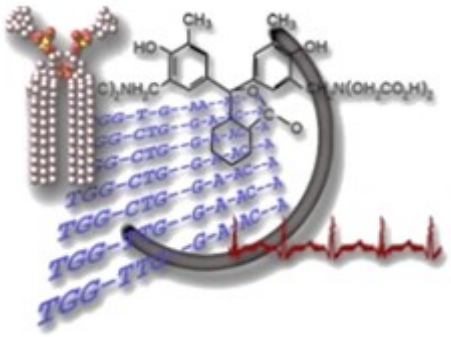
# Pharmaceutical and Pharmacological Sciences Research Retreat

May 19, 2017

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

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|---|---|
| **8:00 - 9:00 AM** | Continental Breakfast and Poster Set up |
| **9:00 - 9:15 AM** | DEO's Welcome |
| **9:15 - 10:40 AM** | **Session I: Faculty and Trainee Oral Presentations** |
| 9:15 - 9:45 AM | Greg Friestad, Ph.D., Associate Professor, Department of Chemistry |
| 9:45 - 10:05 AM | Rondine Allen, graduate student (Rice Lab), MNPC |
| 10:05 - 10:25 AM | Brittany Ripley, graduate student (Washington Lab), Department of Biochemistry |
| **10:25 - 10:40 AM** | Break |
| **10:40 - 12:00 PM** | **Session II: Faculty and Trainee Oral Presentations** |
| 10:40 - 11:10 AM | Kevin Rice, Ph.D., Professor, MNPC |
| 11:10 - 11:30 AM | Josie Schamp, graduate student (Doorn Lab), MNPC |
| 11:30 - 12:00 PM | Catherine Musselman, Ph.D., Assistant Professor, Department of Biochemistry |
| **12:00 - 1:00 PM** | Lunch Break |
| **1:00 - 2:00 PM** | **Poster Session** |
| **2:00 - 3:40 PM** | **Session III: Faculty and Trainee Oral Presentations** |
| 2:00 - 2:30 PM | Ethan Anderson, Ph.D., Associate Professor, PSET |
| 2:30 - 2:50 PM | Christy Heidema, graduate student (DeMali Lab), Molecular & Cellular Biology Program |
| 2:50 - 3:10 PM | Kai Rogers, graduate student (Maury Lab), Department of Microbiology |
| 3:10 - 3:40 PM | Maria Spies, Ph.D., Associate Professor, Department of Biochemistry |
| **3:40 - 4:00 PM** | **Awards and Wrap-up** |

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

Trainee Presentations are 15 minutes + 5 minutes for Q & A

# Poster Presentations

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# Abstracts

**mTORC1 is involved in the regulation of Bardet-Biedl syndrome (BBS) 1 gene expression**

**Deng-Fu Guo, PhD**, Balyssa Bell, MS, John Reho, PhD and Kamal Rahmouni, PhD

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

The high prevalence of obesity is associated with increased health risk factors such as cardiovascular diseases and diabetes. A combination of genetic and environmental factors such as availability of nutrition-rich food contributes to the development of obesity. Recent studies from our group have demonstrated the importance of hypothalamic *Bardet-Biedl syndrome (BBS)* genes and the BBSome for the regulation of energy homeostasis. We hypothesized that regulation of *BBS* genes in the hypothalamus may be altered in mice fed with high fat diet. To test this, we investigated the expression of different *BBS* genes in the hypothalamus of mice fed with normal diet (ND) or 60% high fat diet (HFD) for 36 weeks. Expression of *Bbs1* gene, but not *Bbs2, Bbs4, Bbs5, Bbs6, Bbs7, Bbs8 and Bbs9*, was significantly decreased in the hypothalamus of mice fed HFD compared to mice fed with ND, by qRT-PCR (n=6 each, $p<0.05$). Additionally, we found that *Bbs1* gene expression was significantly down-regulated in the hypothalamus of mice fasted for 16 hrs compared to fed mice (n=6 each, $p<0.05$). In order to study molecular mechanisms involved in the down-regulation of *Bbs1* gene expression, we tested its expression in the cultured mouse hypothalamic GT1-7 cells. We found that *Bbs1* gene expression was significantly decreased in the cells treated with 10 mM leucine or 4.5 g/dl of high glucose for 16 hrs, but not in the cells treated with 10 nM insulin for 16 hrs (n=3,$p<0.05$). Furthermore, icv injection of 2 μg of leucine was associated with a trend towards decreased *Bbs1* gene expression in the hypothalamus compared to icv PBS injection after 8 hrs (n=3, $p=0.053$). Inhibition of mTOR complex 1 (mTORC1) with rapamycin (100 nM) restored *Bbs1* gene expression in GT1-7 cells treated with 10 mM leucine, indicating that the mTORC1 pathway plays an important role in the regulation of *Bbs1* gene expression in GT1-7 cells. Given that mTORC1 interacts with IKKα/β, we then tested IKK inhibition with BMS 345541 (500 nM). We found that this also restored *Bbs1* gene expression in GT1-7 cells treated with 10 mM leucine. In contrast, NFkB p65 inhibition (NBP2-29321, 25 μM) did not alter leucine-induced down-regulation of *Bbs1* gene expression in GT1-7 cells. We conclude that decreased *Bbs1* gene expression in the hypothalamus may impair the BBSome function leading to the development of obesity.

**RhoBTB1 is a Novel Gene Protecting Against Hypertension**

**Masashi Mukohda**, Stella-Rita C. Ibeawuchi, Chunyan Hu, Ko-Ting Lu, Anand R. Nair, Larry N. Agbor, Jing Wu, Frederick W. Quelle and Curt D. Sigmund

Department of Pharmacology, Roy J. and Lucille A Carver College of Medicine, University of Iowa, Iowa City, IA

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand activated transcription factor regulating metabolic and vascular function. We previously reported that mice (S-DN) expressing dominant-negative PPARγ in smooth muscle cells (SMC) are hypertensive, exhibit impaired vasodilation, augmented vasoconstriction and reduced expression of a novel PPARγ target gene, RhoBTB1. RhoBTB1 belongs to class of proteins which act as substrate adaptors for the Cullin-3 E3 ubiquitin ligase. Delivery of substrates to the Cullin-3 CRL:3 complex leads to ubiquitination of the substrate protein targeting it for degradation by the proteasome. We hypothesized that loss of RhoBTB1 expression in S-DN mice may be responsible for impaired vasodilation. In this study, we tested the hypothesis that RhoBTB1 may play a protective role by preventing hypertension in response to stressors which cause hypertension, such as Angiotensin-II (Ang-II). To test this, we generated double transgenic mice with tamoxifen-inducible, Cre-dependent overexpression of RhoBTB1 specifically in SMC (S-RhoBTB1). S-RhoBTB1 and non-transgenic (NT) mice were treated with tamoxifen (Tx; 75 mg/kg, ip, 5 days) or vehicle (corn oil) and then Ang-II was infused (490 ng/kg/min, 2 weeks). Overexpression of RhoBTB1 did not alter baseline blood pressure (BP) in the absence of Ang-II. However, increased BP caused by Ang-II infusion was significantly attenuated by RhoBTB1 overexpression in S-RhoBTB1 with Tx compared to Ang-II-infused control mice (either NT with Tx, NT with corn oil, or S-RhoBTB1 with corn oil) in which RhoBTB1 was not overexpressed ($p<0.05$, n=4-5). We also observed increased heart weight in Ang II-infused control mice, which was reversed in S-RhoBTB1 with Tx (n=4). Thoracic aorta, carotid and basilar artery from Ang-II-infused control mice showed impaired acetylcholine (ACh)-induced endothelial-dependent relaxation ($p<0.05$, n=4-5), which was reversed by overexpression of RhoBTB1 in SMC ($p<0.05$, n=4-5). Aorta and carotid artery from Ang-II-infused control mice also displayed decreased sodium nitroprusside (SNP)-induced endothelial-independent relaxation with a right-shifted dose-response ($p<0.05$, n=4-5), which was reversed in Tx-treated S-RhoBTB1 mice ($p<0.05$, n=5). Despite the marked improvement in vasodilation, augmented vasoconstriction to serotonin (5-HT) was preserved in aorta and carotid artery from Ang-II-infused mice and was not reversed in Ang-II-infused S-RhoBTB1 with Tx (n=4-5). We conclude that the novel PPARγ target gene, RhoBTB1, functions in SMC to specifically facilitate vasodilation and through this mediates a protective anti-hypertensive effect. We are currently assessing which proteins may be substrates for Cullin-3 through RhoBTB1.

# Abstracts

**Rad6-18, the Master Regulator of Translesion Synthesis**

**Brittany M. Ripley**, Dr. M Todd Washington

Department of Biochemistry, University of Iowa, Iowa City, IA

Replication of DNA is a vital part of cell growth and division. Any damaged DNA in the path of the replication machinery may derail the replication fork which could cause cell death. Translesion synthesis is a mechanism which avoids derailing DNA replication by recruiting specialized polymerases such as Rev1 and Pol eta to bypassing the damaged DNA. These are thought to be recruited by ubiquitylation of the DNA replication hub protein, PCNA by the ubiquitin conjugate and ligase complex, Rad6-Rad18 (Rad6-18). Rad18 also interacts with Rev1 and Pol eta, but the purpose of these interactions in the TLS mechanism is unknown. Our research indicates that Rad6-18 is the master regulator of TLS. Rad6-18 binds both Rev1 and Pol eta in a manner regulated by a Rad18 autoinhibitory domain. This suggests that Rad6-18 may recruit TLS polymerases by triggering release of the autoinhibitory domain at stalled replication forks. Furthermore, though Rad18 is predicted to be highly disordered, small angle x-ray scattering reveals that the Rad6-18 complex is mostly ordered. This indicates that binding of Rad18 to other proteins induces folding in Rad18, thereby enabling unique interactions with multiple binding partners in TLS. These conclusions shed new light on the importance of Rad6-18 to the regulation and function of TLS.

**RhoBTB1, a Novel PPARγ Target Gene Regulates Vascular Function**

**Masashi Mukohda**, Stella-Rita C. Ibeawuchi, Chunyan Hu, Debbie R Davis, Deng Fu Guo, Kamal Rahmouni, Frederick W. Quelle and Curt D. Sigmund

Department of Pharmacology, Roy J. and Lucille A Carver College of Medicine, University of Iowa, Iowa City, IA

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand activated transcription factor regulating metabolic and vascular function. We previously reported that mice (S-DN) expressing dominant-negative PPARγ in smooth muscle cells (SMC) are hypertensive and exhibit impaired vascular relaxation and enhanced contraction due to increased RhoA/Rho kinase (ROCK) activity, and display reduced expression of a novel PPAR γ target gene, RhoBTB1. We hypothesized that RhoBTB1 may play a protective role in vascular function that is disrupted in S-DN mice. We generated transgenic mice (termed R+) with tamoxifen-inducible, Cre-dependent expression of RhoBTB1 in SMC. R+ mice were crossed with S-DN to produce mice (S-DN/R+) in which tamoxifen-treatment (75 mg/kg, ip, 5 days) restored the deficiency in RhoBTB1 expression observed in the aorta of S-DN mice to normal. Thoracic aorta and basilar artery from S-DN showed impaired acetylcholine (ACh)-induced endothelial-dependent relaxation ($p<0.01$, n=7-9), which was completely reversed by rescue of RhoBTB1 expression in SMC ($p<0.01$, n=9). On the contrary, S-DN mice exhibited enhanced contraction of aorta to serotonin (5-HT) and endothelin-1 (ET1) ($p<0.01$, n=6-9), but this was not reversed by replacement of RhoBTB1 in SMC (n=6-9). Importantly, rescue of RhoBTB1 expression reversed the hypertensive phenotype observed in S-DN mice ($p<0.01$, n=8-10). To identify the mechanism of RhoBTB1, RhoA/ROCK activity was assessed. A ROCK inhibitor, Y-27632, completely improved the impaired ACh relaxation and enhanced contraction induced by 5-HT or ET-1 in S-DN aorta ($p<0.05$, n=4-6), and also reversed the enhanced contraction in S-DN/R+ ($p<0.05$, n=4-6). Consistent with this, increase ROCK-dependent phosphorylation of myosin phosphatase targeting protein (MYPT) was observed in S-DN and this was preserved S-DN/R+ aorta, indicating that restoration of RhoBTB1 did not affect increased RhoA/ROCK activity in S-DN. Since the NOS inhibitor, L-NAME, abolished ACh relaxation in S-DN/R+ and phosphorylation and expression of eNOS were not difference in S-DN/R+ aorta compared to NT and S-DN, we next examined the SMC-dependent relaxation pathway. Aorta from S-DN mice displayed severely decreased sodium nitroprusside (SNP)-, soluble guanylate cyclase (sGC) stimulator, BAY 41-2722- or a cGMP analog, 8-Bromo-cGMP-induced endothelial-independent relaxation with a right-shifted dose-response ($p<0.01$, n=7-9), which was reversed in tamoxifen-treated S-DN/R+ mice ($p<0.01$, n=9). Finally, we found that PDE5 inhibitor, zaprinast improved ACh-induced relaxation in S-DN, suggesting that PDE5 might be target of RhoBTB1. We conclude that loss of RhoBTB1 function explains the impaired vasodilation and hypertension observed n response to interference with PPARγ in smooth muscle. Moreover, these studies define RhoBTB1 as a novel PPARγ target gene that plays an important role in selectively facilitating vasodilatation.

# Abstracts

### The Effect of Macrophage M2 Polarization on Ebola Virus Infection

**Kai Rogers**[1], Bethany Rhein[2], Natarajan Ayithan[3], Wendy Maury[1]

[1]Department of Microbiology, University of Iowa, Iowa City, IA [2]Department of Molecular Medicine, Mayo Clinic, Rochester, MN [3]Institute of Human Virology, University of Maryland, Baltimore, MD

*Ebola virus* (EBOV), a negative sense RNA virus and member of the *Filoviridae* family, is capable of causing tremendous morbidity and mortality. It is appreciated that cells of the innate immune system are important target populations in the initial stages and dissemination of EBOV infection. Our findings indicate the activation status of macrophages significantly impacts the susceptibility of these cells to EBOV infection. Ex vivo, M-CSF-matured resident peritoneal macrophages from C57BL/6 interferon α/β receptor (IFNAR) knockout mice were treated with well-established activation cytokines to elicit an anti-inflammatory M2a phenotype, which was confirmed by qRT-PCR for expression of appropriate marker genes. Cells were infected with a BSL-2 model virus (EBOV/rVSV) expressing GFP and infection was quantified at 24 hours by flow cytometry. We demonstrate that macrophages generated by IL4/IL13 treatment were significantly more susceptible to EBOV/rVSV infection than M-CSF-treated cells. We found that IL4/IL13-treated macrophages bound more EBOV/rVSV than M-CSF-treated cells, suggesting that the enhancement of infection of these cells may be due to enhanced receptor expression. To explore this, expression of C-type lectins and phosphatidylserine receptors that might mediate EBOV entry were assessed. We found RNA levels of SIGNR3 (a murine ortholog of human DC-SIGN) to be significantly higher in the IL4/13 treated macrophages than in M-CSF treated cells. Competitive inhibition studies using mannan reduced EBOV/rVSV entry in IL4/13-stimulated macrophages and ectopic SIGNR3 expression enhanced EBOV/rVSV infection, consistent with a role for SIGNR3-dependent uptake in M2a macrophages. In vivo studies demonstrated that mice exposed to IL4/IL13 24 hours prior to infection showed greatly elevated morbidity and mortality compared to PBS-treated littermates. These results demonstrate that M2a polarization enhances infection at least in part by elevating expression of the C-type lectin, SIGNR3. Further, this is the first demonstration that murine SIGNR3 serves as a receptor for EBOV.

