP between Ren-b KO cohorts that can be attributed to different levels of environmental stress. Thus, BP and heart rate (HR) from five separate experimental cohorts which were subjected to different levels of stress were re-analyzed and compared. In cohorts that were not subjected to any surgical procedures, Ren-b KO mice housed in a stressful environment exhibited higher systolic BP (cohort 1, KO: 130 ± 1 vs WT: 122 ± 1 mmHg, $p<0.05$) in comparison with animals housed in a quiet and more regulated environment (cohort 2; KO: 116 ± 1 mmHg vs WT: 116 ± 1). BP was selectively higher in Ren-b KO mice subjected to a surgical procedure required to study a BP mechanism (Cohort 3, KO: 137 ± 2 vs WT: 117 ± 3, $p<0.05$; Cohort 4, KO: 139 ± 2 vs WT: 112 ± 6, $p<0.05$; Cohort 5, KO: 137 ± 3 vs WT: 113 ± 3 mmHg, $p<0.05$). Similarly, elevation of BP was associated with increased HR. Although it is likely that other factors might also contribute to phenotypic differences between these cohorts, namely genetic drift or changes in bedding and microbiome, this reanalysis of pre-existing data suggests that Ren-b KO mice might be sensitive to environmental stressors. Next, we hypothesized that Ren-b KO mice are sensitized to high salt diet. Ren-b KO fed 4 % NaCl diet did not manifest an increase in mean BP (WT: 113±2 vs KO: 109±2 mmHg, $p=0.18$), indicating that Ren-b ablation does not cause salt sensitivity. Finally, we evaluated whether Ren-b KO are sensitized to Ang II. Continuous Ang II infusion (400 ng/kg/min; subcutaneous) induced a similar increase in tail cuff BP between WT vs Ren-b KO, but Ren-b KO exhibited a prominent elevation in water intake (WT: 3.3±0.3 vs KO: 5.1±0.5 ml/day, $p<0.05$) and urinary volume excretion (WT: 1.3±0.1 vs KO: 2.7±0.6 ml/day, $p<0.05$). We conclude that disinhibition of brain RAS by the ablation of Ren-b leads to enhanced sensitivity to hypertensive challenges that elevate circulatory/peripheral Ang II.

24 **Epigenetic Combination Therapy for the Treatment of Soft Tissue Sarcoma**

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Sarcomas are a diverse group of connective tissue tumors that comprise approximately 1% of adult cancers and 15% of pediatric cancers. The profound heterogeneity of sarcomas presents a unique challenge when trying to understand and treat these malignancies. Surgical resection is often a first-line treatment, followed by adjuvant radiotherapy. To date, few molecular targets have been identified for chemotherapeutic treatment. Epigenetic therapeutics, a group of pharmacologic agents that alter gene expression at the level of transcription, have recently been trialed and shown promise in the treatment of several types of cancer. Decitabine (DAC) is an epigenetic drug that inhibits DNA methyltransferase 1, resulting in hypomethylation of genes in cycling cells. We are investigating DAC as part of a combination therapy for the treatment of solid cancers using a unique primary mouse model of sarcoma developed by our lab. Our preclinical data show that DAC combined with Gemcitabine (Gem), an antimetabolite routinely used in the treatment of solid malignancies, slows tumor growth and extends survival in our sarcoma mouse model better than single-agent treatment alone. We aim to elucidate the mechanisms behind the combination therapy using both our mouse model and patient samples from an ongoing Phase 1b clinical trial at the University of Iowa.

Abstracts

25 **Interference with PPARγ in the Endothelium Produces Endothelial Dysfunction in the Cerebral Circulation in Response to Activation of the Endogenous Renin-Angiotensin System**

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Low salt diet (LSD) is beneficial in salt-sensitive hypertension but may provoke cardiovascular risk in patients with heart failure, diabetes, or other cardiovascular abnormalities because of renin-angiotensin system (RAS) activation. PPARγ is a transcription factor which promotes an anti-oxidant pathway in the endothelium. We studied transgenic mice expressing a dominant-negative mutation in PPARγ selectively in the endothelium (E-DN) to test the hypothesis that endothelial PPARγ plays a protective role in response to LSD-mediated RAS activation. Plasma renin and angiotensin were significantly and equally increased in all mice fed LSD for 6-weeks. Vasorelaxation to acetylcholine was not affected in basilar artery from E-DN at baseline, but was significantly and selectively impaired in E-DN after LSD (33±5 vs 69±2%, $p<0.05$, n=6). Unlike basilar artery, LSD was not sufficient to induce vascular dysfunction in carotid artery. Endothelial dysfunction in the basilar artery from E-DN mice fed LSD was attenuated by scavengers of superoxide (improved from 29±5% to 55±7%, n=6), inhibitors of NADPH oxidase (improved from 23±3% to 54±7%, $p<0.05$, n=6), or blockade of the angiotensin-II AT1 receptor (improved from 31±5% to 64±9%, $p<0.05$, n=5). Gene expression levels of Nox2 was elevated (2.1±0.3 vs 0.4±0.1, $p<0.05$, n=7) while those of antioxidant enzymes catalase and SOD3 were blunted in cerebral vessels of E-DN mice on a LSD (catalase: 0.5±0.1 vs 2.5±0.2; SOD3: 0.2±0.1 vs 1.1±0.1, $p<0.05$, n=7). Simultaneous AT1 and AT2 receptor blockade revealed the restoration of endothelial function after AT1 receptor blockade was not a consequence of AT2 receptor activation (59±10 vs 48±2, $p<0.05$, n=4). We conclude that interference with PPAR γ in the endothelium produces endothelial dysfunction in the cerebral circulation in response to LSD-mediated activation of the endogenous RAS, mediated at least in part, through AT1 receptor activation and perturbed redox homeostasis.

26 **The Angiotensin AT_{1A} Receptor Couples to G-alpha-i in Agouti-Related Peptide-Expressing Neurons to Control Resting Metabolic Rate**

Guorui Deng, PhD., Sarah A. Sapouckey, BS., Eva N. Rodriguez-Cruz, PhD., Charles A. Warwick, PhD., Yuriy M. Usachev, PhD., Julien A. Sebag, PhD., Huxing Cui, PhD, Justin L. Grobe, PhD.

We recently demonstrated that leptin stimulates resting metabolic rate (RMR) through a mechanism that requires angiotensin (ANG) type 1A receptors (AT_{1A}) localized to neurons of the arcuate nucleus (ARC) which express Agouti-related peptide (AgRP). Genetic disruption of AT_{1A} in AgRP neurons results in the loss of RMR responses to leptin, high fat diet, and various other stimuli which correlates with the disinhibition of AgRP, neuropeptide Y (NPY), and production enzymes and transporters (GAD1, GAD2, VGAT) for γ-aminobutyric acid (GABA) within the ARC. We hypothesize that AT_{1A} activation in AgRP neurons causes disinhibition of AgRP, NPY, GAD1, GAD2 and VGAT expression and thus increased inhibitory neurotransmission to pre-autonomic nuclei. To understand the second-messenger network activated by AT_{1A} which mediates transcriptional control of these genes, intracellular calcium ($[Ca^{2+}]_i$), cyclic AMP (cAMP), receptor surface localization, and gene expression responses to ANG were examined in immortalized mouse hypothalamic cells that express typical markers of AgRP neurons. ANG (0.1 and 1 μM) had no effect to modulate $[Ca^{2+}]_i$, but caused a dose-dependent reduction in forskolin-stimulated cAMP which could be blocked by losartan. ANG increased GTP-bound Gαi (vehicle = 0.57±0.08, ANG 1 μM = 0.94±0.07 ratio vs IgG, $p < 0.05$), reduced cell-surface localization of HiBiT-tagged AT_{1A} (vehicle = 0.22±0.02, ANG 0.1 μM = 0.15±0.01, $p < 0.05$), and significantly reduced AgRP and GAD1 expression by 43% and 27%, respectively. Lastly, preliminary studies suggest that pretreatment with pertussis toxin (PTX), an inhibitor of Gαi, abrogated AgRP and GAD1 suppression by ANG. Collectively these findings support the novel concept that within immortalized cells that express markers of AgRP neurons, AT_{1A} couples to Gαi to reduce cAMP, which suppresses AgRP, NPY and GABA. This should disinhibit pre-motor, pre-autonomic circuits within the hypothalamus, resulting in increased thermogenic sympathetic nerve activity, ultimately increasing RMR and energy expenditure.

