Mayo Graduate School 25th Anniversary
2014 Fall Symposium Schedule

September 30 – Phillips Hall, Siebens Building

9:00 – 10:30 a.m.  Poster Session 1
Even Numbered Abstracts

10:45 – 11:45 a.m.  Oral Presentations – Morning Sessions

10:45  Virology & Gene Therapy
Catie Crosby

11:00  Neurobiology of Disease
Chelsea Vadnie

11:15  Molecular Pharmacology & Experimental Therapeutics
Ruisi Wang

11:30  Immunology
Puspa Thapa

12:00 p.m. – 1:00 p.m.  Alumni Panel – Lunch and Learn
Phillips Hall, Siebens Building
Goldstein Hall, Guggenheim Building
(Box lunches provided on a first come/first serve basis)

1:00 – 2:30 p.m.  Poster Session 2
Odd Numbered Abstracts

2:45 – 3:45 p.m.  Oral Presentations – Afternoon Sessions

3:30  Clinical & Translational Science
Beth Cloud

3:45  Biomedical Engineering & Physiology
Kay Pepin

4:00  Biochemistry & Molecular Biology
Sabriya Syed

6:00 - 8:00 p.m.  Wine Reception and Dinner/Marriott Ballroom (By invitation)
October 1 – Phillips Hall, Siebens Building

8:30 - 11:20 a.m. MGS Alumni Presentations

8:30 – 9:05 Kim Butts Pauly, Ph.D.
9:15 – 9:50 Atta Behfar, M.D., Ph.D.
10:00 – 10:35 Eduardo Davila, Ph.D.
10:45 – 11:20 James Patton, Ph.D.

11:30 a.m. – 12:30 p.m. Lunch Break

11:30 a.m. – 12:30 p.m. Alumni Association Sponsored Lunch (By invitation)

1:00 – 2:00 p.m. Distinguished Findling Lecturer
Randy Schekman, Ph.D.

2:15 - 4:20 p.m. MGS Alumni Presentations

2:15 – 2:50 James Hagstrom, Ph.D. – Alumni Presentation
3:00 – 3:35 Noah Gray, Ph.D. – Alumni Presentation
3:45 – 4:20 Liewei Wang, M.D., Ph.D. – Alumni Presentation
MORNING SESSION

10:15-10:30 a.m.
**Virology & Gene Therapy**
*Catherine M. Crosby*
Single-Cycle Replicating Adenovirus (SC-Ad) Platform for Mucosal Vaccination

10:30-10:45 a.m.
**Neurobiology of Disease**
*Chelsea Vadnie*
Activation of Neurotensin Receptor Type 1 Suppresses Locomotion and Glycogen Synthase Kinase-3 Activity

10:45-11:00 a.m.
**Molecular Pharmacology & Experimental Therapeutics**
*Ruisi Wang*
Sphingosine kinase containing exosomes regulate hepatic stellate cell activation

11:00-11:15 a.m.
**Immunology**
*Puspa Thapa*
The transcriptional repressor, NKAP is required for invariant Natural Killer T (iNKT) cells development and differentiation.

AFTERNOON SESSION

3:30-3:45 p.m.
**Clinical & Translational Science**
*Beth A. Cloud*
Quantifying spinal posture and shoulder kinematics with changes to wheelchair seating in individuals with spinal cord injury

3:45-4:00 p.m.
**Biomedical Engineering**
*Kay Pepin*
MR Elastography in Glioma: A Noninvasive Classification of Tumor Grade

Biochemistry & Molecular Biology
4:00-4:15 p.m.
*Sabriya Syed*
Epigenetic Control of GI Electrical Pacemaker Cell Fate & Function in Aging
Title: Single-Cycle Replicating Adenovirus (SC-Ad) Platform for Mucosal Vaccination

Authors: Catherine M Crosby and Michael A Barry, Ph.D.

Advisor: Michael A Barry, Ph.D.

Track: Virology and Gene Therapy

Vaccines that can drive T cell responses and antibodies can be made by attenuating the pathogen. While these attenuated vaccines can be potent, they have a real risk of causing the disease they aim to prevent. An alternate approach is to transfer pathogen genes to another platform to make a gene-based vaccine that mimics a pathogen infection without the risks. Adenoviruses are some of the most potent virus vaccine platforms. However, scale up to large animals and humans has been a significant challenge. Current adenovirus (Ad) vectors fall broadly into two categories: replication defective (RD) and replication competent (RC). RC-Ad vectors can undergo genome replication and progeny virion production. These vectors mediate robust transgene expression and immune responses, but risk causing adenovirus infections. To increase safety, RD-Ad vectors were engineered by deleting E1 genes to disable the viral life cycle. While RD-Ad vectors are safer, they produce significantly less transgene protein and immune responses as vaccines. To create robust but safer vectors, we created “single cycle adenovirus” vectors. We engineered this by deleting a key capsid cement protein IIIa, in lower seroprevalence adenovirus serotype 6 (Ad6-ΔIIIa). Deletion of IIIa rendered the virus incapable of generating infectious progeny in normal cells, but infectious Ad6-ΔIIIa could be produced in IIIa-expressing cell lines. In normal mouse, human, ferret, and macaque cells, Ad6-ΔIIIa replicated its genome, and expressed transgene 10 to 100-fold more than RD-Ad6. Electron microscopy of infected normal cells revealed immature, empty virus particles packing the nucleus. CsCl gradient separation and protein gel analysis confirmed the production of particles lacking genomic DNA, and resembling the intermediate particles formed during wild type adenovirus infections. After intranasal immunization of Syrian hamsters, both SC-Ad and RC-Ad expressed transgenes hundreds of times higher than RD-Ad. This translated into higher serum and vaginal wash antibodies for both vectors. Interestingly, SC-Ad, but not RC-Ad, generated the highest antibody levels that notably climbed in serum and vaginal wash samples for over 12 weeks after single immunization. When RD-Ad and SC-Ad were compared by sublingual immunization in Rhesus macaques, SC-Ad generated higher IFN-γ responses in peripheral blood mononuclear cells and higher serum antibody levels. These data suggest that Ad6-ΔIIIa can be used as a safer “single-cycle” vaccine vector that amplifies transgenes and immune responses, but avoids the production of frank adenovirus infections.
Title: Activation of Neurotensin Receptor Type 1 Suppresses Locomotion and Glycogen Synthase Kinase-3 Activity

Authors: Chelsea Vadnie, Osama Abulseoud, M.D., Sun Choi, David J. Hinton, YuBin Choi, Christina L. Ruby, Ph.D., Alfredo Oliveros, Miguel L. Prieto, M.D., Jun Hyun Park, Ph.D., and Doo-Sup Choi, Ph.D.

Advisor: Doo-Sup Choi, Ph.D.

Track: Neurobiology of Disease

Neurotensin (NT) suppresses locomotor activity and has antipsychotic-like effects, similar to dopamine D2 receptor (D2R) antagonists. Therefore, NT signaling is an attractive target for the treatment for movement and psychiatric disorders. However, little is known about the molecular mechanisms in the brain underlying the behavioral effects of NT. NT-induced hypolocomotion may occur by inhibition of D2R through a receptor-receptor interaction with NT receptor type 1 (NTS1). We investigated the effect of systemic and brain region-specific NTS1 activation on locomotion using a blood brain barrier permeable, selective NTS1 agonist, PD149163. Systemic administration of PD149163 (0.05-0.5 mg/kg) dose-dependently attenuated the locomotor activity of mice and rats. Subsequently, we examined whether PD149163 blocks dopamine receptor-mediated hyperactivity. Pretreatment with 0.1 mg/kg and 0.05 mg/kg of PD149163 inhibited D2R agonist bromocriptine (8 mg/kg)-mediated hyperactivity. However, only 0.1 mg/kg of PD149163 prevented D1R agonist SKF81297 (8 mg/kg)-induced hyperlocomotion. Since the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) have been implicated in the behavioral effects of NT, we examined whether microinjection of PD149163 into these regions reduces locomotion. Microinjection of PD149163 (2 pmol) into the NAc, but not the mPFC suppressed locomotion. Thus, our results suggest that activation of NTS1 in the NAc is sufficient to suppress locomotion and NTS1 activation selectively inhibits D2R-mediated hyperactivity. Lastly, PD149163 (0.5 mg/kg) reduced glycogen synthase kinase-3 (GSK-3) signaling in the NAc of mice and rats. Inhibition of D2R and potential downstream GSK-3 signaling are associated with reduced movement. Thus, our results provide a novel role for NAc NTS1 in suppressing movement through possibly inhibition of D2R-mediated Akt/GSK-3 signaling. Increased GSK-3 signaling has also been implicated in bipolar disorder. Hyperactivity and increased dopaminergic signaling in the NAc are thought to be key features of mania in bipolar disorder. Our work may lead to the development of new therapeutics for bipolar disorder with minimal side effects.
Title: Sphingosine kinase containing exosomes regulate hepatic stellate cell activation

Authors: Wang, Ruisi1; Cao, Sheng2; Yaqoob, Usman2; De Assuncao, Thiago M2; Shah, Vijay M.D.2

Track: Molecular Pharmacology and Experimental Therapeutics

Background/Aims: Hepatic stellate cell (HSC) activation is required for fibrogenesis therefore understanding mechanisms governing HSC activation are important. Exosomes are cell derived extracellular vesicles thought to promote intercellular communication by interacting with recipient cells to deliver specific content. The aim of this study was to determine whether exosomes contribute to HSC activation during liver fibrosis.

Methods/Results: Exosomes were isolated from human serum and conditional media of TSEC (immortalized mouse liver endothelial cells) by differential ultracentrifugation, and characterized based on size (90-110 nm) and marker (TSG101, LAMP1, CD63 and CD81) characteristics. Incubation of LX2 HSC cell line with human serum derived exosomes was associated with increased AKT phosphorylation (8.3-fold, n=6, p<0.05), increased mRNA levels of smooth muscle actin (1.7-fold, n=3, p<0.05), fibronectin (1.8-fold, n=3, p<0.05) and collagen (1.7-fold, n=3, p<0.05), and increased cell migration (2.5-fold, n=3, p<0.05) in Transwell assays. Exosome activation of LX2 cells required exosome endocytosis since inhibition of endocytosis with transfection of the dominant negative dynamin GTPase construct Dyn2K44A or by the pharmacological inhibitor, dynasore significantly attenuated exosome-induced AKT phosphorylation (72% and 90%, respectively, n=3, p<0.05). Exosome biotinylation studies showed that internalized exosomes target initially to early endosomes and subsequently to lysosomes based on double immunofluorescence staining using early endosome marker, EEA and lysosome marker, LAMP1 (Pearson coefficients of colocalization= 0.32 and 0.36, respectively, n=5). Western blot analysis of exosomes for enrichment of molecules implicated in HSC activation revealed presence of sphingosine kinase 1 (SK1), an enzyme that produces sphingosine-1 phosphate (S1P). Indeed, exosomes derived from conditioned media of TSEC overexpressing SK1 further increased LX2 cell S1P levels (2-fold, n=6, p<0.05) and LX-2 migration (2-fold, n=3, p<0.05) suggesting that S1P generated by exosomes may promote HSC activation. Finally, S1P levels were increased in serum of mice with CCl4 induced liver fibrosis (1.4-fold, n=17, p<0.05) and SK1 mRNA levels were upregulated in human liver cirrhosis patient samples (2.5-fold, n=3, p<0.05).

Conclusion: These findings advance our understanding of exosome-mediated HSC activation and identify potential molecular targets for attenuating this process.
In order for the immune system to function, the development of lymphocytes in the bone marrow (B cells) and thymus (T cells) is critical, yet incompletely understood. Studies in our lab have shown that a transcriptional repressor, NKAP, is required for lymphocyte development. NKAP is required for T cell development and maturation; and is also important for maintenance and survival of hematopoietic stem cells (HSCs). NKAP interacts with HDAC3 for its repressor function. Invariant Natural Killer T (iNKT) cells are innate lineage of lymphocytes that can produce copious amount of cytokines within hours of stimulation. In the thymus, positive selection into the iNKT cell lineage occurs at the double positive (DP) stage, and iNKT cells go on to complete development, where they go through various stages (stage 0-3).

After positive selection into iNKT lineage at the DP stage, iNKT cells mature and differentiate into their functional subsets known as NKT1, NKT2 and NKT17.

Previously using CD4-cre NKAP cKO mice, we demonstrated that the transcriptional repressor NKAP is required for positive selection of DP thymocytes into the iNKT cell lineage. To study the role of NKAP in later iNKT cell development and differentiation, we generated PLZF-cre NKAP cKO mice with NKAP deletion occurring after entry into the iNKT lineage at stage 0, bypassing the previous block in iNKT development at the DP stage. In these mice, there was a significant decrease in the absolute number, which was not due to decreased proliferation or increased apoptosis. In the PLZF-cre NKAP cKO mice, there are very few T-bet expressing NKT1 cells, almost no ROR-γt expressing NKT17 cells and decreased number of Gata3 expressing NKT2 cells present. Concurrently with defect in NKT1 and NKT17 lineages, there is lower production of IFN-gamma and a defect in production of IL17. Interestingly, in PLZF-cre NKAP cKO mice, the early stage 0-1 iNKTs have lower PLZF and T-bet expression, which could account for the early block in development and decreased differentiation. Deletion of NKAP associated protein Hdac3 using PLZF-cre also shows an iNKT cell developmental defect, with decreased expression of Tbet and its regulated gene CXCR3. Thus, NKAP together with Hdac3 may regulate iNKT cell development and differentiation.
Title: Quantifying spinal posture and shoulder kinematics with changes to wheelchair seating in individuals with spinal cord injury

Authors: Beth A. Cloud, D.P.T., Kristin D. Zhao, Ph.D. and Kai-Nan An, Ph.D.

Advisor: Kai-Nan An, Ph.D.

Track: Clinical and Translational Science

Up to 70% of individuals who use manual wheelchairs report having shoulder pain. The number and type of upper extremity tasks that wheelchair users must complete on a daily basis are thought to be the reason for pain development. Avoidance of upper extremity activity is not a viable option; rather it is imperative to determine ways to facilitate a reduction of pain and pathology while maintaining independence. Our overall objective is to test the hypothesis that changes to wheelchair seat inclination (dump angle) will change shoulder kinematics due to the likely effect on spinal posture. Specifically, we hypothesize that 14° vs. 0° dump angle will create more advantageous spinal postures and shoulder kinematics during propulsion in individuals with spinal cord injury who use manual wheelchairs.

Measurement of spinal posture and shoulder kinematics in the two dump angle conditions, 0° and 14° (with respect to horizontal), was completed. Spinal posture was quantified with a fiber optic sensor system placed on the skin along the spinous processes of the first sacral vertebra to the seventh cervical vertebra. Shoulder kinematics were quantified with an electromagnetic system. Electromagnetic sensors were placed on the thorax, scapula, and humerus and the anatomical coordinate system for each was defined according to ISB standards. Spinal posture and shoulder kinematics, glenohumeral (GH) and scapulothoracic (ST), were recorded during propulsion in each dump angle condition. Spinal posture (lordosis and kyphosis) was quantified as angles within the regions of curvature. Spinal posture and GH and ST rotations were quantified at 5% increments across the propulsion cycle. Results from one male participant with tetraplegia are presented.

Differences in spinal curvature and GH and ST kinematics are presented as the mean difference across the propulsion cycle. In 14° compared to 0° dump angle the participant had an average of 25.5° more lordosis and 2.2° less kyphosis. The GH joint had 3.0° more abduction, 3.7° more horizontal abduction, and 1.4° more internal rotation in 14° dump angle versus 0°. In terms of ST motion, the 14° condition resulted in 9.0° more internal rotation, 10.2° less upward rotation, and 2.4° more anterior tilt compared to 0° dump angle.

With this individual, the change from 0 to 14° seat dump angle most notably increased spinal lordosis and ST internal rotation while decreasing ST upward rotation. The change in lordosis indicates that this seating adjustment may, as previously suggested, influence the position of the pelvis in the seat and subsequently affect spinal posture. Additional participants will be recruited, with both tetraplegia and paraplegia, to better understand this seating effect across a group of individuals with spinal cord injury.
Gliomas are the most common form of primary brain tumors. The most common type of gliomas are astrocytomas which are classified as pilocytic astrocytoma (grade I), low-grade astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (grade IV). To date no non-invasive technique exists for classification of glioma tumor grade. Instead, biopsy followed by pathologic evaluation of the tumor for classification of grade based on the World Health Organization (WHO) grading system is considered the current gold standard. Magnetic resonance elastography (MRE) is a noninvasive imaging technique capable of quantifying tissue stiffness. Previous studies have shown the feasibility of using MRE to characterize both tumors and global brain stiffness with good reproducibility. The purpose of this project is to investigate the feasibility of using MRE to evaluate gliomas and the potential of MRE-derived shear stiffness for use in tumor classification. Eight patients with previously identified gliomas scheduled for resection were recruited for this study. The average tumor stiffness was 1.8 kPa (range = 1.0-2.8 kPa) while the average stiffness in normal brain from the contralateral side was 3.2 kPa (range = 2.6-3.9 kPa). Two patients had WHO grade II tumors, one grade III, and three grade IV tumors (glioblastoma). Tumor shear stiffness values for the glioblastomas were all less than 2.0 kPa and demonstrated an inverse relationship between stiffness and grade (stiffness decreased with increasing grade). In this pilot study, we have shown the feasibility of quantifying shear stiffness in the most common form of malignant brain neoplasms - gliomas. These tumors are softer than the surrounding normal brain tissue and are clearly distinguishable on the MRE-derived stiffness maps. Higher grade gliomas (glioblastomas) were softer than lower grade gliomas. In this feasibility study, the results suggest that MRE-derived tumor shear stiffness may be correlated with tumor grade in gliomas.
Interstitial cells of Cajal (ICC) are mesenchymal cells that express the receptor tyrosine kinase (RTK) Kit and function as electrical pacemakers and neuromodulators of the gastrointestinal (GI) tract. ICC are reduced or their functions are dysregulated in GI motor disorders and aging. ICC depletion may reflect phenotypic changes to a Kit<sub>low</sub>- state rather than cell death. We hypothesized that epigenetic repression of gene transcription underlies ICC loss in aging and these changes may be reversible. We tracked the fate of ICC in vivo using Kit<sup>Cre<sub>ERT2</sub></sup>/+;R<sup>26</sup>tm<sup>mT-mG</sup>/mT-mG mice and inducing mG (membrane-targeted EGFP) expression by tamoxifen administration on postnatal days (PD) 7-10. By flow cytometry, phenotypically identified (Kit<sup>+</sup>CD34<sup>-</sup>) and genetically tracked (mG<sup>+</sup>Kit<sup>+</sup>CD34<sup>-</sup>) ICC declined by 57% and 50%, respectively, between PD11 and PD163. These changes were accompanied by an increase in mG<sup>+</sup> cells in the Kit<sub>low</sub>-CD34<sup>-</sup> fraction indicating ICC transition. The age-related decline in Kit expression was accompanied by a >13-fold increase in the frequency of mG<sup>+</sup>Kit<sub>low</sub>CD34<sup>-</sup> cells expressing the related RTK Pdgfra. We also observed a 2.3-fold increase in the proportion of Kit<sub>low</sub>CD34<sup>-</sup>Pdgfra<sup>+</sup> cells in mice between 6 and 104 weeks of age. Together, these data indicated that aging-associated ICC depletion reflects a phenotypic transition involving reduced Kit and increased Pdgfra expression. To further study these changes we established a cell model for dysfunctional ICC (e.g., ICL2A) that replicated the phenotypic changes and dysfunction seen in ICC with aging. ICL2A cells showed elevated expression of the epigenetic regulators Ehmt2, Suv39h1, HP1 isoforms, Hdac8 and Dnmt1, as well as members of polycomb repressive complexes PRC1 and 2 including Ezh2. ChIP-seq revealed that compared to the entire genome, repressed ICC genes had a ~2.24-fold reduction of promoter occupancy by the activating histone mark H3K4me3; whereas binding of the inhibitory H3K27me3 and H3K9me3 marks increased 1.43- and 1.18-fold, respectively. Inhibition of Ezh2 with adenosine dialdehyde (Adox) reduced occupancy of the Kit promoter by H3K27me3 and increased Kit mRNA and protein expression. The latter effect could be reproduced with the specific Ezh2 inhibitor EPZ-6438 and siRNA-mediated Ezh2 knock-down, but not with the Ehmt2 inhibitor BIX-0129, the Hdac inhibitor SAHA or the Dnmt blocker RG-108. 2-week exposure to Adox increased the proportion of cells displaying electrical pacemaker activity from 8.5% (6/71) to 62.5% (10/16). We conclude that polycomb-mediated epigenetic repression plays a key role in aging-associated ICC depletion and dysfunction. Inhibition of these repressive mechanisms may help restore ICC function in GI motor disorders and aging. Support: NIH R01 DK58185, Mayo Clinic Center for Individualized Medicine.
### Biochemistry and Molecular Biology

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### Immunology

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### Clinical & Translational Science

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### Biomedical Engineering & Physiology

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### Neurobiology of Disease

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### Virology & Gene Therapy

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Histone Deacetylase 3 Suppresses Erk Phosphorylation and Subsequent Matrix Metalloproteinase (MMP)-13 Activity in Chondrocytes during Endochondral Ossification

Authors: Lomeli R. Carpio, Elizabeth W. Bradley, Ph.D., Meghan E. McGee-Lawrence, Ph.D., Jennifer J. Westendorf, Ph.D.

Advisors: Jennifer J. Westendorf, Ph.D.

Track: Biochemistry and Molecular Biology

Histone deacetylase (Hdac) inhibitors are used extensively for treating cancer, arthritis, and epilepsy; however, these drugs inhibit multiple Hdacs, are teratogens, and have detrimental effects on the skeleton. Several Hdacs contribute to endochondral bone formation. In this study, we defined the cell autonomous role of Hdac3 in chondrocyte maturation by deleting it pre- and post-natally in type II collagen alpha 1 (Col2a1)-Cre expressing chondrocytes. Hdac3-CKO_{Col2} mice rarely survived embryogenesis. The few that did survive were hypomorphic for Hdac3 expression, runted, and had severely reduced cancellous and cortical bone density. Postnatal Hdac3 deficiency was induced with a tamoxifen-inducible Col2a1 Cre model (Hdac3-CKO_{Col2ERT}). At 4-weeks of age, these animals had residual hypertrophic cartilage and increased osteoclast activity in the primary spongiosa. By 8 weeks, these animals had compromised bone architecture, with significant decreases in cancellous bone but increases in cortical bone thickness, which is a compensation for disrupted growth plate maturation during development. Phosphorylation of Erk1/2, as well as its substrate Runx2, was elevated in Hdac3-depleted immature mouse articular chondrocyte (IMAC) micromass cultures. Activated Erk and Runx2 directly stimulate matrix metalloproteinase (MMP)-13 gene expression. MMP-13 mRNA and active enzymatic levels were higher in Hdac3-deficient IMAC cultures, as measured by QPCR and type I collagen zymography of conditioned media, respectively. U0126, an ERK inhibitor, returned MMP-13 expression to control levels in Hdac3-deficient IMAC cultures. In control mice, phosphorylated Erk and MMP-13 expression are restricted to the pre-hypertrophic zone of chondrocytes. With Hdac3 deficiency, phosphorylated Erk and MMP-13 are prematurely and prominently expressed in proliferative, pre-hypertrophic and hypertrophic chondrocytes. Together, these results indicate that Hdac3 controls the temporal and spatial regulation of Erk phosphorylation and subsequent MMP-13 expression to ensure proper chondrocyte maturation and ossification during long bone development.
Atherosclerosis is the primary risk factor for adverse events resulting from cardiovascular disease, such as strokes and heart attacks. Senescent cells have been identified in atherosclerotic lesions (plaques) but their role in the progression of the disease is unknown. Senescent cells have the potential to influence their neighbors by releasing soluble protein factors, collectively known as the ‘senescence-associated secretory phenotype (SASP).’ The SASP includes chemokines and cytokines that have been largely identified at pro-atherosclerotic, as well as metaloproteinases the cause plaque instability by promoting extracellular matrix degradation. Therefore, we hypothesized that clearing p16\(^+\)-senescent cells would reduce the progression of atherosclerosis and decrease inflammatory markers in the vasculature in a mouse model of atherosclerosis. To test this, we generated low-density lipoprotein knockout (LDL\(^r\)) as atherosclerotic controls and bred these animals to the p16-3MR mouse, which uses the p16 promoter to drive expression of both RFP as a marker and viral thymidine kinase as a drug-inducible killing gene. When ganciclovir is administered, p16\(^+\) cells are killed. We find that with ganciclovir administration, LDL\(^r\)\(^{-}\) animals have significantly higher plaque load compared to LDL\(^r\)\(^{-}\)3MR by both en face staining of plaques in the descending aorta with Sudan IV and cross-sectional measurement of plaques in the brachiocephalic artery, without alteration in atherogenic lipids in the plasma. Future work will include identifying the p16\(^+\) cell populations generated in atherosclerotic plaques using the RFP tag to isolate cells by flow cytometry and determining which cell types are susceptible to ganciclovir- or senolytic-mediated cell killing. We are also conducting studies to examine the effect of senescent cell clearance on established plaques.
Title: Identification and Characterization of a Novel Role for MMSET in Cell Cycle Progression

Authors: Debra L. Evans, Huadong Pei, Ph.D., JungJin Kim, Ph.D., SeungBaek Lee, Ph.D., and Zhenkun Lou, Ph.D.

Advisor: Zhenkun Lou, Ph.D.