### mTORC1 Signaling Is Required for Leucine to Induce Endothelial Dysfunction in the Mouse Aorta

**John J. Reho**, Deng-Fu Guo, Kamal Rahmouni

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

Obesity-induced hypertension is associated with vascular endothelial dysfunction. Our laboratory has previously demonstrated a critical role of the mammalian target of rapamycin complex 1 (mTORC1) in the regulation of the cardiovascular system and arterial pressure. mTORC1 serves as an intracellular energy sensor regulating protein synthesis through its downstream signaling components S6-kinase and the ribosomal S6 protein. We tested the hypothesis that activation of the mTORC1 signaling pathway in the vasculature is involved in the regulation of vascular endothelial function. Aortic rings isolated from wildtype male mice were stimulated with the branched chained amino acid leucine *ex vivo* (10mM; 24hrs) to activate mTORC1 signaling. Leucine activated mTORC1 signaling in the aorta as indicated by the increased phosphorylation of the downstream S6 via Western blot (2.0±0.2AU vs 1.0±0.2AU in unstimulated controls; $p<0.05$). Immunohistochemistry revealed that mTORC1 signaling was increased in both the endothelium and the smooth muscle of aortas exposed to leucine stimulation. Leucine stimulated aortic rings exhibited impaired acetylcholine induced relaxation compared to control (Max. relaxation: 71±4% vs 56±3%; $p<0.05$) with no changes in relaxation responses evoked by sodium nitroprusside (Max. relaxation: 84±2% vs 78±4%) indicating endothelial but not smooth muscle dysfunction. We next infected leucine stimulated aortic rings with an adenoviral S6-kinase dominant negative construct (Ad-DNS6K; 24hrs) and this rescued the endothelial dysfunction associated with leucine induced mTORC1 activation in aortic rings (Max. relaxation: 69±2%; $p<0.05$) with no effect on relaxation responses to sodium nitroprusside (Max. relaxation: 81±3%). To test if direct activation of mTORC1 signaling was involved in vascular endothelial function, we used an adenoviral construct of a constitutively active S6-kinase (Ad-CAS6K) to recapitulate leucine-induced endothelial dysfunction in aortic rings. Infection of aortic rings with Ad-CAS6K increased the phosphorylated S6 protein compared to adenoviral GFP (Ad-GFP) control (2.1±0.1AU vs 1.0±0.1AU; $p<0.05$). This activation of mTORC1 signaling resulted in impaired acetylcholine-induced relaxation (Max. relaxation: 67±5 vs. 81±3%; $p<0.05$) without altering the relaxation evoked by sodium nitroprusside (Max. relaxation: 90±1% vs. 90±2%) recapitulating the vascular phenotype in leucine stimulated aortic rings and obese mice. We conclude that mTORC1 signaling is a novel regulator of vascular endothelial function and that dysregulation of this signaling pathway may be involved in the endothelial dysfunction associated with states of overnutrition such as obesity.

# Abstracts

**Endothelial Cullin3 Mutation Causes Vascular Dysfunction, Arterial Stiffening, and Hypertension**

**Jing Wu**[1], Larry N. Agbor[1], Shi Fang[1], Chunyan Hu1, Ko-Ting Lu[1], Xuebo Liu1, Masashi Mukohda[1], Anand R. Nair[1], Curt D. Sigmund[1, 2]

[1]Department of Pharmacology, [2]UIHC Center for Hypertension Research, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City 52242

Frame-shift mutations in Cullin-3 (causing skipping of exon 9, CUL3Δ9) were previously identified in pseudohypoaldosteronism type II (PHAII) patients that exhibit increased renal NaCl reabsorption, hyperkalaemia, metabolic acidosis and hypertension. The hypertension phenotypes were initially found to be driven by renal tubular mechanisms, but we have recently shown that smooth muscle expression of Cul3Δ9 causes vascular dysfunction and elevation of arterial pressure via augmented RhoA/Rho-kinase signaling. Whether other extra-tubular mechanisms contribute remains unknown. We generated a model of selective and inducible expression of CUL3Δ9 in the endothelium using a construct (CAG-Cul3D9), which induces the expression of Cul3D9 (and TdTomato reporter) in response to Cre-recombinase. In primary aortic endothelial cells isolated from CAG-CUL3Δ9 mice, CUL3Δ9 expression was robustly induced by adenovirus carrying cre recombinase gene. Cul3Δ9 acted in a dominant negative manner by interfering with expression and function of endogenous Cullin-3, leading to impaired turnover of Cullin-3 substrate protein phosphatase 2A (PP2A), a marked decrease in phosphorylated eNOS, and reduced nitric oxide (NO) bioavailability. Treatment with PP2A inhibitor Calyculin A (5 nM) rescued CUL3Δ9-induced impairment of eNOS activity in these cells. Likewise, inhibition of Cullin activity using MLN4924 (1 μM) caused a marked reduction in phosphorylated eNOS and NO production in mouse lung endothelial cells (MLECs), while these changes were prevented by Calyculin A. To test the importance of endothelial Cul3 in vivo, we bred CAG-Cul3D9 mice with Tek-CREERT2 mice specifically expressing a tamoxifen inducible Cre-recombinase in the endothelium. The specificity of transgene expression was confirmed by western blot analysis of sorted endothelial and non-endothelial fractions of the aortas from the resultant mice (termed E-Cul3D9) 4 weeks following tamoxifen injection. E-Cul3D9 mice exhibited impaired endothelial-dependent relaxation in the carotid artery (relaxation to acetylcholine at 30 μM: 69.9 ± 5.7 % in E-Cul3D9 vs. 85.6 ± 4.3 % in control littermates, $p<0.05$, mean ± SEM), while no differences were seen in non-endothelial dependent relaxation. Moreover, E-Cul3D9 mice exhibited nocturnal hypertension as determined by radio telemetry (systolic pressure at 11pm, 143.7 ± 2.8 mmHg E-Cul3D9 vs. 118.5 ± 3.4 control littermates, $p<0.01$) and arterial stiffening as indicated by elevated pulse wave velocity (4.0 ± 0.5 E-Cul3D9 vs. 2.9 ± 0.2 control littermates, $p<0.05$). These data define a novel pathway involving Cullin-3/PP2A/phopho-eNOS in the regulation of endothelial function. Selective expression of the CUL3Δ9 mutation in the endothelium partially phenocopies the hypertension observed in CUL3Δ9 patients, suggesting that mutations in Cullin-3 cause human hypertension in part through a vascular mechanism featured by impaired eNOS activity in endothelium.

**Inhibiting G protein βγ signaling blocks prostate cancer stem cell-like properties and enhances the efficacy of paclitaxel**

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Aberrant activation of G protein-coupled receptors (GPCRs) is implicated in prostate cancer progression. As a treatment strategy, however, targeting GPCR has been challenging because prostate cancer cells overexpress many GPCRs, which have redundant roles in cancer progression. To address this redundancy, we tried to block signaling via a hub through which multiple GPCRs converge — the G-protein Gβγ subunits. Inhibiting signaling via Gβγ by overexpressing the scavernger, Gat, or the Gβγ inhibitor, gallein, in several castration-resistant prostate cancer cell lines (i.e. PC3, DU145 and 22Rv1), impaired cell growth and migration *in vitro*, and halted tumor growth and metastasis in nude mice. The blockade of Gβγ signaling also diminished prostate cancer stem cell-like activities, by reducing tumorsphere formation *in vitro* and tumor formation in a limited dilution assay in nude mice. Furthermore, Gβγ blockade enhanced the sensitivity of prostate cancer cells to paclitaxel treatment, both *in vitro* and *in vivo*. Together, our results identify a novel function of Gβγ in regulating prostate cancer stem-cell-like activities, and demonstrate that targeting Gβγ signaling is an effective approach in blocking prostate cancer progression and augmenting response to chemotherapy.

# Abstracts

### Smooth Muscle PPARγ Mutation Causes Salt-sensitive Hypertension

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Systemic loss of peroxisome proliferator-activated receptor-γ (PPARγ) function causes hypertension, whereas its activation lowers blood pressure largely via PPAR γ activity in the vasculature. We have recently shown that loss of PPARγ in smooth muscle cells increases mesenteric myogenic tone, promotes vascular remodeling, and predisposes to deoxycorticosterone acetate (DOCA)-salt-induced hypertension in mice. However, it is unclear whether these effects were attributable to altered salt sensitivity. In this study, we examined the role of smooth muscle PPARγ in regulating renal function and salt-sensitivity. Transgenic mice expressing dominant negative PPARγ in smooth muscle cells (S-P467L) and non-transgenic littermates (NT) were fed normal chow diet (containing 0.3% salt) or a 4% salt diet for a total of 8 weeks and metabolic cage studies were performed at baseline, 3 weeks, 6 weeks, and at the end of the protocol. S-P467L and NT mice had similar food intake, feces weight, and weight gain on high salt diet throughout the study. While 24-hour water intake tripled in both strains (6.3 ± 0.21 mL S-P467L vs. 5.9 ± 0.40 mL NT, n=7) when they were switched to high salt diet from normal chow, S-P467L mice excreted 32% less urine in the third week (24-hour urine 1.96 ± 0.13 mL S-P467L vs. 2.90 ± 0.17 mL NT, two-way ANOVA $p<0.01$). In order to assess renal function, mice were subjected to a bolus i.p. injection of normal saline equal to 10% of their body weight and the urine volume excreted in the subsequent 4 hours were plotted as a percentage of saline injected. We observed a marked decline in their capacity to excrete this volume challenge in S-P467L mice (28.2 ± 2.1% S-P467L vs. 39.9 ± 5.1% NT, $p<0.05$) in the third week of high salt diet. Because nitric oxide (NO) is known to regulate sodium and water balance, we determined daily renal NO production as indicated by 24-hour urinary nitrate/nitrite. Three-week dietary salt induced a 4-fold increase in urinary NO metabolites in NT mice, but this was blunted in S-P467L mice (2.82 ± 0.19 μmoL/24 hours S-P467L vs. 4.42 ± 0.27 μmoL/24 hours NT, $p<0.01$). In parallel with these renal phenotypes, S-P467L mice developed marked elevation of blood pressure that peaked in the third week of high salt feeding (telemetry systolic pressure 136.4 ± 3.4 mmHg S-P467L vs. 124.2 ± 2.4 mmHg NT, $p<0.01$) and remained elevated throughout the study. At the end of 8 weeks, vascular function was assessed in freshly isolated carotid and basilar arteries, and flow cytometry analysis was performed in single cell suspensions of aorta and kidney tissues. S-P467L mice, but not NT littermates, exhibited severe impairment of acetylcholine- and sodium nitroprusside-induced vascular relaxation in the carotid and basilar arteries in response to high salt. $CD45^+$ total leukocytes, $CD3^+$ T cells, $CD4^+$/ $CD8^+$ T cell subsets, and $F4/80^+$ monocytes/macrophages were increased in the aortas but were paradoxically decreased in the kidneys of S-P467L mice in response to high salt. These data indicate that loss of PPARγ in smooth muscle cells predispose to impaired vascular relaxation, renal dysfunction, and salt-sensitive hypertension. This study highlights the significance of vascular PPARγ in regulating systemic physiological responses.

### FGF21 Regulates Metabolism Through Adipose-Dependent and –Independent Mechanisms

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FGF21 is an endocrine hormone that regulates energy homeostasis and insulin sensitivity. The mechanism of FGF21 action and the tissues responsible for these effects have been controversial, with both adipose tissues and the central nervous system having been identified as the target site mediating FGF21-dependent increases in insulin sensitivity, energy expenditure, and weight loss. Here we show that, while FGF21 signaling to adipose tissue is required for the acute insulin-sensitizing effects of FGF21, FGF21 signaling to adipose tissue is not required for its chronic effects to increase energy expenditure and lower body weight. Also, in contrast to previous studies, we found that adiponectin is dispensable for the metabolic effects of FGF21 in increasing insulin sensitivity and energy expenditure. Instead, FGF21 acutely enhances insulin sensitivity through actions on brown adipose tissue. Our data reveal that the acute and chronic effects of FGF21 can be dissociated through adipose-dependent and -independent mechanisms.

# Abstracts

**Bardet-Biedl Syndrome 1 (BBS1) protein contributes to vascular endothelial function**

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Most cells in the human body possess primary cilium, a tiny antenna-like projection from the cell surface to the environment. In endothelial cells, the primary cilia function as a sensor of blood flow and sheer stress and contribute to both calcium and nitric oxide signaling. Bardet-Biedl syndrome (BBS) proteins are associated with cilia-related structures such as the basal body. At least 21 *BBS* genes (*Bbs1-Bbs21*) have been identified so far. The BBS1 protein is an important member of the BBSome complex which contain seven additional BBS proteins (BBS2, 4, 5, 7, 8, 9, and 18). The BBSome mediates protein trafficking to the cilia membrane and perhaps to other cellular compartments. To investigate the role of the cilia protein and the BBSome in endothelial cell function, we generated mice lacking the *Bbs1* gene specifically in endothelial cells by crossing mice carrying floxed alleles of the *Bbs1* gene ($Bbs1^{fl/fl}$) with the endothelial –specific $Tie2^{cre}$ mice. The $Bbs1^{fl/fl}/Tie2^{Cre}$ mice developed normally compared to littermate control mice. We assessed vascular function *ex vivo* using aortic rings and resistance-sized mesenteric arteries. Interestingly, we found that loss of the *Bbs1* gene leads to endothelial dysfunction. In aortic rings, relaxation responses to acetylcholine were decreased in female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice compared to controls (Max. relaxation: 40.1±7.7% vs 73.6±15.3%). The male $Bbs1^{fl/fl}/Tie2^{Cre}$ mice displayed a slightly decreased response that was not statistically different (Max relaxation: 70.4±0.6% vs 76.5±1.7%). In the resistance-sized mesenteric arteries, both male and female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice showed significantly decreased in relaxation response to acetylcholine (Max. relaxation: male: 38.3±5.5% vs 67.4±5.5% in controls; female: 27.5±12.9% vs 80.8±11.3% in controls, $P<0.05$). However, the relaxation responses evoked by sodium nitroprusside were not different in male and female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice in both the aorta and mesenteric arteries, indicative of endothelial but not smooth muscle dysfunction in the endothelium-specific Bbs1 null mice. Thus, disruption of the *Bbs1* gene in endothelial cells leads to vascular endothelial dysfunction. Our studies have identified the BBS1 protein as a novel regulator of vascular endothelial function.

**Novel Endocrine Circuit Regulating Sugar Satiety**

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The recent rise in obesity has been greatly influenced by food intake and diet composition. Although multiple studies have explored the complex regulation of macronutrient preference, the molecular mechanisms that govern the appetite for specific macronutrients are largely unknown. Recently, we demonstrated that the pleotropic hepatic endocrine factor, fibroblast growth factor 21 (FGF21), was shown to mediate endocrine control of simple sugar intake and sweet taste preference through its actions in the paraventricular nucleus (PVN) of the hypothalamus. The PVN is comprised of multiple types of neurons, including oxytocin neurons, which project to the ventral tegmental area (VTA), or "reward center" of the brain. In addition, oxytocin neurons are responsible for the synthesis of oxytocin, and recent evidence revealed that oxytocin plays a role in regulating sweet taste preference. Thus, we hypothesized that FGF21 regulates simple sugar intake and sweet taste preference through its actions on oxytocin neurons in the PVN. Using both gain- and loss-of-function experiments, we show that oxytocin is involved in FGF21 mediating sweet intake and preference. Activation of oxytocin neurons specifically in the PVN using designer receptor exclusively activated by designer drugs (DREADDs) was sufficient to decrease sucrose preference in mice. Conversely, administration of the blood-brain barrier penetrant oxytocin receptor antagonist, L368,899, abrogated the suppression of sugar preference mediated by FGF21. Through these studies, we have identified new mechanistic information into this liver-brain hormonal axis regulating central pathways controlling energy homeostasis and reward.

# Abstracts

**Mechanisms of complement C5a-induced mechanical sensitization in mouse: The roles of macrophages, cytokines and TRPV1**

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The complement system is a principal component of innate immunity. Recent studies have highlighted the importance of C5a and other components of the complement system in inflammatory and neuropathic pain, although the underlying mechanisms are largely unknown. In particular, it is unclear how the complement system communicates with nociceptors and which ion channels and receptors are involved. Here we propose that the complement fragment C5a triggers macrophage-to-neuron signaling which involves TRPV1 sensitization and ultimately leads to thermal and mechanical sensitization. The inflammatory thermal and mechanical hyperalgesia induced by intraplantar injection of complete Freund's adjuvant (CFA) was accompanied by upregulation of C5a in the hindpaw and was markedly reduced by C5a receptor (C5aR1) knock-out or treatment with the C5aR1 antagonist PMX53. Administration of C5a into the mouse hindpaw produced mechanical and thermal hyperalgesia in a dose-dependent manner with C5aR1 KO mice showing no hyperalgesia. Immunohistochemistry of mouse plantar skin revealed that C5aR1 was expressed primarily in resident skin macrophages. Additionally, C5a evoked strong $Ca^{2+}$ mobilization in cultured macrophages dependent upon C5aR1 activation of Gβγ-phospholipase Cβ signaling and $Ca^{2+}$ mobilization from ER calcium stores. Drug-induced macrophage depletion in transgenic macrophage Fas-induced apoptosis (MAFIA) mice abolished C5a-dependent thermal and mechanical hyperalgesia. Examination of inflammatory mediators following C5a injection revealed a rapid upregulation of numerous factors including NGF, a mediator known to sensitize TRPV1. Preinjection of an NGF-neutralizing antibody or Trk inhibitor GNF-5837 prevented C5a-induced thermal hyperalgesia. Notably, NGF-induced thermal hyperalgesia was unaffected by macrophage depletion. Interestingly, both thermal and mechanical hyperalgesia produced by C5a were absent in TRPV1 knock-out mice, and were blocked by coadministration of TRPV1 antagonist AMG9810. As TRPV1 is a noxious heat sensor and is unlikely to directly mediate mechanical sensation, these data suggest that C5a produces heat hyperalgesia by sensitizing TRPV1 to heat stimuli while C5a-induced mechanical hyperalgesia is potentially dependent upon neurogenic inflammation originating from TRPV1 containing fibers. The potential for C5a induced neurogenic inflammation is currently being tested. Collectively, our findings highlight the importance of macrophage-to-neuron signaling in pain processing and identify C5a, NGF, and TRPV1 as key players in this cross-cellular communication.