Abstracts

27 Elucidating the mechanism of how phosphorylation of GR by Erk2 Attenuates function

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Glucocorticoids (GCs), are among the most widely prescribed drugs and are used to treat a variety of disorders from asthma to blood cancers. Although highly effective GCs have limited use due to severe side effects. Recently, we have explored limiting side effects by potentiating GC activity in the tissue of interest. Specifically, we are working on understanding how GCs work to kill leukemia cells in B-cell acute lymphoblastic leukemia (B-ALL) patients. Understanding how this works has the potential to help create more efficient drugs and therapies. We identified a set of proteins in the RAS/MAPK pathway that when knocked down sensitize cells to dexamethasone (a GC). This sensitizing happens by affecting the glucocorticoid receptor (GR), a GC-activated transcription factor. Inhibition of the lymphoid-restricted PI3Kδ reduces phosphorylation of GR and results in enhanced GR function. Also, inhibition of the terminal kinase in the RAS/MAPK pathway, Erk2, also enhanced GR function, suggesting that it is the kinase that modifies GR. The goal of this project is to understand how phosphorylation of GR by Erk2 inhibits its function. I have expressed and purified Erk2 and GR from *E. coli* and used the kinase to phosphorylate GR in vitro. I have conducted preliminary binding assays which suggest phosphorylated GR has a decreased affinity for DNA. Further experiments will help elucidate the mechanism of this so that we can start to understand how GR phosphorylation by Erk2 changes the transcription factors activities and how that may work to specifically kill leukemia cells.

28 Modulatory Role of RGS2 in MC4R Signaling Pathway for Metabolic Regulation

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The prevalence of obesity and associated disorders, such as diabetes and hypertension, has reached alarming levels worldwide. While there is a consensus that the central nervous system (CNS) plays a key role in these pathological processes, the underlying neural substrates mediating these effects remain incompletely understood. Central melanocortin signaling, mainly via acting on melanocortin-4 receptor (MC4R), is one of the key signaling pathways in the brain essential for homeostatic regulation of energy balance, as loss of function of MC4R develop severe obesity in both humans and rodents. MC4R is a G protein-coupled receptor (GPCR) exclusively expressed in the CNS including those hypothalamic nuclei known to regulate energy balance. In spite of tremendous interests in developing anti-obesity therapeutics by targeting brain MC4R signaling, our current knowledge about the downstream signaling cascades and potential modulators of this GPCR is still surprisingly limited. Regulators of G-protein signaling (RGSs) are known to negatively modulate GPCR signaling by accelerating the hydrolysis of GTP bound to an active Gα subunit. Here we found that one of RGS family members, RGS2, is co-expressed with MC4R in the paraventricular nucleus of hypothalamus (PVN) which is major site where MC4R acts to control whole body energy homeostasis. Additionally, the expression level of RGS2, but not other RGS members, was significantly elevated in the hypothalamus of obese MC4R-null mice, but not in similarly obese wild-type mice fed high-fat diet. Interestingly, RGS2-null mice, which were lean and had elevated basal metabolic rate compared to their control littermates, exhibited an exaggerated response to intracerebroventricular administration of MC4R agonist to increase the resting metabolic rate. These observations suggest that RGS2 may act downstream of MC4R to negatively regulate MC4R signaling and thereby affect whole energy metabolism. Detailed mechanistic study aimed at understanding the potential modulatory role of RGS2 in MC4R signaling pathway is underway.

Abstracts

29 **RGS6 protein profiling reveals multi-isoformic expression in mouse tissues and a novel brain-specific isoform**

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RGS proteins modulate the magnitude and duration of G protein-coupled receptor (GPCR) signaling by facilitating heterotrimeric G protein inactivation, a function bestowed by their RGS domain. RGS6 is a member of the R7 subfamily distinguished by two additional domains, DEP and GGL, which target these proteins to the membrane and promote their stability, respectively. RGS6's cellular roles are also likely affected by mRNA splicing and alternative domain inclusion/exclusion. Indeed, we previously identified multiple RGS6 splice variants predicted to produce 36 distinct RGS6 protein isoforms containing either long (RGS6L) or short (RGS6S) N-terminal domains, an incomplete or intact GGL domain, and 9 alternative C-termini. While sequence similarities have complicated the study of individual RGS6 protein isoforms, we and others have demonstrated that RGS6-specific inhibition of GPCR-$G\alpha_{i/o}$ signaling is critical for the modulation of several CNS disorders and cardiac function/dysfunction. Furthermore, RGS6 has unique G protein-independent functions required for its beneficial tumor suppressor role as well as its detrimental roles in mediating alcohol-induced peripheral toxicity. Given the complexity in RGS6 protein structure and its diverse functions, it seemed imperative to conduct a comprehensive analysis of mouse whole-body RGS6 protein isoform expression. Therefore, we developed RGS6-specific antibodies that recognize all RGS6 protein isoforms (RGS6-fl), that selectively detect the N-terminus of RGS6L isoforms (RGS6-L), and that detect an 18 amino acid alternate C-terminal sequence (RGS6-s) present in 25% of predicted RGS6 proteins. Using these antibodies we demonstrated that RGS6 proteins are most highly expressed in CNS tissues, but are also expressed in: lung, kidney, bladder, prostate, heart, omental fat, stomach, intestine, and breast. Furthermore, western analysis revealed that while RGS6 proteins with MWs corresponding to RGS6L+GGL isoforms were expressed in multiple tissues, RGS6L-GGL and RGS6S isoforms were not detected, suggesting they are less stable or are expressed at much lower levels than RGS6L+GGL isoforms. Finally, western analysis identified two novel brain-specific RGS6 protein isoforms of unknown origin and function that are larger (~61 and 69kDa) than the ubiquitously expressed ~56kDa RGS6L proteins. Both brain-specific RGS6 isoforms are recognized by the RGS6-L and RGS6-fl antibodies while only the 69kDa isoform is detected by RGS6-s. Together this data begins to define the functional significance behind the complexity of RGS6 gene processing and further clarifies RGS6's role in normal physiology and pathophysiology by resolving tissue-specific RGS6 protein expression.