Track: Biochemistry and Molecular Biology

MMSET is a histone methyltransferase with diverse implications in human disease, ranging from Multiple Myeloma to the developmental disorder Wolf Hirschhorn Syndrome. Interestingly, we have observed a defect in cell-cycle progression upon MMSET depletion. Furthermore, we have observed an S-phase-specific degradation of this histone methyltransferase. The work presented will address the role of MMSET in normal cell cycle progression as well as the mechanism by which it is faithfully degraded during each S phase. The contribution of deregulated MMSET levels in human disease will also be addressed.
A key step in the lysosomal degradation of membrane proteins is sorting into the Multivesicular Body (MVB), a process wherein a region of the endosomal membrane buds into the lumen of the endosome to form an intraluminal vesicle (ILV). This sorting process is mediated by Endosomal Sorting Complexes Required for Transport (ESCRT-0, I, II and III). Ubiquitination of MVB cargo and recognition of Ub-cargo by ESCRT-0, I and II permit entry into the MVB pathway; however, Ub is typically removed from the MVB cargo prior to cargo inclusion into the ILV. The coordination of deubiquitination of MVB cargo with ESCRT-III disassembly and ILV formation are not clear. Bro1 appears to play a critical role in coordinating cargo de-ubiquitination and ILV formation through interactions with ESCRT machinery, the Ub peptidase, Doa4, ubiquitin-modified cargo, and regulation of the ESCRT-III-associated AAA ATPase Vps4. These data suggest that members of the Bro1 family may be similarly impacting ESCRT-dependent processes in a number of contexts.
Phosphorylation at serine 448 regulates lipid kinase activity of Phosphatidylinositol-4-phosphate5-kinase type 1 γ

Authors: Nisha C. Durand, Ligia I. Bastea and Peter Storz Ph.D

Advisor: Peter Storz Ph.D.

Track: Biochemistry and Molecular Biology – Cancer Biology

Phosphoinositide kinases are enzymes that phosphorylate phosphatidylinositol and its derivatives. Phosphatidylinositol-4-phosphate5-kinase type 1 γ (PIP5K1γ) is a class I Phosphoinositide kinase that generates the lipid messenger PIP2. PIP5K1γ is recruited to the Focal Adhesions by an association with the FERM domain of talin, an interaction which enhances the kinase activity of PIP5K1γ. Here we show that PIP5K1γ kinase activity is also regulated by phosphorylation of serine residue 448 which lies adjacent to its kinase core domain. This phosphorylation event is mediated by Protein Kinase D 1 which functions downstream of RhoA signaling. Phosphorylation of PIP5K1γ at S448 attenuates PIP5K1γ lipid kinase activity. Spatio-temporal generation of PIP2 at the focal adhesions could be a critical regulator of Focal Adhesion turnover.
Small Angle X-ray Scattering Analysis of the Machinery Involved in Mitochondrial Iron-Sulfur Cluster Biogenesis

Authors: Belinda K Galeano, and Grazia Isaya, M.D., Ph.D.

Advisors: Grazia Isaya, M.D., Ph.D.; James Thompson, Ph.D.

Track: Biochemistry and Molecular Biology

Iron sulfur clusters (ISCs) are essential co-factors for a large number of cellular enzymes involved in the Kreb’s cycle, the electron transport chain, DNA synthesis and repair, and iron homeostasis among other processes. In eukaryotic cells, mitochondria are the primary site of ISC biogenesis. The proteins involved in ISC biogenesis inside mitochondria are highly conserved from yeast to humans and oversee the assembly of [2Fe-2S] and [4Fe-4S] clusters, along with the transfer of these clusters to mitochondrial apo-proteins.

The current model of ISC biogenesis proposes ISC synthesis is initiated on a scaffold protein (yeast Isu1/mammalian ISC) and involves (i) a cysteine desulfurase (yeast Nfs1/mammalian NFS1, stabilized by a small binding partner, Isd11/ISD11) that serves as the sulfur donor; (ii) frataxin (yeast Yfh1/mammalian FXN) that serves as the iron donor; and (iii) the electron donor chain formed by ferredoxin reductase and ferredoxin (yeast Arh1-Yah1/mammalian FDXR-FDX2). Subsequently, ISC are thought to be released from Isu1/ISCU and transferred to apo-enzymes by various chaperone and co-chaperones. In this model, Isu1/ISCU is thought to cycle between the early and the late components. However, recent studies suggest that mitochondrial ISC biogenesis occurs on stable complexes composed of at least four components: Nfs1, Isd11, Isu1 and Yfh1 in yeast; NFS1, ISD11, ISC and FXN in humans. Given the crucial function controlled by these complexes, it is important to identify their structural architecture and molecular interactions in order to elucidate their mechanisms.

Using single particle reconstruction from transmission electron microscopy (TEM), we have recently obtained three-dimensional models of oligomeric Yfh1 bound to Isu1; these models consistently indicate that six Isu1 oligomers bind symmetrically to the 24-subunit Yfh1 oligomer (Ranatunga et al. manuscript in preparation). Little is known, however, about the structure of Isu1 monomer and oligomers and the mechanism of Isu1 oligomerization. Therefore, we have used small angle x-ray scattering (SAXS) to characterize purified Isu1 protein in solution. We have determined that Isu1 can exist as a flexible, stable monomer or as dynamic oligomers with varying subunit number. Ab initio modeling of Isu1 monomer indicates a compact, folded region and with a disordered terminus. Oligomeric Isu1 exhibits significant concentration dependent higher order oligomerization. Flexibility analysis reveals greater flexibility in Isu1 monomer as compared to Isu1 oligomers. In addition, the rigidity of Isu1 oligomers increases with each subsequent higher order oligomerization. Based on our understanding of the Yfh1-Isu1 complex and of Isu1 in solution, we propose that Isu1 oligomerization is normally induced by a conformational change that occurs upon Isu1 binding to Yfh1 oligomer.
Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited nephropathy causing 4-10% of end stage renal disease. Mutations to PKD1 or PKD2 cause ADPKD, with PKD1 associated with significantly more severe renal disease. The PKD1 and 2 proteins, polycystin-1 (PC1) and -2 (PC2), complex and have been proposed to form a functional channel. The severity of disease in PKD1 is associated with the level of the mature PC1 glycoform. Here we show that PC1/PC2 first interact in the ER before GPS/GAIN cleavage with PC2 acting as a chaperone that is essential for PC1 maturation and ciliary and plasma membrane localization. This function of PC2 depends on complexing with PC1 via its distal coiled-coil domain. In Pkd2-/- mice, complete loss of PC2 prevents PC1 maturation and secretion. In heterozygotes, a 50% PC2 reduction results in a non-equimolar reduction (20-25%) of the mature PC1 glycoform. Interbreeding between various Pkd1 and Pkd2 models shows more severe disease in bilineal animals, illustrating phenotypically the importance of complexing of these proteins for function. Since PC2 partly acts by regulating maturation of PC1, the level of mature PC1 is a disease determinant in PKD2 as well as PKD1.
Gold nanoparticles (AuNPs) have been widely studied for use in disease therapeutics as targeting and imaging agents, drug delivery vehicles and as self-therapeutics. When AuNPs interact with the biological milieu they form a corona layer, which is predominantly composed of proteins. The outer “soft” layer is dynamic and here proteins are free to exchange over time. The inner “hard” layer on the other hand consists of proteins firmly bound to the NP surface. The Vromans effect predicts that given a limited surface area, low affinity, high abundance proteins, which first attach to the surface, are over time replaced by high affinity, low abundance proteins. Our aim was to enrich low abundance proteins on the AuNP surface to probe for differentially expressed proteins that are not detected by traditional methods of protein identification. We studied the binding of ovarian cell lysates (cancerous and non cancerous) to 10 nm sized positively and negatively charged AuNPs. The formation of corona around the AuNPs was characterized by dynamic light scattering and zeta potential measurements. The composition of the protein corona was identified using mass spectrometry. Proteins were also identified from the cell lysate pool to serve as a control to assess protein enrichment on NP surface. Using this approach, Hepatoma Derived Growth Factor (HDGF) was identified as a low abundance protein that was detected by mass spectrometry in cancer cells only after enrichment on the surface of positively charged AuNPs. HDGF is overexpressed in multiple ovarian cancer cells lines and knock down using siRNA shows decrease in cell proliferation, G2/M arrest and apoptosis. This suggests an important role of HDGF in the tumorigenicity of ovarian cancer, which could serve as an important therapeutic target for the disease.
Title: Adipose lipolysis and the free fatty acids released are essential in regulating hepatic expression of the G0/G1 Switch Gene 2 (G0S2) during fasting

Authors: Bradlee L. Heckmann, Xiaodong Zhang, Xitao Xie, Alicia M. Saarinen, and Jun Liu M.D. / Ph.D.
Advisors: Jun Liu M.D. / Ph.D.
Track: Biochemistry and Molecular Biology

Lipid metabolism is a vital mechanism of global energy homeostasis. During periods of nutrient deprivation, including normal fasting periods, increased energy demand is met through the induction of triglyceride hydrolysis in adipose tissue. This lipolytic event releases free fatty acids (FFAs) into general circulation for use as energy substrates in the periphery, with specific trafficking for oxidation in the liver. While our understanding of lipid metabolism in adipose tissue is fairly well comprehensive, our knowledge in peripheral tissues including liver is still limited. In both rodent and human, upon fasting, FFAs from adipose tissue begin to accumulate in liver, forming lipid droplets. In addition to lipid accumulation, increased oxidative metabolism is also observed. The accumulation of FFAs in the form of lipid droplets is most likely a protective and/or rationing mechanism to prevent FFA toxicity or immediate consumption of FFA, allowing for sustained energy availability. The G0/G1 Switch Gene 2 (G0S2) is the primary lipolytic inhibitor, as it directly binds to and prevents the enzyme activity of adipose triglyceride lipase, the rate-limiting enzyme in the lipolytic machinery. During fed periods in adipose tissue we show G0S2 expression is high, however upon fasting, G0S2 expression is rapidly decreased allowing for increased lipolysis and subsequent FFA release. Interestingly, the exact opposite is witnessed in liver. During fed periods hepatic G0S2 expression is essentially undetectable, whereas there is a dramatic and rapid induction upon fasting or chemical lipolytic stimulation as we demonstrate herein. This observation appears to connect adipose lipolysis to hepatic G0S2 expression in a very intimate manner. Moreover, we demonstrate that this phenomenon is ablated in mice that have a constitutive inhibition of adipose lipolysis through adipose specific transgenic expression of G0S2. This confirms that G0S2 expression in liver is dependent on both adipose lipolysis and trafficking of lipolytically released FFAs to the liver. Furthermore, we utilize global G0S2 knockout mice to demonstrate promoter activation and provide evidence of protein stability. When combined, these data allow us to conclude that the activation of G0S2 expression is occurring at the message level, and is not a result of protein stabilization by FFAs or other lipolytic constituents. Clearance of accumulated lipid in liver during fasting is necessary to return to a healthy state. Upon refeeding, we demonstrate G0S2 levels in liver decrease and lipid droplets are cleared rapidly. While further study is necessary, this interplay between liver and adipose tissue and the subsequent regulation of metabolic proteins including G0S2, is a vital relationship for regulating the liver’s normal response to FFAs and for rationing of energy substrates to maintain global energy balance during normal and prolonged fasting periods.
Title: Oxygen concentration controls oncogenic epigenetic effects in models of familial paraganglioma

Authors: Yeng F. Her, Molly Nelson Holte, and Jim Maher, Ph.D.

Advisors: Jim Maher Ph.D.

Track: Biochemistry and Molecular Biology – Cancer Biology

Our laboratory is interested in understanding how defects in metabolism can promote tumorigenesis in familial paraganglioma (PGL), a neuroendocrine cancer. Familial PGL displays autosomal dominant inheritance and is due to loss of both copies of genes encoding subunits of succinate dehydrogenase (SDH), a tricarboxylic acid cycle enzyme. Loss of SDH function is particularly interesting because it exemplifies the Warburg effect of aerobic glycolysis, a mysterious feature of many cancers. Interestingly, it has been reported that mammals living at high altitude have an increased incidence of carotid body hyperplasia and PGL (1-4) and increased penetrance of PGL among SDH carriers (5). These observations suggest that chronic hypoxia accelerates the development of familial PGL. However, to date there has been no clear explanation for how loss of SDH function and hypoxia interact to cause neuroendocrine tumorigenesis. Here, we examine how low oxygen concentration modifies loss of SDH function to increase oncogenic epigenetics effects implicated in the development of paraganglioma. Our central hypothesis is that dioxygenase inhibition by succinate accumulation causes oncogenic epigenetics effects including activation of Hypoxia Inducible Factor (HIF), accumulation of histone methylation, and depletion of 5-hydroxymethyldeoxycytosine (5hmC) in DNA. Because oxygen is also a substrate in these reactions, succinate inhibition should be more profound at lower oxygen concentrations. We examined how succinate accumulation influenced HIF, methylated histone, and 5hmC levels in cell culture models in the presence of 21% oxygen (room air) and 10% oxygen (closer to physiological O₂ concentrations of 2-5%). Tested models included SDHB shRNA lentiviral knockdown human tissue culture cells and primary cultured cells from a novel SDHC conditional knockout mouse. We show for the first time that dioxygenase inhibition by SDH loss is sensitive to oxygen concentration, as expected, offering an explanation for the clinical observation that hypoxia promotes familial paraganglioma.
Title: **IGF-1 mediates TGFβ’s pro-fibrotic activity**

Authors: Danielle M Hernandez, and Edward B Leof, PhD

Advisors: Edward B Leof, PhD

Track: Biochemistry and Molecular Biology – Cancer Biology

Idiopathic Pulmonary Fibrosis (IPF) scars the lungs and irreversibly cripples the body's air exchange system in approximately 50,000 new individuals resulting in the death of 40,000 others (the equivalent of breast cancer) in the United Stated every year [1, 2]. With no known etiology, or FDA approved therapy, two-thirds of patients die within five years from this chronic, life threatening, interstitial lung disease [3, 4]. Utilizing a murine IPF model, we identified Insulin-like Growth Factor-1 (IGF-1) as a novel target supporting Transforming Growth Factor β (TGFβ) dependent pro-fibrotic responses. TGFβ signaling is known to impact a plethora of cell activities in a unique cell type specific manner, from growth arrest and apoptosis in epithelial cells, to promoting fibroproliferative phenotypes in mesenchymal cells [5]. In addition, IGF-1 contributes to both mitogenic and metabolic signaling, utilizing similar signaling pathways as TGFβ [6]. We provide evidence that TGFβ treated fibroblasts, but not epithelial cells, upregulate IGF-1 expression as well as induce auto-phosphorylation of the IGF1 Receptor-beta (IGF1Rβ). In addition, immunohistochemical analysis of tissue from Belomycin-treated mice with induced fibrosis [7], confirms the IGF-1 upregulation in fibrotic tissue in vivo. Furthermore, pharmacological inhibition of IGF-1 signaling diminishes TGFβ-stimulated morphologic transformation and growth in 3D culture. From these preliminary studies indicating the biological and physiological relevance of IGF-1 we sought to determine how IGF-1 bioavailability was maintained to regulate TGFβ-induced fibrotic responses. As Insulin-like Growth Factor Binding Proteins (IGFBPs) are known to sequester IGFs, bind Extracellular Matrix (ECM), and independently control cell proliferation, differentiation, and survival [8], we determined that IGFBP-3 & IGFBP-5 are additionally upregulated by TGFβ and sequester IGF-1 within the ECM. These findings set the stage to continue testing the central hypothesis that IGF-1 contributes to profibrotic TGFβ signaling and addresses a critical void in our inability to treat patients with fibrosis [9]
Pancreatic cancer is a very aggressive cancer with a 5-year survival rate of only 6%. Acinar to ductal metaplasia, known as ADM, is a phenomenon that is documented in early stage pancreatic cancer in which the secretory acinar cells alter their cell architecture and overall phenotype to become more ductal in nature. Using whole transcriptome sequencing we have found that this reprogramming is reminiscent of a cell reprogramming event called epithelial to mesenchymal transition (EMT). EMT occurs in development, wound healing and cancer cell invasion from many different tissues wherein changes to the cellular architecture and signaling events enable the cells to become more motile. ADM in pancreatic cancer does not show all the features of EMT, with a clear upregulation of mesenchymal markers without a complete downregulation of epithelial markers. Upon closer examination, we have found that early stage pancreatic cancers initiated from pancreatic acinar cells give rise to a population of not only duct-like cells, but also cells expressing neuroendocrine markers. Using the neuroendocrine marker, chromogranin A, we have confirmed the presence of these cells within the tumor, and in a subpopulation of delaminated and circulating tumor cells. Neuroendocrine tumor cells tend to be highly invasive and the phenotypic plasticity that is found in pancreatic cancer is based on the unique properties of the pancreatic progenitor phenotype taken on during ADM and may contribute to the aggressiveness of this disease as a whole. Thus, we are examining the function of proteins known to contribute to pancreatic development, especially the transcription factor Pdx1, with regards to the various paths of tumor cell differentiation. Understanding the full impact and contribution of a reprogramming event in pancreatic cancer may provide new targets to treat this deadly disease.
Title: Pancreatic Cancer-Induced β cell Dysfunction: Role of Exosomes and Adrenomedullin

Authors: Naureen Javeed, Gunisha Sagar, Ph.D., Shamit K. Dutta, Julie S. Lau, Gloria M. Petersen, Ph.D., Suresh T. Chari, M.D., Debabrata Mukhopadhyay, Ph.D.

Advisor: Debabrata Mukhopadhyay, Ph.D.

Track: Biochemistry and Molecular Biology – Cancer Biology

It has been reported that ~85% of pancreatic cancer (PC) patients have an abnormal fasting glucose, and nearly half develop type II diabetes (DM), which is frequently new-onset (i.e. of < 36 months duration). Although the correlation between diabetes and pancreatic cancer is well known, the pathophysiology is still not well defined. It is believed that new-onset diabetes in pancreatic cancer (PC-DM) is a paraneoplastic phenomenon that is caused by tumor secreted products. In recent work by our group, candidate mediators of β cell dysfunction were identified in PC-DM. A 52 amino acid peptide, adrenomedullin (AM), was found to be upregulated in PC cell lines and capable of inducing β cell dysfunction both in vitro and in vivo. Though plasma AM levels are elevated in PC, it is unlikely that it is acting as a hormone. Therefore, we hypothesize that AM is being packaged and carried to target β cells by biologically active molecules called exosomes through the blood. Exosomes are 30-100 nm endosomal-derived nanovesicles that are secreted by many different cell types and contain a variety of biological molecules. Our data have shown that PC exosomes (PC-Exo) isolated from commercially available PC cell lines and from PC patient-derived cell lines showed the presence of AM through Western blot analysis. Additionally, PC-Exo from cell line as well as from PC patient plasma were able to decrease insulin secretion from β cells, whereas AM inhibitory peptide can reverse the process. In contrary, we found that AM peptide as well as PC-Exo promotes insulin mRNA levels in β cells.

To address this counterintuitive result, we observed that the mRNA of ER stress markers were upregulated in the presence of AM, suggesting failure of the Unfolded Protein Response (UPR) in disposing of excess misfolded/unfolded insulin in the ER, leading to a decrease of insulin exocytosis. Taken together, our results suggest a novel mechanism by which PC causes diabetes by shedding PC-Exo that contain critical cargo, which can impair β cell function. Understanding the mechanism and mediators of PC-DM could lead to future biomarker development to detect this cancer in its infancy.
Roles of the kinase, Pak2, in Cellular Senescence
Jong-Sun Lee and Zhiguo Zhang, Ph.D.

Advisor: Zhiguo Zhang, Ph.D.

Track: Biochemistry and Molecular Biology

Cellular senescence, triggered through telomere erosion and oncogene activation, is a cell growth arrest state that causes loss of tissue function in mammals. Cellular senescence contributes to organism aging and tumor suppression. Therefore, it is critically important to understand the molecular mechanisms of cellular senescence. Cellular transition from proliferation to senescence is established by increased expression of the cell cycle inhibitor p16\(^{\text{INK4A}}\) and by formation of concentric chromatin complexes, named senescence-associated heterochromatin foci (SAHF), which in turn repress expression of genes involved in proliferation. It is known that the histone H3.3-H4 chaperone HIRA plays an important role in the formation of SAHF and senescence. However, its mechanism associated with chromatin organization and gene expression during senescence remains enigmatic. Recently, we found that the p21-activated serine/threonine protein kinase 2 (Pak2) regulates HIRA-mediated nucleosome assembly through phosphorylation of histone H4 serine 47 (H4S47ph), promoting H3.3 incorporation into nucleosomes. We showed that depletion of Pak2 results in global gene expression changes, and the magnitude of these changes correlate inversely with H3.3 density at the promoter. A previous study observed that Pak2 is activated via the RAS/ERK/PAK2 pathway in PC12 cells. Furthermore, normal fibroblast cells undergo premature senescence by activation of oncogenic Ras. Thus, we hypothesize that Pak2 may be an important regulator of cellular senescence, in part through regulating SAHF formation. To test this hypothesis, we asked whether Pak2 is involved in oncogene-induced senescence (OIS). We depleted Pak2 in oncogenic H-RasV12-induced senescent IMR90 human fibroblasts and observed changes in cellular senescence markers. Our results show that depletion of Pak2 during senescence progression seemingly delays OIS establishment as detected by reduced expression of p16\(^{\text{INK4A}}\) and formation of SAHF. Interestingly, depletion of Pak2 after establishment of OIS in oncogenic H-RasV12-induced senescent IMR90 human fibroblast reverses characteristic cellular senescence markers. Given these observations, we are pursuing mechanistic studies in OIS models to determine how Pak2’s role in HIRA-mediated nucleosome assembly regulates cellular senescence. Furthermore, we are employing accelerated aging mouse models to determine Pak2’s role in replicative cellular senescence.
Title: **Spartan deficiency causes genomic instability and progeroid phenotypes**

Authors: Reeja Maskey, Myoung Shin Kim, Ph.D., Darren Baker, Ph.D., Bennett Childs, Liviu Malureanu, M.D., Jan M. van Deursen, Ph.D. and Yuichi Machida, Ph.D.

Advisor: Yuichi Machida, Ph.D.

Track: Biochemistry and Molecular Biology - MPET LAB

Spartan (also known as DVC1 and C1orf124) is a PCNA-interacting protein implicated in translesion synthesis, a DNA damage tolerance process that allows the DNA replication machinery to replicate past nucleotide lesions. However, the physiological relevance of Spartan has not been established. Here we report that Spartan insufficiency in mice causes chromosomal instability, cellular senescence, and early onset of age-related phenotypes. Whereas complete loss of Spartan causes early embryonic lethality, hypomorphic mice with low amounts of Spartan were viable. These mice were growth retarded and developed cataracts and lordokyphosis at a young age. Cre-mediated depletion of Spartan from conditional knockout mouse embryonic fibroblasts resulted in impaired lesion bypass, incomplete DNA replication, formation of micronuclei and chromatin bridges, and eventually cell death. These data demonstrate that Spartan plays a key role in maintaining structural and numerical chromosome integrity, and suggest a previously unknown link between Spartan insufficiency and progeria.
Title: An experimental approach to measuring protein-mediated looping in vitro

Authors: Lauren S. Mogil and Jim Maher Ph.D

Advisors: Jim Maher Ph.D

Track: Biochemistry and Molecular Biology – Biochemistry

Double-stranded DNA is the genetic material of most living things. Understanding DNA mechanical properties is crucial for understanding nuclear structure and gene regulation. DNA must complex with proteins for packaging and condensation within cells. Persistence length is a physical property that defines the stiffness of a polymer, governing the scale over which the polymer behaves in a rod-like vs. string-like manner. The persistence length of double-stranded DNA is ~150 bp (~50 nm) making it among the stiffest biopolymers. It is hypothesized that the two main contributors to DNA stiffness are mutual charge repulsions along the DNA backbones and attractive base stacking interactions. T4 DNA ligase-mediated cyclization kinetics experiments are used to measure the physical properties of DNA, specifically DNA longitudinal and torsional flexibilities and helical repeat. This method estimates the stiffness of the polymer under the restriction that the DNA must form a perfect circle with ligation of properly-aligned termini. However, real DNA looping is mediated by proteins. Currently, there are few quantitative methods to measure DNA flexibility by protein-mediated looping analysis in vitro. We propose to solve this problem using a novel massively parallel DNA/protein conjugate approach and deep sequencing. We will simultaneously measure DNA loop probabilities as a function of loop length and test the results against existing theory.

Nhp6A is a sequence nonspecific yeast DNA binding protein that provides an ideal probe for measuring probabilities of different intramolecular DNA loop sizes after formaldehyde cross-linking. A DNA fragment (~1,000 bp) carrying a pendant amino group will be covalently linked to a myc-tagged Nhp6A variant carrying a C-terminal cysteine using a heterobifunctional cross-linker NHS-maleimide reagent. The protein will then be allowed to randomly bind the tethered DNA via intramolecular looping. A modified ChIP-exo protocol and deep sequencing will then be used to measure the probability of each possible loop size at single base pair resolution, all in a single experiment. The DNA length-dependent looping probability can then be estimated and compared to theoretical predictions. These experiments provide a first step to improve in vitro modeling of in vivo DNA looping.