**RABL6A, a novel critical regulator of Akt-mTOR signaling in pancreatic neuroendocrine tumor cells**

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A better molecular understanding of pancreatic neuroendocrine tumors (PNETs) is needed to improve patient diagnosis and treatment. PI3K/Akt/mTOR signaling is aberrantly activated in PNETs resulting therapies targeting the pathway. Our data suggests that RABL6A, a novel oncoprotein amplified in PNETs, is a key regulator of this clinically relevant pathway. We found loss of Akt Ser-473 phosphorylation following RABL6A depletion along with impaired S6K phosphorylation, downstream target of Akt-mTOR signaling. Multiple mechanisms control Akt-S473 phosphorylation. We demonstrated mTORC2, the kinase that phosphorylates Akt at Ser-473, remains intact and active in RABL6A deficient cells. Moreover, overexpression of Nek2 kinase, which promotes Akt-S473 phosphorylation and is downregulated by RABL6A loss, was unable to rescue the RABL6A knockdown phenotype. Our findings suggest protein phosphatases (other than PP1) are activated by RABL6A loss and reduce Akt-S473 phosphorylation. Given the central role of Akt in tumorigenesis, we hypothesized that reinstating its activity may rescue the arrest phenotype caused by RABL6A loss. Restoration of Akt in RABL6A-depleted cells partially rescued the G1 phase arrest and induced S phase entry but was insufficient to allow mitosis, suggesting RABL6A regulates other factors required for cell division. Finally, as predicted from our results RABL6A loss desensitized PNET cells to the Akt inhibitor, MK-2206. Thus, RABL6A controls multiple cancer pathways necessary for PNET cell cycle progression and survival. Overall, this work identifies RABL6A as an essential activator of Akt-mTOR signaling, suggesting it is a new potential biomarker and target for anticancer therapy in PNET patients.

# Abstracts

**Vasopressin Induces Discrete Symptoms of Preeclampsia through Receptor- and Gestational Age-Specific Mechanisms**

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Preeclampsia (PE) is a common gestational disorder characterized by new onset hypertension, proteinuria, intrauterine growth restriction (IUGR), and renal glomerular endotheliosis (RGE) that presents in the 2nd or 3rd trimester. We have demonstrated that arginine vasopressin (AVP) secretion is elevated as early as the 6th week of gestation in women who later develop PE and also that chronic low-dose AVP infusion during pregnancy causes elevated systolic blood pressure (SBP), proteinuria, IUGR, and RGE in C57BL/6J mice. Here we further characterize the AVP mouse model of PE and use the model to address the hypothesis that AVP induces individual PE phenotypes through distinct receptor types during distinct phases of gestation. By telemetry, AVP infusion resulted in isolated systolic hypertension, consistent with large elastic artery stiffness. AVP infusion also resulted in significantly increased hypoxia-inducible factor 1 (HIF1)-alpha binding to chromatin (saline n=5, 0.31±0.01; AVP n=5, 0.34±0.01 AU, $p<0.05$) within the placenta, consistent with placental hypoxia. To examine receptor involvement, we infused combinations of saline or AVP (24 ng/hr), with conivaptan (non-selective V1A/V2 antagonist, 22 ng/hr) or relcovaptan (V1A antagonist, 22 ng/hr) throughout gestation. As previously reported, AVP infusion increased SBP (GD18: saline n=35, 111±1.7; AVP n=23, 119±1.9 mmHg, $p<0.01$) and proteinuria (saline n=27, 48±4.5; AVP n=28, 67±5.6 mg/mL, $p<0.01$), caused IUGR (saline n=20, 0.80±0.03; AVP n=20, 0.72±0.03 g/fetus, $p=0.055$), and RGE. Conivaptan prevented elevations in SBP (n=11, 110±3.5 mmHg, $p<0.05$ vs AVP), but provided no protection from IUGR (n=3, 0.76±0.05 g/fetus), proteinuria (n=11, 73±12.3 mg/mL) or RGE. Relcovaptan prevented proteinuria (n=15, 45±3.9 mg/mL, $p<0.05$ vs AVP) and RGE, but had no effect on SBP (n=9, 118±3.2 mmHg) or IUGR (n=16, 0.77±0.05 g/fetus). Preliminary studies with tolvaptan (V2 antagonist, 22 ng/hr) show an attenuation of SBP (GD18: n=4, 114±3.8). To examine timing of AVP action, we infused AVP only up to GD3 or GD10. AVP infusion through GD3 had no significant effect on any measured endpoint, but AVP infusion through GD10 elevated SBP through GD10 (which returned to control levels by GD18) and caused significant proteinuria at GD18 (n=19, 88±9.2 mg/mL, $p<0.05$ vs saline), in the absence of evidence of RGE by electron microscopy. Overall, these data support the concepts that during pregnancy, elevated AVP causes (i) isolated systolic hypertension, (ii) placental hypoxia, (iii) proteinuria and RGE, and (iv) IUGR. Whereas increased SBP appears to be sustained throughout gestation and requires V2 receptor activation, renal damage requires V1A receptor activation. These data establish selective timeframes and receptors which mediate the generation of PE phenotypes by AVP. Given that PE phenotypes can be mechanistically dissociated in humans (eg – a diagnosis of PE does not necessitate IUGR or proteinuria and modulating blood pressure does not modify other phenotypes), the current study may explain this mechanistic divergence and identify selective receptor targets to treat individual symptoms.

**Oxidative route to *N,O*-acetals linked to the amide nitrogen of peptides**

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The *N,O*-acetal moiety is present in several natural products. Linking the *N,O*-acetal to the amide nitrogen of short peptidic structures enables traceless linkages to other components of interest. In this context, we present methodology to prepare *N,O*-acetals from a wide array of peptides. Installation of an *N*-silylmethyl group onto various amino acids and subsequent peptide coupling of the resulting *N*-silylmethyl amines provides dipeptides poised for *N,O*-acetal formation. These *N*-silylmethyl peptides can then be subjected to Tamao-Fleming oxidation to provide the desired *N,O*-acetals. We detail the results and scope of this strategy to install *N,O*-acetal linkers onto a variety of dipeptides.

# Abstracts

**Access to the C15–C40 Fragment of Tetrafibricin via Configuration-Encoded 1,5-Polyol Methodology**

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The potent fibrinogen receptor antagonist tetrafibricin contains multiple chiral 1,5-polyol moieties which are also present in a variety of biologically active natural products, and the isolated stereogenic centers present challenges in synthesis and stereochemical characterization. We previously introduced a synthetic strategy toward 1,5-polyols with unambiguous stereocontrol using enantiopure building blocks with alcohol configurations previously encoded in *O*-silylcyanohydrin functionality and exploiting iterative Julia-Kocienski couplings. We have merged this approach with the Evans tactic of intramolecular benzylidene acetal construction via conjugate addition to access the *anti,syn*-1,5,7-triol which appears in the C15–C25 segment of tetrafibricin. Now we present the application of this configuration-encoded 1,5-polyol methodology to the C26–C40 fragment, and its stereoselective coupling to the C15–C25 fragment via Mukaiyama aldol addition, generating the C15–C40 fragment of tetrafibricin.

**Deletion of brain-specific isoform of renin (renin-b) increases resting metabolic rate by stimulating brown adipose tissue sympathetic nerve activity, but not insulin sensitivity**

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Activation of the brain renin-angiotensin system (RAS) stimulates energy expenditure through increasing sympathetic nerve activity (SNA). The brain specific isoform of renin, termed renin-b (ren-b), has been proposed as a negative regulator of the brain RAS. Deletion of ren-b induces central RAS activation and hypertension. However, the metabolic effects of ren-b deletion have yet to be described. We generated a mouse model with a selective deletion of ren-b while preserving expression of renin-a (Renin-b$^{Null}$ mice). Under basal conditions, Renin-b$^{Null}$ mice do not show differences in body weight, food consumption, and physical activity, but did show a resistance to high fat diet-induced weight gain in males (36.8 ± 1.2 in Renin-b$^{Null}$ vs. 41.9 ± 1.4 in control, $p<0.03$), but not in females. Ren-b$^{Null}$ mice exhibited increased resting metabolic rate (0.156 ± 0.005 kcal/h in Renin-b$^{Null}$ vs. 0.145 ± 0.003 kcal/h in control littermates, $p<0.015$, mean ± SEM) concomitant with an increased sympathetic nerve activity (SNA) in brown adipose tissue (BAT) (40.8 ± 3.1 in Renin-b$^{Null}$ vs. 27.2 ± 3.1 in control, $p<0.003$). This was associated with an increase in uncoupled protein 1 (UCP1) expression in the subscapular BAT (2.1 ± 0.5 fold increase in Renin-b$^{Null}$ vs. control, $p<0.05$). Compared to control mice, glucose tolerance test revealed a mild but significant glucose intolerance in Renin-b$^{Null}$ mice and no significant differences in the insulin tolerance. All together, these data indicate that renin-b is involved in maintaining energy homeostasis likely by preventing central RAS activation. The activation of BAT SNA and the subsequent UCP1 overexpression, but not increased insulin sensitivity, are likely involved in the increased metabolic phenotype observed in Renin-b$^{Null}$ mice. Downregulation of renin-b and the consequent brain RAS activation may be an important pathophysiological mechanism involved not only in the development of neurogenic hypertension, but also in obesity and other metabolic diseases.

# Abstracts

**The reactive dopamine metabolite 3,4-Dihydroxyphenylacetaldehyde (DOPAL) is a potent inducer of collagen secretion in human cardiac fibroblasts**

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Spontaneous generation of reactive sugar- and lipid-derived aldehydes occurs *in vivo* as a byproduct of oxidative stress, and these aldehydes form protein adducts via electrophilic attack on cysteine and lysine residues. Catecholaldehydes are biogenic aldehydes formed as products of catecholamine metabolism by monoamine oxidase (MAO). MAO catalyzes the oxidative deamination of dopamine to generate 3,4-dihydroxyphenylacetaldehyde (DOPAL), a reactive molecule that has been shown to be neurotoxic stemming from the reactivity of both catechol and aldehyde groups. 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, and that MAO activity in this tissue is associated with postoperative atrial fibrillation. Increased collagen deposition in the heart (i.e., fibrosis) is a well-known risk factor for arrhythmias, and is also associated with diabetes. Here, we tested the hypothesis that DOPAL-adducts 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. Collagen content in the media increased with CML-, HNE- and DOPAL-BSA ($P<0.05$), as compared with BSA alone (control). Surprisingly, treatment with DOPAL increased type I and type III collagen secretion by 11-fold and 32-fold, respectively, compared to vehicle-treated cells ($P<0.05$), an effect that was >5-fold greater than with either CML or HNE. These findings suggest that reactive aldehydes, particularly catecholaldehydes, may significantly contribute to fibrosis in the heart via increased collagen secretion. Further, they provide evidence that compounds with aldehyde-scavenging capacity may have therapeutic value in mitigating cardiac fibrosis.

**Identifying a Negative Regulator of E-Cadherin Force Transduction**

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All cells are subjected to mechanical force. These forces are sensed by cell surface adhesion receptors. Upon sensing force, adhesion receptors trigger robust actin cytoskeletal rearrangements and growth of the adhesion complex—a process called stiffening. Phosphorylation of vinculin Y822 by Abelson kinase (Abl) is a critical component of E-cadherin-mediated force transduction. This residue is also implicated in tumorigenesis. Analysis of The Cancer Genome Atlas reveals that a small number of metastatic breast cancers harbor a Y822C mutation, rendering it non-phosphorylatable. Given this rationale, a negative regulator of vinculin Y822 phosphorylation could produce parallel effects. This project seeks to identify the negative regulator of vinculin Y822 phosphorylation and assess its physiological effects on cellular stiffening. Here, we place SHP-2 (PTP1d) as a candidate phosphatase that targets vinculin Y822. We demonstrate that SHP-2 is activated in response to force on E-cadherin in a temporal manner that would negatively regulate cell stiffening. Moreover, active SHP-2 localizes to sites of E-cadherin mechanotransduction in response to force. Finally, we demonstrate that a SHP-2 trapping mutant more robustly traps vinculin in response to force. These observations align with other evidence showing that vinculin contains a consensus binding site for SHP-2, and that SHP-2 inhibition produces phenotypes that are similar to cells expressing phosphorylated vinculin Y822. Future studies will be directed at potentiating the E-cadherin force transduction pathways by studying the effect of a constitutively phosphorylated vinculin at Y822.

# Abstracts

**The effect of polyanhydride chemistry in particle-based cancer vaccines on the magnitude of the anti-tumor immune response**

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**Study background and purpose:** Immunotherapies and in particular cancer vaccines represent a viable approach aiming at recognizing and destroying tumor cells through stimulating effective anti-tumor immune responses without causing deleterious side effects associated with conventional cancer therapies. With a steady growth in research towards finding a feasible approach to treat cancer, vaccines for cancer treatment have attracted a significant amount of attention in the past few years. The goal of this research was to design and develop a cancer vaccine formulation in a prophylactic setting using biodegradable polyanhydride polymers as a delivery system. Polyanhydrides have been reported to have inherent immunologic adjuvant properties and polyanhydride particles can act as a Toll-like receptor (TLR) agonists.

**Methods:** To achieve our goal, three polyanhydride polymers (CPTEG, CPH, and SA) were used to synthesize three different chemistries of copolymers (50:50 CPTEG:CPH, 20:80 CPTEG:CPH, and 20:80 CPH:SA) using a melt polycondensation technique. These copolymers were characterized for their degree of hydrophobicity by measuring the contact angle between water droplets and films of polyanhydride copolymers. Thereafter, particles of polyanhydride copolymers encapsulating a tumor-associated antigen, ovalbumin (OVA), were synthesized using a double emulsion solvent evaporation technique. Prepared particles were characterized for their size, shape, and surface charge. The ability of prepared formulations to stimulate immune responses was investigated using a mouse model. In addition, the impact of CpG ODN, a TLR9 agonist, on the immune response was also studied. Immune responses (cellular and humoral) to cancer vaccine formulations were measured after treatment of C57BL/6J female mice with two subcutaneous injections, seven days apart, of 50 µg OVA encapsulated in particles composed of different polyanhydride copolymers with or without 25 µg soluble CpG ODN. Mice were challenged with E.G7 tumor cells on day 35 post-prime vaccination and the tumor progression was monitored for the subsequent 2 months.

**Results:** Contact angle measurements showed that the CPH content in polyanhydride copolymers was directly correlated with the hydrophobicity. Prepared particles had an average size of 1 µm with narrow size distribution (PDI < 0.2), and had negatively charged surfaces. SEM photomicrographs revealed that polyanhydride particles encapsulating OVA had a spherical shape with smooth surface morphology. In vivo studies showed that 20:80 CPTEG:CPH particles encapsulating OVA significantly stimulated the highest level of CD8+ T lymphocytes, generated the highest serum titers of OVA-specific IgG antibodies, and produced longer survival in comparison to formulations of other polyanhydride copolymers. Interestingly, mice vaccinated with 20:80 CPTEG:CPH/ OVA displayed a substantial delay in the onset of tumor growth, whilst other formulations not involving 20:80 CPTEG:CPH did not delay the onset of tumor appearance compared to the naïve mice. The results also revealed that co-delivery of CpG ODN with polyanhydride particles encapsulating OVA did not enhance the anti-tumor immunity. These results accentuate the crucial role of the polyanhydrides copolymer composition in stimulating the immune response, and provide important insights on rationally designing efficacious cancer vaccines.

**Conclusions and future directions:** Treatment of mice with cancer vaccine formulations based on different polyanhydride copolymer chemistries encapsulating OVA resulted in a stimulation of the tumor-specific immune response with different magnitudes. Vaccination with 20:80 CPTEG:CPH/OVA, the most hydrophobic formulation, stimulated the strongest cellular and humoral immune responses and provided the longest survival outcome without adding any other adjuvant. The most important finding in this study was that the copolymer chemistry of polyanhydride particle -based cancer vaccine formulations can have a direct effect on the magnitude of the anti-tumor immune response and should be selected carefully in order to achieve optimal cancer vaccine efficacy. In the future work, other immunologic adjuvants such as PET-lipid A and poly I:C will be used to further improve and optimize the anti-tumor immune response.