30 **Detecting Aldehyde Conjugates Using Near-Infrared Fluorescence**

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Medicinal and Natural Products Chemistry

Parkinson's Disease (PD) is a degenerative disease of the central nervous system characterized by bradykinesia and tremors with a loss of dopaminergic cells in the brain, particularly in the substantia nigra. Dopamine, an important neurotransmitter, is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) by monoamine oxidase and further biotransformed to an acid or alcohol product. DOPAL is a highly reactive metabolite that is toxic to dopaminergic cells, where it is produced. Under normal conditions, DOPAL is further metabolized to the nontoxic acid or alcohol products, however, under pathological conditions or following insult, DOPAL can increase to harmful levels. DOPAL and other biogenic aldehydes are hypothesized as chemical triggers of disease (catecholaldehyde hypothesis) that cause cell death, protein aggregation and oxidative stress. Identifying targets of and quantifying dopamine metabolite protein adducts is valuable because of their implication in PD and may yield elucidation of biomarkers for earlier diagnosis or mechanistic targets for drug discovery. Such findings may yield novel biotechnology to diagnose disease (e.g., PD) earlier and development of therapeutics which address the pathogenic process. This project focuses on the identification of viable biomarkers for determination and quantification of DOPAL conjugates in neuronal cells using near-infrared fluorescence.

Abstracts

31 **Regulator of G protein signaling 6 (RGS6) modulation of pathological α-synuclein accumulation and Parkinson's disease**

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Parkinson's disease (PD) is a devastating, primarily non-familial, age-related neurodegenerative disorder characterized by the progressive loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). The reason why these SNc DA neurons degenerate and why their loss is largely associated with aging is unknown. However, accumulation of α-synuclein aggregates in Lewy bodies is believed to play a crucial role in PD pathogenesis. In support of this hypothesis, β-agonists dramatically and concomitantly reduce α-synuclein expression and the incidence of human PD as well as repress MPTP-induced SNc DA neuron loss in mice (Mittal *et al.*, Science 2017). RGS proteins modulate G protein-coupled receptor (GPCR) signaling by facilitating heterotrimeric G protein inactivation through their GTPase-activating activity (GAP) toward Gα subunits, a function bestowed by their RGS domain. RGS6 ($G\alpha_i$-GAP), a member of the R7 RGS protein subfamily, is restrictively expressed in SNc DA neurons that undergo degeneration in PD and knockout of RGS6 in mice leads to several PD hallmarks, including: late-age onset SNc DA neuron degeneration, reduced nigrostriatal DA, motor deficits, and pathological accumulation α-synuclein in the SNc. $RGS6^{-/-}$ mice also exhibit hyperactive DA D_2 autoreceptor (D_2R) signaling and late-age onset reduction in cAMP/PKA-mediated Drp1 phosphorylation (S656) in SNc DA neurons resulting in smaller and fewer mitochondria, two more hallmarks of PD. Given that β-agonists repress α-synuclein expression through their activation of β2-adrenorecetor-$G\alpha_s$, a GPCR that increases cAMP, and RGS6's role in inhibiting D_2R-$G\alpha_i$ signaling, a GPCR that reduces cAMP, we hypothesized that RGS6, like β-agonists, may function to prevent the pathological accumulation of α-synuclein through modulation of cAMP signaling. Here, we show that RGS6 plays a critical role in protecting against age-onset pathological α-synuclein accumulation in the SNc of 12 and 18mo mice. RGS6 suppression of late-age onset α-synuclein expression and PD is likely due to its ability to increase cAMP signaling through inhibition of D_2R-$G\alpha_i$ signaling in SNc DA neurons. These findings demonstrate that RGS6 is a critical neuroprotective protein in PD pathogenesis and illuminate an entirely novel signaling pathway underlying pathological α-synuclein accumulation in PD.

32 **A catecholamine metabolite induces collagen secretion in human cardiac fibroblasts via RAGE activation: Implications for cardiac fibrosis with diabetes**

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Obesity/diabetes is associated with a hyper-adrenergic state, characterized by substantially increased levels of circulating catecholamines and adrenergic activation. Catecholaldehydes are biogenic aldehydes formed as products of catecholamine metabolism by monoamine oxidase (MAO). Oxidative deamination of norepinephrine and dopamine by MAO generates the catecholaldehydes 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL) and 3,4-dihydroxyphenylacetaldehyde (DOPAL), respectively, and H2O2. These highly reactive electrophiles have been implicated as causal factors in the etiology of neurodegenerative diseases and cardiac injury from ischemia and diabetes. Our lab has recently reported that diabetic patients have higher content and activity of MAO in atrial myocardium as compared with age-matched nondiabetic patients. Further, diabetics have >3-fold more hydroxyproline in their atrial tissue, a marker of fibrosis. Here, we tested the hypothesis that catecholaldehydes induce production and secretion of collagen in human cardiac fibroblasts. Fibroblasts were isolated and cultured from right atrial appendage samples obtained from patients during during cardiac surgery. Cells were then treated with bovine serum albumin (BSA) conjugated with DOPAL, N(6)-Carboxymethyllysine (CML, an advanced glycation end product), and 4-hydroxynonenal (HNE, a n6 polyunsaturated fatty acid-derived aldehyde). Collagen type I and III were measured in the media via immunoblot & ELISA. Surprisingly, treatment with DOPAL increased type I and type III collagen secretion by >5-fold greater than with either CML or HNE ($P<0.05$). Co-treatment with an antagonist for the receptor for advanced glycation end products (RAGE) mitigated the DOPAL-adduct effects. These findings suggest that reactive aldehydes, particularly catecholaldehydes, may contribute to fibrosis in the heart via RAGE-mediated mechanisms.

Abstracts

33 Neuroanatomical basis of PVN MC4R-expressing neurons for sympathetic cardiovascular control

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It is well established that the central melanocortin system is critical for autonomic functions and energy homeostasis mainly via signaling at melanocortin-4 receptor (MC4R). Importantly, while obesity is commonly associated with elevated sympathetic tone and blood pressure, severely obese humans and rodents due to genetic MC4R deficiency exhibit normal to low sympathetic tone and blood pressure, suggesting a significant role of MC4R pathway in mediating obesity-associated sympathoexcitation and hypertension. MC4R is widely expressed in the brain including hypothalamic paraventricular nucleus (PVN) which regulates feeding and sympathetic traffic. However, the neuroanatomical basis of PVN $MC4R^+$ neurons for sympathetic regulation is unclear. The goal of current study is to map the PVN MC4R neural circuits affecting sympathetic tone in mice. To this end, we injected Cre-dependent AAV driving ChR2-eYFP expression into the PVN of MC4R-t2a-Cre knock-in mice, which allow targeted anterograde tract-tracing of PVN $MC4R^+$ neurons throughout the brain. In addition to known brain region for feeding behavior (i.e. parabrachial nucleus), we found broad innervations of PVN $MC4R^+$ neurons to various brain regions important for autonomic-cardiovascular control, including, but not limited to, nucleus of solitary tractus, dorsomotor nucleus of vagus, and ventrolateral medulla. Considerable innervation was also evident in spinal cord, which is further confirmed by Fluoro-gold (FG)-mediated retrograde tracing in the spinal cord (thoracic T6-10) of MC4R-GFP transgenic mouse. Double immunofluorescence labeling of GFP and FG revealed that ~50% (58 out of 116) $MC4R^+$ neurons in the posterior PVN project to thoracic spinal cord. Furthermore, microinjection of synthetic MC4R agonist (MTII) into the PVN evokes ~35% (from baseline) increase in renal sympathetic nerve activity in anesthetized mice. These results provide important insights into understanding the divergent neural circuits by which PVN MC4R signaling differentially regulates metabolic and cardiovascular functions. Functional dissection of these diverging neural pathways using optogenetic/ chemogenetic approaches is ongoing.