The results to date will be reviewed with respect to the hypothesis in question.
Overexpression of the nuclear transport protein Nup88 is a common feature of many cancers. Furthermore, the extent to which Nup88 is overexpressed within a tumor has been linked with tumor aggressiveness and reduced survival. However, whether and how Nup88 overexpression drives multistage tumorigenesis is unknown. Here we show that Nup88 transgenic mice develop tumors, including tumor types where Nup88 serves as a prognostic biomarker. Nup88 overexpression was found to induce aneuploidy and prevent securin and polo-like kinase 1 (Plk1) from accumulating in late G2 phase. Further investigation revealed that Nup88 sequestered the anaphase-promoting complex/cyclosome (APC/C) inhibitor Nup98-Rae1 away from Cdh1-activated APC/C, thereby promoting the unscheduled proteosomal destruction of securin and Plk1. Securin and Plk1 insufficiency were associated with chromosome segregation defects including lagging chromosomes caused by delayed centrosome separation as well as chromatin bridge formation resulting from low separase activity. Collectively, our data demonstrate a critical role for Nup98-Rae1 in maintaining genomic stability by inhibiting the precocious degradation of key mitotic regulators and establish a causal role for Nup88 overexpression in promoting chromosomal instability that may contribute to the pathogenesis of many human cancers.
Autosomal Recessive Polycystic Kidney Disease (ARPKD) is a rare infantile-onset ciliopathy (incidence ~1:20,000) that is characterized by bilateral cystic kidneys and congenital hepatic fibrosis (CHF). The disease is caused by mutations to PKHD1 (Polycystic Kidney and Hepatic Disease 1, Chr 6p12) with known genotype-phenotype correlations: patients with two truncating alleles manifest with in utero/neonatal lethality, while patients with at least one in-frame allele have milder renal disease (ranging from childhood end-stage kidney disease to adult onset disease with severe CHF). The PKHD1 gene product, fibrocystin (FC), is a large 447 kDa single-pass type I transmembrane protein, containing 64 putative N-linked glycosylation sites, a 420 kDa ectodomain (NT) and a small 22 kDa C-terminal cytoplasmic tail (CT).

To date, little is known about FC localization and function or the pathomechanism associated with the various truncating/non-truncating mutations. Therefore, we have developed and optimized an assortment of tools to study the trafficking/localization of FC. First, we knocked-in a N-terminal V5 tag at the murine FC WT locus, which allows endogenous analysis of the protein using tissue samples or immortalized epithelial cell lines that we have generated from homozygous V5-FC/H2Kb mice. This highlighted FC localization to the primary cilia and secretion in exosome-like vesicles (ELVs). Second, the GeneSwitch System™ containing an inducible C-terminal V5 epitope tagged FC allows for the examination of FC trafficking/localization and interactors. This system revealed that induction of exogenous FC leads to three large protein products detectable by western blotting. Lastly, optimization of immunoprecipitation and immunofluorescence protocols confirmed the localization and shedding of FC.

Together, we believe that the experimental models/methods are a crucial tool to elucidate normal/mutant localization and function of FC. Further, we believe that these tools will allow analysis of the various pathomechanisms underlying different mutations, potentially providing insight into affected pathways and personalized therapeutic avenues based on mutation and pathomechanism type.
Title: Transitions between "closed" and "open" conformations of the ESCRT-III subunit, Ist1, lead to distinct modes of Vps4 regulation

Authors: Jason A Tan, Brian A. Davies, Johanna A. Payne, David Katzmann, Ph.D

Advisor: David Katzmann, Ph.D

Track: Biochemistry and Molecular Biology

Subunits belonging to the endosomal sorting complexes required for transport-III (ESCRT-III) act in concert with the AAA-ATPase, Vps4, to mediate important cellular activities, including viral budding, cytokinesis, and multivesicular body (MVB) sorting. Common to all of these activities is the requirement for membrane budding away from the cytoplasm, a process that is mediated by the assembly of monomeric ESCRT-III subunits (Snf7, Vps20, Vps24, Vps2, Did2, Ist1, and Vps60) in the cytoplasm into a membrane-bound ESCRT-III polymer, as well as stimulation of Vps4 ATPase activity. Previously, it was shown that ESCRT-III subunits can regulate Vps4 activity (stimulation by Vps2, Did2, and inhibition by Ist1), but the mechanisms underlying these distinct modes of regulation, and their relevance to ESCRT-III function in vivo remain poorly understood. Here, we report that Ist1 is able to both inhibit and stimulate Vps4 activity, and that transitions between these distinct modes of regulation are related to conformational changes in Ist1. By performing site-directed mutagenesis of Ist1, we have determined that (1) the interaction of the Ist1 MIT-interacting motif 1 (MIM1) element with the Vps4 MIT domain is essential for both inhibition and stimulation of Vps4 ATPase activity, (2) Vps4 inhibition also requires a secondary interaction with the Ist1 ELYC motif, and (3) a lower level of Vps4 stimulation that is not dependent on MIM1-MIT interactions. In addition, using limited proteolysis and analytical gel filtration, we provide evidence that Ist1 inhibition occurs in a “closed” conformation, whereas Ist1 stimulation occurs in an “open” conformation, and that transitions between these conformations involve rearrangement of multiple surfaces on Ist1 in both the Ist1 NTD and CTD. Lastly, we demonstrate that interactions between wt Ist1 and its ESCRT-III binding partner, Did2, are sufficient to convert Ist1 from an inhibitor to a stimulator of Vps4 activity. This finding suggests that transitions between “closed” and “open” Ist1 mutants conformations in vitro may be induced during assembly into the ESCRT-III polymer in vivo. Together, these findings lead to model whereby monomeric Ist1 inhibits Vps4 activity in the “closed” conformation in the cytoplasm, and converts to a stimulator of Vps4 activity following association with Did2 during its incorporation into the ESCRT-III polymer at sites of membrane budding.
Title: **BubR1 insufficiency selectively inhibits lung development at the sacculation stage**

Authors: Robbyn L. Weaver, and Jan M. van Deursen, Ph.D.

Advisor: Jan M. van Deursen, Ph.D.

Track: Biochemistry and Molecular Biology – Cancer Biology

BubR1 is a mitotic checkpoint protein whose role within the mitotic checkpoint complex (MCC) helps ensure genetic stability by preventing the onset of anaphase until sister chromatids are properly attached to their respective spindle poles. Complete knockout of BubR1 is early embryonic-lethal at the blastocyst stage, and while BubR1 hypomorphic mice (BubR1^{H/H}) with 10% BubR1 are viable, they develop a variety of rapid aging phenotypes with progressive aneuploidy. Mice with 5% BubR1 (BubR1^{-H}) are growth retarded at birth, gasping and pale, and die within several hours. To investigate the cause of death, we evaluated late-stage (embryonic day 18.5) BubR1^{-H} embryos using histology. We found that lung development of these embryos had arrested prior to sacculation, a stage where the respiratory epithelium differentiates into type I and type II cells to form terminal saccules that establish the blood-air barrier. Importantly, no overt developmental defects were observed in other tissues. Although lung epithelium of BubR1^{-H} mice does differentiate into type I and type II cells, the relative abundance of these cells was significantly altered. Specifically, the number of type I cells, which provide surface area and structure for gas exchange, was significantly decreased, while the number of type II cells, which secrete pulmonary surfactant, was increased. These findings uncover a selective developmental requirement for BubR1 in the differentiation of lung epithelial progenitor cells that seemingly is independent of its established roles mitotic checkpoint control and chromosome segregation.
Title: Differential Regulation of HP1 Isoforms by Aurora Kinases During Mitosis

Authors: Monique Williams, Angela Mathison, Gwen Lomberk and Raul Urrutia, M.D.

Advisor: Raul Urrutia, M.D.

Track: Biochemistry and Molecular Biology – Cancer Biology

Previous elegant studies performed in the fission yeast Schizosaccharomyces pombe have identified a requirement for Heterochromatin Protein 1 (HP1) in spindle pole formation and appropriate cell division. However, the regulation of HP1 isoforms by kinases critical for supporting mitotic progression remains to be fully characterized. We report for the first time the phosphorylation of both HP1γ and HP1α by the mitotic kinase Aurora A and B, respectively. Our results demonstrate that EGF, working through the RAF-MEK-ERK pathway, ultimately activates Aurora kinases, which in turn phosphorylate HP1γ at serine 83 and HP1α at serine 92. Notably, these phosphorylation events occur within Aurora consensus sites located within the linker region of these proteins. Using double-thymidine block in HeLa cells, we define that the phosphorylation of both HP1 isoforms takes place during mitosis, concordant with the time that the phosphorylated serine10 mark is deposited on Histone H3. Congruently, the phosphorylated form of these proteins localize to the mitotic apparatus. Experiments employing pharmacological inhibitors, dominant negative proteins, and siRNA-based knockdown of Aurora A and B confirm that these enzymes are the responsible HP1 kinases. Utilizing HP1γ as a model for mechanistic studies, we demonstrate that the Aurora-dependent phosphorylated form of this protein is necessary for cell proliferation. Combined, these results demonstrate that posttranslational modifications in histone code readers serve as distinct signals to endow these proteins with specific functions.
Title: Viscoelastic characterization of transverse isotropic tissue mimicking phantoms and muscle

Authors: Sara Aristizabal, Carolina Amador, Ph.D., Bo Qiang, Ivan Z. Nenadic, Ph.D., James F Greenleaf, Ph.D. and Matthew W. Urban, Ph.D.

Advisors: Matthew W. Urban, Ph.D. and James F Greenleaf, Ph.D.

Track: Biomedical Engineering and Physiology – Ultrasound Research

Assessment of the mechanical properties of soft tissues has become an important physiological parameter of the status of different body tissues under normal and abnormal conditions. Several ultrasound radiation force-based methods have the ability of determining tissue mechanical properties when the tissue has isotropic characteristics but these methods face challenge when the tissues under evaluation such as kidney, myocardium, and skeletal muscle have properties that are directionally dependent, a phenomenon known as anisotropy. To study the viscoelastic characteristics of this phenomenon in a laboratory setting, we designed a transverse isotropic (TI) phantom incorporating fibrous material with preferential orientations embedded in tissue mimicking gelatin, and we evaluated a sample of excised pork tenderloin in a saline bath at 30°C.

Measurements were made in the phantom at two gelatin concentrations (8% and 14%) and the sample of pork muscle at different angles by placing each individual phantom and the pork tenderloin on a rotating platform with a rotation range oscillating between 0° to 360° in 10° steps. The phantom and excised pork muscle were rotated with respect to the transducer, where 0° and 180° were defined along the fibers, and 90° and 270° were defined across the fibers. Shear waves were generated and measured by a Verasonics ultrasound system equipped with a linear array transducer operating at 4.1 MHz center frequency. The shear wave speed was evaluated from the distribution of particle displacement, which was estimated by a two-dimensional in-phase/quadrature auto-correlation method with spatial and temporal averaging of the compounded echoes from three different angled plane waves detected at an effective frame rate of 4.16 kHz. To estimate the shear elasticity ($\mu_1$) and viscosity ($\mu_2$), a Voigt model was fit to the shear wave dispersion curves of the phase velocity within 100-700 Hz. The values for $\mu_1$ and $\mu_2$ increase as the transducer is placed along the fibers (0°) and decrease as the transducer is placed across the fibers (90°). When looking at their behavior in a polar coordinate system, it is possible to appreciate that $\mu_1$ and $\mu_2$ distribution approximates the profile of an ellipse. This behavior indicates that $\mu_1$ and $\mu_2$ are angularly dependent, and exhibit a TI behavior that can be studied using viscoelasticity measurements.
Neurons in the medial temporal lobe structures respond to specific places, objects and persons. Selective responses to multi-modal representations of famous individuals or landmarks were recorded in the human hippocampus and proposed to underpin perceptual and memory traces for declarative episodic memories (Quiroga 2012). It remains unclear how these selective neuronal activities initially develop in response to novel, unfamiliar visual stimuli. Here we employed International Affective Picture Set (IAPS) of unpublished, protected images in a visual recognition memory task to investigate how new stimuli are encoded and recalled by medial temporal lobe neurons.

Neuronal extracellular unit activity was recorded from the hippocampus, amygdala and parahippocampal cortex of four epilepsy patients undergoing seizure monitoring. The putative action potentials were sorted into individual single units using a semi-automated clustering approach. Firing of individual single units as well as cross-correlated activity of single unit pairs was assessed during the encoding of specific IAPS images and their subsequent recall 24h later.

Individual neurons did not show selective firing responses to the IAPS images across the two task stages. There was no significant effect of the affective charge, novelty, memory accuracy or subjective ratings of the images on the single unit activity. On the population level, however, presentation of the images modulated unit firing, which ramped up during picture viewing. Furthermore, cross-correlation analysis revealed pairs of significantly correlated single unit firing on trials with specific images during the encoding and recall stages of the task. These significant cross-correlations were higher in the hippocampus than in the parahippocampal cortex or the amygdala (p < 0.001, Wilcoxon rank sum test) and were stronger for unit pairs from the same brain structure than from two different structures (p < 0.001, Wilcoxon rank sum test). Overall, the correlated unit pairs constituted approximately 5% of all possible cell pair combinations.

Our results show that presentation of visual stimuli is associated with significantly cross-correlated neuronal activity in the medial temporal lobe structures. We speculate that the encoding of novel, unfamiliar visual stimuli is performed on the population level of correlated firing of neurons, which would support a Hebbian mechanism for the emergence and development of neuronal ensembles underlying specific memory representations.
Heart failure is a leading cause of death and disability in the United States. A common hallmark of chronic heart failure is development of pulmonary congestion and/or edema. Pulmonary congestion refers to the accumulation of fluid in the pulmonary circulation due to decreased function of the heart, whereas pulmonary edema is the transudation of fluid into the interstitial and alveolar space. While these two conditions are generally thought to be equivalent, each uniquely and individually contribute to decreased pulmonary function. Increases in thoracic fluid volume may cause engorgement of the pulmonary vasculature, potentially limiting lung volumes and increasing work of breathing. Evaluating the contributions of these two distinct mechanisms to impaired lung function will provide a better understanding of how to control the pulmonary symptoms of heart failure. The protocol was reviewed by the Mayo Clinic Institutional Review Board and written consent was obtained from all subjects. First, subjects performed a forced vital capacity maneuver. Next, an esophageal balloon was inserted via the nose into the lower third of the esophagus. Respiratory mechanics, gas exchange, and lung diffusion capacity were measured in the seated upright, and supine positions. Subjects performed vital capacity maneuvers at 10-100% maximal expiratory effort, and the chest wall recoil was recorded using the quasi-static relaxation technique. Gas-exchange, heart rate, esophageal and mouth pressures, blood pressure, and pulse oximetry were recorded during 6 min of quiet breathing in the seated upright. These variables were also measured within the initial 10 min of moving to the supine position, and once again after 20 minutes in the supine position. Diffusion capacity for carbon monoxide (DLCO): DLCO was obtained 2-3 times at the end of each experimental condition (i.e., seated upright, immediate supine, 30 mins supine). DLCO was used to determine changes in lung capillary volume and gas-exchange surface area. On the same day, a full thoracic scan was obtained of the pulmonary and cardiac anatomy. A second scan was then taken with the bed stationary in the CT scanner at the midlevel of the heart. An iodine contrast-agent was injected and scans were taken during diastole. Transit of the contrast through the heart afforded the determination of thoracic and pulmonary blood volumes. Extravascular lung water was determined by the equation, \[ \frac{H_u_{roi} - H_u_{air}}{H_u_{blood} - H_u_{air}} \], where \( H_u \) is the hounsfield unit density of the area described by the subscript.
Lung diffusing capacity is the ability of the lungs to transfer gases from the alveoli to the blood and is generally measured via uptake of carbon monoxide (CO) during one or more breaths. Lung diffusing capacity of CO (DLCO) is considered to be dependent on the pulmonary alveolar surface area (Dm) available for gas exchange and the pulmonary capillary blood volume (Vc) available to take up oxygen and deliver it to the tissues. These factors can be modeled as two resistances in series, where $\theta_{\text{CO}}$ is the reaction rate of CO with hemoglobin:

$$\frac{1}{DLCO} = \frac{1}{Dm} + \frac{1}{\theta_{\text{CO}} \ast Vc}$$

There are multiple techniques used to determine Dm and Vc from measurements of DLCO, including the 'gold-standard' multiple FIO2 method and the more recent single FIO2 (rapid CO/NO) method. Because CO and oxygen competitively bind hemoglobin, the multiple FIO2 method utilizes multiple oxygen concentrations to modify available binding sites for CO and therefore vary DLCO, allowing for calculation of Dm and Vc via a graphical method by plotting the equation above. On the other hand, the single FIO2 method utilizes lung diffusing capacity of nitric oxide (DLNO), a gas whose diffusion is dependent only on Dm; a so-called $\alpha$-ratio is then used to convert from Dm(NO) to Dm(CO), and Vc is then calculated from these values. However, two assumptions used in these calculations can greatly vary the outcome: $\theta_{\text{CO}}$ can be calculated via seven different equations, and the $\alpha$-ratio has not been consistently measured, yielding a range of values which exist in the literature. Therefore, the purpose of this study was to determine both the optimal $\theta_{\text{CO}}$ equation and $\alpha$-value to be used in the single FIO2 technique which give (1) physiologically reasonable values of Dm and Vc and (2) agreeable values to the multiple FIO2 ("gold-standard") technique. Seven young, healthy males performed DLCO/DLNO maneuvers in duplicate while breathing 20, 40, or 60% oxygen during rest and submaximal cycling exercise (80 Watts). Dm and Vc were then calculated via both methods using seven $\theta_{\text{CO}}$ equations and a range of $\alpha$-ratios used in previous literature. The concordance correlation coefficient and t-Statistic were then used to determine the similarity of Dm and Vc values between the two methods. With the exception of the “RF7.4” $\theta_{\text{CO}}$ equation, the multiple FIO2 method gave physiologically reasonable data for Dm and Vc. At rest, the optimal $\theta_{\text{CO}}$ equation was inconsistent, while an $\alpha$-ratio of 2.5 yielded most similar values for Dm and Vc calculated via the single FIO2 method as compared to the multiple FIO2 method. During exercise, the “Hol” and “RF2.5” $\theta_{\text{CO}}$ equations showed the best agreement for values of Dm and Vc; however, the optimal $\alpha$-ratio was variable.
Intramuscular pressure (IMP) has been shown to increase linearly with increasing muscle force. However, regional variations in IMP have been observed within the muscle. The cause of these geometric variations has yet to be defined. Local fluctuations in fluid content may contribute to these pressure gradients. The purpose of this study was to validate a technique for tracking interstitial fluid flow during muscle lengthening using fluorescent microspheres.

Fluorescent microspheres were injected into the rat tibialis anterior (TA) pre- and post-lengthening, n = 4. The TA was lengthened to 10% strain, which was measured with the knee and ankle joint at 90°. Two microsphere diameters, 0.1 and 0.2 µm, were injected into individual muscles post-dissection to evaluate the effects of bead size. 20 µm muscle cross-sections were stained and imaged using confocal microscopy to evaluate the microsphere dispersion axially along the length of the muscle.

The fluorescent microspheres were concentrated at the injection site and flowed with the interstitial fluid staying outside the muscle fascicle. The microspheres spread radially away from the injection site, primarily in the axial direction along the length of fibers. As a percent of the original length, the beads were distributed axially 19% (0.1 µm beads) and 29% (0.2 µm beads) for muscles lengthened pre-injection and 44% (0.1 µm beads) and 45% (0.2 µm beads) for muscles lengthened post-injection.

This study illustrates that injection of fluorescent microspheres is a viable technique to evaluate intramuscular interstitial fluid flow. The microspheres stay in the interstitial space and do not penetrate the fascicle. The initial distribution of the beads is likely a result of the injection force. The expanded distribution of microspheres in the post-injection lengthened specimens indicates interstitial fluid flow occurs during muscle lengthening.
INTRODUCTION: Dystonia is a neurologic movement disorder characterized by sustained, involuntary muscle contractions that result in repetitive motions or abnormal positions of involved body parts. Symptom onset is activity-dependent, and prolonged dystonic postures considerably limit normal movements. Diagnosis of dystonia relies solely on clinical assessment and is therefore subjective. We have recently reported that spectral analysis of surface and fine-wire electromyography (EMG) signals provides a quantitative and objective measure of dystonic behavior in lower extremity muscles. In this case report, we will describe an individual where spectral analysis of surface EMG signals quantitatively confirmed the presence of dystonia.

MOTION DATA: All data were analyzed at four time-points: quiescent, prior to the walking trials; an early walking trial; a late walking trial; and quiescent, after conclusion of the walking trials. Spectral analysis was performed on the EMG signals and the median power frequency (MdPF) was quantified for each spectrum. The kinematic data demonstrated a lack of dorsiflexion in stance (5° peak dorsiflexion), which the multisegment foot model identified as a 10° decreased calcaneal-tibial dorsiflexion. Dynamic EMG signals exhibited nearly continuous activity in all of the muscles tested. Spectral analysis showed that the MdPF shifted progressively to lower frequencies over the course of the gait study in the bilateral gastrocnemius and hamstrings (Figure 1). This shift was associated with symptom onset and severity.

TREATMENT DECISIONS AND INDICATIONS: Changes in the kinematics were attributable to the abnormal muscle activation patterns and joint motion limitations. The changes persisted throughout the study. The spectral analyses, which showed that the MdPF gradually shifted to lower frequencies over the course of the gait study, were suggestive of a task-specific lower extremity dystonia. Possible treatment options for dystonia include administration of Parkinson’s disease medications (e.g. levodopa) or intramuscular injection of neurotoxins (e.g. botulinum toxin). A trial course of levodopa was prescribed for this patient due to the generalized nature of his symptoms.

SUMMARY: A 14-year-old male was referred for clinical evaluation of fatigue and shaking with exertion. Physical examination and motion analysis findings were suggestive of a task-specific lower extremity dystonia, which was confirmed with spectral analysis of the EMG signals collected from the lower extremities. This clinical case demonstrates the utility of EMG spectral analysis as a quantitative tool for the objective diagnosis of dystonia.
Diseases of the central nervous system (CNS) commonly affect motoneurons. In particular, spinal cord injury, aging and other congenital and neurological disorders can cause motoneuron dysfunction or loss. At present there are no effective diagnostic tools or treatments that specifically target motoneurons. Nanotechnology has revolutionized drug delivery systems. We hypothesized that nanoparticles can be used to target drug delivery to motoneurons. Specifically mesoporous silica nanoparticles (MSNPs) may be ideal for this application since they can be independently modified (pore size, surface chemistry) allowing loading of various cargos; and surface-engineered for bio-functionality and biocompatibility. These properties make MSNPs a suitable treatment vehicle to target the CNS. The aim of this study was to characterize the role of nanoparticle size, concentration, time of incubation and surface coating on uptake by cultured motoneurons (mouse motor neuron-like hybrid cell line: NSC-34 (Cellutions Biosystems Inc.)). Four different fluorescently-labeled MSNPs were evaluated: 1) PEG-PEI-Ace MSNPs of two different sizes: 150 nm and 2) 50 nm, 3) lipid coated MSNPs (protocell - 140 nm) and 4) PEG-PEI MSNPs (50nm). A FlexStation microplate reader was used to evaluate the uptake of the MSNPs by measuring the fluorescence intensity per cell after treatment with different MSNP concentrations and incubation times. Uptake of MSNPs by differentiated motoneurons was determined with immunohistochemistry and confocal imaging, allowing selection of differentiated motoneurons based on morphology and expression of beta III tubulin. CytoTox-Glo (Promega, Madison, Wisconsin) cytotoxicity assay was used to measure dead-cell protease activity and quantify the death rate of cells after MSNPs treatment. After three hours of incubation, PEG-PEI-Ace MSNPs and protocells showed significant motoneuron uptake (p<0.05), whereas minimal uptake was evident with PEG-PEI MSNPs. Uptake of MSNPs appeared to be saturable for all four MSNPs. Differentiated motoneurons in culture also displayed significant MSNPs uptake at both dendrites and cell soma. Interestingly, evidence of uptake of PEG-PEI MSNPs was present at both differentiated and undifferentiated motoneurons. Regardless of the cell density and duration of treatment, there was minimal evidence of cytotoxicity by any of the MSNPs. In conclusion, MSNPs can be used to target drug delivery to motoneurons. Specifically, MSNPs coating, size and concentration have an effect in motoneurons uptake, with smaller (~50 nm) and coated PEG-PEI-Ace MSNPs showing substantial uptake. More studies are needed to evaluate the endocytosis process of the MSNPs in motoneurons, and the intracellular localization over time. These results elucidate the appropriate characteristics of the MSNPs for specific in-vivo applications.
Delivery system characteristics limit the minimum number of protons that can be delivered per spot, resulting in a min-MU limit. Plan quality is dependent on the min-MU limit. The purpose of this study was to investigate the influence of the minimum monitor unit (MU) on the quality of clinical treatment plans for scanned proton therapy.

To investigate the potential impact of this min-MU on the quality of treatment plans two sites were studied: a pediatric brain tumor at a depth of 5-10 cm; a head and neck tumor at a depth of 5-20 cm. Intensity modulated spot scanning proton plans were created for the following parameter variations: min-MU limit range of 0.0000-0.006; and spot spacing range of 0.5-2.0 $\sigma$ of the spot size at isocenter in water ($\sigma=4$ mm in this work). The treatment plans were analyzed by comparing each plan’s ability to avoid dose to critical structures while maintaining homogeneous target coverage.

The increase of the min-MU with a fixed spot spacing decreases plan quality both in homogeneous target coverage and in the avoidance of critical structures. The head and neck plans showed over a 20% increase in relative dose for the hottest spot in the CTV when comparing min-MU limits of 0.0000 and 0.0060 with a fixed spot spacing of 1 $\sigma$. Figure 1 shows the difference between the two plans, where the non-color washed portions represent areas of dose above 110%. Similar trends were seen in the case of the pediatric brain. The mean and maximum dose for critical structures surrounding the tumor also followed the trend of increasing as the min-MU limit increased with a constant spot spacing. Figure 2 is of the DVHs that show the variation across all min-MU limits as spot spacing is held constant (1 $\sigma$). The DVHs of CTV coverage show min-MU limits of 0.0000 and 0.0010 produce the same plan quality and that quality is much worse with min-MU of 0.0020.