# Abstracts

**Molecular cloning of a novel 69 kDa brain-specific isoform of Regulator of G protein Signaling 6 (RGS6)**

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RGS proteins play critical roles in modulating the magnitude and duration of signaling through many G protein-coupled receptors (GPCRs) due to their ability to facilitate inactivation of heterotrimeric G protein signaling. RGS proteins terminate both Gα and Gβγ signaling through their GTPase-activating activity towards specific Gα subunits, an activity bestowed by their semiconserved RGS domain. RGS6 is a member of the R7 subfamily of RGS proteins distinguished by two additional domains, DEP/DHEX and GGL, which target R7 members to the membrane and regulate protein stability, respectively. We have demonstrated that RGS6-specific inhibition of G αi/o is critical for the modulation of several CNS disorders for which we suggest RGS6 is a novel therapeutic target. Remarkably, we have discovered that RGS6-/- mice have reduced behavioral correlates of anxiety and depression, exhibit diminished alcohol seeking and reward behaviors, and develop late age-onset Parkinson's disease. The role of RGS6 in these disorders is dependent on its ability to inhibit the signaling of various brain GPCRs, including: 5-$HT_{1A}$ serotonin receptors in hippocampal and cortical neurons (anxiety/depression), $GABA_B$ receptors in mesolimbic dopaminergic neurons (alcoholism), and D2 receptors in dopaminergic neurons of the substantia nigra pars compacta (Parkinson's disease). Potentially key to the ability of RGS6 to regulate these various GPCRs are previously unidentified domains that arise via alternative mRNA splicing. Our initial cloning efforts identified 36 distinct splice-forms in human brain encoding RGS6 isoforms ≤ 56 kDa. Recently, we identified, in both mouse and humans, at least two additional uniquely brain-specific RGS6 protein isoforms that are larger (~61 and 69 kDa) than the ubiquitously expressed 56 kDa forms. The functions of these RGS6 variants and how they arise is unknown, but they may be critical for our understanding CNS pathology as both isoforms are highly expressed in the brain regions affected by the CNS disorders referred to above. Here we report PCR amplification and cloning of six novel RGS6 cDNAs from a human brain library that arise by alternative mRNA splicing and inclusion of novel RGS6 exons. One of these novel cDNAs encodes a 69 kDa protein that co-migrates with the large brain-specific RGS6 protein present in human and mouse. This RGS6 splice-form exhibits a near exclusive CNS pattern of expression and generates an RGS6 protein with a predicted C-terminal extension near the RGS domain that may represent a novel protein interaction site. Other identified RGS6 cDNAs include those with novel exons that lead to early stop codons yielding RGS6 proteins lacking all but the N-terminal domain and those with novel 3' exons that generate RGS6 splice-forms with unique C-terminal domains. Interestingly, several of the newly identified exons are shared primarily between humans and other primates. Together, this research lays the foundation for experiments to elucidate the functional significance of RGS6 alternative mRNA splicing in normal brain function as well as pathology. (NIH CA161882, MJ Fox 11551)

**Applications of the Radical-Polar Crossover Reaction to Medicinal Chemistry**

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Radical-polar crossover (RPC) chemistry holds great promise as a synthetic method due to its ability to incorporate two separate bond formations into a single reaction. Previous work in our group illustrated the synthesis of piperidine motifs through Mn-mediated RPC chemistry.1 Radical addition to *N*-(2-phenylethyl)-enamines can be used to initiate the formation of a carbon framework of a highly substituted isoquinoline structure.2 Herein we expand the scope of the Mn-mediated RPC reaction. We also present the synthesis of a 3-(aminylmethlyl)-isoquinoline compound that could potentially be used in the treatment of obsessive-compulsive disorder (OCD).

# Abstracts

**Effects of endothelial-specific interference with PPARγ activity in offspring born from AVP-induced preeclamptic pregnancies**

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PPARγ is a ligand-activated transcription factor known to regulate metabolic and vascular function. Dominant-negative (DN) mutations in PPARγ result in hypertension, and synthetic agonists of PPARγ have been shown to reduce blood pressure. Previously we found that mice expressing DN-PPARγ specifically in the endothelium (E-DN) exhibit vascular dysfunction. Preeclampsia (PE) is a hypertensive disorder of pregnancy which programs cardio/metabolic disease in offspring. PE is associated with vascular dysfunction, and we therefore hypothesized a role for endothelial PPARγ in the pathogenesis of PE and its sequelae. To test this, C57BL/6J dams were bred with E-DN sires, and symptoms of PE were induced by the infusion of vasopressin throughout gestation. We assessed phenotypes of PE first in pregnant dams, and then in offspring as adults. Compared to saline infusion (SAL), AVP induced significant elevations in maternal blood pressure and urinary protein levels. Offspring to these pregnancies were then genotyped and allowed to grow to adulthood to assess cardio-metabolic function.

Systolic blood pressure measured in adult offspring born to AVP-infused pregnancies was similar to mice born from SAL-infused pregnancies. Vascular relaxation responses to acetylcholine and sodium nitroprusside were not different in adult offspring born from either SAL- or AVP-infused pregnancies. Urinary protein levels were significantly elevated in adult male and female E-DN offspring born to preeclamptic pregnancies compared to genotype-matched controls born from SAL-infused pregnancies. Body weight measured over time showed significantly increased gain in body weight in male E-DN exposed to preeclampsia compared to those exposed to saline in utero. These data highlight the impact of *in utero* exposure to elevated AVP upon cardiovascular function in the mother, and the adverse renal and metabolic consequences of this manipulation upon offspring. Moreover, our data suggests that interference with endothelial PPARγ in pups born from preeclamptic pregnancies increases the risk for cardio-renal and metabolic dysfunction.

**RABL6A-dependent regulation of c-Myc expression and activity is essential for cell cycle progression and survival of pancreatic neuroendocrine tumor cells**

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Neuroendocrine tumors (NETs) are challenging, indolent malignancies whose incidence has risen significantly. A better understanding of molecular mechanisms underlying NET pathogenesis is needed to identify drug targets and develop improved therapies. We recently showed that the novel GTPase, RABL6A, is required for pancreatic NET (PNET) cell proliferation and survival. We compared gene expression profiles of RABL6A-depleted PNET cells to that of patient-derived primary PNETs and found significant overlap in the dysregulated genes, with factors involved in c-Myc signaling prominently altered in both datasets. Given the central role of c-Myc in cancer, we investigated its status in RABL6A-depleted PNET cells. RABL6A loss moderately diminished c-Myc mRNA expression while dramatically reducing c-Myc protein levels and activity. Proteasome inhibition failed to increase c-Myc protein, suggesting that RABL6A regulates c-Myc protein expression through other post-transcriptional mechanisms. We hypothesized that reinstating c-Myc activity would rescue the growth arrest caused by RABL6A loss. However, exogenous c-Myc only partially rescued the G1 phase arrest in RABL6A depleted cells. This correlated with increased S-phase entry, reduced expression of the cell cycle inhibitor, p27Kip1, and increased levels of CKS1B, a c-Myc transcriptional target that promotes p27 degradation. Notably, c-Myc overexpression was unable to promote mitosis following RABL6A depletion since cells arrested in S and G2/M phases, likely due to activation of DNA damage and mitotic checkpoints. These studies reveal RABL6A is a new essential regulator of c-Myc expression and activity, advancing our understanding of Myc regulation and strengthening the potential value of therapeutically inhibiting RABL6A function in PNET patients.

# Abstracts

**Arginine vasopressin acts directly upon human placental trophoblasts to elicit calcium responses**

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Previously we demonstrated that preeclampsia, a hypertensive disorder of pregnancy, is associated with elevated secretion of arginine vasopressin (AVP) as early as the sixth week of gestation. In addition, chronic low-dose infusion of AVP into pregnant wildtype C57BL/6J is sufficient to initiate all of the cardinal phenotypes of preeclampsia including gestational-dependent de novo hypertension, renal glomerular endotheliosis, placental hypoxia and intrauterine growth restriction. We hypothesize that AVP acts directly upon placental trophoblasts to cause placental dysfunction, and that this mechanism contributes to placental dysfunction during preeclampsia. First, we performed in silico re-analyses of a published microarray dataset (GSE75010). In this dataset (n=77 controls, n=80 preeclampsia), all five types of AVP receptors were abundantly expressed at the mRNA level in placental tissue. Interestingly, placentas from human pregnancies complicated by preeclampsia exhibited increases in expression of the AVPR1A ($p=0.03$) and OXTR ($p<0.01$) receptors, but no significant changes in the other AVP-sensitive receptors including AVPR1B ($p=0.14$), AVPR2 ($p=0.98$), or CUL5 ($p=0.09$). Second, we confirmed that all five receptor subtypes are expressed at the mRNA level in cultured immortalized human first-trimester trophoblasts (HTR8/SVneo cells). Third, we examined second-messenger signaling mechanisms, focusing on intracellular Ca2+ release, as the Gαq-calcium pathway is commonly activated by AVPR1A, AVPR1B, and OXTR. Increases in intracellular Ca2+ concentration ([Ca2+]i) with graded doses of AVP were examined in HTR8/SVneo cells pre-loaded with the fluorescent Ca2+-sensitive indicator FURA-2. AVP evoked [Ca2+]i responses of increasing amplitude at 10, 100 and 1000 nM. Fourth, we examined AVPR1A receptor-dependence of these [Ca2+]i responses. Pre-treatment of the HTR8/SVneo cells with the AVPR1A-selective antagonist relcovaptan (1 uM) abolished [Ca2+]i responses to AVP (100 nM), and subsequent washout of the antagonist restored responsiveness. These findings lead us to conclude that human placental trophoblasts express AVP receptors which functionally couple to Gαq-calcium signaling. Together, the findings that (i) AVP secretion is elevated during preeclampsia, (ii) AVP is sufficient to initiate preeclampsia phenotypes in mice, (iii) AVPR1A appears to be increased in placenta from preeclamptic pregnancies, and (iv) AVPR1A activation functionally couples to Ca2+ signaling in trophoblasts supports the overall concept that AVP-AVPR1A-Ca2+ signaling in the trophoblast may contribute to placental dysfunctions in preeclampsia.

**Regulatory T cell memory in MHV infection**

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Regulatory T cells (Tregs) are critical for the maintenance of peripheral tolerance. There is currently limited evidence in support of the existence and role of Treg memory, as memory has primarily been studied in effector T cell populations. The primary challenge in identifying Treg memory lies in the fact that most Tregs are thought to be self-antigen-specific. Because most of these self-antigens are constitutively expressed, it is difficult to study memory populations that persist in the absence of cognate antigen. Mice infected with the neurotropic strain of the murine coronavirus, mouse hepatitis virus (MHV), develop acute encephalitis as well as immune-mediated acute and chronic demyelinating diseases. Tregs recognizing the immunodominant epitope of MHV have been identified and shown to suppress T cell responses during MHV infection. These virus-specific Tregs play a role in ameliorating immunopathology in MHV infection, as adoptive transfers of Tregs prior to infection increase survival and reduce demyelination. Because MHV infection represents a scenario in which Tregs of known antigen-specificity encounter non-persistent cognate antigen, this represents an ideal model to study the development of Treg memory. Preliminary data shows that adoptively transferred virus-specific Tregs persist in the periphery following MHV infection, suggesting that these cells may represent a memory population.

# Abstracts

**Smooth Muscle-Specific Deletion of Cullin-3 Causes Severe Early Onset Hypertension**

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Dominant *de novo* Cullin-3 mutations resulting in the skipping of exon 9 (CUL3Δ9) causes early onset human hypertension. We showed previously that expression of the CUL3Δ9 protein in smooth muscle interferes with expression and function of endogenous Cullin-3, resulting in hypertension. Here, we tested the hypothesis that deletion of Cullin-3 specifically in smooth muscle would cause severe hypertension. We bred CUL3 "floxed" mice with mice expressing tamoxifen-inducible $CRE^{ERT2}$ under control of the smooth muscle promoter. Tamoxifen (75 mg/kg) was administered *i.p* to generate smooth muscle specific CUL3 knockout mice (S-CUL3KO). Molecular analysis in aorta showed that Cullin-3 protein was undetectable whereas Cullin-1 was abundantly expressed in S-CUL3KO mice. S-CUL3KO mice exhibited increased systolic BP (SBP) at 2 weeks and 4 weeks post tamoxifen administration compared to control mice (2 weeks SBP: 145±1 S-CUL3KO vs 115±2 control, $p<0.001$; 4 weeks SBP: 169±1 S-CUL3KO vs 115±3 control, $p<0.001$). Aorta from S-CUL3KO mice exhibited significantly impaired vasorelaxation to acetylcholine (ACh) (at 100 μM: 1.0±3% S-CUL3KO vs 77±5% control, $p<0.0001$), and to the nitric oxide donor sodium nitroprusside (SNP) (at 100 μM: 15±4% S-CUL3KO vs 96±1% control, $p<0.001$). Consistent with data from aorta, cerebral basilar artery from S-CUL3KO mice also exhibited severe impairment to ACh- and SNP-mediated vasorelaxation compared to controls. As index of aortic stiffness, pulse wave velocity was significantly increased in S-CUL3KO mice (3.7±0.1 S-CUL3KO vs 2.2±0.1 control, $P<0.001$). Total RhoA, a Cullin-3 substrate was increased in adventitia-free media aorta from S-CUL3KO mice (1.8±0.1 S-CUL3KO vs 1.0±0.1 control, $P<0.001$). Interestingly, captopril administration (120 mg/kg/day) in drinking water for 5 days normalized BP in hypertensive S-CUL3KO mice to baseline pre-tamoxifen levels (SBP captopril: 116±1 S-CUL3KO) suggesting a role for the renin-angiotensin system. We conclude that smooth muscle Cullin-3 is a major determinant of vascular tone and BP regulation.

**Histone deacetylase 4 in steroidogenic factor-1 neurons of the ventromedial nucleus of hypothalamus is required for normal glucoregulation in female mice**

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Studies suggest that histone modification is a key process to modulate chromatin condensation and subsequent gene repression and class IIa Histone deacetylases (HDACs) directly mediate the action of several key metabolic hormones including insulin, glucagon, and leptin. Glucose and energy homeostasis are largely governed by distinct types of hypothalamic neurons. However, whether and how individual member of class IIa HDACs in key hypothalamic neurons contribute to glucose metabolism and energy homeostasis is not understood. Here we found that female, but not male, mice lacking a class IIa HDAC, HDAC4, specifically from steroidogenic factor-1 (SF-1)-expressing neurons ($HDAC4^{SF1KO}$) of the ventromedial nucleus of hypothalamus displayed elevated blood glucose levels compared to their littermate controls under long-term high-fat challenge. Despite elevated glucose levels, however, $HDAC4^{SF1KO}$ mice showed comparable food intake, body weight, and glucose tolerance compared to their littermate controls. Consecutive monitoring of blood glucose levels at 4-hour interval over 24-hour period revealed that female, but not male, $HDAC4^{SF1KO}$ showed significantly high glucose level at the beginning of the dark cycle and maintained higher glucose levels throughout the day. In addition, co-immunoprecipitation in hypothalamic extracts demonstrates that HDAC4 directly binds to estrogen receptor alpha, a main mediator of estrogenic action on metabolic regulation. Our results indicate that the gene repression in SF-1 neurons by HDAC4 may represent a novel mechanism for rhythmic regulation of glucose metabolism in female mice. Understanding the metabolic regulation by HDAC4 in this brain site should facilitate the identification of sexually dimorphic mechanisms relevant in the glucoregulation.

# Abstracts

**Relief of Paclitaxel-induced Peripheral Neuropathy by Nicotinamide Riboside, an NAD+ Precursor**

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Chemotherapy-induced peripheral neuropathy (CIPN) is a common and disabling adverse effect of many chemotherapeutic drugs, for which an effective treatment remains elusive. We recently reported that nicotinamide riboside (NR), a naturally occurring vitamin precursor of NAD+, can prevent and relieve established CIPN induced by paclitaxel in tumor-naïve rats. The clinical relevance of such studies in tumor-naïve rats has been questioned. Moreover, it is critical that drugs that alleviate CIPN do so without interfering with chemotherapy and there are concerns that agents that increase NAD+ will promote tumorigenesis. This study 1) determined whether NR treatment facilitates tumorigenesis or interferes with the chemotherapeutic effect of paclitaxel and 2) sought to replicate the findings in tumor-bearing rats. Female rats were injected intraperitoneally with 50 mg/kg NMU (N-methyl-N–nitrosourea) at 3 weeks of age to induce tumors of the mammary gland. To evaluate if NR promotes tumorigenesis, a subset of rats was dosed daily with NR (200 mg/kg) or Vehicle (water) starting at 6 weeks after NMU when tumors began to appear. To evaluate if NR interferes with chemotherapy, a second subset of rats was injected intravenously with paclitaxel when a palpable tumor was detected and concomitant treatment with NR or vehicle was begun. Daily gavage with 200 mg/kg of NR did not facilitate tumor growth (size or number) compared to vehicle-treated rats. Also, paclitaxel reduced tumor size to an equivalent extent in NR and vehicle-treated rats. Finally, NR diminished mechanical hypersensitivity and prevented cold sensitivity induced by paclitaxel in these rats as it did in tumor-naïve rats. Studies are underway to determine whether the mechanism of action entails protection of nerve fibers, possibly by preventing mitochondrial dysfunction, or preventing activation of glia and macrophage. These finding provide additional support for a clinical trial of NR for the relief of taxane-induced peripheral neuropathy.