34 The Mechanistic Role of Metal Ions, Ca^{2+} and Mg^{2+}, in RGS: G-Protein Interactions

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Regulator of G protein signaling (RGS) proteins are negative regulators of G protein-coupled receptor (GPCR) signaling through their ability to act as GTPase activating proteins (GAPs) for some Ga subunits. The RZ subfamily, of which RGS17 is a member, binds to activated Ga_o, Ga_z, and Ga_{il-3} proteins to modulate downstream pathways, including those involved in formation of cyclic AMP. In contrast to other RGS proteins, less is known about the regulation of RZ family members. Both Crystallization and ^1H-^{15}N 2D HSQC NMR experiments revealed an interaction of the metal ion Ca^{2+} with RGS17 at a defined binding site. Subsequent protein-protein interaction experiments, using AlphaScreen were used to assess the impact of the ions Ca^{2+} and Mg^{2+} on the RGS17 interaction with activated Ga_o. The results indicate that both Ca^{2+} and Mg^{2+} have an effect of promoting the RGS17-Ga interaction. These studies will extend to examining the selectivity and affinity of RGS17 for other physiologically relevant divalent metal cations, such as Zn^{2+}, Cu^{2+}, and Mn^{2+}. In addition, the residues of RGS17 that bind Ca^{2+} are conserved in multiple RGS proteins. The functional impact of metal ion binding is likely not limited to RGS17 and a more in-depth evaluation of these proteins for metal binding deserves further attention.

Abstracts

35 **Molecular Cloning of a Novel Brain-specific RGS6 Isoform Preferentially Expressed in the Substantia Nigra of Humans with Parkinson's Disease**

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RGS proteins modulate G protein-coupled receptor (GPCR) signaling by facilitating heterotrimeric G protein inactivation through their GTPase-activating activity toward Gα subunits, a function bestowed by their RGS domain. RGS6, a member of the R7 subfamily, is critically involved in several CNS disorders where it may be a novel therapeutic target. Remarkably, $RGS6^{-/-}$ mice have reduced anxiety/depression, exhibit diminished alcohol seeking/reward behaviors, and develop Parkinson's disease (PD). RGS6's role in these disorders depends on its ability to inhibit various GPCRs, including: cortical and hippocampal $5\text{-}HT_{1A}$Rs (anxiety/depression), mesolimbic $GABA_B$Rs and D_2Rs (alcoholism), and D_2Rs in the substantia nigra (SNc, PD). Potentially key to RGS6's ability to regulate numerous GPCRs are unidentified domains arising via alternative mRNA splicing. Our initial cloning effort identified 36 RGS6 mRNAs in human brain encoding proteins ≤56kDa. Recently, we identified, in mouse and human, additional brain-specific RGS6 protein isoforms that are larger (~61, 69kDa) than the ubiquitously expressed ~56kDa RGS6L isoforms. The function of these RGS6 isoforms is unknown, but they may be critical for normal CNS function and pathology as both are expressed in brain regions affected by the disorders described above. Here we report the cloning of a novel RGS6 transcript arising via novel exon (Alternative 3, A3) inclusion. This transcript was named RGS6LA3α1 to indicate it resembles the RGS6Lα1 transcript identified in our initial cloning effort. RGS6LA3α1 exhibits near exclusive CNS expression, encodes a protein that co-migrates with the 69kDa brain-specific RGS6, and has a C-terminal extension near the RGS domain that may be a novel protein interaction site or regulatory domain. Of particular interest is the role of the 69kDa RGS6 in PD pathogenesis as its expression is upregulated in the SNc of PD patients relative to other RGS6L proteins which are down-regulated. This latter finding is consistent with our evidence that RGS6 is required for SNc dopamine (DA) neuron survival. However, these data also raise the intriguing possibility that while RGS6L isoforms promote SNc DA neuron survival, RGS6LA3α1/the 69kDa brain-specific RGS6 isoform may fail to do so or even contribute to neuron death. We hypothesize that dysregulated RGS6 transcript splicing, and A3 exon inclusion, could lead to both a physical loss of RGS6L isoforms as well as a functional loss of RGS6-mediated GAP activity due to RGS domain interference caused by C-terminal extension, a prediction supported by molecular modeling. We predict that decreased RGS6L isoform expression and increased expression of the 69kDa protein disinhibits SNc D_2R signaling culminating in cytotoxic DA byproduct accumulation and ultimately SNc DA neuron death. Together, this research begins to elucidate the functional significance of RGS6 alternative mRNA splicing in brain function and pathology.

Abstracts

36 **Reduced Placental Expression of Regulator of G Protein Signaling-2 (RGS2) in Preeclampsia: Association, Consequence, and Cause**

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To date, the early-gestational mechanisms driving pathogenesis of preeclampsia (PreE) remain largely unclear. However, altered G protein signaling has been implicated in PreE, including alterations in GPCR agonists such as vasopressin, endothelin, and angiotensin. Regulator of G protein Signaling 2 (RGS2) is an endogenous terminator of GPCR signaling, and mutations in the *Rgs2* gene are linked to hypertension and increased risk of developing PreE. Therefore, we hypothesized reduced placental *RGS2* may increase the risk of developing PreE by disinhibiting Gαq signaling. *In silico* reanalysis of a publicly-available microarray dataset (GSE75010) revealed a significant reduction in *Rgs2* mRNA in placentas from PreE pregnancies compared to controls (Con n=35, PreE n=49, $p<0.05$). We confirmed this reduction in *Rgs2* mRNA by qPCR using human placental tissue samples (PreE 19% of Con, n=11 vs 9, $p<0.05$) from the University of Iowa Maternal-Fetal Tissue Bank. To examine if reduced feto-placental *RGS2* was sufficient to induce PreE phenotypes, wildtype C57BL/6J female mice were mated with *Rgs2*-deficient (*Rgs2*-KO) sires or their wildtype littermate sires. Compared to dams mated with littermate control sires, dams mated with *Rgs2*-KO sires developed increased diastolic blood pressure (Con 92 ± 2 vs *Rgs2*-KO 98.2 ± 2 [24 hr avg mmHg]; $p<0.05$) and increased proteinuria (18.2 ± 2.2, n=7 vs 28.4 ± 2.8, n=10 mg/day, $p<0.05$). Preliminary histological analysis of placentas from dams mated with an *Rgs2*-KO sire indicate decreased spiral artery number (Con 7.7 ± 0.2 vs *Rgs2*-KO 5.6 ± 0.4) and diameter (Con 128±3 vs *Rgs2*-KO 86±12 μm). Previous studies have identified a CRE sequence in the *Rgs2* promoter that is critical for transcriptional regulation of *Rgs2*. Thus, we hypothesized loss of cAMP/CREB-mediated stimulation may lead to the observed reduction in *RGS2* expression during human PreE. Indeed, reduced phosphorylated CREB (p-CREB) binding was observed in PreE placentas (Con 0.146 ± 0.024 vs PreE 0.070 ± 0.021 % Input; $p<0.05$). However, PreE was not associated with decreases in placental cAMP levels (Con 1.71E-14 ± 3.45E-15 vs PreE 2.40 ± 3.46E-15 mol/mg), leading us to suspect changes in *RGS2* promoter function during PreE. Bisulphite PCR analysis of the *RGS2* promoter revealed no changes in methylation of the RGS2 promoter in PreE versus control placentas. To determine if acetylation of the *RGS2* promoter can regulate expression of *RGS2*, we treated samples with the pan-HDAC inhibitor, SAHA. Preliminary data indicate treatment with SAHA blocks the effect of forskolin (FSK) to stimulate *RGS2* mRNA expression (Veh 1.00±1.34; FSK 2.61±1.45; SAHA 1.0±1.24; FSK+SAHA 1.38±1.44 fold) and p-CREB binding to the *RGS2* promoter (Veh 0.011±0.004; FSK 0.050±0.012; SAHA 0.010±0.002; FSK+SAHA 0.025±0.005 % Input) in immortalized HTR8/SVNeo human trophoblast cells, indicating that the activity of a SAHA-sensitive HDAC is required for p-CREB-mediated stimulation of *RGS2* transcription in trophoblasts. Ongoing experiments are focused on the identification of the specific HDAC involved, and preliminary findings point toward a class II HDAC. Overall, these findings support a role for reduced placental *RGS2* in the pathogenesis of PreE, which appear to involve changes in p-CREB stimulation of the *RGS2* promoter due to altered HDAC activity.