Given a fixed spot spacing of $\leq \sigma$ of the spot size in water, plan quality decreases as min-MU increases greater than 0.001. The effect of min-MU needs to be taken into consideration while planning proton therapy treatments.

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INTRODUCTION: Cine Phase Contrast (CPC) MRI is used in biomechanics for measuring skeletal muscle strain distribution to enhance understanding of localized muscle function and mechanical failure modes. Muscle strain is obtained by integrating CPC-encoded velocity. The error of the measured velocity, which has not been previously quantified, is of interest because it provides a lower bound to the derived strain error. The aim of this study was to quantify the accuracy and precision of the CPC-encoded velocity measurements. It was hypothesized that measurement accuracy would improve with systematic error correction and that precision would follow the thermal noise equation: \( \sigma = (\sqrt{2} \cdot V_{\text{enc}}) / (\pi \cdot \text{SNR} \cdot \sqrt{N}) \), where \( V_{\text{enc}} \) is encoding velocity, SNR is signal-to-noise ratio, and \( N \) is number of independent pixels averaged.

METHODS: A custom MRI-compatible jig was designed to move a B-gel phantom at five velocities (0, ±20, and ±40mm/s) using a linear stepper motor. Velocity data were collected with a 1.5T MRI system (GE Medical Systems) using the commercially available Fast 2D Phase Contrast sequence. The three encoding directions (through plane, frequency, and phase) were evaluated independently. Post-processing was performed using custom Matlab (The Mathworks) code. Two types of systematic error were removed from the data: eddy current-induced bias and calibration-type error. Accuracy and precision, defined as the mean and standard deviation of the error between the measured and true velocities, were quantified before and after removal of systematic error.

RESULTS: This study demonstrated greater than 70% improvement in accuracy of the through plane- and frequency-encoded data (error reduced from 1.1 to 0.1 and from 1.3 to 0.4mm/s, respectively) after removal of systematic error. The primary contributor to this change was the calibration-type error, which has not been previously addressed in the literature. Only minor improvement occurred in the phase-encoded data (error reduced from 1.4 to 1.3mm/s).

The predicted random error from the thermal noise equation was \( \sim \)1.2mm/s, based the parameters of this experiment (\( V_{\text{enc}}=50\text{mm/s}, N=9, \text{SNR}=6.2 \)). The measured random error ranged between 1 to 1.4mm/s, depending on the encoding direction.

SUMMARY: By correcting systematic error – including a previously undescribed calibration-type error – CPC velocity measurement accuracy may be increased by 70%, except for the phase-encoded measurements. Experimental random error matches the theoretical prediction.

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Title: Therapeutic Delivery of TrkB by Adeno-Associated Virus Enhances Respiratory Recovery after Upper Cervical Spinal Hemisection

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Track: Biomedical Engineering and Physiology

Brain derived neurotrophic factor (BDNF) acting through its full-length tropomyosin related kinase receptor subtype B (TrkB) has been shown to be necessary and sufficient to enhance spontaneous recovery of ipsilateral diaphragm muscle (DIAm) EMG activity following cervical spinal cord hemisection at C2 (C2SH). The purpose of this study was to determine if therapeutic delivery of TrkB to phrenic motoneurons via an adeno-associated viral vector following spinal cord injury increases functional recovery of ipsilateral DIAm activity post-C2SH.

On the third day following C2SH, adult male rats were randomized to receive intrapleural delivery of adeno-associated virus serotype 7 (AAV7) encoding TrkB or green fluorescent protein (GFP). Recovery of ipsilateral DIAm EMG activity was assessed using chronically implanted EMG electrodes. The proportion of animals displaying recovery of ipsilateral DIAm EMG activity was significantly greater in the group treated with AAV-TrkB (10/15) compared to those treated with AAV-GFP (2/11; p=0.01). The extent of recovery quantified using the root-mean square (RMS) amplitude of ipsilateral hemidiaphragm EMG activity 40% of pre-injury values in animals treated with AAV-TrkB (vs. 10% in those treated with AAV-GFP). Given that during the time course of spontaneous recovery after C2SH, expression of excitatory glutamatergic (GluR) and serotonergic (5-HTR) receptors increases in phrenic motoneurons, their expression was quantified by real time RT-PCR at 21 days post-C2SH. No difference was found between the AAV-TrkB and AAV-GFP treated groups 21 days post-C2SH. These results suggest that viral delivery of AAV-TrkB below the level of spinal cord injury is sufficient to promote functional recovery of ipsilateral hemidiaphragm EMG activity, even when administered post injury.
Dixon-based methods for fat suppression avoid errors by accounting for main magnetic field inhomogeneities during the separation of the fat and water signals. Additionally, when applied to contrast-enhanced MR angiography, Dixon methods have been shown to demonstrate improved signal-to-noise ratio compared to background suppression via subtraction\(^1,2\). Single-echo Dixon techniques avoid extending the scan time, but require that phase parameters be known \textit{a priori}\(^3\)\(^-\)\(^5\). Partial Fourier sampling can reduce scan time by up to a factor of two, but has not yet been applied to single-echo Dixon imaging. The purpose of this work is to theoretically derive and experimentally demonstrate partial Fourier single-echo Dixon with homodyne reconstruction.

Single-echo Dixon imaging assumes that both the water and the fat signals are real and have known initial phase, time-dependent \(\Delta \theta_0\)-induced phase, and chemical shift-induced phase. To this end, a phase constrained reconstruction\(^6\) was used. The homodyne processing used here is identical to the standard process\(^7\), except i) it is performed within a phase constrained reconstruction and ii) the usual lumped-phase term is separated into unique phase terms. A fat-water phantom was constructed to simulate an abdomen (bovine gelatin) with subcutaneous fat (vegetable shortening) and an enhanced abdominal aorta (~0.4 mmol gadolinium-doped bovine gelatin). Fully-sampled single- and dual-echo axial images were acquired. Phase-constrained Dixon reconstruction was performed on i) fully-sampled, ii) retrospectively undersampled and zero filled, and iii) retrospectively homodyne filtered single-echo images.

Water images from the single-echo Dixon reconstruction show good fat suppression when fully sampled, zero filled, and reconstructed with homodyne processing. The corresponding fat images depict the vegetable shortening layer of the phantom very well with only slight water signal leakage within the gadolinium-doped gelatin.

The single-echo Dixon reconstruction with zero filling shows noticeable blurring compared to the fully-sampled image. Homodyne processing sharpens the image and shows good fat suppression and minimal artifacts. The slight undersampling artifacts can potentially be reduced by using a more random high-pass region or with an iterative approach to partial Fourier reconstruction (e.g. POCS). Future work will investigate the use of this method in vivo and in conjunction with parallel imaging.
Title: Moderate Hyperoxia Induces Extracellular Matrix Remodeling in Developing Human Airway Smooth Muscle

Authors: Elizabeth R. Vogel, MD, Rodney D. Britt, PhD, Arij Faksh, MD, Hitesh Pandya, MD, YS Prakash, MD, PhD, Richard J. Martin, MD and Christina M. Pabelick, MD

Advisors: Christina M. Pabelick, MD; YS Prakash, MD, PhD

Track: Biomedical Engineering and Physiology: Physiology

Chronic wheezing and asthma remain significant causes of morbidity and mortality in the pediatric population. Premature infants are at increased risk due to early exposure to hyperoxia and/or mechanical ventilation. As in adult asthma, a thickened airway contributes to limited airflow. However, the mechanisms underlying these changes and the potential impact of hyperoxia in this context are unknown. Airway remodeling involves airway smooth muscle (ASM) cell proliferation and increased extracellular matrix (ECM) production. We hypothesized that even moderate hyperoxia (40-50%) enhances remodeling in developing airways contributing to neonatal/pediatric asthma. Human fASM cells were enzymatically dissociated from canalicular stage (18-22 weeks gestation) fetal tracheobronchial trees (de-identified samples, in vitro only; MRC-Wellcome Trust; approved by UK Ethics Committees, considered exempt by Mayo Institutional Review Board). Cells were exposed to normoxia or moderate hyperoxia for 24-72 hours. Additional samples were pretreated with 10 mM n-acetylcysteine (NAC; ROS scavenger) for 60 min prior to hyperoxia exposure. Western analysis for collagens, fibronectins, and matrix metalloproteinases (MMPs) was performed. Semi-quantitative immunofluorescence was used to analyze extracellular ECM deposition. MMP activity was determined using gelatinase zymography. Exposure of fASM cells to moderate hyperoxia significantly increased expression and deposition of ECM proteins collagen I and collagen III while fibronectin deposition was decreased. NAC pretreatment prevented hyperoxia-induced increase in collagens, but did not reverse changes in fibronectin deposition. Hyperoxia increased MMP-2 and -9 activity while endogenous MMP inhibitors (TIMPs) were decreased. NAC pretreatment prevented these increases in MMPs. These results demonstrate that moderate hyperoxia enhances ECM deposition and remodeling in developing airways by altering the balance between MMPs and their inhibitors and increasing collagen deposition. Taken in conjunction with prior data demonstrating increased fASM proliferation with hyperoxia, these data further demonstrate that hyperoxia is an important instigator for remodeling in the developing airway. Understanding the mechanisms by which hyperoxia affects the airway may lead to new therapies for prevention and treatment of airway diseases such as asthma in former preterm infants.
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common form of PKD, with an estimated prevalence of 1:400 - 1:1,000 births, and is characterized by progressive renal cyst formation resulting in end stage kidney failure during the 5th decade of life. Since patients with ADPKD exhibit significant phenotypic variability and a detailed mechanism of renal cyst initiation is still unknown, more research is need to determine pathways that contribute to cyst development. Analysis of cyst linings from ADPKD patients and PKD animal models suggests that Wnt signaling is upregulated. Due to the role of Wnt signaling in renal epithelial cell proliferation during development and after injury, it is possible that the Wnt pathway becomes reactivated in PKD to contribute to the proliferation of renal epithelial cells encompassing a developing cyst. The protein products of ciliary genes mutated in other forms of PKD, such as NPHP2, MKS3, and NPHP3, have been shown to interact directly with the Wnt pathway and regulate its activity in vitro. However, the majority of animal models used in these studies are embryonic lethal, and do not address the role of Wnt signaling at later stages of kidney development. Therefore, it is critical to examine the role of Wnt signaling in a more slowly progressing animal model of ADPKD, which more accurately reflects the pathological state observed in the majority of PKD patients.

To address this important question, our laboratory has generated and extensively characterized a model of slowly progressing ADPKD. I will use this model to address the following specific aims: (1) Determine if and how Wnt signaling is altered in cysts by utilizing RT-PCR and transcriptome analysis of cyst linings, (2) Determine if altered Wnt signaling in PKD can affect cystic disease in vivo by analyzing cyst formation in our PKD model with additional kidney-specific alterations in Wnt signaling, and (3) Identify direct protein interactions between components of the Wnt signaling pathway and Pkd1, a gene mutated in ADPKD. Ultimately, completion of these studies will help to establish if modulation of Wnt signaling can ameliorate cystic disease in ADPKD patients, as well as potentially other cystic diseases where Wnt signaling is up-regulated.
Title: Utilizing TALENs and transposons for individualized medicine: modeling a private mutation in a family with early onset cirrhosis and hepatocellular carcinoma

Authors: Patrick R. Blackburn, Raymond Hickey, Nasra Giama, Rebecca Nace, Andrew Bordner, Dimitar Gavrilo, Mounif El-Youssef, Wafa’a Al-Qabandi, Karl Clark, Eric Klee, Lewis Roberts, and Stephen C. Ekker, Ph.D.

Advisors: Stephen C. Ekker, Ph.D.

Track: Clinical and Translational Sciences – Cancer Biology

The massive accumulation of genetic data is outpacing our ability to use this information to make meaningful healthcare decisions. There is a very clear need for a rapid system that would enable the functional assessment of candidate mutations and provide actionable information for physicians.

Hepatocellular carcinoma (HCC) is the most common solid tumor of the liver and usually occurs in older adults with a history of liver disease. Non-viral associated HCC is rare in children but has been linked to certain autosomal recessive disorders. Two children, aged 12 and 13, were diagnosed with HCC secondary to hepatosplenomegaly and cirrhosis during infancy. One child died within three months of diagnosis of HCC, and the younger sibling received a liver transplant at the Mayo Clinic. Standard laboratory testing did not reveal any possible causes of cirrhosis. Based on the consanguinity of the parents, it was hypothesized that the children suffered from an autosomal recessive disorder and whole exome sequencing was done.

This analysis identified a candidate mutation in FAH that results in a missense mutation in a conserved residue in the catalytic pocket of the enzyme. Mutations in FAH are found in patients with type I tyrosinemia. We used TALENs, a novel genome engineering tool, to knockin the mutation into zebrafish to functionally characterize the novel variant in vivo. We also used a bi-functional transposon system to rescue the defect in Fah KO mice through co-expression of the mutant or wild-type human FAH protein with luciferase and compared the repopulation kinetics of hepatocytes expressing either form of FAH. The patients in this family were found to have an atypical ‘silent’ form of type I tyrosinemia which was undetectable using the gold standard biochemical approaches that are routinely used to diagnose the disorder.
Title: **Characterization of SEMA3A-Encoded Semaphorin as a Naturally Occurring Kv4.3 Protein Inhibitor and its Contribution to Brugada Syndrome**

Authors: Nicole J. Boczek, Dan Ye, Eric K. Johnson, Wei Wang, Lia Crotti, David J. Tester, Federica Dagradi, Yuka Mizusawa, Margherita Torchio, Marielle Alders, John R. Giudicessi, Arthur A.M. Wilde, Peter Schwartz, Jeanne M. Nerbonne, Michael J. Ackerman

Advisor: Michael J. Ackerman

Track: Clinical and Translational Science

Rational: Semaphorin 3A (SEMA3A)-encoded semaphorin is a chemorepellent that disrupts neural patterning in the nervous and cardiac systems. In addition, SEMA3A has an amino acid motif that is analogous to hanatoxin, an inhibitor of voltage-gated K(+) channels. SEMA3A-knockout mice exhibit an abnormal ECG pattern and are prone to ventricular arrhythmias and sudden cardiac death.

Objective: Our aim was to determine whether SEMA3A is a naturally occurring protein inhibitor of Kv4.3 (Ito) channels and its potential contribution to Brugada syndrome.

Methods and Results: Kv4.3, Nav1.5, Cav1.2, or Kv4.2 were coexpressed or perfused with SEMA3A in HEK293 cells, and electrophysiological properties were examined via whole-cell patch clamp technique. SEMA3A selectively altered Kv4.3 by significantly reducing peak current density without perturbing Kv4.3 cell surface protein expression. SEMA3A also reduced Ito current density in cardiomyocytes derived from human-induced pluripotent stem cells. Disruption of a putative toxin binding domain on Kv4.3 was used to assess physical interactions between SEMA3A and Kv4.3. These findings in combination with coimmunoprecipitations of SEMA3A and Kv4.3 revealed a potential direct binding interaction between these proteins. Comprehensive mutational analysis of SEMA3A was performed on 198 unrelated SCN5A genotype-negative patients with Brugada syndrome, and 2 rare SEMA3A missense mutations were identified. The SEMA3A mutations disrupted SEMA3A’s ability to inhibit Kv4.3 channels, resulting in a significant gain of Kv4.3 current compared with wild-type SEMA3A.

Conclusion: This study is the first to demonstrate SEMA3A as a naturally occurring protein that selectively inhibits Kv4.3 and SEMA3A as a possible Brugada syndrome susceptibility gene through a Kv4.3 gain-of-function mechanism.
Title: **Targeting mitochondrial genes in zebrafish using TALENs**

Authors: Jarryd M. Campbell, Tanya Poshusta, Ester Perales-Clemente, Ph.D., Karl J. Clark, Ph.D., Grazia Isaya, M.D., Ph.D., Timothy J. Nelson, M.D., Ph.D.

Advisor: Stephen C. Ekker, Ph.D.

Track: Clinical and Translational Sciences

Mitochondrial disorders are collectively prevalent in the population (~1/8500) but have limited treatment options. There remains a need for a model that will allow for understanding of 1) the developmental consequence of mitochondrial disease and 2) provide an *in vivo* context for disease that display phenotypes observed in patients, yet remain high-throughput for drug screening possibilities. The zebrafish is able to accommodate both of these, and we believe that this model can be a link to the identification of new treatment options for mitochondrial disease. Manipulation of the zebrafish nuclear genome has become a reality in recent years, primarily because the tools for genome editing are becoming more accessible and more efficient. The malleability of the zebrafish genome opens the possibility to knockout several nuclearly-encoded mitochondrial genes. Our approach was to make TALENs against nuclear genes in each of the complexes of the electoral transport chain (complex I, II, III, IV, V, and coenzyme Q) as well as against proteins in the calcium uniporter, in mitochondrial DNA expression and maintenance, and in others for a total of 29 genes. We have successfully made TALENs and recorded somatic activity against all 29 genes targeted, and these fish are currently being screened for germline mutagenesis. Once we get fish lines, we will identify phenotypes related to each mutation and test a panel of FDA approved drugs for phenotype improvement to identify candidates for drug repurposing. Alternatively, patient genotyping can identify genetic mutations that can be reverse engineered into the zebrafish genome. By replicating the patient-specific mutations *in vivo*, these model zebrafish will more faithfully recapitulate the disease and become a complementary model to yeast or mice for drug screening studies of a particular disease. Freidreich’s Ataxia (FRDA) is the most commonly inherited ataxia, and there is no treatment. FRDA is caused by a trinucleotide repeat expansion in the first intron of frataxin, so we are attempting to knock-in trinucleotide repeats into fish frataxin. Our strategy is to do this in two generations: for the first, we have successfully knocked-in a PhiC31 integrase aatB recognition site in the first intron of zebrafish frataxin using TALENs and a single stranded oligonucleotide; in the second we will introduce a (GAA)$_{500}$ repeat using PhiC31 integrase and a receiver plasmid containing the repeat and an aatP site, which is needed to complete the integration reaction into the genome. As an alternative approach, we are knocking-out zebrafish frataxin and will use transposition for introducing the diseased human frataxin gene. If we are able to observe FRDA-like phenotypes, we will use the same drug panel to identify any that will ameliorate the condition. Finally, we have been working on tools to target mitochondrial-encoded genes in order better understand and treat mitochondrial DNA disorders, another class of mitochondrial diseases.
Title: Biomarkers significantly improve the performance of the Milan criteria in predicting hepatocellular carcinoma (HCC) recurrence post liver transplantation (LT)


Advisors: Lewis R. Roberts, MB. ChB. PhD.

Track: Clinical and Translational Science

HCC recurrence is a major impediment to effective treatment of HCC by LT. Despite using the Milan criteria for candidate selection, up to 20% of HCC patients develop recurrence after LT and consequently have poor survival. This limits the benefit/risk ratio of LT for HCC patients compared to patients with benign liver disease. In order to optimize organ allocation strategies, other objective preoperative parameters that can reliably predict the risk for recurrence post-LT are needed. We aimed to determine the association between pre-LT alpha-fetoprotein (AFP), lens culinaris agglutinin-reactive AFP (AFP-L3) and des-gamma-carboxy prothrombin (DCP) alone, or in combination with other biomarkers or Milan criteria and risk of HCC recurrence after LT. A retrospective cohort study of HCC patients undergoing LT between 2000 and 2008 was conducted (n=127). Serum AFP, AFP-L3% and DCP were blindly measured using the µTASWako i30 immunoanalyzer. The hazard ratio (HR) and 95% confidence interval (95%CI) were calculated using Cox Proportional Hazards analysis. Of the variables examined, tumor size, the Milan criteria and high levels of biomarkers were significantly associated with HCC recurrence. HRs (95%CI) were 1.4 (1.1–1.7), 2.6 (1.4–4.7), 2.8 (1.4–5.4), 3.2 (1.7–6.1), and 3.5 (1.9–6.7) for tumor size, tumor stage outside Milan criteria, AFP ≥250 ng/mL, AFP-L3 ≥35%, and DCP ≥7.5 ng/mL; p=0.004, 0.003, 0.002, 0.0003 and <0.0001, respectively. The HR (95%CI) increased to 5.2 (2.3–12.0) for patients with both AFP ≥250 ng/mL and DCP ≥7.5 ng/mL, p<0.0001. Among patients with tumors within the Milan criteria, the HRs (95%CI) were 3.1 (1.3–7.5), 4.3 (1.8–10.1) and 4.5 (1.9–10.6) for AFP ≥250 ng/mL, DCP ≥7.5 ng/mL, and AFP-L3 ≥35%; p=0.01, 0.0008, 0.0005, respectively. The HR (95%CI) for tumors outside the Milan criteria increased from 2.6 (1.4–4.7) to 8.6 (3.0–24.6), and 7.2 (2.8–18.1) when combined with AFP ≥250 ng/mL, and DCP ≥7.5 ng/mL respectively (p<0.0001 for both). The concordance index (95%CI) of the Milan criteria increased from 0.63 (0.56–0.70) to 0.68 (0.60–0.76), 0.70 (0.62–0.78) and 0.70 (0.62–0.78) when combined with AFP, DCP and AFP-L3%, respectively, suggesting that combining the biomarkers with the Milan criteria was more predictive of recurrence than the Milan criteria alone. **Conclusions:** Using both biomarkers and Milan criteria may be better than using the Milan criteria alone in optimizing the decision of liver transplantation eligibility. Our findings could potentially improve the organ allocation algorithm for LT.
Cigarette smoking is the leading preventable cause of death worldwide with no successful treatment options for the majority of individuals wishing to quit. Substance use disorders are associated with specific endophenotypes and comorbid conditions that are not represented in all patients, but important to health and treatment outcomes. Comorbid alcohol abuse is predominant among these, as a significant proportion of smokers are also heavy drinkers, and a leading cause of death of alcoholics is tobacco-related disease. To improve treatment efficacy and individualize treatment strategies in patients wishing to quit smoking and/or drinking, the etiology of this coincident behavior must be more clearly understood, and the genetic correlates of nicotine/ethanol drug dependence and treatment response identified.

Key components of substance use disorders include drug-seeking behavior, molecular neural adaptations including nicotinic acetylcholine receptor (nAChR) upregulation, difficulty achieving abstinence, and comorbid drug abuse; each of which may have a significant genetic contribution. We used a zebrafish preclinical model to study these aspects of nicotine and ethanol substance abuse. We developed assays to measure the behavioral response associated with nicotine and ethanol co-administration, the transcriptional changes associated with chronic nicotine and ethanol exposure, and the corresponding effect of nicotine/ethanol exposure on nAChR protein levels. Furthermore, we used distinct zebrafish behavioral response assays to identify novel treatment options for tobacco and alcohol dependence. Together these assays provide a multifaceted view of these addictive drugs, and the integration of this data enables us to interrogate the molecular and genetic contributors to the actions of nicotine and ethanol. In doing so, we will be able to identify novel genes implicated in these responses. Nucleotide variants in the human orthologs of these genes could describe, in part, the variability in both the manifestation as well as the treatment of tobacco dependence. Our future work will assess the functional relevance of human variants in genes identified through our studies as well as additional polymorphisms previously identified in GWAS to be associated with tobacco or alcohol related phenotypes. The goal of these studies is to have a positive impact on the treatment of the individual with tobacco dependence.
Title: Overcoming tumor induced chronic inflammation through immunoregulatory mechanisms seen during late pregnancy

Authors: Elizabeth Ann Enninga, Wendy Nevala and Svetomir Markovic MD, PhD

Advisor: Svetomir Markovic MD, PhD

Track: Clinical and Translational Science

The systemic immune system of metastatic melanoma patients exists in a state of chronic inflammation/immune exhaustion (Th2 bias) which correlates to a poor prognosis. This state of Th2 biased systemic immunity is very similar to that of early normal pregnancy. However, unlike metastatic melanoma, an unknown mechanism during late pregnancy switches systemic chronic inflammation (tolerance) back to a normal, proinflammatory (Th1) state prior to labor. We hypothesize that the switch towards normalization of systemic immunity in pregnancy is due to haploidentical fetal cells leaking into maternal circulation causing a strong proinflammatory signal to the maternal immune system, promoting immune recovery and delivery (rejection) of the fetus (labor). We are testing this hypothesis in an ongoing clinical study of pregnant women and applying these findings towards a therapeutic strategy in metastatic melanoma (allogeneic lymphocyte transfusion). We have tested whether or not allogeneic stimulation (transfer from a genetically non-identical donor) can “recover” the function of peripheral blood immune cells in patients with metastatic cancer. Preliminary data suggests that in vitro exposure of peripheral blood immune cells from patients with untreated metastatic melanoma to allogeneic stimuli “recovers” proinflammatory characteristics of immune cells. We also looked at cellular proliferation in the presence sex hormones, which are strong drivers of immunity, to determine other important modulators that could be targeted to improve the acute immune response. This work will serve as the basis for an interventional phase I clinical trial using allogeneic lymphocyte infusion for the treatment of patients with metastatic melanoma.
Title: The Risks of Stepping Down from Scheduled Inhaled Corticosteroid to as-needed Inhaled Corticosteroid in Individuals with Stable Asthma: A Systematic Review

Authors: Michael R. Gionfriddo

Advisors: Victor Montori M.D. MSc, Matthew A Rank M.D.

Track: Clinical and Translational Science

Introduction: In the United States, asthma affects nearly 19 million adults and 7 million children, causing significant morbidity, mortality, and cost. While under-treatment of asthma remains a problem, many patients with asthma are overtreated. Overtreatment in asthma exposes patients not only to unnecessary cost, but unnecessary exposure to drug which may result in adverse events. Therefore, reducing or “stepping down” the amount of medication patients with asthma are on may reduce the patient’s chance of experiencing an adverse event and reduce the financial burden of care. This systematic review aimed to examine the effect of stepping down from scheduled inhaled corticosteroids (ICS) to as-needed ICS in patients with stable asthma.