**Reduced Placental Regulator of G-Protein Signaling-2 (RGS2) and Preeclampsia**

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Though mid- and late-gestational mechanisms of preeclampsia (PreE), a late-pregnancy cardiovascular disorder, are relatively well characterized, the early gestational pathogenesis of PreE remains unclear. Various hormone activators of Gαq second-messenger signaling pathways have been implicated in PreE. Regulator of G-protein Signaling 2 (RGS2) acts as an endogenous terminator of Gαq signaling, and a SNP in the RGS2 gene, resulting in reduced RGS2, is associated with increased risk for developing PreE. We hypothesized RGS2 is expressed in placental trophoblasts, and reduced expression of RGS2 in placental tissue may represent a risk factor for the development of PreE due to disinhibited Gαq signaling. Placental tissues from PreE and normal human pregnancies were examined for mRNA of RGS proteins. Of the RGS members examined, RGS2 is among the highest expressed. In PreE placenta, RGS2 mRNA may be suppressed. Immunohistochemical detection confirms localization of RGS2 protein in trophoblasts of placentas from control and PreE human pregnancies. RGS2 positive trophoblasts are found in the junctional zone/labyrinth of placentas from wildtype C57BL/6J mice. Loss of RGS2 in the feto-placental unit of pregnant wildtype mice induced HIF1α binding to chromatin, supporting placental hypoxia. Further, dams exhibited isolated diastolic hypertension by telemetry, supporting microvascular dysfunction. We conclude RGS2 is present in trophoblasts, placental RGS2 is suppressed in PreE, and loss of feto-placental RGS2 is sufficient to cause placental hypoxia and maternal diastolic hypertension. These studies provide evidence suggesting disinhibited Gαq signaling in the placenta may contribute to PreE, and a father's genetics may contribute to risk for PreE.

# Abstracts

**Characterization of the RBP7 dependent PPARγ antioxidant hub in endothelium**

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand activated nuclear receptor, characterized as a master regulator of adipogenesis and glucose metabolism. Dominant negative PPARγ in the vascular endothelium leads to hypertension, increased oxidative stress, and decreased nitric oxide signaling. PPARγ is dispensable until stress is added, such as high fat diet or angiotensin II. Our laboratory's endothelial specific dominant negative PPARγ mutant (E-V290M) mice develop endothelial dysfunction when challenged with stressors. These effects are mitigated with expression of exogenous ROS scavengers. PPARγ, therefore, plays a pivotal role in protecting the endothelium under stress conditions via anti-oxidant transcriptional activity. Novel bioinformatic work in our lab identified retinol binding protein 7 (RBP7) as a PPARγ target gene, exclusively expressed in the endothelium. RBP7 belongs to the fatty acid binding protein family, which has been shown to act as intracellular shuttles of fatty acid ligands, and has conserved residues for ligand binding. Endothelial specific RBP7 KO in mice resulted in the same endothelial dysfunction as E-V290M mice; also mitigated with ROS scavengers. We hypothesize that PPARγ and RBP7 form a transcriptional regulatory loop in endothelial cells, required for an anti-oxidant state.

To test this hypothesis analysis of: cellular localization of RBP7 with ligand, determination of PPARγ transcriptional responses, and epigenetic alterations are studied. A series of RBP7 mutants have been constructed to define the mechanism of the RBP7 and PPARγ interaction. We have determined trends in subcellular localization of RBP7 with ligand treatment. Moreover, differences in mRNA expression were found between RBP7 KO and WT mice and cells.

**Lateral Hypothalamic Regulation of Energy Homeostasis and Sympathetic Outflow: The Role of Leptin and Melanocortin Signaling**

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Neurons in distinct hypothalamic nuclei play a fundamental role in both body weight homeostasis and sympathetic regulation, but the underlying neural basis of this association remains incompletely understood. The lateral hypothalamic area (LHA) has long been considered as a "feeding center" in the brain as animals with either electronic or excitotoxic lesion of LHA exhibit aphagia and sympathetic overactivity, leading to a dramatic body weight loss or even death. Among the different types of neurons residing in the LHA, we have recently found a unique subpopulation of GABAergic neurons in the LHA that are distinct from MCH and orexin neurons, but coexpress both leptin receptor (LepR) and melanocortin 4 receptor (MC4R), two well-established regulator of feeding, energy homeostasis, sympathetic traffics, and cardiovascular function. Using *in vivo* Cre/loxP system coupled with chemogenetics, here we show that LHA GABAergic neurons are critical for feeding, locomotor activity, sympathetic traffics, and blood pressure regulation. Selective chemogenetic activation of LHA GABAergic and LepR+ neurons increase locomotor activity and decrease food intake. While the change of blood pressure is somewhat dependent on locomotion, depressor effect was observed by selective activation of LHA LepR+ neurons, but not GABAergic neurons. Furthermore, targeted microinjection of leptin into the LHA dose-dependently increase renal SNA, and re-expression of endogenous MC4Rs in the LHA of severely obese MC4R-null mice restores the blunted responses of MC4R agonist (MTII)-mediated increase in sympathetic outflow to the kidney. Our findings highlight an important role of LHA GABAergic neurons which engage leptin and melanocortin signaling to regulate energy homeostasis and sympathetic traffics.

# Abstracts

**Examination of the NCLX Knock-out Effects on Mitochondrial and Cytosolic $Ca^{2+}$ signaling in Hippocampal Neurons**

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Mitochondrial $Ca^{2+}$ transport plays an important role in regulating various physiological and pathological processes in neurons including excitability, synaptic transmission, ATP synthesis, gene regulation and neuronal response to excitotoxic stress. Recently, NCLX ($Na^+$/ $Ca^{2+}$/$Li^+$/ eXchanger, also known as SLC8b1) has been identified as an essential molecular component of mitochondrial $Na^+$/ $Ca^{2+}$ exchange, the system that mediates $Ca^{2+}$ extrusion from the mitochondrial matrix. However the role of NCLX in neurons is not well understood. Here, we used NCLX knock-out (KO) mice (Jackson Lab, C57BL6J background) to examine the role of NCLX in the control of mitochondrial and cytosolic $Ca^{2+}$ signaling in hippocampal neurons. We simultaneously monitored cytosolic and mitochondrial $Ca^{2+}$ concentrations ($[Ca^{2+}]$cyt and $[Ca^{2+}]$mt, respectively) using Fura-2 and mitochondria-targeted $Ca^{2+}$ indicators mt-R-GECO1 or mt-LAR-GECO1.2. Our experiments showed that NCLX KO increased the amplitude of $[Ca^{2+}]$mt elevation (by ~2-fold) produced by strong depolarization (50 mM KCl) and also slowed its recovery toward the baseline compared to hippocampal neurons from WT mice. In contrast, NCLX KO did not have a significant effect on either the amplitude or duration of $[Ca^{2+}]$mt responses evoked by mild depolarization (20 and 30 mM KCl) or by 100 μM glutamate . A characteristic feature of NCLX is that it can use Li+ instead of Na+ to transport $Ca^{2+}$. Notably, we found that replacement of Na+ with Li+ halted $Ca^{2+}$ removal from mitochondria in WT hippocampal neurons. In contrast, Na+ replacement with Li+ did not reduce $Ca^{2+}$ extrusion from mitochondria in WT sensory dorsal root ganglion (DRG) neurons. Under excitotoxic conditions caused by prolonged exposure to 100 μM glutamate, the latencies of delayed $Ca^{2+}$ deregulation and strong mitochondrial depolarization were shorter in NCLX KO hippocampal neurons than in those from WT mice. We also found that the distribution of overexpressed NCLX-Flag was different from that of mitochondria-targeted EGFP in hippocampal neurons. Collectively, our data suggest that NCLX only mildly contributes to mitochondrial $Ca^{2+}$ signaling under some conditions, e.g., strong depolarization, but has little role in regulating $Ca^{2+}$ extrusion from mitochondria under other conditions, such as stimulation by glutamate or mild depolarization. Our findings strongly imply the existence of other molecule distinct from NCLX that mediate $Ca^{2+}$ extrusion from mitochondria in central neurons.

**Study on the compatibility of Manganese mediated radical addition reaction with thiazole**

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Tubuvaline is a fragment of the Tubulysins, which are a family of natural products with remarkable cytostatic activities. Our group developed a Manganese mediated radical addition reaction that can synthesize chiral amines with excellent diastereoselectivities. Such methodology has already been utilized in the synthesis of Tubuvaline by previous graduate students. Here we report the progress we made in a revised approach to Tubuvaline utilizing the same methodology. We report the construction of the amine substructure by radical addition reaction with the presence of thiazole group, and how the compatibility is influenced by the substituent groups on the thiazole.

# Abstracts

**Functional Characterization of the Mitochondrial $Na^+$-$Ca^{2+}$ Exchanger (NCLX) in the Peripheral Nervous System using NCLX Knock-out Mice**

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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 such as excitability, neurotransmission, gene expression and neuronal survival. Past research has identified solute carrier from family 8 member B1 (SLC8B1, also known as NCLX) as the mitochondrial $Na^+$-$Ca^{2+}$ exchanger. NCLX has been proposed to be one of the mechanisms involved in mitochondrial $Ca^{2+}$ extrusion found in the inner mitochondrial membrane. However, the role of NCLX in neurons remains largely unknown. Using RT-PCR, we found that NCLX is expressed throughout the brain, and is also present in dorsal root ganglia (DRG) neurons. By simultaneously monitoring $Ca^{2+}$ concentration in the cytosol ($[Ca^{2+}]_{cyt}$) and mitochondria ($[Ca^{2+}]_{mt}$) of cultured DRG neurons, we found that NCLX knockout (KO; Jackson Lab, C57BL/6J background) did not significantly alter the amplitude of depolarization-induced $[Ca^{2+}]_{cyt}$ or $[Ca^{2+}]_{mt}$ elevation as compared to WT animals. However, $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mt}$ recovery to baseline in response to moderate and strong depolarization (30 mM, 50 mM KCl respectively, both for 30 s) was slowed by 2-3 fold, but not halted, in NCLX KO as compared to WT animals. In contrast, when $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mt}$ elevations were elicited by trains of action potentials (5-8 Hz, 4-8 s) using extracellular field stimulation no significant difference was found between NCLX KO and WT DRG neurons in either recovery kinetics or amplitudes. In control experiments, we found that the pharmacological inhibitor of mitochondrial $Na^+$-$Ca^{2+}$ exchanger, CGP37157 (3 and 10 $\mu$M) markedly slowed the recovery of $[Ca^{2+}]_{mt}$ to the baseline in both WT and NCLX KO. In addition, replacement of $Na^+$ with $Li^+$ did not significantly alter the ability of mitochondria to extrude $Ca^{2+}$ from the mitochondrial matrix in DRG neurons. Collectively, our data suggest that NCLX contributes to $Ca^{2+}$ extrusion from mitochondria in DRG neurons following large depolarization-induced $[Ca^{2+}]_i$ transients, but not smaller ones evoked by electrical field stimulation. Our findings also point to the existence of additional, distinct from NCLX transporter(s) that mediate $Ca^{2+}$ efflux from mitochondria in a CGP37157-sensitive manner in DRG neurons.

Key words: NCLX, mitochondria, Ca2+, DRG, CGP37157

**Determining the role of the AT-hook and bromodomain in mediating chromatin signaling to the Brahma Subunit of the SWI/SNF Complex**

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The packaging of eukaryotic DNA into chromatin acts to both compact it into the nucleus as well as provide a mechanism of regulation for all DNA-templated processes. The switching/sucrose non fermenting (SWI/SNF) remodeling complex plays an important role in the restructuring of chromatin. The Brahma related gene 1 (BRG1) subunit provides the ATP-dependent helicase activity of SWI/SNF. Bromodomains (BD) are known to bind acetylated lysines on histone tails, however, the BRG1 BD displays weak affinity and poor specificity for acetylated histone tails in vitro. We propose that the targeting of SWI/SNF to chromatin is modulated by the concomitant activity of BRG1 BD and an adjacent AT-hook motif. We aim at understand the molecular mechanisms by which BRG1 associates with acetylated histones thus modulating the association of SWI/SNF. In order to elucidate such mechanisms, we are studying the functions of the BRG1 BD and AT-hook in the context of the full nucleosome. Our preliminary studies with Electromobility Shift Assays (EMSA) and NMR have revealed a nonspecific binding of BRG1 BD for DNA which is a novel property of this bromodomain. Furthermore they have demonstrated that addition of the AT-hook increases the affinity and specificity for DNA elements. For future research, we propose a stepwise process to elucidate the mechanisms of BRG1 AT-BD association with nucleosomes by performing experiments with separate AT-BD and BD constructs. Different biophysical techniques will be used such as NMR spectroscopy, SAXS, and single molecule TIRF microscopy to assess the interactions of AT-BD and BD with DNA.

# Abstracts

**Regulator of G-protein signaling 6 (RGS6) expression in human substantia nigra pars compacta (SNc) and loss in Parkinson's disease (PD)**

**Zili Luo**[1], Katelin E. Ahlers-Dannen[1], Jianqi Yang[1], Bandana Chakravarti[1], Hanna E. Stevens[2], Nandakumar S. Narayanan[3], and Rory A. Fisher[1]

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Parkinson's disease (PD) is a devastating neurodegenerative disorder characterized by the progressive loss of dopamine (DA) neurons in the SNc. The reason for the greater sensitivity of these neurons to degeneration and their loss with aging in PD is unknown. Although there are treatments targeting symptoms of this disease there are no interventions that protect DA neurons from degeneration. We and our collaborators have recently discovered that RGS6 is restrictively expressed in and required for the adult survival of SNc DA neurons that undergo degeneration in PD. Mice lacking RGS6 exhibit a late onset age-dependent loss of SNc DA neurons accompanied by reduced striatal and SNc DA content and motor deficits as well as increased sensitivity to 6-OHDA-induced neurodegeneration and behavioral deficits. These findings suggest a critical pathogenic link between RGS6 loss and DA neuron degeneration in PD. Here we tested this hypothesis by a quantitative analysis of RGS6 expressing neurons in the SNc of human control and PD patients (n = 5 each). Immunohistochemical analysis of RGS6 and tyrosine hydroxylase (TH) expression and unbiased sterology was used to quantify the numbers of RGS6+ and TH+ SNc neurons. We found that RGS6 is exclusively expressed in DA neurons in the SNc of humans and there is a dramatic (73%) and selective loss of these RGS6+ TH+ neurons in PD patients. Consistent with our hypothesis, all surviving SNc TH+ neurons in normal and PD patients express RGS6. Given our evidence that RGS6 is required for survival of SNc DA neurons in mice, these findings suggest that RGS6 loss in human SNc DA neurons renders them susceptible to neurodegeneration characteristic of PD. These findings provide new evidence suggesting that RGS6 may have a critical neuroprotective role in PD in both mice and humans. Together our findings identify RGS6 as a potential novel therapeutic target for PD treatment (Supported by NIH CA161882, MJ Fox 11551).

**Regulation of Mitochondrial Form and Function by Splice Variants, Phosphorylation and Magnesium**

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Mitochondria are very dynamic organelles that undergo the process of fission and fusion which is tightly regulated. Dynamin related protein 1 (Drp1), a large GTPase, is targeted to the mitochondria where it mediates fission by oligomerizing into rings around the mitochondria and physically dividing it through GTP hydrolysis. Mitochondrial fission factor (Mff) promotes mitochondrial fission by targeting Drp1 to the outer mitochondrial membrane (OMM). Mff has many alternatively spliced exons and the shortest variant, 0000, is the most commonly expressed across different tissues and the most effective at driving fission. In neurons, the most abundant splice variant is 0011, which promotes less fission than Mff 0000. In vitro and in cell data indicate Mff 0000 forms dimer/tetramers that turnover rapidly while Mff 0011 forms very stable oligomers of tetramers. Mff is a phosphorylated protein and this modification opens the tetramer to enhance Drp1 recruitment and fission. Additionally, phosphorylation of Mff 0011 prevents oligomerization driving fission and leads to an increase to neuronal sensitivity in a culture model of stroke. However, magnesium addition, in vitro, promotes oligomerization. These data indicate that the fission activity of Mff 0011 is increased by phosphorylation but decreased by magnesium in the neuron enriched Mff splice variant. Since Mff is predicted to be highly disordered, we have examined the complex by small angle X-ray scattering (SAXS) to better understand its function. Excessive fission may underlie the mitochondrial dysfunction seen in diseases such as Parkinson's disease and Alzheimer's disease and may offer new targets for therapeutic treatments.