Abstracts

37 Investigating the Role of TBX1 in Beige Adipocyte Development

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Beige, UCP1-positive adipocytes emerge in white adipose tissue in response to adrenergic stimulation. UCP1 activity uncouples oxidative phosphorylation from ATP synthesis allowing beige adipocytes to dissipate lipid and glucose as heat thereby increasing energy expenditure. Since adiposity increases significantly during obesity, it has been postulated that increasing the amount of active beige adipocytes could be therapeutically used to counter the development of obesity. Despite this premise, much remains unknown regarding the transcriptional mechanisms underlying beige adipocyte development although two models have been proposed. In model 1; mature, white adipocytes are induced to express UCP1. In model 2; beige, UCP1 positive adipocytes develop via de novo adipogenesis. T-box transcription factor-1 (TBX1) has been identified as a marker of beige adipocytes in both rodents and humans. However, nothing is known regarding the function of TBX1 in these cells. To test if TBX1 is both sufficient and necessary to convert mature white adipocytes into beige adipocytes, we developed two novel mouse models allowing for adipocyte specific overexpression of TBX1 (TBX1 AdipoTG) and adipocyte specific deletion of TBX1 (TBX1 AdipoKO) in vivo. We discovered that adipocyte TBX1 expression is not sufficient or necessary for the conversion of mature white adipocytes into beige, UCP1 positive adipocytes. Rather, adipocyte TBX1 regulates adipocyte hypertrophy and hyperplasia which may play a role in mitigating the development of glucose intolerance and insulin resistance during diet induced obesity. While continuing to determine the mechanisms by which TBX1 regulates adipocyte size and number, we have also begun to test the role of TBX1 in de novo beige adipogenesis by inducibly overexpressing or deleting TBX1 expression in adipocyte stem cells. The development and characterization of these novel mouse models uniquely positions us to fully elucidate the role of TBX1 in all models of beige adipogenesis.

38 Investigating the Effects of Exercise on Adipose Tissue Metabolism

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Exercise has been reported to induce the beiging of white adipose tissue which is characterized by the induction of UCP1 positive, multilocular adipocytes in classically white adipose depots. UCP1 is localized to the inner mitochondrial membrane and its activity results in the dissipation of the mitochondrial membrane proton gradient. This results in the uncoupling of oxidative phosphorylation from ATP production. The increased uncoupling activity results in enhanced uptake of circulating glucose and lipid, enhanced oxygen consumption and the dissipation of energy as heat; essentially energy wasting. In addition to increasing energy expenditure, exercise initiates catabolic processes in order to maintain circulating energy levels thus sustaining work. During exercise, adipocyte triglycerides are hydrolyzed to release free fatty acids into circulation which are known activators of UCP1. Exercise also stimulates proteolysis resulting in increased circulating amino acids. However, the role of circulating amino acids in exercise induced beiging and UCP1 activity has not been tested. We have initiated a series of studies to test if exercise increased circulating amino acids contribute to the beiging of adipose tissue. We have discovered that voluntary wheel running of wild type, littermate male mice for two weeks at thermoneutrality (30°C) is sufficient to increase UCP1 protein content in subcutaneous white adipose tissue, reduce body weight and increase circulating amino acids. We are now initiating studies to test the potential link between exercise, adipose tissue beiging and amino acid metabolism. These physiological studies position us to determine novel effects of exercise and will provide new insight regarding the relationship between exercise and the beiging of white adipose tissue.

Abstracts

39 **PPARγ in the endothelium protects against endothelial dysfunction induced by Angiotensin II and mitochondrial uncoupling**

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PPARγ is a nuclear receptor transcription factor involved in the regulation of metabolism and vascular function through regulation of target genes. We have previously reported that interference with PPARγ in the endothelium promotes endothelial dysfunction and increases oxidative stress in response to a high fat diet or Angiotensin II (Ang II). We hypothesize that PPARγ plays a role in mitochondrial uncoupling thereby protecting against endothelial dysfunction. We evaluated vascular responses to acetylcholine (ACh) in basilar and carotid arteries from transgenic mice expressing dominant-negative mutation in PPARγ specifically in the endothelium (E-V290M), infused with saline or a sub pressor dose of Ang II (120ng/kg/min) for 2 weeks. As expected, Ang II infusion did not cause any changes in systolic blood pressure after 2 weeks either in wild type (WT) or E-V290M mice. Under baseline conditions, ACh-induced endothelial dependent relaxation was not affected in E-V290M and WT mice. After 2 weeks of Ang II, E-V290M mice exhibited a trend for reduced relaxation in the basilar artery. To examine if PPARγ plays a role in mitochondrial uncoupling, the vessels were next pre-incubated with the mitochondrial uncoupler CCCP (Carbonyl cyanide m-chlorophenyl hydrazone, 10^{-6} M for 30 minutes). After CCCP, the relaxation responses to ACh in basilar artery from saline-infused E-V290M mice were reduced, reaching similar levels as observed in Ang II–treated E-V290M. Consistently, in the carotid artery CCCP induced an impairment in vasorelaxation in both E-V290M groups infused with either saline or Ang II, with no effects in WT groups. We conclude that endothelial PPARγ is required to mediate vascular protection against Ang II and mitochondrial uncoupling in E-V290M mice seems to promote deleterious effects in endothelial function independent of Ang II.

40 **Determination of Loperamide Solubility in Co-Solvent Systems Suitable for Application to Open Wounds**

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Background: Chronic wounds are painful, particularly during dressing changes, and patients are often given systemic opioids or non-steroidal anti-inflammatory drugs (NSAIDs) for analgesia. However, long-term opioid administration and long-term oral NSAID administration are associated with adverse effects including addiction and peptic ulcers respectively. Loperamide offers a potential alternative as an opioid drug that provides local analgesia with minimal systemic effects, but its water solubility is low, limiting its potential for incorporation into topical delivery vehicles suitable for open wound application.

Methods: Loperamide solubility was determined in a variety of solvents including propylene glycol and a variety of PEGs. The best performing solvents, PEG200 and propylene glycol, were chosen and combined in ratios of 3:1, 1:1, and 1:3. These combinations were then mixed with water in ratios of 3:1, 1:1, and 1:3 to create nine co-solvent systems (Systems A-I), in which the solubility of loperamide was determined.