Methods: Several electronic databases were systematically searched in April 2014. Articles were screened independently in duplicate. Articles that reported randomized controlled trials or observational studies of at least 12 week follow-up duration that compared stepping down from scheduled ICS to as needed ICS, to maintenance of scheduled ICS were included. Patients were required to have stable asthma as defined by at least 4 weeks without asthma exacerbation prior to intervention.

Results: 3025 articles were retrieved initially, 75 of which were retrieved for full text screening. Of these, only 2 articles were found to be eligible for inclusion, both randomized controlled trials. Using random effects meta-analysis, the relative risk of an asthma exacerbation for individuals who step down from scheduled to as needed ICS was 1.32 (95% CI, 0.81-2.16; P = 0.27, i² 0%). However, those who stepped down had slight decrease in symptom free days (Standard mean difference -.16 [95% CI -0.49, -0.02; p=0.03, i² = 22%]).

Conclusion: There is currently not enough evidence to associate stepping down from scheduled to as needed ICS with an increased risk for exacerbations, although it may lead to fewer symptom free days in those who step down.
Title: **Characterization of pre-clinical melanoma models to predict response to therapy**

Authors: Antoneicka L. Harris, Laura Marlow, Svetomir Markovic, Michael Thompson, Brian Netzel, Dragana Milosevic, Stefan Grebe, and John A. Copland, Ph.D.

Advisor: John A. Copland, Ph.D.

Track: Clinical and Translational Science

Skin cancer is the most common type of cancer, contributing to more than one million cases annually in the United States. Malignant melanoma (MM) is the most aggressive form of skin cancer accounting for approximately 60% of lethal skin tumors. A few common risk factors include genetic mutations, alterations in skin cells, and various phenotypic characteristics, all of which can be attributed to intense and intermittent exposure of ultraviolet radiation. Several standard therapeutic treatment options are available for MM including surgery, chemotherapy, and radiotherapy; however tumors from this disease have been reported to be resistant to both chemotherapy and radiotherapy and neither of the two forms of therapy increase overall survival. Emerging therapies such as immunotherapy and molecular targeted therapy have shown tumor regression and improvement in patient survival. Yet, over time, these patients relapse and become resistant to therapy. Due to its aggressive nature and drug resistance to both standard and molecular therapy there is a critical need to develop novel therapies for MM that will improve overall and progression free survival rates.

Recent reports show that patient derived xenograft (PDX) models have the ability to mimic human response to therapy and more faithfully recapitulate the molecular diversity, cellular heterogeneity and histology seen in patient tumors. The use of these models allows the benefit of performing studies to define favorable therapeutic options that will closely mirror results expected in patient therapy, thus reducing both the amount of funds lost and the number of failed and incomplete clinical trials. Moreover, these models may potentially allow us to answer the unanswered question, “how best to care for patients?”

In an effort to develop novel courses of therapy as well as individualized treatment regimens for patients, we have developed pre-clinical MM PDX models, cell-line derived xenograft models and cell line models that are not only more representative of the diverse clinical appearances of MM, but are also more predictive of patients response to therapy. Our next course of action is to test the responsiveness of established models to treatment regimens received by the patient to determine if the models mimic patients’ response to therapy. Once the models show that they mimic patients’ response to therapy further genetic sequencing as well as functional characterization of established models will be employed as a means of identifying novel, targetable molecular factors. Innovative therapeutic strategies will be tested for efficacy in these preclinical models with the goal of establishing personalized treatment regimens.
Title: Development, testing, and refining the severe sepsis and septic shock sniffer

Authors: Andrew M. Harrison and Vitaly Herasevich, MD, PhD

Advisors: Brian W. Pickering, MD, MSc and Ognjen Gajic, MD, MSc

Track: Clinical and Translational Science

Background: From 1979-2000, the incidence of sepsis in the US increased from 164,000 to 660,000 cases. In 2011, sepsis was also reported as the most expensive condition treated in US hospitals with an aggregated cost of $20.3 billion. However, current sepsis detection algorithms have not considered alert action in the context of failure to recognize and treat.

Objective: Develop and test an automated surveillance algorithm (“sniffer”) for the detection of severe sepsis and monitoring failure to recognize and treat severe sepsis in a timely manner.

Methods: Retrospective diagnostic performance study using independent derivation and validation cohorts. We examined all adult first-admissions to the medical ICU at Mayo Clinic in Rochester, MN, from January through March 2013 (N = 587). Algorithm validation was performed against the “gold standard” of manual chart review by two trained reviewers, with one super-reviewer for cases of disagreement. Algorithm development and testing was performed using iterative recursive data partitioning and critical appraisal of false positive and negative alerts. The algorithm is based on the following variables: suspicion of infection, systemic inflammatory response syndrome, organ dysfunction, shock, and failure to recognize and treat.

Results: The ability of the first technical iteration of the severe sepsis sniffer on the derivation cohort to detect severe sepsis and/or septic shock was suboptimal: 59% sensitivity, 97% specificity, 92% Positive Predictive Value, and 83% Negative Predictive Values. Critical appraisal of false positive and negative alerts, along with iterative introduction of new clinical variables (mean arterial blood pressure, bilirubin, platelets, INR, mechanical ventilation, creatinine, PaO2/FiO2 ratio, urine output, and GCS score) into the algorithm, was then performed, which resulted in an increased sensitivity of 82%. Testing this algorithm on the validation cohort shows similar diagnostic performance. Lastly, 68% of alert positive patients had failure to recognize and treat, defined as no lactate and central venous pressure measurement from ICU admission to alert time plus two hours.

Discussion: Current detection performance of our sepsis alert systems is similar to previous reports. Recursive data partitioning and validation of the failure to recognize and treat domain are anticipated to further refine the sniffer for eventual implementation in the clinical setting.

Conclusion: The validated sepsis sniffer showed sensitivity in good agreement with final derivation algorithm. However, this sniffer could be improved further by adding a failure to recognize and treat component to the algorithm to reduce information overload, interruption, human error, and alert fatigue.
Uterine fibroids, benign tumors of the myometrium, affect up to 70% of reproductive-aged women and can cause symptoms of menorrhagia, pelvic pain, and miscarriage. Uterine fibroids have been associated with hypertension; however, this relationship is not understood. Because the risk of hypertension is associated with sympathetic hyperactivity, we tested the hypothesis that normotensive, premenopausal women with uterine fibroids would have greater muscle sympathetic nerve activity (MSNA) than women without uterine fibroids. We measured intra-arterial blood pressure and MSNA using microneurography in women with uterine fibroids (40±5 yr) and women without uterine fibroids (43±1 yr). These women did not differ in age or body mass index. Mean arterial pressure was not statistically different between women with uterine fibroids and women without uterine fibroids (100±9 vs. 88±3 mmHg, respectively; p>0.05). MSNA burst frequency (7±2 vs. 17±3 bursts/min, fibroid vs. non-fibroid; p=0.03) and burst incidence (10±4 vs. 29±5 bursts/100 heartbeats, fibroid vs. non-fibroid; p=0.03) were significantly decreased in women with uterine fibroids. These data suggest that women with uterine fibroids may have lower MSNA than women without uterine fibroids. Further investigation is needed to understand how MSNA signal transduction affects blood pressure and uterine fibroid formation and growth in women with this condition.
Over one million young children are exposed to drugs used to induce and maintain general anesthesia each year in the United States. Mounting evidence shows that exposure of the developing brains of animals to anesthetic and sedative medications may cause neurodegenerative changes with adverse effects on learning and behavior. The clinical significance of these observations is a topic of intense debate and concern, as there are very few human studies of how anesthetic exposure may affect neurodevelopment. Our group conducted a matched cohort study in which children (N=8548) born between January 1, 1976, and December 31, 1982, in Rochester, Minnesota, were the source of cases and controls. Those exposed to anesthesia (n=350) before the age of 2 were matched to unexposed controls (n=700) on the basis of known risk factors for learning disabilities. Multivariable analysis adjusted for the burden of illness, and outcomes including learning disabilities, receipt of an individualized education program, and the results of group administered tests of cognition and achievement were outcomes. We found that exposure to multiple, but not single, anesthetic/surgery significantly increased the risk of developing learning disabilities (hazard ratio: 2.12 [95% confidence interval: 1.26 –3.54]), even when accounting for health status. A similar pattern was observed for decrements in group administered tests of achievement and cognition. However, exposure did not affect the rate of children receiving an individualized education program. We concluded that exposure to anesthesia and surgery before the age of 2 was a significant independent risk factor for the later development of learning disabilities but not the need for educational interventions related to emotion/behavior. We cannot exclude the possibility that multiple exposures to anesthesia/surgery at an early age may adversely affect human neurodevelopment with lasting consequence.
Title: **Identifying new genetic modifiers for cardiomyopathy**

Authors: Xiao Ma, Yonghe Ding and Xiaolei Xu, Ph.D.

Advisor: Xiaolei Xu, Ph.D.

Track: Clinical and Translational Science

Cardiomyopathy and the related heart failure affect millions of Americans. Despite the progress in identifying causative genes, it is well recognized that cardiomyopathy patients with the same disease-causing mutations can exhibit highly variable phenotypes. Because the variation can be ascribed to modifier genes in different genetic background and environmental factors, identification of genetic modifiers for each particular cardiomyopathy will greatly improve risk stratification, prognostic test development and personalized therapy.

One novel approach to identify new genetic modifiers is based on an efficient strategy to generate and enrich zebrafish insertional cardiac (ZIC) mutants, as well as the development of a simple cardiomyopathy-inducing method in adult zebrafish via doxorubicin (DOX) injection. By applying DOX into 21 homozygous ZIC mutants, we performed a pilot mutagenesis screen and identified gene-break transposon GBT419, GBT136 and GBT002 that significantly alter the rate of DOX-induced cardiomyopathy. Further test showing that at the molecular level, GBT419 disrupts *Retinoid X Receptor, Alpha a (rxraa)* gene that encodes a nuclear receptors that mediate the biological effects of retinoid by their involvement in retinoic acid-mediated gene activation. Interestingly, instead of being knocked down, the expression of *rxraa* gene is actually enhanced after interruption by GBT. In addition, the downstream target gene of *RXRAA* also showing variation after GBT interruption. Those genes we tested are *ALDH1A2, CYP26B, RBP1*. These results indicate that the resistance to DOX-induced cardiomyopathy is a gain of function phenotype. And *rxraa* could be a potential modifier gene for cardiomyopathy. Combining with previous finding of Dnajb6b (Ding, et al, *Circ. Res.* , 2013), which is another novel genetic modifier for cardiomyopathy, the genetic screening using ZIC mutants and DOX injection to find modifiers for cardiomyopathy is proved to be effective and more modifier genes is being found by this methods.
Title: Novel three dimensional imaging analysis of the distribution of intratumoral oncolytic virus infection

Authors: Amber C. Miller, Rebecca Nace, and Stephen J. Russell, M.D.,Ph.D.

Advisors: Stephen J. Russell, M.D.,Ph.D.

Track: Clinical and Translational Science

Oncolytic virotherapies like vesicular stomatitis virus (VSV) are experimental cancer therapies in which naturally evolved or engineered viruses selectively infect tumor cells resulting in tumor cell death and tumor regression. The probability of achieving tumor cure from direct oncolysis has been mathematically shown to be dependent on the amount and distribution of intratumoral infection, with an increase in number, size, and independence of intratumoral infectious centers resulting in a higher probability of tumor cure. We have shown that intratumoral infection is heterogeneously distributed, resulting in intratumoral infection voids. These voids are predicted to be a result of barriers to intratumoral virus delivery and infection spread including extravasation, transvascular transport and interstitial transport, all of which have the potential to be targeted in order to optimize current oncolytic regimens. Optimization will require the ability to accurately visualize and analyze intratumoral infection as it occurs in live animals over time. Dynamic radiohistology using recombinant viruses expressing the thyroidal sodium-iodine symporter (NIS) reporter gene with noninvasive SPECT/CT imaging to detect radiotracer uptake at centers of active NIS expression identifies centers of infection. This technique has proven to be a useful tool in observing the location, size, and evolution of intratumoral infectious centers. While dynamic radiohistology allows qualitative observation of intratumoral infection unobtainable with standard immunohistochemical (IHC) techniques and has been used for basic quantitation of radiotracer uptake, quantitative analysis of infection distribution is still necessary to understand barriers to therapeutic success.

The main objective of this work was to therefore develop a method to quantitatively describe intratumoral infection distribution using three dimensional dynamic radiohistology to visualize intratumoral infection noninvasively in tumor bearing animals administered oncolytic VSV-NIS. Algorithms derived for analysis include a distributive distance transformation to describe the proportion of tumor tissue at a given distance from any infected area. The distance transformation can be used to create a color-map that visualizes three dimensional data in two dimensions, providing a novel visualization of nearby infectious centers not possible with planar SPECT/CT images or tradition IHC techniques. The distance transformation is also use to identify non-overlapping spherical intratumoral infection voids to describe the location of volumes of uninfected tumor tissue and the uniformity of distribution. These analysis methods describe infected and uninfected regions of tumors in a meaningful way that will have real application in designing ways to increase tumor infection and probability of tumor cure by oncolytic viruses.
Liver Health Disparities among Somali Immigrants in Minnesota

In the US the prevalence of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in the general population is relatively low. However, African Americans and Hispanics have been shown to have higher prevalence of chronic hepatitis viral infections than Caucasians. HBV and HCV infection are a major risk factor for chronic liver diseases, particularly cirrhosis and hepatocellular carcinoma (HCC). In areas with high prevalence of this chronic HBV and HCV infections, including sub-Saharan Africa is a major cause of liver cancer-related mortality. In the US, although the incidence of many cancers is declining, HCC incidence rates are rising, and have tripled in the past 30 years. The increase in US incidence of HCC is likely partly due to increased immigration from sub-Saharan Africa and Asia. The number of African immigrants in the US rose from approximately 40,000 in 1960 to 1.4 million in 2007, with most of the increase occurring since 1990 (Migration Policy Institute). One such African immigrant population is the Somali community, the majority of whom (approximately 70,000) have settled in Minnesota either directly from refugee camps in Kenya or Ethiopia or by secondary migration from other US states. Since advanced HCC is a highly lethal cancer, it is critical to institute measures to educate immigrants, particularly those from Africa, about chronic HBV and HCV infections, to screen immigrants for chronic HBV and HCV infections, and to establish effective mechanisms to link chronic HBV and HCV subjects with care to secure long-term survival. Until recently, no studies had specifically focused on chronic hepatitis infection rates in Somali immigrants in the US, and their contribution to cirrhosis and HCC. Shire et al. determined the frequency of HBV and HCV infections in Somali patients seen at the Mayo Clinic. Non-Somali residents in Olmsted County (90% of whom are Caucasians) served as controls. Among Somalis, the adjusted frequency of HBsAg positivity and Anti-HCV positivity were 10-fold and 3-fold higher, respectively, than in controls. In addition, the study showed that chronic HCV was a primary risk factor driving development of liver cancer among Somali immigrants. Since the Shire et al. study was hospital-based, additional studies focused on the general Somali immigrant population are needed, however, no state-wide epidemiological work has been done to elucidate the actual burden of this disease in this newly arrived African community. In addition, the incidence of hepatobiliary cancers among Blacks in Minnesota is the highest compared to other racial groups, and since Somalis come from regions endemic for viral hepatitis, we hypothesize that Somalis may bear and contribute to a significant portion of the increasing morbidity and mortality associated with chronic hepatitis infection and HCC. Thus, the long-term goal of this project is to improve the health of immigrant populations in the US as it relates to chronic liver diseases and associated complications.
Cellular senescence has come to the forefront of aging biology research as a significant cause of tissue dysfunction with age. Cellular senescence is an essentially irreversible growth arrest that occurs when cells encounter significant stress, including oxidative damage, metabolic stress, oncogene activation, or telomere shortening due to intensive replication. Senescent cells have a unique phenotype, characterized by an enlarged cell size, accumulation of tumor suppressor proteins p16 and p21, and secretion of a host of cytokines, growth factors, and matrix remodeling proteins collectively known as the senescence-associated secretory phenotype, or SASP. The SASP may contribute to chronic, sterile inflammation that may play a key role in age-related disease. Senescent cell burden is known to increase with age in many tissues and has been associated with disease states such as diabetes, primary sclerosing cholangitis, and dementia. In our laboratory, we have found that high fat feeding in mice causes increased senescence in adipose tissue including both subcutaneous and visceral depots. In these studies, we tested the hypothesis that clearing senescent cells from high-fat fed mice would improve metabolic phenotypes associated with obesity. In order to test this hypothesis, we used the p16-3MR mouse model, which contains a viral tyrosine kinase gene driven by the p16 promoter, allowing clearance of senescent cells expressing p16 by the administration of Ganciclovir. With Ganciclovir treatment, we see improvements in glucose and insulin tolerance, enhanced active energy expenditure, and reduced senescent cell abundance in adipose tissue. These results suggest that senescent cells play a role in metabolic phenotypes associated with high fat diet, and that clearance of senescent cells, or ablation of the SASP, might prove to be an interesting therapeutic target in obesity and diabetes.
Pancreatic ductal adenocarcinoma (PDAC) is a lethal and aggressive disease and is the fourth leading cause of cancer death in the United States largely due to difficulty in early diagnosis and lack of effective treatment. Although progress has been made in the histological classification and staging of PDAC, the origin of this disease has yet to be defined. One known early hallmark of PDAC is its association with a massive fibrotic response marked by extensive collagen deposition. Coinciding with the overexpression of collagen fibers and inflammation is the differential expression of discoidin domain receptors (DDRs). DDR1 and DDR2 are the only known receptor tyrosine kinases (RTKs) to specifically bind and be activated by collagen, thus stimulating the activation of downstream targets associated with cell proliferation including PI3K, Akt, Ras, MAPK, and Erk. To date, the role of DDRs has not been studied in PDAC. Our preliminary studies utilize the oncogenic KRAS\textsuperscript{G12D} knock-in mouse to initiate pancreatic tumorigenesis, including the fibrotic response. Typically DDR1 is expressed in cells with an epithelial phenotype and DDR2 is expressed in cells with a mesenchymal phenotype. In the tumor cells of the KRAS\textsuperscript{G12D} mouse model, we found an upregulation of DDR2 and a downregulation of DDR1 as tumors progressed. However, in both human and mouse PDAC, we found a substantial upregulation in metaplastic ducts near the tumor tissue. Our laboratory has previously determined that metaplastic ducts are a preneoplastic lesion in the pancreas. Currently, DDR1 and DDR2 knockdown cell lines are in progress as are knockout mouse models to determine a definitive role of DDRs in ductal metaplasia and PDAC progression. We anticipate that DDRs may serve as potential therapeutic targets and help with the prevention and treatment of PDAC.
Title: Generation of pluripotent stem cells from patients with familial dilated cardiomyopathy due to a novel RBM20 mutation

Authors: Saranya P. Wyles, Sybil Hrstka, Saji Oommen, Santiago Reyes Ramirez, Jeanne L. Theis, Andre Terzic, Timothy M. Olson, Timothy Nelson, M.D., Ph.D.

Advisors: Andre Terzic M.D, Ph.D, Timothy M. Olson M.D., Grazi Isaya M.D., Ph.D., and Timothy Nelson, M.D., Ph.D.

Track: Clinical and Translational Sciences

Dilated cardiomyopathy (DCM) is a leading cause of heart failure with an estimated prevalence of 36.5 per 100,000 individuals. Currently, it is the most common indication for heart transplantation and is associated with substantial mortality. Recently, genetic linkage analysis in families with autosomal-dominant DCM led to the discovery of the distinct heterozygous missense mutation in the highly conserved arginine-serine (RS)-rich region of exon 9, known as ribonucleic acid binding motif protein 20 (RBM20). This particular mutation is strongly associated with morbidity and mortality. Pluripotent stem cells generated from patients with RBM20-associated DCM would be useful in disease modeling to better understand the underlying pathology and to develop clinical-grade therapeutics. In this study, skin fibroblasts from RBM20 mutation-associated DCM patients and controls were derived from explants of 3-mm dermal biopsies after informed consent under approved protocols by Mayo Clinic. We show here that induced pluripotent stem cells (iPSCs) can be generated from patients with RBM20-associated DCM by reprogramming their adult fibroblasts with four transcription factors (Sox2, Oct4, Klf4, and c-Myc). RBM20-DCM-specific iPSCs have the hallmarks of pluripotency as shown by immunofluorescence analysis, gene-specific quantitative PCR, spontaneous differentiation into embryoid bodies, and karyotyping. Additionally, patient-specific iPSC cardiomyocytes were characterized for structural abnormalities using transmission electron microscopy, RT-PCR, and microarray. RBM20 cardiomyocytes show an elongated and narrow sarcomeric morphology compared to healthy control cardiomyocytes. Functional defects were assessed by Fluo-4-AM labelled calcium handling. Abnormal calcium handling due to calcium overload was noted in RBM20 cardiomyocytes compared to control. These results are a step toward using iPSCs for hereditary DCM disease modeling, as well as providing the foundation to bioengineer gene therapy approaches in the era of individualized medicine.
Asthma is characterized by an increased number of Th2- and Th17-cells in the lungs. Cytokines produced by these cell types induce features of asthma, such as airway hyperresponsiveness (AHR), airway remodeling (thus increased mucus secretion) and eosinophilic airway inflammation. Eosinophils are produced in the bone marrow and are of the myeloid lineage. In asthma, eosinophils accumulate in the lungs and release pro-inflammatory granule proteins and chemokines. The receptors in the lungs that are responsible for recruitment of eosinophils have been discovered. However, little information is available on how airway exposure to allergens affect the dynamics of eosinophils in bone marrow and peripheral circulation. Airway epithelial cells, in addition to acting a physical barrier, have the ability to act as first-responders against acute injury and pathogens. Therefore, the aim of this study was to examine the connection between lung epithelial cells and eosinophil maturation.

We used an established model of asthma that involves intranasal (I.N.) administration of the fungal extract of *Alternaria alternaria* (ALT). Upon lung exposure to ALT, IL-33 was released by epithelial cells within one hour, followed by increased production of IL-5 in the lungs. We also found elevated serum levels of IL-5 in response to ALT exposure, which reached a peak at 6 hours post ALT administration. After prolonged exposure to ALT over 6 days, a significant increase in eosinophil maturation was observed in bone marrow. In addition, the serum from mice challenged with ALT was able to stimulate bone marrow cells to undergo eosinophil maturation *in vitro*. Administration of IL-5-blocking antibody *in vivo* decreased eosinophilpoiesis in bone marrow in response to ALT below PBS baseline levels. We also found that IL-33 release serves a possible positive feedback mechanism because mice deficient in IL-33 receptor (Il1rl1−/−) expressed significantly less IL-33 mRNA in response to ALT than their wild type counterparts. We demonstrated innate IL-5 production was produced by type 2 innate lymphoid cells (ILC2’s) directly from IL-5 reporter mice and indirectly measuring serum from *IL-7Rα−/−* mice. Finally, we demonstrated that our model was dependent on IL-33 signaling as *Il1rl1−/−* mice failed to induce eosinophilpoiesis after six days of ALT administration. Taken together, this study illustrates that the type 2 innate immune responses in the lungs leads to enhanced production of eosinophils in bone marrow through the increase in lung and blood levels of IL-5, dependent on IL-33 signaling and ILC2’s. Thus, distant organs communicate during allergic inflammation by using cytokines as a mediator. This concept will aid in the identification of novel therapeutic strategies to alleviate symptoms of asthma and allergic diseases.
Innate lymphoid cells (ILCs) are emerging as important effector cells in innate immunity and tissue homeostasis. Group 2 ILCs (ILC2s) have been identified in organs and tissues, such as adipose tissue, small intestine, lungs and skin, where they are involved in helminth immunity, type 2 immune responses, and tissue pathology and recovery. Using flow cytometry, we identified ILC2s in mouse uteri and confirmed IL-5, but not IL-13, production in response to IL-33 both in vivo and in vitro. Furthermore, intraperitoneal administration of IL-33 induced recruitment of activated ILC2s to uteri as well as to lungs. To investigate the possibility that uterine ILC2s might play a role in maintaining a healthy pregnancy, we mated ST2−/− females (on a Balb/c background) with ST2−/−, MHC-matched Balb/c and MHC-mismatched C57B6 males (ST2 is the IL-33 receptor). Balb/c, C57B6 and Balb/c x C57B6 pairs were used as controls. Litter sizes were not significantly different among the pairings. We defined viable pups as those still alive 24 hours after parturition. Total numbers of non-viable pups, percent of litters with at least one non-viable pup and percent of non-viable pups per litter were significantly increased in ST2−/− x C57B6 pairs when compared to control pairs. Thus, IL-33-responsive ILC2s are present in murine uterine tissue and may play roles in successful reproduction.
Title: **CXCR4 Participates in T Cell Receptor Signaling in the Absence of SDF-1**

Authors: Brittney Dinkel, Kim Kremer, and Karen Hedin, Ph.D.

Advisor: Karen Hedin, Ph.D.