# Abstracts

**Neurons of the arcuate nucleus in mice express angiotensinogen**

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Recent studies from our laboratory have demonstrated that angiotensin II (ANG) type 1A receptors (AT1A) specifically localize to the subset of neurons which express both the leptin receptor (LepR) and agouti-related peptide (AgRP). These are critically involved in the control of thermogenic adipose sympathetic nerve activity (SNA) and thereby resting metabolic rate (RMR).This mechanism appears to involve AT1A-mediated suppression of gamma-aminobutyric acid (GABA) synthesis and packaging in AgRP neurons of the arcuate nucleus (ARC). It remains unclear, however, how LepR signaling results in AT1A activation within AgRP neurons. We hypothesize that activation of LepR in POMC and/or AgRP neurons results in de novo synthesis and local release of angiotensinogen (AGT) within the ARC, and consequently increased autocrine or paracrine ANG signaling within the ARC. To test this hypothesis, we examined the localization of AGT mRNA expression within the ARC of wildtype C57BL/6J mice by fluorescent in situ hybridization methods (RNAscope) and confocal fluorescent microscopy. Consistent with our working hypothesis, cells within the ARC expressing AGT mRNA colocalize with both AgRP and POMC. Cells within the ARC expressing insulin II (INS2) also expressed AGT. Finally, as expected, AGT was also expressed in astrocytes, colocalizing with glial fibrillary acidic protein (GFAP). We and others have previously demonstrated that LepR activation increases phosphorylation of the signal transduction and activator of transcription-3 (STAT3) transcription factor, and that p-STAT3 appears to stimulate AGT expression in other cell types including cardiac myocytes and hepatocytes. We therefore hypothesize that the phosphorylation of STAT3 by LepR activation is mechanistically involved in the regulation of AGT expression by leptin in POMC and AgRP neurons. Ongoing studies are aimed at (i) examining AGT expression control by pSTAT3 in POMC and AgRP neurons of the ARC, and (ii) using Cre-lox recombination methods to selectively disrupt the AGT gene in LepR, POMC and AgRP neurons to study the functional significance of AGT in these cells in the control of SNA and RMR, and thereby energy homeostasis.

**Cardiovascular development defects produced by combined loss of RGS6 and oxidizable CaMKII due to defective Notch signaling**

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Congenital heart defects (CHDs) are the most common birth defects with nearly 40,000 infants born annually with a CHD in the U.S. alone. There is a critical lack of understanding of the etiology of CHDs and therefore, effective therapeutic strategies for this disease. Emerging evidence has implicated defective Notch signaling as the major cause of embryonic death of mice due to cardiovascular defects. RGS6 is abundantly expressed in the heart and mediates doxorubicin (Dox)- and alcohol-induced myocardial cell apoptosis and cardiomyopathies by functioning as an upstream activator of NADPH oxidase (Nox) and consequent reactive oxygen species (ROS) generation. Oxidative activation of the Ca2+/calmodulin-dependent protein kinase II δ (CaMKIIδ) on two key methionines in response to ROS generation in heart promotes cardiac disease including cardiomyopathy and cell death. While interrogating the relationship between RGS6 and CaMKII oxidation in heart we discovered a severe defect in embryonic cardiovascular development due to combined loss of RGS6 and ox-CaMKII. To determine the function of RGS6 and ox-CaMKII in embryonic cardiovascular development and the underlying mechanisms responsible for cardiovascular defects due to their combined loss, we developed double mutant ($RGS6^{-/-}$/$CaMKII^{VV}$) mice by intercrossing of knock-in mice expressing an oxidation resistant form of CaMKIIδ ($CaMKII^{VV}$) and $RGS6^{-/-}$ mice. Here, we show that $RGS6^{-/-}$/$CaMKII^{VV}$ mice undergo midgestational embryonic lethality associated with cardiac, hematopoietic, and vascular remodeling defects in placentas, yolk sacs, and embryos. Combined loss of RGS6 and ox-CaMKII promoted endothelial cell apoptosis in hearts and decreased the expression of arterial markers and Notch signaling genes including Efnb2, Gja4, Gja5, Notch1, Jag1, Hey1, Hey2 and Hey1L in both yolk sacs and embryos. In conclusion, RGS6 and ox-CaMKII are required together for cardiovascular development and function in parallel pathways as critical upstream modulators of Notch signaling.

# Abstracts

**Investigating a Novel Mechanism for the Beiging of White Adipose Tissue**

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Beige, UCP1-positive adipocytes are detectable 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 or increasing the "beiging" of white adipose tissue could be therapeutically used to counter the development of obesity. Despite this premise, much remains unknown regarding the molecular and transcriptional mechanisms underlying beige adipocyte development. T-box transcription factor-1 (TBX1) has been identified as a beige adipocyte transcription factor found in both rodents and humans. TBX1 is a member of the T-box family of transcription factors yet, aside from its expression in beige adipocytes, nothing is known regarding its specific function in these cells. To test the role of TBX1 in beige adipocyte development and metabolism we have generated two novel mouse models allowing for adipose specific overexpression of TBX1 (TBX1 AdipoTG) and adipose specific deletion of TBX1 (TBX1 AdipoKO). Using these mouse models and a combination of complementary molecular techniques we are able to test the sufficiency and necessity of TBX1 expression during the beiging of white adipose tissue.

**Rescuing Mitochondrial Division in Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) via Chemically-Inducible Drp1 Recruitment**

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First identified in the Charlevoix-Saguenay region of Quebec, ARSACS is now recognized as the second most common recessive spastic ataxia. There are no treatments for this devastating disorder, which is caused by mutations in the *SACS* gene and which strikes homozygous carriers in early in life. The recently characterized *SACS-/-* mouse recapitulates many of the cardinal features of ARSACS, including gait abnormalities and loss of cerebellar Purkinje cells. These studies also implicated mitochondrial dysfunction resulting from impaired mitochondrial division as a pathogenic mechanism. Regardless of whether dysregulated mitochondrial fission/ fusion is a primary event (as suggested by the interaction between sacsin and the mitochondrial fission enzyme Drp1) or occurs downstream of cytoskeletal abnormalities, interventions that restore healthy levels of mitochondrial division hold promise for the treatment of ARSACS. In our project we have planned to: 1) directly assess the contribution of mitochondrial dysfunction to neurological phenotypes in *SACS-/-* mice and to 2) provide proof-of-concept evidence for the therapeutic potential of drugs that stimulate mitochondrial division. To this end, we developed a novel transgenic technology, which we refer to as chemically-inducible Drp1 recruitment (CIDR), to drive mitochondrial division, thereby accelerating mitochondrial biogenesis and clearance of dysfunctional mitochondria via mitophagy. Under control of a conditional promoter for brain-region specific expression, the CIDR transgene encodes a two-component Drp1 receptor that can be activated with a dimerizing drug. Currently, our efforts are to generate mice with CIDR in cerebellar Purkinje neurons, cross them with *SACS -/-* mice, and assess dimerizer-dependent improvements in histopathology and motor coordination.

# Abstracts

**WDR26 promotes AKT2-driven breast cancer cell growth and migration**

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The phosphoinositide 3 kinase (PI3K)/AKT pathway governs cell growth and migration and is one of the most deregulated pathways in breast cancer. AKT assumes three isoforms that share a high sequence homolog but play distinct functions in breast cancer progression. Although both AKT1 and AKT2 are required for tumor growth, AKT1 inhibits tumor metastasis, while AKT2 promotes tumor invasion and metastasis. It remains largely unknown the mechanisms by which AKT isoforms function differently in breast cancer metastasis. Previously we identified WDR26 as a novel scaffolding protein that is upregulated in breast cancer and promotes G protein-coupled receptor-mediated PI3K/AKT activation. Here, we show that WDR26 preferentially interacts with AKT2 in vitro and in vivo. Knockdown of WDR26 in breast cancer cells largely reduced AKT2 activation but had a minor effect on AKT1 activation. Overexpression of an active AKT2, but not AKT1, rescued the defect in MDA-MB 231 cell proliferation and migration induced by WDR26 knockdown. Taken together, our results have demonstrated a key role of WDR26 in promoting AKT2-driven breast cancer cell growth and migration.

**Contribution of POMC and AgRP neurons to the control of metabolic autonomic nerve activity by leptin**

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Central action of the adipocyte-derived hormone leptin is critical for metabolic regulation. Leptin promotes energy expenditure by increasing sympathetic nerve activity (SNA) to thermogenic brown adipose tissue (BAT). Leptin also increases hepatic SNA and parasympathetic nerve activity (PNA) to modulate glucose production by the liver. We previously demonstrated the importance of the hypothalamic arcuate nucleus (ARC) in underlying leptin -induced increases in BAT SNA as well as liver SNA and PNA, but the contributions of specific neuronal populations within the ARC are not fully understood. Here, we tested the hypothesis that proopiomelanocortin (POMC) and agouti-related peptide (AgRP) neurons of the ARC mediate regional SNA and PNA responses evoked by leptin. To test this, we used direct multifiber nerve recording to assess the effects of intracerebroventricular (ICV) leptin (2 μg) on regional nerve activity in mice lacking leptin receptors on POMC ($POMC^{Cre}/LepR^{fl/fl}$) or AgRP neurons ($AgRP^{Cre}/LepR^{fl/fl}$). We observed a partially blunted BAT SNA response to ICV leptin in both $POMC^{Cre}/LepR^{fl/fl}$ (148±29%, $p<0.05$) and $AgRP^{Cre}/LepR^{fl/fl}$ mice (172±62%, $p<0.05$) as compared to littermate controls (327±62%), suggesting a distributed control of BAT thermogenesis by leptin across neuronal populations. Conversely, the hepatic SNA response to leptin was significantly reduced in $AgRP^{Cre}/LepR^{fl/fl}$ mice (76±21%, $p<0.05$), but not in $POMC^{Cre}/LepR^{fl/fl}$ mice (120±49%) relative to controls (196±36%). However, the hepatic parasympathetic response to leptin was completely abolished in mice lacking leptin receptors on either POMC (-17±17%) or AgRP (20±15%) neurons vs control mice (108±24%, $p<0.05$), indicating that both of these populations are required for leptin to suppress hepatic glucose production. Together, these findings support a critical role for hypothalamic neurocircuitry in dissociating the regional autonomic effects of leptin, in particular with respect to thermogenic control and glucose homeostasis.

# Abstracts

**Role of BBS proteins in insulin receptor trafficking and signaling**

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Background: Insulin and it receptor plays an important role in glucose homeostasis but the mechanisms underlying insulin resistance remain poorly understood. Bardet Biedl syndrome (BBS) is a human genetic disorder associated multiples features including insulin resistance and obesity. BBS is considered as a prototypical example of a ciliopathy – cilia-related disease and the mechanisms of insulin resistance in BBS remains elusive.

Objective: Test the hypothesis that BBS proteins mediate insulin receptor trafficking to the plasma membrane and impairment of BBS proteins leads to insulin resistance.

Design/Methods: 1) Cultured IMCD and HEK 293 cells in which the Bbs1 gene was deleted using CRISPR-Cas9 were used, 2) Confocal microscopy was used to study the membrane localization of the insulin receptor and glucose transporter (GLUT 4), and 3) Western blots used to study the insulin receptor signaling capacity (AKT).

Results: Cells lacking the Bbs1 gene displayed decreased amount of insulin receptors in the plasma membrane relative to control cells. Consistent with this observation, Bbs1 deficient cells treated with insulin exhibited a decreased activation of the downstream AKT signaling. We also found that the amount of GLUT4 in the plasma membrane is decreased in the cells lacking the Bbs1 gene as compared to wild type cells

Conclusion(s): Our findings highlight the importance of BBS proteins for the membrane localization of the insulin receptor, its downstream signaling pathway as well as the membrane localization of the glucose transporter, GLUT4. A disrupted insulin receptor trafficking and signaling may thus account for insulin resistance in BBS patients.

**Investigation of RTEL1 DNA Maintenance Mechanisms**

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Abstract: Telomeres act as a buffer region at the end of chromosomes, preventing degradation of the genetic code during replication. In humans this repetitive TTAGGG sequence is vital to keeping our genome intact. Regulator of telomere length 1 (RTEL1), an iron-sulfur cluster helicase, is an important factor in maintaining telomere length. RTEL1 acts to resolve specific DNA structures that form at telomeres that can lead to telomere loss during replication. RTEL1 also acts throughout the genome as an antagonist to homologous recombination. While it is clear that RTEL1 plays an important role in DNA maintenance, due to a lack of biochemical studies on RTEL1, many questions remain regarding the mechanisms of RTEL1. Lack of biochemical studies is driven in part by the challenge of purifying the protein. Our current work involves optimizing the purification of active RTEL1 from HEK293 cells. Single molecule total internal reflection microscopy (SM TIRFM) experiments allow for the use of very little protein while yielding a great deal of information on DNA binding and remodeling activity of helicases such as RTEL1. We aim to address questions of RTEL1 mechanism of dismantling specific DNA structures, including G-quadruplexes and displacement loops, as well as the role of interactions with other proteins.

# Abstracts

**CDK8-Selective Kinase Inhibitor, Senexin A, in Regulation of Heart Disease**

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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 overexpression 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.

**Sunitinib-loaded poly(lactic-co-glycolic acid) nanoparticles to deplete myeloid-derived suppressor cells**

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Immunotherapy has recently emerged as an outstanding option to treat cancer. This treatment strategy is focused on stimulating immune cells to recognize and kill tumor cells. However, this treatment strategy can be problematic since tumor cells develop a variety of mechanisms to suppress host immune responses. Myeloid-derived suppressor cells (MDSC) are a group of heterogeneous immature myeloid cells that inhibit T cell responses and reduce the efficacy of cancer immunotherapies. In tumor bearing mice, this cell population include a granulocytic (gMDSC) and a monocytic (mMDSC) subset that accumulates the lymph nodes, spleen, and tumor.

In this project, we propose the depletion of MDSC using poly(lactic-co-glycolic acid) nanoparticles functionalized with pepH6 (a MDSC-binding peptide sequence) and loaded with sunitinib, exploring the potential ability of these particles to accumulate in sites where MDSC is known to be present. We found that both pepH6 functionalized and non-functionalized sunitinib nanoparticle formulations accumulated in the spleen and depleted gMDSC in this organ. Moreover, biodistribution studies showed the ability of nanoparticles to accumulate in the lymph nodes and tumor, suggesting its potential to deplete MDSC in these tissue compartments. Even though further work is needed to determine and improve the effect of nanoparticle functionalization on MDSC targeting, our results suggests that these formulations may be useful in improving the efficacy of an immunotherapy, as reduction of MDSC levels can attenuate T cell suppression.

# Abstracts

**Characterization of epidermal properties and skin surface analytes in children with infantile hemangiomas**

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Introduction: Infantile hemangiomas are benign vascular lesions which may have different epidermal properties and skin surface cytokines, chemokines, and biologic analytes (CCBAs) compared to non-lesional skin. The objective of this study was to characterize the epidermal properties and CCBA expression of normal and lesional skin in infantile hemangiomas patients.

Methods: All procedures were approved by the University of Iowa Institutional Review Board. Children diagnosed with a hemangioma who had not received prior treatment were enrolled. Barrier function (measured by transepidermal water loss (TEWL)), hydration, color, and pH were measured on the hemangioma and on nearby normal skin. CCBAs from both sites were collected via skin wash sample and quantified using a multiplexed fluorescent bead-based immunoassay. The sites were compared using a paired t-test, and hemangiomas classified by different covariates were compared using a one-way ANOVA with Tukey's multiple comparison ($p \leq 0.05$ was considered significant).

Results: Twenty-two children (median age: 3.7 months, range 1.1-96.0 months) were enrolled in the study. For the majority of subjects, TEWL and pH of hemangioma skin was not significantly different than normal skin ($p > 0.05$). Hydration and color were significantly different ($p < 0.05$) between sites in 68.4% and 80.0% of subjects, respectively. CCBA concentrations on the hemangioma were significantly different between hemangioma locations and growth stages ($p < 0.05$); sex and hemangioma depth did not influence CCBA concentrations ($p > 0.05$).

Conclusions: Hemangioma skin has similar barrier function and pH as non-lesional skin. CCBA expression is related to various covariates; a generalized linear mixed model will be used to further evaluate these trends.

**Discovery and characterization of two drivers of hepatocellular carcinoma: *Gli2* and *Fign***

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Liver cancer is globally the sixth most commonly diagnosed and the third most deadly cancer. The most common form, hepatocellular carcinoma (HCC), predominately occurs due to liver cirrhosis. HCC is a difficult cancer to treat due to inadequately understood pathways involved in tumorigenesis that interact with the cirrhotic microenvironment. To discover genetic drivers of HCC, we performed a forward genetic screen using Sleeping Beauty (SB) transposon mutagenesis in a damaged liver microenvironment. Briefly, SB screens allow us to analyze common transposon insertion across tumors to determine candidate cancer driver mutations.

This SB screen identified *Gli2* and *Fign* as novel driver mutations in a significant subset of the tumors. *Gli2* is a Hedgehog signaling transcription factor that promotes transcription of cell proliferation genes. *Fign* is a relatively uncharacterized protein with the ability to sever microtubules. In human HCC, both GLI2 and FIGN protein levels are elevated as compared to normal tissue. We validated *Gli2* as a driver of HCC by generating hepatocyte-specific *Gli2*-overexpressing mice using hydrodynamic tail vein injection of *Gli2* cDNA into *FAH-/-* mice. Mice underwent rapid tumorigenesis, demonstrating that *Gli2* is a strong oncogene. Additionally we have demonstrated that overexpression of *Fign* in cultured hepatocytes promotes invasion after organoid formation. Current projects focus on characterizing the tumorigenic mechanism of these two genetic drivers.

The discovery of two novel HCC driver mutations, validation of *Gli2* as a bona fide HCC oncogene, and mechanistic insight into these drivers will allow for future research into understanding HCC tumorigenesis and potential therapeutic options.