Results/Discussion: Loperamide solubility was found to be highest in PEG200 and propylene glycol, which showed solubilities of 29.65±8.57 mg/mL and 69.21±2.44 mg/mL respectively. For the co-solvent systems made using these solvents, loperamide solubility was found to vary inversely with water content, with the systems containing 25% v/v water, systems A, D, and G, showing the highest loperamide solubilities by far, at 75.59±2.83 mg/mL, 71.76±2.46 mg/mL, and 73.40±1.55 mg/mL respectively compared to 11.13±1.05 mg/mL, 12.74±0.39 mg/mL, and 14.99±0.76 mg/mL for the 50% v/v water systems, B, E, and H respectively, and 3.86±0.40 mg/mL, 3.61±0.36 mg/mL and 4.39±0.19 mg/mL for the 25% systems, C, F, and I respectively. Systems A, D, and G were chosen for further development. These systems showed the highest loperamide solubility and will therefore be able to contain the highest concentrations of drug. Future studies include diffusion tests to determine the effects of varying propylene glycol and PEG200 concentration on loperamide permeability.

Abstracts

41 **Induction of Endoplasmic Reticulum Stress Upregulates Renin Expression in Immortalized Mouse Hypothalamic N43/5 Cells**

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Dysregulation of the brain renin-angiotensin system (RAS) has been implicated in many forms of hypertension, including resistant hypertension. However, the mechanism by which the brain RAS is activated is unknown. We have recently published on the activation of brain RAS following disinhibition of renin-a expression after deletion of the brain-specific renin-b isoform. Endoplasmic reticulum (ER) stress, which is an accumulation of unfolded proteins in the ER, has been linked to hypertension as central angiotensin II administration induced ER stress markers. We hypothesize that ER stress induces brain RAS activation by upregulating renin expression. We first tested if ER stress could induce renin expression *in vitro* in a mouse neuroblastoma hypothalamic cell line, N43/5 cells. After treating cells with ER stressor thapsigargin, a SERCA pump inhibitor, for 8 hours we observed transcriptional upregulation of ER stress marker Bip. This same treatment also induced an increase in renin expression. We have previously shown that deletion of renin-b in the brain causes an increase in renin-a expression specifically in the rostral ventrolateral medulla (RVLM). This data suggests that ER stress may induce RAS activation through a similar renin-a disinhibitory mechanism. Under basal conditions, the RVLM of renin-b knockout mice showed no difference in gene expression of ER stress marker Bip or Chop. This suggests that ER stress may be upstream of renin induction. To test this, we treated C57BL/6 mice with tunicamycin via intracerebellar ventricle cannula. We detected a positive endogenous renin transcript signal via *in* situ hybridization bilaterally in the medulla in the vicinity of the RVLM from vehicle and tunicamycin treated mice. We are currently testing if ER stress quantitatively increases renin expression using qPCR. These data extend previous studies showing that RAS activation in the brain causes ER stress by suggesting that ER stress may also induce the brain RAS.

42 **Determining the Role of Presynaptic Ca^{2+} Signaling in the Spinal Cord in Pain Sensitization**

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Chronic pain affects approximately 100 million Americans and only a minority of patients experience satisfactory relief of their pain with currently available pharmaceutics. One type of chronic pain caused by direct injury to the nerve is called neuropathic pain, and it affects ~10% of the overall population. Despite the prevalence, the underlying mechanisms of neuropathic pain are not well-defined and better understanding of the mechanisms that promote sensory sensitization after injury could lead to better treatment of this condition. We hypothesized that one of the potential mechanisms promoting neuropathic pain is central sensitization, or the enhancement of synaptic transmission in the spinal cord. Sensory afferents, responsible for transducing painful stimuli, terminate in the spinal cord at what is called the first sensory synapse. This synapse is a key regulator of pain signaling and aberrant processes at this synapse can lead to an amplification of pain. As most researchers have only examined the post-synaptic signaling of this synapse via patch-clamp recordings, we aim to address a gap in the current knowledge about the potential role of presynaptic signaling in sensory sensitization. Utilizing a unique mouse line that expresses GCaMP specifically in the presynaptic sensory neurons, an *ex vivo* intact spinal cord preparation, and multiphoton microscopy to image presynaptic Ca^{2+} signaling, we are able to evaluate presynaptic Ca^{2+} signaling in the spinal cord with high spatial (~0.5 mm) and temporal (~10 ms) resolution. Preliminary results indicate that neuropathic injury alters basal signaling and responsiveness to sensory modulators.

Abstracts

43 **Mitochondrial Ca2+ Uniporter (MCU) Knockout (KO) Protects Against Neural Network Hyperexcitability and Seizures**

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During neuronal activity, mitochondria buffer cytosolic Ca^{2+} that is subsequently released back to the cytosol. This mitochondrial Ca^{2+} cycling shapes Ca^{2+} signaling and regulates processes such as neurotransmission, gene expression, excitability and cell survival. Critical for mitochondrial buffering is the protein CCDC109A, also known as the mitochondrial Ca^{2+} uniporter (MCU), the pore forming subunit of a greater Ca^{2+} transport complex that allows Ca^{2+} uptake into mitochondria. Previously, we have shown that MCU knockout (KO) significantly alters cytosolic and mitochondrial Ca^{2+} signaling in peripheral and central neurons. Using MCU KO mice and Ca^{2+} imaging we investigated neuronal network excitability *in vitro* using two convulsants, the $GABA_A$ receptor antagonist, bicuculline and an inhibitor of A-type voltage-gated K^+ channels, 4-Aminopyridine (4-AP). Both convulsants (0.2-4 mM for bicuculline and 0.5-10 mM for 4-AP) induced prominent oscillations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) in wild type (WT) cultured hippocampal neurons (12-16 DIV). These $[Ca^{2+}]_{cyt}$ oscillations are known to be driven by bursts of action potentials and synaptic activity, and are synchronized throughout the neuronal network. We found that hippocampal neurons from MCU KO mice were highly resistant to the induction of $[Ca^{2+}]_{cyt}$ oscillations by both convulsants. Given that epileptiform activity *in vitro* was inhibited by MCU KO, we hypothesized that MCU KO mice would be more resistant to seizures. To test this hypothesis, we compared the susceptibility of WT and MCU KO mice to electroshock-induced seizures. We found that WT mice developed maximal tonic hind limb extension seizures with a threshold of 9 +/- 1 mA. In contrast, stimulations up to 30 mA failed to induce maximal seizures in MCU KO mice. Interestingly, a broad panel of behavioral testing failed to detect any sensory, motor or cognitive deficits in MCU KO mice. Patch-clamp examination of synaptic activity showed that frequency of glutamate AMPA receptor-mediated miniature EPSCs significantly decreased whereas frequency of $GABA_A$ receptor-mediated miniature IPSCs significantly increased in MCU KO hippocampal neurons compared to that from WT mice. Our research suggests that MCU regulates neural network (hyper)excitability and identify MCU as a potential new therapeutic target for the treatment of epilepsy and seizures.

Key words: Mitochondria, Calcium, Seizures

44 **WDR26 regulates an AKT-Gsk3-Wnt/b-catenin signaling cascade to maintain the breast cancer stem cell population and controls cancer metastasis**

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Cancer metastasis is the major cause of tumor mortality and has been attributed in part to the presence of a minority subpopulation of cancer stem cells (CSCs) in the bulk of tumor cells. We showed previously that WDR26, a scaffolding/adaptor protein that is highly upregulated in breast cancer, promotes breast cancer growth and metastasis. Here we show WDR26 was required for maintaining the CSC populations in breast cancer cells and the formation of lung metastases. Downregulation of WDR26 in breast cancer cells impaired the CSC-like activities and reduced the CSC population. Mammary gland-specific deletion of WDR26 in the MMTV-PyMT mouse model of breast cancer did not affect primary tumor formation but abolished spontaneous lung metastasis. WDR26 promoted β-catenin activation via AKT and GSK3, and the activity of GSK3 and β-catenin was required for maintaining the CSC population in breast cancer cells. Our results have identified a novel, WDR26-dependent pathway that may link breast CSC activities to tumor metastatic potential.