Track: Immunology

The chemokine receptor CXCR4 binds to its ligand SDF-1 to signal T cell chemotaxis and gene expression. CXCR4 has been shown to physically associate with the T-cell receptor (TCR) upon stimulation by SDF-1 and this leads to a prolonged ERK activation and co-stimulation of IL-2 and IL-10. Here we show that CXCR4 plays a critical role in TCR signaling even in the absence of SDF-1. Using purified normal human T cells cultured in vitro, we first found that including AMD3100, a CXCR4 antagonist, reduced production of both IL-2 and IL-10 cytokines following either TCR or TCR+CD28 stimulation. Using the Jurkat T cell line, we additionally found that luciferase assays showed that TCR mAb-initiated transcription from the AP1-NFAT binding site of the IL-2 promoter is inhibited by AMD3100, while transcription from the NFκB binding site was not significantly affected. In addition, either treatment with AMD3100 or knockdown of CXCR4 via shRNA, inhibited TCR-mediated activation of N-Ras, K-Ras and the ERK MAP kinase pathway. CXCR4 knockdown did not impair synapse formation in Jurkat T cells stimulated by superantigen on antigen presenting cells. Together these results indicate a critical role for CXCR4 in TCR signaling. Future experiments will determine if CXCR4 is required for antigen-MHC signaling and will explore whether the TCR-CXCR4 heterodimer is induced by TCR signaling. These results may explain the T cell immune defect in WHIM patients and the lack of pre-TCR survival signals in Lck-Cre CXCR4 knockouts.
While nodal metastasis remains a critical prognostic factor in patients with melanoma, the mechanisms contributing to lymphatic involvement remains elusive. Recent work in our lab has identified regional immunosuppression to be present in the sentinel lymph nodes of patients prior to clinical evidence of nodal metastasis. Cellular interactions and the contribution of soluble factors in facilitating this Th2-dominant, immunosuppressive immune profile has been extensively studied; however, an appreciation for the role of subcellular counterparts remains to be defined. Our proposed model seeks to describe a role for melanoma-derived vesicles in harboring regional immunosuppression prior to clinical evidence of nodal metastasis. In this study, we investigated the generation, protein content and immune regulating properties of membrane-bound nanovesicles secreted from human melanoma cell lines and primary melanocytes. Utilizing recent technology advances in Nanoparticle Tracking Analysis (NTA), quantification of melanoma derived vesicles revealed an increase in secreted vesicles in response to the cellular stresses of hypoxia and serum deprivation. Proteomic analysis of the vesicle cargo by mass spectrometry and cytokine, chemokine and angiogenesis specific proteome arrays identified unique signatures for each of the cultured cell lines evaluated. Functional assessment revealed that dendritic cells matured with either CD40L or heat shock in vitro in the presence of melanoma-derived vesicles have significantly reduced surface marker expression of co-stimulatory markers CD80 and CD86 compared to controls matured in the absence of melanoma vesicles. Taken together, this study demonstrates a critical function for melanoma-derived vesicles in immunosuppression. This immune modulating role of melanoma-derived vesicles suggests one mechanism by which regional immunosuppression precedes nodal involvement and may foster an environment for metastasis to occur.
Adipocyte Derived Mesenchymal Stem Cells Modulated the T-cell Response during Chronic Renal Injury

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Advisor: Joseph P. Grande, M.D., Ph.D.

Track: Immunology

Background: Atherosclerotic renal artery sclerosis (RAS) is a significant public health concern, affecting 7% of the population over 65 years old and 45% of patients with coronary artery or aorto-iliac disease. Recent reports are unable to identify the benefits of revascularization versus current medical therapies. Our previous studies have shown significant benefit from inhibiting inflammatory Th1 T-cells and M1 macrophages in the murine model of RAS.

Objective: Our aim was to determine the potency of a novel mesenchymal stem cell (MSC) based therapy in modulating the T-cell response and slow the progression of renal injury within RAS.

Methods: MSCs were isolated and cultured from murine adipocyte tissue and injected via the carotid artery into C57BL/6 mice at 2 and 3 weeks after RAS was induced. Mice were sacrificed at 1 and 2 weeks post MSC injection. Kidneys were evaluated by histology, RT-PCR, and flow-cytometry to determine the T-cell response.

Results: Conjugated microsphere labeling and flow cytometry illustrated that the MSCs traveled to the stenotic kidney. Histology and immunohistochemistry detected a bimodal separation within the MSC treated animals' renal atrophy and the presence of infiltrating T regulatory cells (Tregs) on mice treated at 3 weeks post-RAS. 50% of the treatment group demonstrated a 90% reduction in atrophy and a lower level of Tregs infiltration while the other 50% demonstrated no change when compared to un-treated RAS mice. PCR array studies determined a significant down-regulation of NOS2, RORc, and CCR4 within MSC treated mice with low vs. high atrophy. In contrast, all of the mice treated at 2 weeks post-RAS shown response to MSC therapy. Flow cytometry studies shown that MSC treatment resulted in the down-regulation of Th1 T-cells and up-regulation of Tregs in the stenotic kidney.

Conclusion: The results suggest that MSCs have an effect on the inflammatory environment present in RAS under certain conditions. Further studies are needed to determine the effective treatment window for MSC administration.
Title: In-trans provision of CD3 Conformational Change increases T cell receptor reactivity to poorly immunogenic antigens

Authors: Michele M. Hoffmann, Carlos Molina Mendiola, Michael J. Hansen, Edwin E. Reyes, Christopher A. Parks, Govindarajan Rajagopalan, Larry R. Pease, Ph.D., Adam G. Schrum, Ph.D., Diana Gil Pages, Ph.D.

Advisor: Diana Gil Pages, Ph.D.

Track: Immunology

Adaptive immune functions of mature T cells are initiated by binding of the T cell antigen receptor (TCR) to agonist peptide-MHC (pMHC) ligands on the surface of antigen presenting cells (APCs). TCR interactions with agonist pMHCs induce a conformational change in the tails of the associated CD3 complex (CD3Δc) that has been postulated to contribute to efficient TCR/CD3 triggering and productive T cell responses. In contrast, TCR engagement by antagonistic pMHCs does not induce either CD3Δc or full TCR/CD3 signaling in mature T cells. Here, we have used a monovalent Fab fragment (Mono-Fab) specific for the murine CD3 complex to provide in-trans induction of CD3Δc to mature T cells engaging pMHC ligands. This Mono-Fab does not interfere with TCR capacity to bind pMHC and fails to elicit CD3 signaling events in the absence of TCR/pMHC interactions. Using OT-I transgenic T cells and a collection of altered peptide ligands derived of the agonist peptide OVA (pOVA), we show that in-trans CD3Δc addition to mature OT-I T cells using Mono-Fab enables T cell responses to antagonist ligands that fail to induce CD3Δc on their own. Moreover, using a mouse model of a poorly immunogenic melanoma, we show that Mono-Fab promotes in vivo T cell dependent anti-melanoma responses that are antigen-specific. Our data suggests that in-trans CD3Δc provision decreases the activation threshold of T cells, thereby promoting adaptive immune responses to poorly immunogenic antigens. We propose that targeting the CD3 complex for the induction of CD3Δc could be exploited to achieve efficient TCR/CD3 triggering by poorly immunogenic antigens in the context of vaccination against cancer and infectious diseases.
Recent thymic emigrants (RTEs) must undergo phenotypic and functional maturation to become long-lived mature naïve T cells. In CD4-cre NKAP conditional knockout mice, NKAP-deficient RTEs fail to complete T cell maturation. Here, we demonstrate that NKAP-deficient immature RTEs do not undergo apoptosis, but are eliminated by complement. C3, C4 and C1q are bound to NKAP-deficient peripheral T cells, demonstrating activation of the classical arm of the complement pathway. As thymocytes mature and exit to the periphery, they increase sialic acid incorporation into cell surface glycans. This is essential to peripheral lymphocyte survival, as stripping sialic acid with neuraminidase leads to the binding of natural IgM and complement fixation. NKAP-deficient T cells have a defect in sialylation on cell surface glycans, leading to IgM recruitment. We demonstrate that the defect in sialylation is due to aberrant α2,8-linked sialylation, and the expression of three genes (ST8sia1, ST8sia4 and ST8sia6) that mediate α2,8 sialylation are downregulated in NKAP-deficient RTEs. The maturation of peripheral NKAP-deficient T cells is partially rescued in a C3-deficient environment. Thus, sialylation during T cell maturation is critical to protect immature RTEs from complement in the periphery.
The most severe complication of *Plasmodium falciparum* infection, human cerebral malaria (HCM), leads to coma, neurological deficits, and potentially death. A hallmark of cerebral malaria is vascular leakage into the brain parenchyma caused by blood-brain barrier (BBB) disruption. Due to this association between BBB disruption and HCM, we investigated cerebral endothelial cell (CEC) tight junction protein alteration at the BBB in the *Plasmodium berghei ANKA (PbA)* model of experimental cerebral malaria (ECM). The BBB disruption in ECM is dependent at least partially on CD8 T-cells and the expression of their effector molecule perforin. In this study, we investigated the localization and mechanism behind the vascular leakage during ECM. *PbA* infected C57BL/6 mice presented with areas of vascular leakage that co-localized with dysregulation of the BBB tight junction proteins claudin-5 and occludin on microvessels as shown using confocal microscopy. T1 weighted gadolinium enhanced MRI revealed that the vascular leakage is specific to the hypothalamus, olfactory bulb, thalamus, corpus callosum, and brain stem. The localization of this leakage within critical brain regions is consistent with the neurologic deficit and morbidity associated with ECM. Meanwhile, *PbA* infected perforin deficient mice retained tight junction integrity and displayed significantly reduced vascular leakage. We propose that perforin contributes to BBB tight junction disruption and vascular permeability within vital regions of the brain. This likely contributes to the pathology and morbidity associated with ECM.
Title: Multiphoton Microscopy to Image Blood Brain Barrier Disruption

Authors: April M. Huseby, Fang Jin and Aaron J. Johnson, Ph.D.

Advisor: Aaron J. Johnson, Ph.D.

Track: Immunology

Theiler’s murine encephalomyelitis virus (TMEV) infection in mice has been used as an animal model for investigating acute events of neuroinflammation. The immune mediated events that follow result in permeability and immune infiltration by day 7 post-infection. The contribution and dynamics of immune cell subtypes that mediate protection in the C57BL/6 chronic disease resistant mice and conversely, the events that lead to substantial permeability in susceptible mice are not completely understood. Our lab has previously investigated the role for CD8+ T cells directly interacting with virally infected neurons. More recently we have observed CD4+ cells in tight apposition to neuronal cells. The role for these immune cells and other types observed has largely been assayed with ex vivo methodology. With the advent of multiphoton/2 photon laser scanning microscopy (2PLSM) we can visualize T cell-neuronal interactions and vascular permeability in real time. Adoptive transfer of green fluorescent protein (GFP) expressing T cells and Q dot labeling of the vasculature could be visualized in vivo. Future studies with 2PLSM will allow us to measure the cell dynamics and motility of the immune components that contribute to the protection or pathogenesis during neuroinflammation.
The human gut contains bacteria on the order of $10^{14}$ and recent studies have indicated these gut microbes play an important role in both health and disease. There is mounting evidence that specific communities of bacteria can induce substantial proliferation of colonic T regulatory cells and thus can modulate inflammatory diseases. Therefore to identify novel human commensal with disease protective property, we isolated number of human gut commensal bacteria of Bacteroidetes family and tested their ability to suppress disease in experimental autoimmune encephalomyelitis (EAE), an experimental model of MS. Here we report identification of *Prevotella histicola* (*P. histicola*), an anaerobic Gram negative bacterium that appear to have potent systemic immunomodulatory effects. Many species of *Prevotella* are found within the human oral cavity and intestine, and are increased in the guts of persons eating veggie rich diets. *P. histicola* has shown a disease suppressive effect, especially when administered as live bacteria to the HLA-DR3.DQ8 transgenic mouse in the EAE model. We are currently working on characterizing the optimal growth conditions for the bacteria and their requirements to generate a disease suppressive effect. *P. histicola* is also being investigated in the Caco2 cell line, a human colonic epithelial tissue for potential mechanisms of action.
Tumor immunotherapies have been shown to be an effective addition to standard cancer treatments, demonstrating promise in both model systems and clinical trials. These therapies target a variety of aspects of the immune response, including the innate and adaptive immune systems, with the goal of overcoming the low immunogenicity of the tumor environment. A recent focus has been placed on adjuvant therapies stimulating Toll-like receptors (TLRs), which activate tumor-infiltrating dendritic cells (DCs), which in turn play a role in CD8+ T cell activation and function. One TLR ligand of interest is CpG, a TLR9 agonist. CpG administration has been shown to reduce levels of extracellular programmed cell death protein 1 (PD-1) on CD8+ T cells in an IL-12 dependent manner; this protects CD8+ T cells from negative regulation via an interaction between PD-1 and programmed death-ligand 1 (PD-L1), often present on tumor cells. **The aim of this study was to determine the role of IL-12 in downregulation of PD-1 in CD8+ T cells.** When CpG was administered to WT and IL-12 KO mice, there was significantly more PD-1 expressed on the surface of CD8+ T cells of the IL-12 KO mice, indicating a role of IL-12 in CpG-mediated downregulation of PD-1. However, when IL-12 alone was administered to splenocytes in culture, there was no significant decrease in PD-1 expression following stimulation of the CD8+ T cells. Similarly, there was not a significant decrease in the PD-1 expression when CD8+ T cells were isolated, stimulated, and treated with IL-12. This suggests that there are differences in IL-12 function between CpG administration in vivo and IL-12 administration in vitro or that IL-12 may need to be administered during a specific time frame in the CD8+ T cell response. Future directions include determining PD-1 levels following IL-12 administration during different phases of the T cell response and exploring the role of IL-12 administration in a tumor model in vivo.
As central mediators of signal transduction, protein-protein interactions (PPI) are thought to coordinate cellular responses through the formation of extensive networks. We hypothesized that measuring PPI network activity in human T cells would reveal immune activation versus tolerogenic signaling profiles. We have designed and mounted a new technological application, multiplex immunoprecipitation detected by flow cytometry (MIF), to empirically measure a significant subset of the signaling PPI network in T-cells. The approach captures protein complexes onto microspheres through immunoprecipitation, and uses fluorescent probes to detect co-associated proteins. A panel of distinct microsphere bead types (Luminex xMAP) allows for capturing many distinct complexes simultaneously. Currently, MIF is capable of 253 unique pair-wise measurements between 22 different proteins that participate in T cell antigen receptor signaling. We have applied MIF to distinguish agonist (pro-immune) versus antagonist (pro-tolerance) activation of the T-cell antigen receptor. To analyze this innovative dataset, we developed new statistical approaches that utilize the full fluorescence distribution and correct for technical variability and multiple hypothesis testing. We compared our approaches to existing ones. Our results favor the use of a novel, empirically derived alpha cut-off correction, and we propose two optimized analytical approaches for MIF data. Furthermore, composite network signatures have been identified by principle component analysis, while the application of other dimensionality reduction and clustering methods continue to be explored. In summary, MIF is providing new insight into the network patterns of shared protein complexes as they translate distinct signals into T-cell function.
Title: Enhancing cancer targeted immunity with structural manipulation of MHC class I

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Track: Immunology

T cells exist in the normal repertoire that are capable of recognizing tumor derived antigens, yet remain unresponsive due to tolerance and immune regulatory mechanisms. We hypothesize that given sufficiently strong stimulation, these T cells may become activated enough to overcome the regulatory environment of cancer, and mount a sterilizing response against the tumor. Knowledge of the fine structure of TCR in complex with peptide-MHC has revealed that interaction strength is governed by the sum of all amino acid contacts between the TCR hypervariable loops, MHC alpha helices, and peptide. We predict that amino acid substitutions within the alpha helices of MHC that make contact with TCR hypervariable loops may increase the binding affinity between the members of the TCR peptide-MHC complex, resulting in enhanced signaling leading to T cell activation. Using modeling algorithms, a set of MHC structural mutants was predicted to have increased binding affinity for the TCR when key residues were altered to tryptophan. Cell lines expressing the predicted altered MHC ligands (altMHC-L) were made and used to evaluate immunogenicity against models of self-antigens, or antagonist peptides. These studies revealed that altMHC-L do have increased binding affinity for the TCR, and that T cells respond to self-peptide-altMHC-L complexes with enhanced activation and proliferation. Current studies are focused on CD8 killer T cell effector function in response to altMHC-L.
Small colony variants (SCVs) are a subgroup of bacteria that are slow-growing, unresponsive to antibiotics and can persist intracellular. Staphylococcal SCVs have been shown to be more successful at intracellular persistence than normal phenotype staphylococci and are associated with chronic prosthetic joint infections (PJI). Staphylococcal bacteria secrete specific peptides called phenol soluble modulins (PSMs), which are regulated by the agr system and assist the organism in both the establishment and the subsequent dissemination of the infection. These peptides are multifunctional and can act to enhance virulence of the organism during invasion or act as an antimicrobial when in direct contact with other competing pathogens. Whole genome expression analysis of two *S. epidermidis* SCVs was previously conducted and demonstrated decreased expression, relative to the parental and control isolates, of genes encoding pro-inflammatory peptides of the PSM family. We sought to validate these results using an RT-PCR assay we designed. Two SCV/parent pairs of *S. epidermidis* isolated from the infected joint prostheses of two patients were studied. Each pair and a control strain RP62A were grown in trypticase soy broth and harvested at mid-logarithmic, late-logarithmic and late-stationary phase. Total RNA was isolated, purified, and quantified. Using the RT-PCR assay, we analyzed the expression of the following genes: PSM-β1\textsubscript{a}, PSM-β1\textsubscript{b}, PSM-β2, PSM-β3, and PSM-γ. Normalized to the control gene, the gene encoding PSM-β1\textsubscript{b} was decreased more than 5 fold, the gene encoding PSM-β1\textsubscript{b} and PSM-β2 was decreased more than 3 fold, and PSM-γ gene expression was unchanged when comparing SCV1 to Parent1. In addition, the gene encoding PSM-β1\textsubscript{b} was decreased more than 15 fold, the gene encoding PSM-β2 was decreased more than 11 fold, the gene encoding PSM-β3 was decreased more than 25 fold, and the gene encoding PSM-γ was decreased more than 3 fold when comparing SCV2 to Parent2. Our findings indicate a possible mechanism of how SCVs allow persistence of infection and how differential expression of PSMs may be related to SCVs. Therefore, we propose the hypothesis that *S. epidermidis* SCVs evade the host immune system by producing less PSM-β (1 & 2) and PSM-γ compared to their isogenic parent strains. To date, PSM-β has been shown to promote biofilm formation at low concentrations and detachment at high concentrations, while PSM-γ has been show to contain antimicrobial and cytolytic activity. However, much of what is known about PSMs are relatively new and so much is yet to be discovered. Future and continual work on this study will help provide an understanding of the effects these SCVs and the effects the PSMs they secrete have on the immune system, in addition to providing insight as to what makes these SCVs less susceptible to death.
Title: **HDAC3 is required for thymocyte positive selection**

Authors: Rachael Philips, Meibo Chen, Doug McWilliams, Paul Belmonte, Megan Constants, Scott Heibert, and Virginia Shapiro.

Advisor: Virginia Shapiro, Ph.D.

Track: Immunology

Proper gene regulation is critical during T cell development in order to generate functional T cells. Development involves both the activation and repression of genes as cells progress through each developmental checkpoint. Histone acetylation/deacetylation is one mechanism of regulating gene expression in which acetylation by histone acetyl transferases (HATs) allows for gene expression and deacetylation by histone deacetylase (HDAC) enzymes promote gene silencing, and their role in T cell development is still under investigation. In this study, our lab focused on HDAC3. We examined the role HDAC3 plays during T cell development by utilizing a CD2-Cre-mediated loss of HDAC3 (CD2icre HDAC3-cKO), which deletes early in thymocyte development. When HDAC3 is absent, there is a developmental block during positive selection, leading to very few CD4 and CD8 SP thymocytes as well as T cells in the periphery. During positive selection, HDAC3-deficient thymocytes fail to up-regulate Bcl-2 and show increased apoptosis. Mechanistically, thymocytes fail to up-regulate the transcription factor c-Rel that regulates Bcl-2 expression after positive selection. This correlates with a failure to down-regulate RORγt, which is required for c-Rel expression. These results suggest that HDAC3 is required for the down-regulation of RORγt during positive selection. This work is supported by NIH RO1 to V.S.S as well as internal funds from the Mayo Clinic.
The T Cell Receptor (TCR) is a multi-subunit protein complex composed of an alpha/beta heterodimer in complex with the CD3 signaling subunits (δζζ). For some time the stoichiometry of the complex was debated within the literature, however a composition of αβεγεδζζ has been agreed upon. Although the stoichiometric make up of the TCR has recently been accepted, the valency of the protein complex remains a controversy. The currently accepted model for the TCR remains as a monovalent complex (αβεγεδζζ); however, previously published work from our lab has proposed a model for a bivalent TCR complex (αβεγεδζζ * 2). To elucidate the valency of the TCR, we have utilized immunoprecipitation detected by flow cytometry (IP-FCM) and size exclusion chromatography shift (SEC-shift) as methods for detecting complex size and subunit composition. The sensitivity of these techniques allows for an accurate determination between the possibility of a monovalent, bivalent and multivalent protein complex. Our results strongly suggest that the TCR exists as a bivalent complex of exactly two αβεγεδζζ in its native state. Further investigation is required to delineate subunit placement within the complex, as well as functional relevance for the existence of a bivalent TCR. With a more sound understanding of the biochemical structure of the TCR, more effective manipulation strategies of T cell mediated immune processes can be achieved.
Title: Investigating Melanoma-Associated Immune Modulation via Analysis of Peripheral Blood T Cell Transcriptome Profiles

Authors: Adam Scheid, Virginia Van Keulen, Sara Felts, Ph.D., Yuji Zhang, Ph.D., Matthew Block, M.D., Ph.D., Svetomir Markovic, M.D., Ph.D., and Larry Pease, Ph.D.

Advisor: Larry Pease, Ph.D.

Track: Immunology

While complex relationships between tumor cells and the immune system have been defined, the nature of tumor-associated systemic immune modulation remains controversial. In order to investigate this controversy we used RNAseq to compare and contrast peripheral blood T cell transcriptomes of healthy blood donors to those of patients with stage IV melanoma. Using this approach we have found that consistent profiles of CD4⁺ and CD8⁺ T cell gene expression differentiate healthy individuals from one another and differentiate melanoma patients from one another as well. Interestingly, these expression profiles also group healthy individuals differently than they do melanoma patients, suggesting that gene expression differs between peripheral blood T cells of healthy individuals and cancer patients. Some of the gene transcripts that are differentially expressed between these groups are known to be involved in immune functions, supporting the possibility that some of the differences in T cell transcriptome profiles could give rise to differences in T cell immune function in cancer patients relative to healthy individuals. Another intriguing finding was the differential expression of long intergenic non-coding (linc)RNAs in CD4⁺ and CD8⁺ T cells between healthy individuals and cancer patients. Although the biological impacts of our findings have yet to be defined, our transcriptome analysis suggests there may be differences in peripheral blood T cells between healthy blood donors and melanoma patients. Investigation of these differences could provide new insights into cancer immunology and lay foundations for more effective clinical cancer immunotherapies.
We have previously described a model in which ganciclovir (GCV) treatment of subcutaneous B16 melanomas expressing the Herpes Simplex Virus thymidine kinase gene (B16tk) induced complete macroscopic regressions resulting in minimal residual disease (MRD) followed by aggressive tumor recurrences. Here we show that B16tk recurrences (B16tk REC) were effectively treated by a VSV-cDNA Ganciclovir Escape Epitope Library (GEEL) derived from the pooled cDNA of three different B16 REC tumors. We identified the murine BRAF gene as a potential immunogen of B16 recurrence. Although parental B16tk cells were consistently wild type for BRAF, three separate B16tk REC tumor explants contained mutated BRAF sequences. Consistent with this, when primary B16tk tumors were induced to regress, enter MRD, then recur by a variety of chemo-, viro-, and immunotherapy; 14/16 recurrences contained a mutated BRAF sequence within the 595-605 region.

We exploited the finding of BRAF as a potential target of immune responses against B16tk recurrences by showing that an immunotherapy strategy including VSV-mediated expression of BRAF prevented B16tk REC recurrences following frontline GCV therapy in 100% of mice. In addition, once B16tk recurrences started to grow again in vivo, although they were insensitive to further GCV, they could be completely eradicated by treatment with the BRAF-specific inhibitor PLX-4720. This therapeutic effect was associated with generation of widespread T cell responses against the emerging relapsing tumor. Taken together, our data suggest that the acquisition of a mutated BRAF is acquired de novo within the small population of B16tk cells which survive frontline therapy as MRD; and is a direct result of genetic plasticity in at least a proportion of these cells which can respond to applied selective pressures. Thus, BRAF mutations represent a powerful effector mechanism by which dormant melanoma cells can progress to actively progressing recurrences in vivo and can be targeted by either immunotherapy or chemotherapy.
Title: The transcriptional repressor, NKAP is required for invariant Natural Killer T (iNKT) cells development and differentiation.

Author: Puspa Thapa¹, Meibo Chen¹, Doug McWilliams¹, Paul Belmonte¹, Megan Constans¹, Scott Hiebert², Derek Sant'Angelo³ and Virginia Smith Shapiro¹ Ph.D.

Advisor: Virginia Smith Shapiro, Ph.D.

Track: Department of Immunology

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In order for the immune system to function, the development of lymphocytes in the bone marrow (B cells) and thymus (T cells) is critical, yet incompletely understood. Studies in our lab have shown that a transcriptional repressor, NKAP, is required for lymphocyte development. NKAP is required for T cell development and maturation; and is also important for maintenance and survival of hematopoietic stem cells (HSCs). NKAP interacts with HDAC3 for its repressor function. Invariant Natural Killer T (iNKT) cells are innate lineage of lymphocytes that can produce copious amount of cytokines within hours of stimulation. In the thymus, positive selection into the iNKT cell lineage occurs at the double positive (DP) stage, and iNKT cells go on to complete development, where they go through various stages (stage 0-3).