# Abstracts

**Extracellular matrix effects on the uptake and toxicity of amyloid-beta**

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Alzheimer's disease (AD) is the most common form of dementia, and currently affects more than 30 million individuals worldwide. Because AD is still poorly understood, there are currently no treatment options to either delay the onset, or slow the progression of the disease. Current research suggests the progression of the disease is caused by the production and aggregation of the amyloid-beta peptide. The monomeric species of this peptide is generally regarded as non-toxic, at physiologic levels, but its presence in aggregated forms, such as oligomers, protofibrils and mature fibrils, are believed to induce neurotoxicity and dystrophy. Spontaneous Aβ fibrillation i*n vivo* remains poorly understood, but it has been suggested that fibril nucleation *in vitro* occurs via an intracellular pathway initiated by monomeric Aβ endocytosis. Furthermore, it has been shown that the brain becomes softer with aging, and even softer in patients with AD. This suggests that the extracellular matrix (ECM) may play a role in Aβ fibril formation, through a change in Aβ endocytosis. This could provide a better approach to determine an in vivo mechanism to underscore Alzheimer's disease.

It has been previously shown that cells grown on softer substrates have a reduced membrane tension, and show a modification of both particle and peptide uptake. To model the varying environment of the ECM, polyacrylamide gels were made with varying mechanical moduli, done by adjusting the percent of polyacrylamide (5-10%) and N,N'-Methylenebisacrylamide (BIS) (0.3-0.03%). The stiffness of each gel was determined by measuring the shear modulus using rheology. An ECM linker protein (fibronectin) was then conjugated to the gel surface using sulfo-SANPAH. SH-SY5Y neuroblastoma and Hep-2 epithelial cells grown on these gels show a sensitivity to their ECM environment. An increase in cell spreading and stress fibers were observed with an increase in the substrate rigidity. The toxicity of Aβ was studied by treating the cells with either monomeric or fibrillated species of Aβ, and a higher toxicity was observed in cells plated on stiffer substrates. Moreover, the extent of Aβ internalization and its intracellular distribution was confirmed with confocal microscopy and further evaluated using a quantitative dot-blot analysis, showing a decrease in Aβ uptake in response to ECM stiffness.

Intracellular Aβ processing remains a central question to understanding the early-stage events in AD pathogenesis. If the ECM plays a role in Aβ endocytosis, and fibril formation occurs, the ECM could also be playing a role in the neurotoxic effects of AD. This work demonstrating the biophysical role of ECM stiffness in modifying Aβ internalization and plaque production offers an improved *in vitro* model to correlate with further *in vivo* studies, and could potentially offers novel avenues of therapeutic intervention once the role of the ECM is understood.

# Abstracts

**Effect of microneedles and formulation parameters on immune response in the elderly**

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Introduction: Aging is associated with a decreased response to vaccination. Microneedles (MNs) can target antigen presenting cells (APCs) in skin to generate a robust immune response. Furthermore, APCs process particulate antigens more efficiently than soluble antigens. The objective of our study was to evaluate the effect of antigen nanoparticle formulation and MN type on immune response in elderly mice.

Methods: Young, adult and elderly mice (2, 10 and 24 months respectively, n=5) were vaccinated with 100µg of soluble ovalbumin (OVA) or OVA-loaded PLGA nanoparticles (PLGA-OVA). Additional group of elderly mice were treated with solid steel MNs (750 µm length), and the formulation (soluble or particulate OVA) was applied over treated skin. IgG1 and IgG2α antibodies were measured at day 21, 28 and 35 using ELISA.

Results: PLGA-OVA generated significantly higher antibody titers (IgG1:211200±158844, IgG2α:7700±5862) than soluble OVA (IgG1:180±40 and IgG2α:10±20) in young and adult mice ($p<0.001$). However, PLGA-OVA produced lower titers in elderly mice (IgG1:2566±2025, IgG2α:1200±400). No significant differences were observed in IgG1 titers with MN treatment. However, MN treatment produced significantly higher IgG2α titers at day 35 (250±58) compared to subcutaneous vaccination (30±27) with PLGA-OVA and soluble OVA ($p<0.01$).

Conclusion: PLGA-OVA produced higher antibody titers in all groups; however, lower antibody titers in elderly mice warrants the need for better vaccination strategies. Higher number of IgG1 antibodies indicates polarization towards Th2 type immunity. MN treatment generated higher IgG2α titers in elderly mice. In the future, PLGA-OVA loaded in dissolving MNs will be evaluated for synergistic effect on immune response.

**Deletion of AKAP1 in mice enhances memory, but increases ischemic sensitivity in the brain**

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Recent work has highlighted the importance of mitochondrial fission and fusion in neuron survival and development. Inhibiting mitochondrial fission in hippocampal neuron cultures is protective against numerous insults, but impairs dendritic spine formation. Similarly, forebrain specific deletion of Dynamin related protein 1 (Drp1), the enzyme which catalyzes fission of the outer mitochondrial membrane (OMM), in mice impairs mitochondrial fission, synaptic transmission, and memory but pharmacological inhibition of Drp1 is protective against cerebral ischemia. Oppositely, promoting mitochondrial fission in hippocampal neuron cultures through knockdown of A Kinase Anchoring Protein 1 (AKAP1), which localizes PKA to the OMM promoting inhibitory phosphorylation of Drp1 @ Serine 637 (S637), sensitizes neurons to insult, but increases dendritic spine density. However, the effects of deleting AKAP1 on mitochondrial fission, neuron survival and development have yet to be investigated *in vivo*. Using mice in which *AKAP1* was deleted we hypothesized increased mitochondrial fission in neurons, increased cerebral ischemic sensitivity, increased dendritic spine density and enhanced memory. We found that *AKAP1* -/- mice exhibit significant mitochondrial fragmentation in hippocampal CA1 neurons, reduced phosphorylation of Drp1-S637 and increased Drp1 localization to mitochondria relative to *AKAP1* +/+ mice. Confirming our hypothesis, *AKAP1* -/- mice were more sensitive to cerebral ischemia as evident by increased cerebral infarct volume following focal occlusion. However, when evaluating dendritic spine density we did not observe an increase in *AKAP1 -/-* mice. Though, we did observe a significant increase in mushroom dendritic spine volume in *AKAP1 -/-* mice. Additionally, phosphorylation of CaMKII @ Threonine 286 was increased in *AKAP1* -/- mice with contextual memory being significantly augmented in *AKAP1 -/-* mice. This work implicates phosphoregulation of Drp1 and mitochondrial fission as important mediators of neuron survival and function.

# Abstracts

**CRISPR-Cas9 Gene Editing Yields a Novel Rat Model of the Metabolic Syndrome**

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Metabolic Syndrome (MetS) is the clinical presentation of three or more risk factors—central obesity, dyslipidemia (elevated triglycerides, low HDL), hyperglycemia and hypertension—each of which contributes to increased risk of heart disease, diabetes and stroke in more than 20% of U.S. adults. There is strong evidence that MetS and its symptoms are highly heritable, yet identification of causative genes remains elusive, likely due to the complexity of the syndrome. Genome-wide association studies have fallen short in determining the causative loci; therefore, we employ the genetically tractable inbred Lyon Hypertensive rat model to tease apart the complex etiology of MetS.

Using a genome-wide approach, we previously identified a completely novel gene on rat chromosome 17 (RNO17) using a combination of QTL and eQTL mapping and gene network analysis, and found that *RGD1562963* (*RGD*) has genetic effects on components of MetS.

CRISPR-Cas9 gene editing was used to introduce insertion and deletion mutations (indels) in exon 2 of *RGD*, and we are currently studying the mutations' effects in the LH-derived rat. Though experiments are ongoing, preliminary data indicates homozygous *RGD* mutants have decreased body weight and abdominal adiposity, and also have increased resting aerobic metabolic rate (RMR) and hypertension.

Our studies suggest *RGD* exerts pleiotropic effects on various components of MetS. Furthermore, inhibition of this gene at the whole body level is tolerated, and induced increased resting metabolic rate and prevention of obesity. 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.

**E3 Ubiquitin Ligase Mutations in X-linked Intellectual Disability**

**Jianing Song, MS**[1], Ronald A. Merrill, PhD[1], Yujia Liu, BS[1], Marie Shaw, BS(Hons)[2,3], Renee Carroll, BS[2,3], Vera Kalscheuer,PhD[4], Fiona McKenzie, PhD[5], Lachlan Jolly, PhD[2,3], Jozef Gécz, PhD[2,3] and Stefan Strack, PhD[1]

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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) 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.

# Abstracts

**Physiological Significance of Angiotensin $AT_{1A}$ Receptors in Vasopressin-Producing Cells**

**Danny W Linggonegoro**[1], Jeremy A Sandgren[1], Kristin E Claflin, PhD[1], Katherine J Perschbacher[1], Jonathan Ni[1], Nicole A Pearson, MPH[1], Gary L Pierce, PhD[2], Mark K Santillan, MD, PhD[3], Justin L Grobe, PhD, FAHA[1]

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Low-renin and salt-sensitive forms of hypertension are characterized by elevated activity of the brain renin-angiotensin system and secretion of arginine vasopressin (AVP). While angiotensin in the brain is a known stimulant of AVP secretion through its AT1 receptor, the localization of relevant AT1 receptors remains unclear. We tested whether $AT_{1A}$ receptors localized to AVP-producing cells are important for AVP secretion. To examine AVP and $AT_{1A}$ co-localization, mice expressing Cre-recombinase via the AVP gene (AVP-Cre) were bred with mice expressing a conditional red fluorescent ROSA-$stop^{flox}$-tdTomato construct and GFP via an $AT_{1A}$ BAC transgene. Dual-fluorescent cells were detected in supraoptic nuclei (SON) but not paraventricular nuclei. Mice lacking $AT_{1A}$ specifically in AVP-producing cells ($AT_{1A}^{AVP\text{-}KO}$) were then generated by breeding AVP-Cre mice with mice harboring a conditional endogenous $AT_{1A}$ gene. $AT_{1A}^{AVP\text{-}KO}$ mice exhibited normal serum (littermate n=18, 302±35 vs $AT_{1A}^{AVP\text{-}KO}$ n=8, 308±42 pg/mL, p=NS) and urine (n=26, 145±33 vs n=11, 170±54 pg/mL, p=NS) copeptin (the stable C-terminal fragment of AVP) as well as hematocrit (n=14, 46.3±0.7 vs n=7, 47.5±1.3 %, p=NS), despite a physiologically and statistically significant increase in serum osmolality (n=33, 324±1.3 vs n=19, 330±1.6 mOsm/kg, $p<0.01$), supporting a role for $AT_{1A}$ in AVP-producing cells in modulating the osmotic control of AVP release. Systolic blood pressure (SBP) (n=18, 109±1.3 vs n=5, 107±1.2 mmHg), urine volume (n=27, 1.1±0.1 vs n=12, 0.9±0.2 mL/d), and fluid intake (n=27, 4.0±0.2 vs n=12, 3.9±0.2 mL/d) were all normal (p=NS) in $AT_{1A}^{AVP\text{-}KO}$ mice. Two-bottle choice between water and escalating concentrations of NaCl uncovered minor alterations in sodium intake behavior. Serum osmolality (n=22, 336±2 vs n=9, 333±3 mOsm/kg), SBP (n=23, +10.4±2.1 vs n=8, +12.9±2.0 mmHg), urine output (n=23, +12.7±0.8 vs n=9, +12.7±1.5 g/day), and fluid intake (n=23, +16.2±1.3 vs n=9, +14.8±2.5 mL/day) all increased normally (p=NS) in response to deoxycorticosterone acetate (DOCA)-salt treatment. Unexpectedly, $AT_{1A}^{AVP\text{-}KO}$ mice (n=12) exhibited decreased body mass compared to littermate controls (n=24) regardless of sex between 5-18 weeks of age (3-way ANOVA; genotype $p<0.05$), yet no major differences in body composition were observed. Between 12-15 weeks of age, there were no differences in calories ingested (n=27, 14.4±0.3 vs n=12, 14.6±0.5 kcal/day) or digestive efficiency (79.3±0.7 vs 77.9±1.1 % ingested), indirectly implicating AVP in the control of energy expenditure. To examine a role for AVP in energy expenditure, resting metabolic rate (RMR) was assessed by respirometry in a cohort of female C57BL/6J mice chronically infused with AVP (24 ng/hr, sc). Preliminary results indicate a possible 20% reduction in RMR with 3 week AVP infusion (n=4, 0.171±0.003 vs n=5, 0.136±0.014 kcal/hr, p=0.06). Collectively these data support a role for $AT_{1A}$ receptors, localized to AVP-expressing cells, in osmotic control of AVP secretion as well as energy homeostasis through the modulation of energy expenditure.

# Abstracts

**Acid-sensing ion channels in behavioral responses to alcohol**

**Gail I.S. Harmata**[1-3], Margaret J. Fuller[3-5], Syed Alam[3,6], Rebecca J. Taugher[3,6], Brian J. Dlouhy[7], John A. Wemmie[1,3-7]

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Alcohol abuse exacts an enormous toll on health and society. Current therapies have limited efficacy, and the mechanisms by which alcohol exerts its effects on behavior and brain function remain incompletely understood. Alcohol's effects are thought to involve multiple neurotransmitter systems in the brain, including glutamate and endogenous opioids. Previous work indicates that these systems are also altered by acid-sensing ion channels (ASICs), members of the DEG/ENaC cation channel family activated by extracellular acidosis and shown to contribute to synaptic transmission at glutamatergic synapses. Disrupting ASIC1A, a critical ASIC subunit, increased behavioral responses in mice 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 compared Asic1a-/- to Asic1a+/+ mice in acute responses to alcohol, alcohol conditioned place preference (CPP), and alcohol consumption. We found that locomotor effects of acute alcohol administration were increased in Asic1a-/- mice. Alcohol CPP also tended to be increased in the Asic1a-/- mice. However, thus far we have not observed any differences between Asic1a-/- and Asic1a+/+ mice in consumption of freely-available alcohol, or in a binge drinking model. Together these results are consistent with the effects of ASIC1A on cocaine and morphine-related behaviors and suggest that ASIC1A may also play a role in alcohol responses. If so, ASIC1A may provide novel insight into the mechanisms underlying the behavioral effects of alcohol. However, more work is needed to better understand the role ASIC1A in responses to these diverse drugs of abuse.

**Retinol Binding Protein 7 Mediates an Anti-oxidant Response to Cardiovascular Stressors by Regulating PPARγ Activity and Adiponectin in Endothelium**

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Impaired PPARγ activity in endothelial cells causes oxidative stress and endothelial dysfunction which causes a predisposition to hypertension, but the identity of key PPARγ target genes which protect the endothelium remain unclear. Retinol binding protein 7 (RBP7) is a PPARγ target gene which is essentially endothelial-specific. Whereas RBP7-deficient mice exhibited normal endothelial function at baseline, they exhibited severe endothelial dysfunction in response to cardiovascular stressors, including high fat diet and subpressor angiotensin-II. Endothelial dysfunction was not due to differences in weight gain, impaired glucose homeostasis or hepatosteatosis, but occurred through an oxidative stress-dependent mechanism which can be rescued by scavengers of superoxide. RNA sequencing revealed that RBP7 was required to mediate induction of a subset of PPARγ target genes by rosiglitazone in the endothelium including adiponectin. Adiponectin was selectively induced in the endothelium of control mice by high fat diet and rosiglitazone whereas RBP7-deficiency abolished this induction. Adiponectin inhibition caused endothelial dysfunction in control vessels, whereas adiponectin treatment of RBP7-deficient vessels improved endothelium-dependent relaxation and reduced oxidative stress. We conclude that 1) RBP7 is required to mediate the protective effects of PPARγ in the endothelium through adiponectin, and 2) RBP7 is an endothelial-specific PPARγ target and regulator of PPARγ activity.

# Abstracts

**PPP2R5D mutation and cellular growth**

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The protein phosphatase 2A (PP2A) is a heterotrimeric holoenzyme consisting of a catalytic C subunit, a scaffolding A subunit and a regulatory B subunit. The B subunit is composed of three subfamilies, B, B' and B''. Recently, de novo germline mutations in PP2A subunits have been identified in intellectual disability (ID) and human overgrowth syndrome. The most commonly mutated subunit is B'δ (PPP2R5D) and most of these mutations switch a negatively charged to the positively charged residue (glutamate to lysine) in the highly conserved acidic loop. While it remains unclear how the charge reversal affects the functional role of PP2A as a phosphatase, our immunoprecipitation data show that the catalytic subunit and the scaffolding subunit still bind to the regulatory subunit despite the mutations, thereby suggesting that PPP2R5D mutations do not appear to alter the assembly and stability of the PP2A holoenzyme. Therefore, in the present study, we examined how PPP2R5D mutations affect the functional role of PP2A as a phosphatase, specifically in regard to human overgrowth and ID. In HEK293 cells stably expressing inducible GFP-tagged B'δ WT and E198K, which is the most severe form of PPP2R5D mutations, we found E198K enhances cell growth as assessed by the MTS assay. When stimulated with isoproterenol, a beta-adrenergic receptor agonist, E198K caused an approximately fourfold increase in both the phosphorylated-to-total MEK1/2 and ERK1/2 ratio. This effect, however, was partially suppressed when the MEK-ERK pathway was inhibited by UO126. These data suggest that PPP2R5D mutation disinhibits MEK signaling, implicating a role of B'δ subunit in regulating MEK signaling. Indeed, aberrant activation of MEK signaling has been shown to promote cell growth. Additional studies are necessary to understand how PPP2R5D mutations promote growth and how this pertains to the ID syndromes.