Abstracts

45 Mapping a novel endocrine circuit regulating alcohol consumption

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In the United States alcohol use disorder (AUD) affects ~15% of adults with the prevalence of binge drinking on the rise in adolescents and young adults. AUD represents a major issue to healthcare given that chronic excessive alcohol consumption in humans is associated with cardiovascular disease, metabolic syndrome, and cancer while acute alcohol intoxication can prove lethal. Economically, AUD represents a massive burden due to loss of productivity and associated healthcare costs. Recently, the endocrine hormone fibroblast growth factor 21 (FGF21), known for its potent metabolic effects, was illustrated to significantly reduce alcohol consumption via an undescribed mechanism requiring expression of the obligate FGF21 co-receptor ß-klotho (KLB) in the brain. Importantly, single nucleotide polymorphisms (SNPs) in both FGF21 and KLB genomic loci are highly associated with increased alcohol consumption in humans. Here we extend those findings illustrating that FGF21 can reverse alcohol consumption even in mice chronically administered ethanol prior to FGF21 administration. Furthermore, excessive alcohol consumption promotes FGF21 secretion from the liver perhaps representing a homeostatic feedback loop to regulate alcohol consumption. However, the target of FGF21 in the brain mediating these effects remains unclear. Excitingly, we have identified KLB expressing neurons activated by FGF21. Additionally, deletion of KLB in glutamatergic neurons significantly increases alcohol consumption in mice. These findings represent a novel endocrine circuit regulating alcohol consumption in an FGF21 dependent manner. Future studies will focus on mapping this circuit and characterization of neurons expressing KLB which are activated by FGF21.

46 Single Molecule Observation of the Rad52 Recombination Mediator Mechanism

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Homologous recombination (HR) is an essential pathway that repairs deleterious forms of DNA damage such as interstrand DNA crosslinks, stalled replication forks and double strand breaks. While deficiency in HR is risk factor in cancer development, cancers may also become dependent on excessive HR, making this process an attractive target for cancer therapy. Following a double strand break and resection of the 5' end in HR, the 3' overhang is immediately coated by the ssDNA-binding protein, replication protein A (RPA). The high affinity of this interaction kinetically blocks the formation of the Rad51 nucleoprotein filament – the active species in the HR. Rapid displacement of RPA requires the action of a recombination mediator, BRCA2 in humans or Rad52 in *Saccharomyces cerevisiae*. The underlying mechanism by which a recombination mediator promotes the formation of the Rad51 nucleoprotein filament has remained elusive. RPA contains four DNA binding domains (DBDs), which have been proposed to undergo dynamic interactions with ssDNA within the RPA-ssDNA complex. We have used RPA with individually labeled DBDs and single molecule total internal reflection fluorescence microscopy (smTIRFM) to elucidate domain level conformational dynamics of RPA on ssDNA. We observe various levels of fluorescence enhancement that are domain-dependent; suggesting microscopic dissociation/association events in the bound complex. Both DBD-A and D of RPA showed 4 states of fluorescence in smTIRFM experiments, though the behavior of the A and D domains are distinct. Addition of Rad52 resulted in a loss of the highest state of RPA DBD-D fluorescent enhancement, though had no effect of DBD-A. The effect of Rad52 on RPA DBD-D dynamics, but not DBD-A, suggests the Rad52 acts to limit the dynamics of RPA DBD-D specifically. The limitation of RPA DBD-D dynamics by Rad52 may allow for nucleation and filament formation of Rad51, thus promoting HR.

Abstracts

47 **Vascular Smooth Muscle RhoBTB1 Protects from Hypertension and Arterial Stiffness by Cullin-3 Dependent Ubiquitination of Phosphodiesterase 5**

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Previously our lab showed expression of dominant negative form of transcriptional factor PPARg (S-P467L) in vascular smooth muscle cells (VSMCs) causes vascular dysfunction and hypertension. Here we assess the physiological role of RhoBTB1, a PPARg target as well as potential Cullin3 substrate adaptor. S-P467L mice exhibiting RhoBTB1 deficiency were bred with tamoxifen-inducible RhoBTB1 expression mice. Intriguingly, restoration of RhoBTB1 in VSMCs fully corrected elevated blood pressure (SBP, 141±6 vs 124±3 mmHg, $p<0.01$, $n=8$-10), arterial stiffness (Aortic Pulse Wave Velocity, 3.8±0.2 vs 2.5±0.1 mm/ms, $p<0.01$, $n=11$-13) and vascular dysfunction (Ach-induced relaxation in aorta, 46±5% vs 80±2%, $p<0.01$, $n=6$-9) in S-P467L mice. Improvement of Ach-induced vasodilation suggested that RhoBTB1 might be able to enhance Ach-nitric oxide-cGMP pathway. Subsequently, PDE5 activity was shown to be increased in S-P467L mice and suppressed upon RhoBTB1 restoration. This is consistent with the observation that aorta from S-P467L mice relaxed to the same extent as control mice in response to a PDE-resistant cGMP analog while the relaxation remained impaired with a cGMP analog which can be degraded by PDE5. Next we sought to determine the molecular function of RhoBTB1. Since E3 ubiquitin ligase Cullin-3 is known to have BTB-domain containing protein as substrate adaptor, we tested the hypothesis that RhoBTB1 promotes PDE5 degradation. It was shown in HEK293 cells that RhoBTB1 interacts with PDE5 through co-immunoprecipitation, while expression of RhoBTB1 increases PDE5 ubiquitination in a Cullin-dependent manner. In addition, Ang II-infusion also results in RhoBTB1 deficiency and hypertension, which can be prevented in VSMCs RhoBTB1 complementation. We conclude that RhoBTB1 acts as a substrate adaptor for Cullin-3 and exerts its cardiovascular protective effect via promoting PDE5 proteasomal degradation and enhancing cGMP mediated vasodilation.

48 **β-klotho in Leptin-Sensitive Cells is Necessary for FGF21-mediated Weight Loss**

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Fibroblast growth factor 21 (FGF21) is an endocrine hormone that controls energy homeostasis by signaling to the central nervous system. Importantly, the metabolic effects of FGF21 on energy expenditure appear to require the adipokine leptin, as mice deficient in leptin or the leptin receptor exhibit significantly attenuated weight loss in response to FGF21 treatment. Moreover, co-administration of FGF21 with a leptin agonist enhances body weight loss in diet induced obese (DIO) mice compared to administration of either alone. Thus, we hypothesize that FGF21 and leptin signaling interact to control energy expenditure and overall body weight. To determine whether leptin-sensitive cells express the FGF21 co-receptor, β-klotho, which confers specificity for FGF21 action and is absolutely required for FGF21 signaling, we administered leptin to β-klotho-Cre mice which conditionally express tdTomato in the presence of Cre recombinase. Leptin-mediated activation of phosphorylation of STAT3 (pSTAT3) co-localized with β-klotho-positive cells in the arcuate nucleus (ARC), a region of the brain critically involved in the control of energy expenditure. To determine whether β-klotho is required in leptin receptor-expressing cells to mediate the metabolic actions of FGF21, we generated a novel mouse model which lacks β-klotho in leptin-sensitive cells ($KLB^{LepR\text{-}KO}$ mice). Intriguingly, DIO $KLB^{LepR\text{-}KO}$ mice have markedly impaired decreases in body weight in response to extended FGF21 administration. However, in contrast to body weight, FGF21-mediated improvements in glucose homeostasis and insulin sensitivity were retained in DIO $KLB^{LepR\text{-}KO}$ mice compared to wildtype littermates as determined by glucose tolerance and insulin tolerance tests, respectively. Taken together, these data suggest that FGF21 signaling to leptin-sensitive cells is critical for its full effects on body weight reduction.