After positive selection into iNKT lineage at the DP stage, iNKT cells mature and differentiate into their functional subsets known as NKT1, NKT2 and NKT17. Previously using CD4-cre NKAP cKO mice, we demonstrated that the transcriptional repressor NKAP is required for positive selection of DP thymocytes into the iNKT cell lineage. To study the role of NKAP in later iNKT cell development and differentiation, we generated PLZF-cre NKAP cKO mice with NKAP deletion occurring after entry into the iNKT lineage at stage 0, bypassing the previous block in iNKT development at the DP stage. In these mice, there was a significant decrease in the absolute number, which was not due to decreased proliferation or increased apoptosis. In the PLZF-cre NKAP cKO mice, there are very few T-bet expressing NKT1 cells, almost no ROR-γt expressing NKT17 cells and decreased number of Gata3 expressing NKT2 cells present. Concurrently with defect in NKT1 and NKT17 lineages, there is lower production of IFN-gamma and a defect in production of IL17. Interestingly, in PLZF-cre NKAP cKO mice, the early stage 0-1 iNKTs have lower PLZF and T-bet expression, which could account for the early block in development and decreased differentiation. Deletion of NKAP associated protein Hdac3 using PLZF-cre also shows an iNKT cell developmental defect, with decreased expression of Tbet and its regulated gene CXCR3. Thus, NKAP together with Hdac3 may regulate iNKT cell development and differentiation.
Pneumocystis pneumonia (PCP) is an opportunistic fungal infection which is mostly tightly linked to the HIV / AIDS epidemic. This fungal pneumonia does not occur in the healthy CD4+ T cell competent hosts, but is potentially fatal to patients with a CD4+ T cell count less than 200 cells/μL, indicating a requirement for CD4+ T cells in defense against PCP. Based on this, one would predict that the CD3δ knockout (CD3δ-/-) mouse should be susceptible to PCP, because this mouse is reported to have a defect in the positive selection step of thymocyte development, leading to CD4+ T cell deficiency in the periphery. However, we have found that the CD3δ-/- mouse is able to survive PCP infection, with no evidence of succumbing to the disease. We know the mice live longer than CD4-deficient susceptible mice, including mice lacking both Major Histocompatibility Complex classes (MHC-/-), and mice missing Rag (Rag-/-). This indicates some level of adaptive immune system activity within the CD3δ-/- mouse. In addition, two labs, including ours, have reported some low level of CD4+ T cells within the CD3δ-/- mouse, but the extent to which those few cells may be immune competent, or dysfunctional “leaked” cells, is incompletely understood. To further illuminate the mechanism of PCP resistance in CD3δ-/- mice, we will be using several other knockout and cell-subset-depletion models to determine the impact of different cell types, and to determine the origin of the cells providing anti-PCP immunity. The information gained in these studies should help us to determine the cause of PCP immunity within a mouse whose T cell development is blocked, with the potential to apply lessons learned to provide immunity to immunodeficient patients. We may also redefine the requirement of CD3δ in thymocyte positive selection, which could contribute to current understanding of T cell development.
Title: Determining the function of an uncharacterized protein, C16orf58

Authors: Carly Baehr and Larry Karnitz, Ph.D.

Advisors: Larry Karnitz, Ph.D., Scott Kaufmann, M.D. Ph.D., Martin Fernandez-Zapico, M.D., Bruce Horazdovsky, Ph.D., Yuichi Machida, Ph.D.

Track: Molecular Pharmacology and Experimental Therapeutics

C16orf58 (C16) is the mammalian ortholog of the plant gene Root UV-Sensitive (RUS3). The loss of members of the RUS family of proteins has been shown to cause growth defects in Arabidopsis thaliana when roots are exposed to low levels of UV light, potentially due to defects in auxin signaling caused by mislocalization of surface-expressed transmembrane proteins. Although the human C16orf58 protein exhibits 37% amino acid sequence identity with the A. thaliana RUS3, no function has been yet elucidated in mammalian cells. To determine the function of the mammalian ortholog in human cells, C16of58 somatic knockout cell lines were generated using TAL-Effecter Nuclease technology, and these lines were characterized in terms of gene expression and sensitivity to various stress conditions. Although loss of C16orf58 does not cause significant growth defects in unstressed cells, the ΔC16orf58 lines showed marked sensitivity to specific chemotherapy agents including doxorubicin, etoposide and paclitaxel; but not to cisplatin, floxuridine or veliparib. The ΔC16 cells were also sensitive to certain types of metabolic stress, including serum starvation and treatment with 2-deoxy D-glucose, and to autophagy induction by chloroquine or mTOR inhibitor treatment. Additionally, ΔC16 cells showed increased signaling through the AMPK pathway in response to metabolic stress as compared to wild-type cells. By peptide mass fingerprinting analysis of proteins co-precipitating with C16orf58, C16 was found to interact with ion and solute transport proteins, including the ER-localized SERCA ATPases and mitochondrial ATPases, as well as proteins involved in endoplasmic reticulum protein trafficking. The ΔC16 cells were also found to have elevated cytoplasmic Ca2+ levels, suggesting that the loss of C16 may reduce the activity or expression of SERCA and potentially other ATP-dependent ion or solute transporters, leading to altered cell signaling responses. Taken together, these data suggest a broad function for C16 in protein trafficking, energy homeostasis and cell metabolism.
Title: IQGAP1 is required for CXCR4 expression, CXCR4 trafficking and signaling in the Jurkat T cell line

Authors: Adebowale O. Bamidele, and Karen E. Hedin, Ph.D.

Advisors: Karen Hedin, Ph.D., Scott Kaufmann, M.D, Ph.D., Larry Karnitz, Ph.D., Adam Schrum, Ph.D and Keith Knutson, Ph.D.

Track: Molecular Pharmacology and Experimental Therapeutics

CXCR4 (CXC chemokine receptor 4) is a G-protein coupled receptor (GPCR) that is activated upon binding its ligand SDF-1, stromal-cell-derived factor 1 to regulate gene expression, cell proliferation and chemotaxis. In T lymphocytes, CXCR4 activation stimulates ERK MAPK phosphorylation, cytokine secretion, cell migration and T cell immune activation. SDF-1/CXCR4-induced signals are dependent on the cell-surface expression of CXCR4, thus identifying mechanisms that modulate CXCR4 expression is vital. IQGAP1 is a cytoskeletal/MAPK scaffold protein and its role in chemokine receptor or GPCR signaling is poorly understood. In addition, the role of IQGAP1 in CXCR4 signaling is unknown. We have identified a novel role for IQGAP1 in mediating CXCR4 expression, trafficking and signaling in Jurkat T lymphocytes. IQGAP1 depletion dramatically reduced the cell-surface expression of CXCR4. The residual CXCR4 on the cell-surface IQGAP1 depleted cells was impaired in several steps of SDF-1-induced CXCR4 trafficking. Forced expression of CXCR4 in IQGAP1 depleted cells restored its cell-surface levels, but not SDF-1-induced CXCR4 endocytosis, recycling, coupling to downstream ERK1/2 MAP kinase activation and directional T cell migration. In contrast, loss of IQGAP1 induced multiple membrane protrusions and increased cell spreading via increased and sustained activation of Rho-GTPases (Rho, Rac1 and CDC42) and F-actin. Rho-GTPases constitutively bind IQGAP1 and their depletion showed defects similar to IQGAP1 depletion. Our data shows that IQGAP1 is essential for many critical aspects of CXCR4 functions by regulating CXCR4 expression, trafficking through early and recycling endosomes, ERK1/2 activation, actin polymerization and chemotaxis. Our findings suggest that IQGAP1 may be an important mediator of tumor promoting functions of CXCR4 in other cell types.
Title: Genes associated with serum estrone, estrone conjugates and androstenedione concentrations in postmenopausal women with estrogen receptor positive breast cancer


Advisor: Richard Weinshilboum, MD

Track: Molecular Pharmacology and Experimental Therapeutics

Estrone (E1), the predominant estrogen in postmenopausal women, is synthesized from androstenedione (A) in the ovaries and adipocytes and can be converted to conjugates (E1Cs). Both circulating E1 and E1Cs are known risk factors for breast cancer; and E1C is present at concentrations up to 10 fold higher in breast cancer tissue than in serum. However, little is known regarding the genes that regulate serum A, E1 or E1Cs concentrations. In a cohort of 776 postmenopausal women with estrogen receptor positive breast cancer, we used a genome-wide association study (GWAS) to identify SNP signals associated with these concentrations. Serum levels of each hormone were measured using GC-MS/MS, and patients were genotyped at ~600,000 SNPs and another 7 million were imputed. Using linear regression models with additive genetic effects, a GWAS was conducted for each hormone level separately as well as the ratio of E1/A, E1Cs/A and E1Cs/E1. SNP effects were adjusted for BMI, population stratification and other relevant variables. Multiple SNPs in \textit{SLCO1B1}, a gene that encodes a transporter, were genome-wide significantly associated with E1C levels, E1Cs/E1 and E1Cs/A ratios. The variant allele of the top genotyped SNP rs4149056 (p value = 3.74x10^{-11}) is a missense mutation that has an established association with statin-induced myopathy and sex hormone binding globulin levels. SNPs in \textit{EMR2} (p=1.39x10^{-07}), \textit{ELMO1} (p=3.19x10^{-07}), \textit{LDB3} (p=5.67x10^{-07}) and \textit{CYP11B1/2} (p=6.65x10^{-07}) genes were associated with androstenedione levels. SNPs downstream of \textit{SYNJ2NP2/ARIP2} and upstream of the testis-specific gene \textit{ADAM21} (p=1.48x10^{-06}) were associated with E1 levels, with a non-synonymous SNP in \textit{ADAM21} showing suggestive association (rs45480894, p=2.58x10^{-06}). The ratio of E1/A was associated with a missense SNP in \textit{ANO7} (rs74804606, p=5.69x10^{-07}). These results, particularly those for \textit{SLCO1B1} that encodes a liver transporter of E1C, point towards mechanisms that may explain variation in these hormones that are associated with increased risk of breast cancer.
Title: Mapping Cell-Autonomous Cardiogenic Signatures of Bioengineered Cardiac Progenitors According to Native Cardiogenesis

Authors: Katherine A. Hartjes

Advisors: Andre Terzic, MD/PhD and Timothy J. Nelson, MD/PhD

Track: Molecular Pharmacology and Experimental Therapeutics

Induced pluripotent stem cells (iPSCs) offer an unparalleled platform to prioritize molecular pathways underlying intrinsic defects in organogenesis and to identify the initial point of divergence between health and disease. By applying disease-in-a-dish strategies, bioengineered systems that produce stage-specific cardiac tissues offer unique opportunities for discovery science focused on cardiac pathobiology. However, the utility of iPSC-derived cardiac progenitors to recapitulate the molecular etiology of complex structural heart defects has yet to be validated. Therefore, validation of this platform with unbiased transcriptional profiles of normal and abnormal heart development is required to calibrate the utility of iPSC technology for molecular disease discovery.

Utilizing a multidimensional expressome approach, we investigated morphological cardiac defects through both in utero cardiogenesis and in vitro cardiac differentiation of iPSCs. Nitric oxide synthase 3 knockout (NOS3-/-) mice served as a model of highly penetrant congenital septal defects. By establishing distinct roadmaps of cardiogenesis in WT and NOS3-/- mice, this bioinformatic platform enables molecular dissection of initial transcriptional divergence points underlying abnormal cardiogenesis.

Herein, we establish the accuracy of iPSC-derived cardiac tissues to model molecular defects of a NOS3+ murine model of congenital heart disease. Transcriptional profiling of cardiac tissues from WT and NOS3+ embryos reveals differential gene expression as an in utero benchmark of health and disease. Calibrated according to this native transcriptional profile, microarray analysis of iPSC-derived cardiac tissue identifies corrupted cell-autonomous pathways within the NOS3+ samples.

Future work will validate the utility of this in vitro platform by pharmacological compensation of nitric oxide levels to restore the transcriptional profile and cellular phenotype of NOS3+ cardiac progenitors. Overall, this study establishes a stem cell-based platform to discover the molecular etiology of complex structural cardiac defects, aiming toward the ultimate goal of targeting therapeutic modifiers based on disease-in-a-dish applications.
Title: Understanding the Mechanisms of PARP Inhibitor Resistance in Ovarian Cancer

Authors: Rachel Hurley; Jill Wagner; Karen Flatten; Anand Patel, MD/PhD; Andrea Wahner Hendrickson, MD; Scott Kaufmann, MD/PhD.

Advisor: Scott Kaufmann, MD/PhD

Track: Molecular Pharmacology and Experimental Therapeutics.

Women carrying mutations in the BRCA1 or BRCA2 genes, key components of the Fanconi anemia/homologous recombination (HR) pathway, have a significantly heightened lifetime risk of ovarian cancer (40-60% & 11-27% respectively) relative to the 1.4% risk of women in the general population. PARP inhibitors have demonstrated selective killing BRCA1 or BRCA2 mutant tumors and further studies have identified defects in other components of the DNA repair pathway also sensitize to PARP inhibitor. In initial clinical trials, PARP inhibitors have exhibited a promising therapeutic response in up to 50% of ovarian cancer patients with BRCA1/2 mutations, highlighting the potential for PARP inhibitors to play a key role in future ovarian cancer therapeutics. With the immense chemotherapeutic potential of PARP inhibitors, understanding resistance mechanisms is critical. Current understanding of PARP inhibitor resistance arises primarily from in vitro and cell culture-based mouse in vivo research. Only secondary mutations that restore BRCA function have been confirmed in patients, accounting for less than 50% of the observed patient resistance. As such, a significant gap in knowledge remains.

We have generated cell lines that demonstrate in vitro resistance to the PARP inhibitor ABT-888, and cross-resistance to other PARP inhibitors. Initial work suggests that up-regulation of Rad51 confers resistance through restoration of the homologous recombination DNA repair pathway. Future work will clarify the role of Rad51 in PARP Inhibitor resistance and elucidate the mechanism of up-regulation. Additionally, patient tumorgrafts will be utilized to develop in vivo PARP inhibitor resistance, which will be utilized to characterize the role of DNA damage repair proteins. Our long-term goal is to elucidate patient-applicable mechanisms of PARP inhibition and identify predictive biomarkers for PARP inhibitor resistance.
PRSS3/mesotrypsin is an atypical isoform of trypsin, whose upregulation has been implicated in promoting tumor progression. To date there are no mesotrypsin specific inhibitors and this impedes deciphering the pathological role of this enzyme. Development of mesotrypsin specific inhibitors would allow interrogation of this isoform's role in tumor progression and could possibly form the basis for novel therapeutic strategies targeting mesotrypsin, but to date the small molecule inhibitors toward the trypsin family have poor affinity and low selectivity. Compounds from an \textit{in silico} screen derived by molecular modeling based on mesotrypsin's crystal structure were analyzed. One hit, diminazine diaceturate, had an inhibitory constant (Ki) of 3.6±0.27 µM and was subsequently co-crystalized with mesotrypsin. We report a solved crystal structure of mesotrypsin complexed with diminazine diaceturate refined to 1.25 Å resolution. Efforts to study other small molecule inhibitors of mesotrypsin are ongoing and in combination with the present structure will inform derivatization and developmental efforts to generate novel and more selective mesotrypsin inhibitors.
Title: MLN4924 Induces Noxa Upregulation in Acute Myelogenous Leukemia and Synergizes with Bcl-2 Inhibitors

Authors: Katherine Lorraine Broin Knorr, Paula A. Schneider, X. Wei Meng, Haiming Dai, B. Douglas Smith, Allan D. Hess, Judith E. Karp, Scott H. Kaufmann M.D.-Ph.D.

Advisor: Scott H. Kaufmann, M.D., Ph.D.

Track: Molecular Pharmacology and Experimental Therapeutics

MLN4924, an inhibitor of the Nedd8 Activating Enzyme (NAE), has exhibited promising clinical activity in acute myelogenous leukemia (AML) and has previously been shown to induce apoptosis in AML cell lines, an effect that typically results from an alteration in the balance between pro- and anti-apoptotic Bcl-2 family members. Here we demonstrate MLN4924 induces apoptosis in AML cell lines and clinical samples through upregulation of the pro-apoptotic Bcl-2 family member Noxa and neutralization of Mcl-1, via a mechanism distinct from those observed in other malignancies. Inactivation of E3 Cullin Ring Ligases (CRLs) through NAE inhibition causes accumulation of the CRL substrate c-Myc, which transactivates the PMAIP1 gene encoding Noxa. Because Noxa neutralizes Mcl-1, an anti-apoptotic Bcl-2 paralog often upregulated in resistant AML, further experiments examined the effect of combining MLN4924 with Bcl-2 inhibitors. In combination with ABT-199 or ABT-263 (navitoclax), MLN4924 exerts a synergistic cytotoxic effect. Further preclinical and possible clinical study of MLN4924 as a single agent and in combination appears warranted in situations where targeting Mcl-1 or multiple anti-apoptotic Bcl-2 proteins simultaneously would potentially be advantageous.
One of the hallmarks of cancer is altered metabolism where cancer cells up-regulates glycolytic pathway and directs glucose metabolism away from mitochondrial oxidation and toward increased lactate production even in the presence of oxygen (the Warburg effect). Although Warburg originally claimed that increased aerobic glycolysis in cancer is due to a defect in mitochondria, later studies showed that mitochondria in most of cancers is intact and produces significant amount of ATP. Thus, it remains unclear how glycolytic and mitochondrial ATP are bioenergetically coordinated by oncogenic events to fill total ATP pool in cancer cells.

HER2+ breast cancer, which compromises 20-25% of total breast cancer patients, exhibits oncogenic tyrosine kinase HER2 amplification that is associated with tumor aggressiveness and poor prognosis. Although the current FDA approved drugs against HER2 such as trastuzumab and lapatinib have shown considerable clinical benefit, significant number of patients relapses, underscoring the need to develop new therapeutic approaches. One such approach is to target HER2-induced metabolic alteration because previous studies by our lab and others have shown that HER2 can translocate to the mitochondria and modulate ATP/ADP ratio to promote drug resistance.

To identify metabolites regulated by HER2 signaling, we performed HPLC-based metabolite profiling of HER2+ BT-474 cells and found that lapatinib treatment significantly decreased ATP and phospho-creatine levels. As phospho-creatine is a bioenergetic molecule used for energy transfer from mitochondria, we next examined whether HER2 might directly phosphorylate mitochondrial metabolic enzymes to regulate phospho-creatine levels in HER2+ breast cancer. We thus performed phospho-proteomic analysis along with biochemical assays of mitochondria-enriched fractions from BT-474 cells and found that mitochondrial creatine kinase 1 (MtCK1), which converts ATP and creatine to ADP and phopho-creatine, is tyrosine phosphorylated and activated by HER2 signaling. Based on these preliminary data, we hypothesize that HER2 may tyrosine phosphorylate MtCK1 to increase its enzymatic activity, thereby supplying cancer cells with phospho-creatine to promote drug resistance. Consistent with this hypothesis, we found that creatine supplementation renders BT-474 cells resistance to lapatinib, implying that reduction in the dietary creatine could act as individualizing prevention of drug resistance in HER2+ breast cancer patients.
Title: Uncovering the Genetic Basis of Sporadic, Pediatric Dilated Cardiomyopathy

Authors: Pamela A. Long, Jared M. Evans M.S., and Timothy M. Olson, M.D.

Advisor: Timothy M Olson, M.D.

Track: Molecular Pharmacology and Experimental Therapeutics

Idiopathic dilated cardiomyopathy (DCM) is a heritable, genetically heterogeneous disorder characterized by progressive degeneration of cardiac muscle. DCM typically exhibits an autosomal dominant mode of inheritance and is clinically silent in childhood, with delayed diagnosis at a mean age of 45 years. However, a rare subset of patients develops symptomatic heart failure at a young age. We sought to identify the genetic basis of sporadic, pediatric DCM in a phenotypically well-characterized cohort consisting of nineteen families. In all families, recruited non-consanguineous parents underwent screening echocardiography and displayed no phenotypic evidence of DCM. Array comparative genomic hybridization (aCGH) was performed on the proband when DNA was available. Cumulatively, aCGH did not detect any chromosomal aberrations, with the exception of one patient who was identified as a 45x/46xx Turner syndrome mosaic. Whole exome sequencing was carried out on recruited family members and data were filtered for rare, predicted deleterious recessive and \textit{de novo} genetic variants. A wide spectrum of candidate genes were identified among the nineteen families. Two families were found to harbor compound heterozygous mutations in three novel candidate genes; $TAF1A$, $DNMBP$, and $ELAC2$. Due to their recessive inheritance, these hypothesized loss-of-function mutations are being modeled utilizing zebrafish knockouts to determine their involvement in DCM pathogenesis. Next, one family was found to harbor a homozygous recessive mutation in $LRRC10$, a novel candidate gene for human DCM, which is reported to be necessary for proper cardiac development and function in zebrafish and mouse. \textit{In vitro} functional studies are currently underway to determine the significance of this exact homozygous mutation. In addition, one family was found to harbor compound heterozygous truncating mutations in $ALMS1$, a known yet rare gene for syndromic pediatric DCM. Finally, four families were found to harbor rare missense \textit{de novo} mutations in established DCM genes, providing evidence for pathogenicity of these mutations. In conclusion, sporadic, pediatric DCM appears to be a genetically heterogeneous disease, similar to what is observed in adult-onset DCM. However, an additional 4 out of 19 cases appear to inherit recessive mutations as a cause for disease, a unique finding that distinguishes pediatric from adult-onset DCM. Whole exome sequencing data are currently being analyzed for the remaining eleven families to gain further insight into the genetic underpinnings of sporadic, pediatric DCM.
Title: Metformin functional pharmacogenomics: Investigating the mechanism of CDC25B regulation of AMP-activated protein kinase and metformin cytotoxicity

Authors: Reynold C. Ly, Nifang Niu, M.D. Ph.D, and Liewei Wang, M.D. Ph.D.

Advisor: Liewei Wang, M.D, Ph.D.
Track: Molecular Pharmacology and Experimental Therapeutics

Metformin is an oral type II antidiabetic drug currently under study for drug repurposing as an anti-neoplastic agent. Its anticancer mechanism involves activation of AMP-activated protein kinase (AMPK) pathway. Downstream effects of AMPK activation include mTOR inhibition which contributes to metformin’s anticancer effect. Despite current knowledge, metformin’s pharmacodynamic action is not fully elucidated as other studies have identified metformin mediated AMPK independent anticancer effects. To gain insight into metformin’s anticancer mechanism, we took a pharmacogenomics approach to identify novel candidate genes associated with metformin cytotoxicity, which might help us to further understand the mechanisms involved in metformin action. A genome wide association study was performed on 300 lymphoblastoid cell lines to correlate SNP, mRNA expression, and CpG methylation data with metformin IC\textsubscript{50}. 62 candidate genes were found highly associated with metformin IC\textsubscript{50}. Following further screening by ingenuity pathway analysis and siRNA knockdown cytotoxicity studies in triple negative breast cancer cells (MDA MB 231 and HS-578t), top three genes with the lowest p-value association were selected including CDC25B. CDC25B is a cell cycle phosphatase protein and is important in facilitating cell cycle progression, specifically at G2-M checkpoint by activating cyclin-B-CDK1 complexes. To determine how CDC25B expression modulates metformin cytotoxicity, we performed CDC25B knockdown and overexpression studies using HS-578t and HEK-293t cells. Results show CDC25B knockdown in HS-578t cells causes resistance to metformin, a phenomenon that was correlated with decreased AMPK phosphorylation, whereas CDC25B overexpressed HEK-293t cells showed increased AMPK phosphorylation (p-AMPK). Metformin treatment further enhances p-AMPK in CDC25B overexpressed HEK-293t cells. Next, we want to identify how p-AMPK is modulated by CDC25B. We hypothesized that CDC25B might directly affect AMPK phosphorylation by regulating either upstream kinases or protein phosphatases such as PP2A. PP2A is known to regulate AMPK by dephosphorylating p-AMPK. During the process of testing these hypotheses, we found that CDC25B and PP2A interact with each other by anti-FLAG IP. This interaction further increases with metformin treatment. Thus, we hypothesize that CDC25B-PP2A interaction block PP2A from interacting with AMPK and prevent AMPK dephosphorylation. Addition of metformin enhances the p-AMPK state and downstream activation of the AMPK pathway, resulting in cells sensitive to metformin. Further studies will be done to further characterize CDC25B-PP2A interaction and its impact on the AMPK pathway in cancer. Our study has clinical relevance as CDC25B expression in cancer might serve as a potential biomarker for selecting patients for metformin treatment.
Title: **Antagonistic Correlation of Neurogranin and Kynurenic Acid in Regulating NMDA Receptor Signaling in Schizophrenia and Alcohol Use Disorders**

Authors: Alfredo Oliveros, Erhardt, Sophie, Hyung W. Nam, D.-S. Choi Ph.D

Advisor: D.-S. Choi, Ph.D

Track: Molecular Pharmacology and Experimental Therapeutics

N-Methyl-D-Aspartate glutamate receptor (NMDAR) signaling plays an essential role in the pathophysiology and treatment mechanisms for psychiatric disorders including schizophrenia (SCZ). Neurogranin (Ng), a critical signaling molecule downstream of the NMDAR, has recently been associated with SCZ, and deficiency of Ng appears to promote schizophrenia-like behaviors in mice. Consistently, kynurenic acid (KYNA), an NMDAR antagonist, is increased in the cerebrospinal fluid of SCZ patients. Our preliminary data demonstrated that Ng deletion in mice increases ethanol drinking and disrupts pre-pulse inhibition (PPI). Moreover, we found that our constitutive Ng deletion model (Ng -/- mice) displays social interaction deficits which can be recovered by ethanol drinking. Taken together, these behavioral phenotypes are similarly displayed by individuals suffering from schizophrenia. Therefore we hypothesize that LPS-induced elevation of KYNA levels in the brain may promote ethanol drinking in addition to disruptions of PPI. Our preliminary data would suggest that disruptions in sensorimotor gating will be induced due to KYNA antagonism at the NMDAR, thus allowing establishment of a potential causal relationship between reduced NMDAR-mediated Ng signaling and increased brain KYNA. To determine this relationship we will present a model of LPS induced increases in KYNA examining PPI levels in 8-week C57BL6/J mice at 24-hr and 48-hr post i.p. LPS treatment (0.83mg/kg). This behavioral analysis will be immediately followed by western blot assessment of Ng expression in the ventral hippocampus and frontal cortex, both regions which are critical to NMDAR signaling and Ng function and have been implicated in the pathophysiology of SCZ. Importantly, our proposed model will elucidate a mechanism linking NMDAR regulated signaling to the pathophysiology of SCZ and its concomitant relationship to alcohol use disorders.
Title: **Therapeutic targeting of \( \text{ER\(\beta\)} \) in triple negative breast cancer**

Authors: Jordan M. Reese, Malayannan Subramaniam, Kevin S. Pitel, Elizabeth S. Bruinsma, Anne Gingery, James N. Ingle, Matthew P. Goetz, John R. Hawse

Advisors: John R. Hawse, Ph.D. and Richard Weinshilboum, M.D.