**Genetic and environmental factors that suppress seizures in a fly model of epilepsy**

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Epilepsy is one of the most common neurological disorders affecting more than 65 million people worldwide. Notably, clinical and experimental studies suggest that seizure severity in epilepsy patients is influenced by various genetic and environmental factors, raising the exciting possibility for better management of epilepsy by controlling these phenotypic modifiers. In this study using the *Drosophila* voltage-gated sodium ($Na_v$) channel mutant, *Shudderer* (*Shu*) ─ a fly model of human epilepsy, we have identified multiple genetic and environmental modifiers of the seizure-like mutant phenotypes. First, unbiased forward genetic screen revealed that phenotypic severity of the mutant is reduced when function of a glutathione transferase gene is suppressed. Interestingly, loss-of-function mutations of the glutathione transferase gene increase the levels of the inhibitory neurotransmitter GABA in the *Shu* brain. Second, we discovered that a simple dietary modification during development drastically suppresses *Shu* phenotypes with concurrent increase in GABA levels in the adult brain. Third, we also found that antibiotic treatment reduces seizures in Shu mutants. We currently focus on elucidating the molecular and cellular mechanisms underlying the seizure suppressing activity of these factors. Based on evolutionary conservation of the basic neurobiological processes between flies and humans, our study is expected to provide fundamental insights into the gene-gene and gene-environment interactions that affect epileptic symptoms. As *Shu* is a mutant for the evolutionarily conserved $Na_v$ channel gene, this study is expected to contribute to a better understanding of etiology and pathophysiology of other disorders that are linked to abnormal $Na_v$ channel functions, including autism, migraine, ataxia and pain syndromes.

# Abstracts

**Myocardial responsiveness to beta-adrenergic stimulation is unaltered by hyperinsulinemia**

**Satya Tadinada**[1], Erin B. Lane[2], Jamie Soto[3,4], Serif Bacevac[3], William Kutschke[3], Kathy Zimmerman[3], Melissa Davis[3], Dale E. Abel[3,4]

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Background and Significance: The incidence of Non-Insulin Dependent Diabetes Mellitus (NIDDM) and obesity are on the rise around the world. One of the hallmarks of both NIDDM and obesity is hyperinsulinemia and both these hyperinsulinemic states are risk factors for heart failure. Previous studies reported that insulin inhibits β adrenergic receptor induced inotropy in cultured cardiomyocytes via GRK2 dependent β2 adrenergic receptor desensitization. The goal of this study was to assess the effects of hyperinsulinemia on cardiac function *in vivo.*

Methods: Cardiac effects of hyperinsulinemia were studied in 2 settings- an acute setting where mice were rendered hyperinsulinemic acutely by jugular injection of 1U/kg insulin and a chronic setting, in which, mice developed hyperinsulinemia during a high fat diet feeding (HFD) protocol that lasted >24 weeks. Cardiac function was assessed by Transthoracic Echocardiography (TTE) and left ventricular (LV) catheterization. Cardiomyocyte specific knockout of GRK2 and β2 adrenergic receptor and βArrestin2 global knockouts were used in the chronic fat feeding study.

Results: Acute hyperinsulinemia did not alter the β adrenergic responsiveness of the myocardium as assessed by LV catheterization. Chronic fat feeding did not alter cardiac function at baseline or in response to β adrenergic stimulation induced inotropy regardless of the genotype as assessed by TTE and LV catheterization.

Conclusions: Acute effects of hyperinsulinemia on cardiomyocyte β adrenergic responsiveness *in vitro* and in a denervated heart preparation *ex vivo* do not reflect the *in vivo* physiology. Our results from chronic fat feeding studies challenge the relevant literature on HFD induced cardiac dysfunction and the relevance of mouse models of diet induced obesity for mechanistic understanding of impaired cardiac function in hyperinsulinemic states in humans.

**Novel Quinazoline-2,4-diones: Bacterial Type II Topoisomerase Inhibitors**

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Fluoroquinolones are a class of synthetic antibiotics used clinically to treat a wide array of bacterial infections. Fluoroquinolones act by forming a ternary complex with bacterial type II topoisomerases (DNA gyrase or topoisomerase IV) and nicked DNA; religation of DNA is subsequently blocked. In ternary complex the keto-acid moiety of the fluoroquinolone is complexed with a divalent magnesium ion, forming a drug-magnesium-water bridge to a serine and an aspartate (or glutamate) residue on helix-4 of the topoisomerase enzyme. Mutation-mediated resistance arises through substitution of the serine or aspartate/glutamate residues, therefore preventing formation of the magnesium-water bridge and reducing stability of the cleaved complex. Quinazoline-2,4-diones (diones) are structurally similar to fluoroquinolones; while diones form ternary complex similar to fluoroquinolones, these complexes are less stable because the quinazoline-2,4-diones do not contain the keto-acid moiety and therefore do not form a magnesium-water bridge to helix-4. While diones are therefore less potent antibiotics, their non-reliance on the magnesium water bridge generally affords equipotent activity with wild -type and fluoroquinolone-resistant strains of bacteria. Thus quinazoline-2,4-diones provide a structural scaffold for further modification to generate novel inhibitors of bacterial type-II topoisomerases that act on wild-type and fluoroquinolone resistant bacteria. In this presentation the design of novel quinazoline-2,4-dione derivatives expected to have additional binding contacts in ternary complex will be discussed, and the synthesis of a panel of these agents bearing novel chemical moieties on the quinazoline-2,4-dione skeleton will be presented.

# Abstracts

### Surgical removal of gut bacteria promotes weight gain via suppression of energy expenditure

**Nicole Pearson**[1], Ruth A Riedl[1], Samantha N Atkinson[3], Tomye L Ollinger[3], Colin ML Burnett[1], Robert A Edwards[4], Mohamad Mokadem[2], John R Kirby[3] and Justin L Grobe[1]

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A role for gut microbiota in energy homeostasis has been supported by studies involving twins, fecal transplants, colonization of germ-free animals, and antimicrobial treatments. Although these studies have documented qualitative changes in digestive efficiency and microbiota-host communication, there have been few attempts to quantitatively assess the relative contributions of these processes. Therefore it remains unclear whether other energy balance mechanisms may also contribute to the observed effects of the gut microbiota upon host energetics. It has been estimated that bacteria contribute 1-3% of adult human body mass, and that bacteria metabolize energy at ~7 kcal/kg/hr; thus bacteria in a 90 kg man should utilize 150-450 kcal/d, which is the equivalent of 7-22% of a 2,000 kcal/d human energy flux. We therefore hypothesize that gut bacteria contribute a physiologically-relevant fraction of the host's total energy expenditure, and that removal of bacterial biomass would lead to increased weight gain despite simultaneously reducing digestive efficiency. To test this hypothesis we surgically removed the cecum (which is filled with bacteria and accounts for ~1% of body mass in mice) from 13 week old female C57BL/6J mice, and serially assessed host energetics by bomb calorimetry for two months. Compared to sham surgery, cecectomized mice gained significantly more weight (at four weeks: sham n=9, +1.72±0.21 vs cecectomy n=9, +2.95±0.30 g, $p<0.05$). Animals appeared healthy and active, with no changes in grooming or other behaviors noted. No differences in food (2920x chow) intake behavior (3.54±0.28 vs 3.30±0.12 g/d, p=NS) were observed. As expected, cecectomy significantly reduced digestive efficiency (85.8±1.1 vs 82.4±0.9, $p<0.05$), but no difference in total caloric absorption was observed (13.71±1.23 vs 12.23±0.50 kcal/d, p=NS). Energy efficiency (weight gain per calorie absorbed) was significantly increased with cecectomy (4.31±0.59 vs 8.15±0.90 mg/kcal, $p<0.05$). Collectively, these data demonstrate an increase in weight gain after removal of gut bacterial biomass in mice, which occurred due to a major suppression of energy expenditure. Notably the weight gain-resisting suppression of digestive efficiency was insufficient to offset the reduction in energy expenditure that also occurred with cecectomy, resulting in a net caloric retention and thereby excess weight gain. These data highlight a large, dominant, quantitative contribution of gut bacteria to total energy expenditure. Additional studies to assess the contribution of the gut bacteria to aerobic vs anaerobic energy expenditure are required.

### Development of a novel porcine model for Neurofibromatosis Type 1

Katherine A. White[1], **Jordan Kohlmeyer**[2], Christopher S. Rogers[3], David K. Meyerholz[4], Johanna Uthoff[5], Judy A. Rohret[3], Frank A. Rohret[3], Jason Struzynski[3], Adam Goeken[4], Mariah Leidinger4, Shaikamjad Umesalma[6], Karin Panzer[7], Benjamin W. Darbro[7], Rajesh Khanna[8], Jessica C. Sieren[5], Dawn E. Quelle[2,4,6], and Jill M. Weimer[1]

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Loss of the *NF1* tumor suppressor gene causes one of the more common human diseases of the nervous system, neurofibromatosis type 1 (NF1). Children and adults with NF1 suffer from pathologies ranging from benign and malignant tumors to cognitive deficits, seizures, growth abnormalities and migraines. The NF1 gene encodes neurofibromin, a Ras-GTPase activating protein (Ras-GAP) whose mutation in NF1 patients results in hyperactivated Ras signaling. Existing genetically modified *NF1* mutant mice effectively mimic individual aspects of NF1, but none comprehensively models the disease. Here, we developed the first large animal, porcine model of NF1 bearing a mutation in the endogenous *NF1* gene (exon 42 deletion) orthologous to one found in NF1 patients. $NF1^{+/ex42del}$ heterozygous pigs phenocopy the wide range of manifestations seen in humans with NF1, including café au lait spots, neurofibromas, auxillary freckling, and neurological defects in learning and memory. Molecular analyses verified reduced neurofibromin expression in porcine $NF1^{+/ex42del}$ cells, as well as hyperactivation of Ras, as measured by increased expression of its downstream effectors, phosphorylated ERK1/2 and the checkpoint regulators, p53 and p21. Thus, these $NF1^{+/ex42del}$ mutant pigs successfully recapitulate the human disease and provide a unique, much needed tool to advance NF1 research and treatment.

# Abstracts

**Design, Synthesis and Structure-Activity-Relationships of Novel Antimalarials Targeting Apicoplast DNA Polymerase (apPOL) from *P. Falciparum***

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*Plasmodium* spp. are the causative agents of malaria, killing nearly 600,000 people each year. Resistance of *Plasmodium* to chloroquine, artemisinin-based therapies as well as other antimalarial agents accentuates the need for new drugs that target novel aspects of the parasite's biology. Nearly all parasites in the phylum *Apicocomplexa* have an unusual organelle called the apicoplast, which is a relic chloroplast, acquired through a secondary endosymbiotic event with algae. It participates in the biosynthesis of fatty acids, heme, iron-sulfur clusters, and isoprenoids and any defect in apicoplast metabolism or failure of the apicoplast to replicate and divide leads to the death of the organism. Additionally, lack of a human counterpart to the apicoplast makes apicoplast functions and enzymes promising drug targets. The 35-kb genome of apicoplast is replicated by select DNA replication enzymes of which the apicoplast DNA polymerase (apPOL) is unique to the parasite. The apPOLs from *P. falciparum* and *P. vivax* have 84% homology, while the most similar human DNA polymerases are the lesion bypass polymerases theta and nu (23 and 22% identity, respectively). This suggests that drugs targeted against the *Pf*-apPOL would also be effective in treating *P. vivax* infections with low human toxicity. Towards identifying inhibitors of apPOL, a high throughput screen of 400 compounds from the Open Malaria Box provided by Medicines for Malaria Ventures identified an inhibitor of apPOL with an $IC_{50}$ of 0.8 ± 0.3 μM (MMV666123, Fig. 1). Preliminary studies indicate that MMV666123 is specific for apPOL, with no inhibition of human DNA Pol or *E. coli* DNA Pol I. Also, MMV666123 inhibits the polymerase activity of apPOL but not its exonuclease activity, suggesting binding to the C-terminal polymerase domain of apPOL. This is supported by initial docking studies of MMV666123 onto apPOL X-ray crystal structure (PDB: **5DKT and 5DKU)**. Additionally, being from the malaria box substantiates anti-malarial activity of MMV666123. Presented here are our current design, synthesis and *in vitro* evaluation efforts toward understanding the structural requirements of MMV6661213 for inhibition of apPOL and identifying more potent and drug-like apPOL inhibitor derivatives.

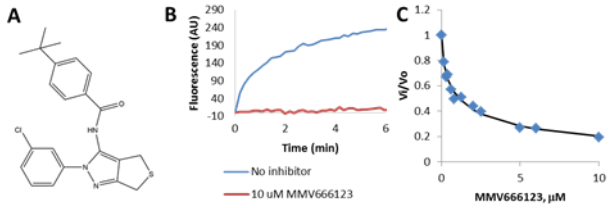


**Figure 1.** *A)* Structure of MMV666123. *B)* Effect of MMV666123 on apPOL activity as measured with the HT substrate. *C)* Dose/Response curve showing $IC_{50}$ of 0.8 μM.

# Abstracts

### Functional Interactions of NIAM and RGS6 Tumor Suppressors in Breast Cancer

**Blake Letney**[1], Chandini Reddi[1], Nicholas Borcherding[2,3], Jesse Gray[1], Jianqi Yang[1], Bandana Chakrvarti[1], Weizhou Zhang[2,3,4], Frederick W. Quelle[1], John G. Koland[1,2], Rory A. Fisher[1], and Dawn E. Quelle[1,2,3,4]

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Breast cancer is the leading cause of cancer death in women worldwide. A better understanding of the molecular pathways underlying breast tumorigenesis will facilitate earlier detection, predict which patients respond better to certain therapies, and provide new targets for cancer treatments. The p53 tumor suppressor plays a major role in preventing breast cancer; therefore, its regulators are potentially meaningful biomarkers and targets of the disease. Our research groups study two new activators of p53, named NIAM (Nuclear Interactor of ARF and Mdm2) and RGS6 (Regulator of G protein signaling 6). Each factor is down-regulated in human breast tumors and RGS6 loss in mice promotes mammary tumorigenesis. In addition, NIAM and RGS6 bind a common partner (the Tip60 acetyltransferase) and both proteins are upregulated by the anti-cancer drug, doxorubicin, to mediate tumor cell arrest and death. Other studies have shown RGS6 impairs Ras and Her2 oncogenic activity while NIAM binds to ARF, the key regulator of oncogene checkpoints. Based on these compelling links, we hypothesized that NIAM and RGS6 act cooperatively through common pathways to suppress breast cancer. In support of that notion, we have found that NIAM and RGS6 proteins form complexes within cells. To test our prediction that combined loss of NIAM and RGS6 will accelerate the rate and incidence of breast cancer, we developed novel *NIAM-/-;RGS6-/-* double-knockout (DKO) mice. We found that loss of *NIAM* or RGS6 alone increased carcinogen-induced breast tumorigenesis relative to wildtype (WT) mice, and that DKO mice display an even greater incidence and rate of carcinogen-induced tumor formation compared to *NIAM-/-* and *RGS6-/-* animals. To complement the in vivo studies, mouse embryo fibroblasts (MEFs) of each genotype are being examined for differences in basal proliferation, replicative senescence, response to DNA damage and susceptibility to oncogenic transformation. Initial results comparing WT and *NIAM-/-* MEFs show that NIAM loss markedly accelerates cellular proliferation and delays senescence. Studies of the *RGS6-/-* and DKO MEFs are ongoing. This work establishes NIAM as a bona fide suppressor of mammary tumorigenesis and shows that it acts cooperatively with RGS6 to prevent breast cancer.

### Assessing the cysteine dependence of small molecule inhibition of RGS4 and RGS14

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Regulators of G-Protein signaling (RGS) proteins act as GTPase activating proteins (GAPs) effecting an increased rate of hydrolysis of GTP to GDP by active Gα subunits, thus terminating their signaling. As there are over 20 different RGS family members, understanding both highly-conserved and unique residues among RGS proteins is of key importance to their therapeutic potential, in order to understand specificity of inhibition. Previous work revealed that cysteine residues may not be crucial to the ability of RGS proteins to act as GAPs, however these sites could be exploited for small molecule targeting and inhibition. Therefore, creating cysteine null and single cysteine mutants will allow for the specific study of interfamily RGS protein cysteine dependence of inhibition. RGS4 and RGS14 were chosen based on previous work detailing that these RGS proteins are highly sensitive to small molecule inhibition. Through monitoring the release of free phosphate, the GAP activity of RGS4 and RGS14 wild type were compared to their respective single cysteine and cysteine null mutants. The results indicate that while there is no cysteine dependence for RGS4 or RGS14 for GAP activity. The characterization of small molecule inhibitors for cysteine dependent mechanisms of action is being evaluated.