Abstracts

49 **Smooth Muscle PPARγ Mutation Causes Impaired Renal Blood Flow and Salt Sensitive Hypertension**

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Mutations in PPARγ cause hypertension (HT) while PPARγ activation lowers blood pressure (BP) in humans. To determine if vascular smooth muscle (VSM) PPARγ regulates salt sensitivity, we studied transgenic mice selectively expressing a HT-causing PPARγ mutant in VSM (S-P467L) and non-transgenic littermates (NT) fed a 4% high salt (HS) diet for 4 weeks. Salt equally suppressed plasma renin in both strains, but S-P467L mice exhibited increased systolic BP (S-P467L 136±3 mmHg vs NT 124±2 mmHg, $p<0.01$) and pulse wave velocity (3.1±0.1 vs 2.7±0.1 m/s, $p<0.01$) in response to HS. The salt-induced HT was not associated with changes in diastolic BP, sympathetic nerve activity, heart rate, or cardiac output. Thus, the pressor effect of HS was likely due to higher peripheral vascular resistance. HS-fed S-P467L mice developed impaired acetylcholine (ACh)- and sodium nitroprusside (SNP)-induced vasorelaxation in carotid (Max ACh relaxation: 31±4.9% vs 90±1.8%, $p<0.01$; Max SNP relaxation: 38±2.8% vs 89±2.6%, $p<0.01$) and basilar artery (Max ACh relaxation: -3.2±9.3% vs 57±5.9%, $p<0.01$). The impaired vasodilation rapidly developed after 3-day HS diet, preceding salt-induced BP elevation. Pre-incubation with a cyclooxygenase inhibitor indomethacin normalized ACh/SNP relaxation responses, and preliminary mass spectrometry indicated HS increased prostaglandin E2 in S-P467L aortas. HS-fed S-P467L mice had smaller renal artery luminal diameter (322±21 vs 389±22 μm, $p<0.05$) and blunted renal blood flow (36±3.6 vs. 50±6.4 μL/min/g, $p<0.05$). During the 4th week of HS diet, S-P467L mice produced 31% less nitrate/nitrite in 24 hour urine compared to NT controls (2.2±0.3 vs 3.2±0.4 μmol, $p<0.05$), suggesting blunted renal bioavailability of nitric oxide, a potent inhibitor of Na-K-2Cl cotransporter (NKCC2). This was associated with a declined capacity of HS-fed S-P467L mice to excrete an acute volume load, which was rescued by an NKCC2 inhibitor furosemide, but not by the Na-Cl-cotransporter inhibitor hydrochlorothiazide. Our data support the novel concept that smooth muscle PPARγ regulates systemic vascular resistance, renal perfusion and tubular sodium transport, and loss of these protective actions of PPARγ predisposes to salt sensitivity and hypertension.

Abstracts

50 Acid-sensing ion channels alter behavioral responses to alcohol

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Alcohol misuse exacts an enormous toll on health and society, and current therapies have limited efficacy. The mechanisms by which alcohol exerts its effects on behavior and brain function remain incompletely understood, including how some individuals are more at risk for alcohol misuse than others. Sensitivity to the acute intoxicating effects of alcohol is believed to represent an important risk factor for subsequent misuse; thus, identifying underlying molecular mechanisms of sensitivity to alcohol could provide vital insight into development of novel therapeutics. Acid-sensing ion channels (ASICs), DEG/ENaC cation channels activated by extracellular acidosis, have effects on neurotransmitter systems affected by alcohol and are known to contribute to synaptic transmission. Disrupting ASIC1A, an important ASIC subunit, in mice increases behavioral responses to other drugs of abuse, such as cocaine and morphine. Therefore, we hypothesized that ASIC1A may also play a role in alcohol-related behaviors. We tested whether disruption of ASIC1A would alter initial responses to alcohol, as sensitivity to the intoxicating effects of alcohol is thought to be clinically relevant for maladaptive alcohol usage. We found that alcohol injection induced increased locomotion in *Asic1a*$^{-/-}$ mice than *Asic1a*$^{+/+}$ mice. Higher normalized activity levels in *Asic1a*$^{-/-}$ mice extended to a range of doses and persisted after repeated doses. Additionally, *Asic1a*$^{-/-}$ mice tended to be less sedated at high alcohol doses. Taken together, these results suggest that ASIC1A is critically involved in responses to acute alcohol exposure. Additional work is underway to clarify how ASIC1A impacts other alcohol-related phenotypes, and whether manipulation of ASIC1A may represent a novel therapeutic target for treatment of alcohol use disorders.

51 High-fat Feeding Induces Cardiac Hypertrophy but does not Consistently Induce Cardiac Dysfunction in Mice

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Diabetic cardiomyopathy characterized by left ventricular hypertrophy predisposes diabetic and obese individuals to development of cardiac dysfunction and subsequently to heart failure. Mouse models of diet induced obesity have been routinely used to study the effects of obesity and diabetes on cardiac dysfunction. Recent evidence from multiple research groups has emphasized the need for evaluation of the utility and relevance of the murine diet induced obesity model for studying cardiovascular abnormalities associated with hyperinsulinemic states including T2DM and obesity. We therefore studied the effect of chronic fat feeding (>20 weeks) on cardiac function in C57BL/6J mice. Different diets were formulated with either lard (32% saturated fat, 68% unsaturated fat) or hydrogenated coconut oil (95% saturated fat) as the source of fat and fatty acids, which contributed 60% of total calories. Both high-fat diets (HFD) induced insulin resistance as assessed by glucose tolerance test and insulin tolerance test. HFD resulted in the development of cardiac hypertrophy (heart weigh/tibia lengths: lard based diet vs control diet- 9.41$\pm$0.35 vs 6.99$\pm$0.19; coconut oil based diet vs control diet- 9.18$\pm$0.23 vs 7.43$\pm$0.43); however cardiac function as measured by B-mode echocardiography and LV catheterization was unaffected in high fat diet groups compared to their respective control diet groups. Further, dietary fat feeding regardless of the source of fat did not alter the gene expression of pathological hypertrophic markers (ANP, BNP, Myh7) or of fibrosis related genes (Ctgf, Col1a1, Col1a2 and Col3a1). However, there was an increase in expression of PPARa target genes such as Pdk4 and fatty acid metabolism genes including CD36, AcadL and Cpt1b. These results suggest that while chronic fat feeding in mice causes cardiac hypertrophy and potentially cardiometabolic remodeling, it might not be sufficient to activate pathological hypertrophic mechanisms that impair cardiac function and cause cardiac fibrosis. Thus, additional factors that are currently not understood may contribute to the reported cardiac abnormalities previously reported by many groups.