Track: Molecular Pharmacology and Experimental Therapeutics

**Background:** Current treatment options for triple negative breast cancer (TNBC) patients are currently limited to non-specific cytotoxic regimens due to lack of tumoral expression of therapeutic targets such as estrogen receptor alpha (ER\(\alpha\)), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). However, recent data from our laboratory has demonstrated that a subset of TNBCs express ER\(\beta\), a closely related family member of ER\(\alpha\). This presence of ER\(\beta\) offers a potential therapeutic target in TNBC.

**Methods:** Expression of ER\(\beta\) protein was determined using a well characterized and validated ER\(\beta\)-specific monoclonal antibody (PPG5/10) in a cohort of 71 TN breast tumors. To further define the biological functions of ER\(\beta\) in TNBC, novel ER\(\beta\) expressing TN cell lines (MDA-MB-231 and Hs578T) were developed and comprehensively characterized at the level of global gene expression profiling, modulation of important biological pathways, cellular proliferation and response to targeted therapies. These cell lines were also used to establish tumor models in athymic nude mice and monitor tumoral responses to ER\(\beta\) targeting agents.

**Results:** In a cohort of 71 patients with TNBC, approximately 25% showed moderate to high levels of ER\(\beta\) protein. In the triple negative MDA-MB-231-ER\(\beta\) and Hs578T-ER\(\beta\) cell lines, expression of ER\(\beta\) led to inhibition of proliferation in response to both estrogen and multiple ER\(\beta\)-specific agonists. Microarray analysis and RT-qPCR profiling of these cells revealed that estrogen and ER\(\beta\)-specific agonists highly induced the expression of multiple cystatins, a family of small secreted cysteine protease inhibitors, while suppressing the expression of many interleukins. Preliminary evidence from **in vivo** tumor models indicates that estrogen treatment of ER\(\beta\) positive TNBC significantly reduces tumor growth.

**Conclusions:** ER\(\beta\) is expressed in a substantial proportion of TNBCs, a type of breast cancer where targeted therapies are currently lacking. Our **in vitro** and **in vivo** data suggests that an ER\(\beta\)-specific agonist or estrogen treatment offers a novel approach to treat this subset of ER\(\beta\) positive TNBC patients and lays the foundation for future work utilizing patient derived xenografts and clinical trials to test this hypothesis.
Title: The Chromatin Remodeler SMARCA2 Cooperates with GLI1 to Regulate Gene Expression in Pancreatic Cancer Cells

Authors: Stephanie L. Safgren, Anne Vrabel, Nelmary Hernandez-Alvarado, Martin E. Fernandez-Zapico

Advisor: Martin Fernandez-Zapico, Ph.D.

Track: Molecular Pharmacology and Experimental Therapeutics

GLI1, a zinc finger transcription factor, is commonly overexpressed in pancreatic cancer and its expression correlates with poor prognosis in this dismal disease. We have demonstrated in a murine pancreatic cancer model that GLI1 is necessary and sufficient to drive transformation through the regulation of the tumor microenvironment. Although the biological role of GLI1 and targets mediating its oncogenic activity are identified, the transcriptional mechanism used by GLI1 to control gene expression remains for the most part unknown. Understanding this mechanism will provide a greater understanding of the basis for Gli1-induced cellular transformation as well as identify regulatory entry points that can be exploited for therapies. To this end, we initially defined the transcriptional regulatory domains of GLI1 using the yeast-derived GAL4 transcription factor system. Deletion fragments of GLI1 were fused to the GAL4 DNA binding domain (DBD) and transfected in cancer cells to screen for the GLI1 domains holding transcriptional activity. We have identified five independent transcriptionally active domains, all of them located in the C-terminal domain of the protein. Further characterization of these domains demonstrates that SMARCA2, an ATPase subunit of the SWI/SNF chromatin remodeler complex, cooperates with GLI1 to regulate gene expression through one of these domains. SMARCA2 increases the transcriptional activity of GLI1, forms a complex with GLI1 and binds to GLI1 target genes in pancreatic cancer cells. Together these findings provide evidence for a role of SMARCA2 as a co-regulator involved in GLI1-mediated gene expression. The knowledge derived from this study will help define the network of events involved in pancreatic transformation and identify regulatory target molecules suitable for directed cancer therapies.
Title: A component of tail-anchored protein insertion machinery is required for leukemia cell growth

Authors: Jennifer C. Shing, Lonn D. Lindquist, and Richard J. Bram, M.D., Ph.D.

Advisor: Richard J. Bram, M.D., Ph.D.

Track: Molecular Pharmacology and Experimental Therapeutics

Calcium-modulating cyclophilin ligand (CAML) is an endoplasmic reticulum (ER) protein that drives tail-anchored (TA) protein insertion into the ER membrane; however, the importance of this process in the context of cancer cell biology is unknown. Using Eμ-myc transgenic mice expressing a tamoxifen-inducible CAML knockout system, we isolated B cell lymphoid malignancies and deleted CAML by Cre-lox mediated recombination ex vivo. Leukemia cells underwent apoptosis following homozygous deletion of CAML, while heterozygous deletion led to reduced rates of proliferation. Cell death caused by loss of CAML expression was attributed to apoptosis by observing hallmarks including DNA fragmentation, phosphatidylserine externalization, and caspase-3 cleavage. Rescue from apoptosis was achieved by overexpression of BCL-2 or BCL-XL, and/or by caspase inhibitor Q-VD-OPh, although rescued cells showed lower proliferation rates compared to control cells. Analysis of ER stress markers, including XBP1 splicing and GRP78 or CHOP upregulation, indicated that the unfolded protein response was not responsible for apoptotic induction. Lastly, studies involving CAML deletion mutants suggest that the interaction between CAML and TRC40, which is important for TA insertion, is not required for cell survival. Taken together, our study indicates that CAML is essential for the growth of c-Myc-induced lymphoblastic leukemias and potentially proposes this protein as a target for anticancer therapy.
The fluorinated pyrimidine 5-fluorouracil (5-FU) and its prodrugs Capecitabine and Tegafur are widely used for the treatment of many aggressive cancers such as colorectal, breast and head & neck cancers. The responses to 5-FU vary among individuals, with some experiencing drug inefficacy and others suffering from 5-FU toxicity. There are an estimated 1300 cases of 5-FU toxicity-related lethality reported each year in the United States. Reduced enzyme activity of dihydropyrimidine dehydrogenase (DPD, DPYD gene), the rate-limiting enzyme that catalyzes the catabolic degradation and inactivation of more than 80% of administered 5-FU, plays a role in the etiology of 5-FU toxicity. Decreased enzyme activity prolongs 5-FU exposure and increases availability of active metabolites resulting in adverse clinical manifestations such as mucositis, neutropenia, nausea, diarrhea, and neurological symptoms. Overall, 30% of observed toxicities are thought to be due to the well-studied *2A (splice variant), I560S and D949V variants; however, the causes of most toxicity remains unknown. Recently our lab reported data for 80 non-synonymous single-nucleotide polymorphisms (SNPs) within the coding region of DPYD gene to identify additional risk variants for 5-FU toxicity. 30 variants with significantly reduced enzyme activity were detected. These variants were shown to be rare in European populations, but more common in other racial groups such as African American and Asian populations. The study highlighted the importance of functionally characterizing additional rare variants. Therefore, we expressed 47 additional protein-coding missense variants in an isogenic mammalian system to measure their enzyme activity. Percent conversion of 5-FU into dihydrofluorouracil, the reaction catalyzed by DPD, was used as a quantitative endpoint. Of the variants studied, 29 showed significantly reduced enzyme activity compared to wild type, 7 of which had enzyme activity similar to *2A (<25% of wild type). Similar to our previously reported study, many of these variants were enriched in non-Caucasian racial groups. These data add to our growing database of deleterious DPYD variations and strongly suggest that genetic testing for not only common variants, but also rare variants, is equally important to improve the success of predictive markers for 5-FU toxicity. These rare variants are particularly important in individualization of 5-FU therapy in non-Caucasian populations.
Title: **Identifying DNMT3B mediated epigenetic mechanisms using cues from the ICF syndrome**

Authors: Joyce J. Thompson, Emily L. Putiri, Gun Eui Lee and Keith D. Robertson, Ph.D.

Advisor: Keith D. Robertson, Ph.D.
Track: Molecular Pharmacology and Experimental Therapeutics

DNA methylation patterns are established and maintained by the DNA Methyl Transferases (DNMTs) DNMT3A/3B and DNMT1 respectively. Through the influence on chromatin structure, DNA methylation patterns drive differentiation and developmental processes. The de novo DNMTs, DNMT3A and DNMT3B show differential expression patterns and exhibit a degree of non-overlapping function evident during hematopoietic differentiation, suggesting a possibility of target preference within the genome. Using cues from the Immunodeficiency Chromosome instability and Facial anomalies (ICF) syndrome we aim to dissect the DNA methylation mechanisms driven by DNMT3B, mutations in which result in manifestation of one class of the syndrome, ICF1. The ICF syndrome is a rare autosomal recessive disorder, characterized by chromosomal instability, pericentomeric decondensation of chromosomes 1, 9 and 16, lack of mature and memory B-cells in peripheral blood resulting in hypogammaglobulinemia or agammaglobulinemia, facial abnormalities and learning disabilities. Mutations in a zinc-finger protein, ZBTB24, was recently identified as the mutation underlying a second class of the syndrome, ICF2. Since the predominant phenotypes in ICF1 and ICF2 are similar despite the difference in genetic cause, we suspect that ZBTB24 is a DNMT3B associated factor, mediating methylation/demethylation and chromatin dynamics at DNMT3B specific DNA elements/genes. The objective of this study is to investigate the role of ZBTB24 in modulating DNMT3B function. Through co-immunoprecipitation studies we show that ZBTB24 exclusively interacts with DNMT3B suggesting the involvement of ZBTB24 in DNMT3B-mediated epigenetic mechanisms. To further investigate the functional role of ZBTB24 in DNMT3B mediated DNA methylation a preliminary study was carried out in the embryonic carcinoma cell line NCCIT. We acutely depleted ZBTB24 and DNMT3B independently using siRNA mediated knockdown and assayed genome-wide methylation using the HumanMethylation450 Bead Chip (450K array). Initial analysis showed that depletion of ZBTB24 did not affect global methylation to the same extent as DNMT3B. However ZBTB24 affects the methylation of specific loci, particularly of genes involved in neuronal differentiation and lymphocyte function. To further assess the role of ZBTB24 in regulating the methylation status of specific loci we first wish to identify genomic sites bound by this zinc-finger protein and specifically study genes bound by both ZBTB24 and DNMT3B. Since patients with ICF syndrome show a defect in B-cell development and function we suspect that the ZBTB24-DNMT3B interaction has cell-type specific roles which we aim to explore using a B-cell developmental model. Our study holds the potential for unraveling the yet unknown function of ZBTB24 in B-cell development and its impact on DNMT3B mediated epigenetic mechanisms.
Title: Acute depletion reveals novel co-regulation of DNA methylation at conserved loci by DNMT1 and DNMT3B

Authors: Rochelle L. Tiedemann, Emily Putiri, Jeong-Hyeon Choi, and Keith D. Robertson, Ph.D.

Advisors: Keith D. Robertson, Ph.D.
Track: Molecular Pharmacology & Experimental Therapeutics, Visiting Graduate Student

DNA methyltransferases (DNMTs) are responsible for establishing (DNMT3A, 3B, 3L) and maintaining (DNMT1) DNA methylation genome-wide. Aberrant DNA methylation is frequently observed in cancer; however, little is known about how regulation of this modification goes awry. In this study, we aim to understand how DNA methylation is regulated by the DNMTs throughout the genome by identifying specific and broad changes in methylation patterning upon depletion of the DNMTs. We utilize siRNA technology to acutely deplete NCCIT embryonic carcinoma cells of DNMT mRNA (individually and in combination), and then assay the impact on genome-wide DNA methylation patterns using the HumanMethylation450 Bead Chip (450K array). Depletion of DNMT1 (individual/combination) resulted in widespread hypomethylation, most notably in gene bodies, 3'UTRs, and intergenic sequences. DNMT3 knockdown resulted in more specific changes in DNA methylation, but surprisingly, more hypermethylation (predominately in gene bodies) than hypomethylation events occurred. These specific hypermethylation events, particularly in samples with DNMT3B KD, significantly overlapped with sites hypomethylated in DNMT1 KD conditions, indicating a potential cross-regulatory role for DNMT1 and DNMT3B in regulating DNA methylation across gene bodies. To gain a more comprehensive genome-wide view of DNA methylation in the absence of DNMT3B, we performed Methyl-CpG-Binding-Domain (MBD)-seq on DNMT3B KD cells. DNA methylation patterns observed by MBD-seq were dynamic across the genome, and overall displayed trends towards hypomethylation. However, analysis of the most significant methylation changes (> 4-fold) revealed that more hypermethylation events occur in intronic sequences, consistent with results obtained using the 450K array. Notably, these hypermethylation events predominately occur in high-expressing genes that are marked by H3K36me3. To further investigate the overlap between DNMT1 hypomethylated and DNMT3B hypermethylated sites, we examined DNA methylation in HCT116 colorectal carcinoma cells lacking (KO) or over-expressing (KI) DNMT1/DNMT3B. Interestingly, a marked number of Cpg sites that gained methylation in the DNMT3B KO overlapped significantly with sites that became hypermethylated in DNMT1 and DNMT3B KI, and hypomethylated in DNMT1 KO. Additionally, these HCT116 hypermethylated Cpg sites gained methylation in NCCIT DNMT3B KD (individual/combination) and lost methylation in DNMT1 KD. Taken together, these results suggest that DNMT1 and DNMT3B co-regulate DNA methylation at conserved loci across cell types in an opposing fashion, providing novel insight into a potential regulatory mechanism for DNA methylation patterning. Further elucidation of this DNMT1 and DNMT3B co-regulation holds the potential to yield novel therapeutic strategies for correcting aberrant methylation events in cancer.
Chronic traumatic encephalopathy (CTE) is a progressive tauopathy resulting from repetitive traumatic brain injury (TBI). CTE pathology can be found concomitantly with other neurodegenerative pathologies, including amyotrophic lateral sclerosis (ALS). To assess the frequency of CTE pathology in ALS, we screened 91 pathologically-confirmed ALS cases in the Mayo Clinic Florida brain bank with tau immunohistochemistry. Six male cases had pathologic features of ALS plus CTE (6.6%). A screen of 95 ALS cases from the Boston VA ALS brain bank found 3 cases of ALS+CTE (3.2%). Clinicopathologic comparison was performed with 8 additional cases with ALS+CTE from the Boston University (BU) CTE brain bank. The BU cohort included 7 American football players (2 NFL, 4 college, 1 high school), 1 professional boxer, and 1 soccer player. All 17 cases of ALS+CTE had marked neuronal cell loss of the anterior horn cells, and neuronal cytoplasmic inclusions immunoreactive for TDP-43 in residual neurons as well as pathologic features of CTE, including prominent focal hyperphosphorylated tau perivascularly and at the depths of cerebral sulci. Of the 17 cases, 2 cases had very mild CTE pathology (Stage I), 13 cases had mild pathology (Stage II), 2 cases had moderate pathology (Stage III), and no cases had severe pathology (Stage IV). Biochemically, TDP-43 protein expression in the frontal cortex and spinal cord was similar between mixed cases and ALS and CTE disease-matched controls. Cognitive and behavioral abnormalities, and history of TBI were not well-documented in the cases screened from ALS brain banks. In summary, the comorbidity of these two neurodegenerative diseases raises the possibility of a clinicopathologic relationship and shared pathogenesis.
Title: Adeno-associated virus mediated C9orf72 expanded GGGGCC repeat expression causes both behavioral and motor impairments in a mouse model of FTD/ALS

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Advisor: Leonard Petrucelli, Ph.D.

Track: Neurobiology of Disease

The C9orf72 hexanucleotide repeat expansion (GGGGCC) has been identified as the major genetic cause underlying both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in 2011, yet the molecular pathogenesis of this expanded repeat remains unknown. To date, there has been no report of a mouse model expressing this pathological repeat expansion that successfully recapitulates the characteristic behavioral/motor deficits associated with c9FTD/ALS. Our preliminary results showed that adeno-associated viral (AAV)-mediated transduction of an expanded number of GGGGCC repeats in mouse primary neurons resulted in accumulation of nuclear RNA foci detected by RNA fluorescent-in-situ hybridization (FISH) and repeat-associated non-ATG (RAN) translated polypeptides which were absent when expressing non-pathogenic number of repeats. We therefore developed a novel mouse model expressing an expanded GGGGCC repeat or a non-pathological repeat length RNA mediated by AAV transduction into the neurons of the central nervous system (CNS) of neonatal mice through intracerebroventricular (ICV) administration. After 6 months of age, these mice were subjected to a battery of behavioral and motor performance tests. Mice expressing an abnormal number of repeats exhibited both behavioral and motor abnormalities when compared to mice expressing non-pathogenic repeats. In addition, affected mice also had a decreased brain weight compared to controls. Therefore these important data suggest that the expression of expanded repeats in the CNS can recapitulate some of c9FTD/ALS-like phenotypes and that further characterization of this mouse model will be a useful tool in elucidating the pathogenic role of expanded repeat expression in c9ALS/FTD.
Title: HDAC6-dependent acetylation and assembly of tau

Authors: Dah-eun Chloe Chung, Casey N. Cook, Ph.D., Yari Carlomagno, Ph.D., and Leonard Petrucelli, Ph.D.

Advisor: Leonard Petrucelli, Ph.D.

Track: Neurobiology of Disease

The microtubule-stabilizing tau protein, upon abnormal hyperphosphorylation and aggregation, can disrupt the microtubule network in the central nervous system. This can contribute to neural dysfunction and cognitive defects in tauopathy, a wide collection of neurodegenerative diseases that share aggregated and accumulated tau as their pathological hallmark. The underlying pathogenic mechanisms to account for tau hyperphosphorylation in diseases remain elusive while a better understanding is necessary in order to develop effective treatments for tauopathy. Our lab previously reported that histone deacetylase 6 (HDAC6) regulates acetylation of tau on its KXGS motifs, which are crucial for the ability of tau to bind and stabilize microtubules. We also discovered that tau acetylation interferes with the ability to assemble into filaments, which was reversed in the presence of HDAC6. This indicates that HDAC6 regulates tau acetylation on residues that are critical for tau assembly. Interestingly, we also found that deacetylation on lysine residues and phosphorylation on serine residues within KXGS motifs compete to modify these motifs. Therefore, enhancing acetylation could be utilized as a novel approach to prevent phosphorylation on these motifs. To provide additional insight into the relationship between HDAC6 and tau, we used mass spectrometry to identify acetylation sites on tau that are specifically regulated by HDAC6 in vitro. We then determined which HDAC6-responsive acetylation sites in tau are critical for assembly. In agreement with previous results, we also found that acetylation on tau's third KXGS motif blocks pathological phosphorylation on the same motif. In future studies, tau acetylation and phosphorylation in human patients and animal models of tauopathy will be assessed by using novel site-specific acetyl-tau antibodies for HDAC6-responsive sites of interest.
Deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) is an emerging therapy for patients with treatment-resistant obsessive-compulsive disorder (OCD). While fronto-striatal dysfunction has been implicated in the pathogenesis of OCD, the mechanism by which VC/VS DBS exerts its effects on disease-associated networks remains unclear. Here, we used fMRI to test the hypothesis that VC/VS DBS exerts its therapeutic effect through modulation of brain areas distal to the site of stimulation. Mayo Clinic IRB approved this study. Patients underwent image-guided stereotactic implantation of bilateral VC/VS DBS leads using the Schaltenbrand and Wahren atlas. During awake surgery, intraoperative monopolar review was performed for each lead. Each patient’s mood, energy, anxiety, level of OCD symptom relief, and presence of side effect was evaluated during high frequency stimulation at each contact from 1 to 8V. Following lead placement and externalization, patients underwent 1.5T fMRI under general anesthesia. During gradient echo echo-planar imaging fMRI acquisition, stimulation was administered in a block design via an external pulse generator, using parameters that caused acute symptom relief or side effect. Intraoperative monopolar review revealed stimulation parameters that caused acute symptom relief, paired with a feeling of “clear-mindedness” or euphoria. Functional MRI data obtained during application of these settings consistently revealed blood oxygen level-dependent (BOLD) signal change in ipsilateral cingulate, putamen, insula, and motor cortices. Parameters that caused patients to feel “foggy-minded” or “scattered” resulted in BOLD signal change predominantly within dorsolateral prefrontal cortex, thalamus, and cingulate cortices. Our results support the hypothesis that VC/VS DBS results in widespread neural circuit modulation in areas distal to the DBS target. Furthermore, the strong dependence on BOLD signal patterns on stimulation parameters and intraoperative response suggest that network modulation may underlie the therapeutic mechanism of this treatment.
Title: MRI-guided, stereotactic delivery of intraspinal stimulating electrodes.

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Advisors: Kendall H. Lee, M.D., Ph.D. and J. Luis Lujan, Ph.D.

Track: Neurobiology of Disease – Neural Engineering

Intraspinal microstimulation (ISMS) is an emerging technique for restoring function lost to paralysis following spinal cord injury. Multiple animal studies have shown ISMS is capable of producing selective control of limb movements while improving fatigue resistance. Nevertheless, several limitations have prevented translation of ISMS technology into humans. Perhaps the most significant obstacle to translation is the difficulty of targeting specific spinal cord regions responsible for selective motor function. Current ISMS methods for restoring locomotion often involve manual implantation of multiple stimulating electrodes or electrode arrays, followed by characterization of stimulation-evoked motor responses. However, electrode targeting relies on external anatomical landmarks, and is thus susceptible to targeting errors due to anatomical differences between animals. Furthermore, there is a large potential for trajectory deviations during electrode insertion due to the mechanical properties of the tissue and inconsistent forces applied during manual electrode insertion.

To minimize these targeting errors and thus improve selectivity, we have designed a frame-based magnetic resonance imaging (MRI)-compatible stereotactic targeting and electrode delivery system. The stereotactic system described herein offers the potential for improved targeting and selective stimulation of neuronal populations responsible for specific motor function, thereby reducing the spatial inaccuracy of existing methods. In turn, use of this system will minimize insertion-related trauma by reducing the number of insertion attempts required to successfully implant electrodes into desired locations.

To test this stereotactic delivery platform, we have established a porcine model of ISMS that offers significant similarities with human anatomy and physiology and is paramount for expedited translation of this technology to human applications. Initial application of the stereotactic system is intended for ISMS. However, the targeting and delivery system described herein will have additional therapeutic applications such as fine needle biopsies, and intraspinal delivery of drugs and stem cells.
We have previously demonstrated that positively charged oligo[poly(ethylene glycol) fumarate] (OPF+) scaffolds loaded with Schwann cells are an optimal combination of polymer and cell type for bridging SCI lesions in rats. However, the regeneration achieved has not been sufficient for inducing functional recovery after complete spinal cord transection. Therefore, in this study we characterized the CNS tissue response to scaffold implantation. When scaffold implanted animals were compared to animals with transection injuries only, collagen scarring, cyst formation, astrocyte reactivity, myelin debris, and chondroitin sulfate proteoglycan (CSPG) accumulation were significantly reduced, accompanied by increased activated macrophages. Although the tissue reaction was beneficial in the short term, we identified a chronic fibrotic host response, resulting in scaffolds surrounded by collagen at 8 weeks. In order to prevent this fibrotic response, poly(lactic-co-glycolic acid) (PLGA) microspheres containing rapamycin were incorporated into OPF+ scaffolds implanted into rats after SCI. Significant functional recovery of the hind limbs was observed in these animals, accompanied by decreased collagen scarring and smaller diameter blood vessels within the scaffold channels. These studies demonstrate that OPF+ scaffolds improve the environment for CNS regeneration, and that preventing the host fibrotic response allows for functional recovery after complete transection of the mammalian spinal cord.
Title: Dysregulated Adenosine Signaling during Adolescence is Associated with Anxiety-, Depression- and Impulsive-Like Behavior in Mice

Authors: David J. Hinton, YuBin Choi, Alfredo Oliveros, Chelsea A. Vadnie, Sun Choi, Doo-Sup Choi, Ph.D.

Advisor: Doo-Sup Choi, Ph.D.

Track: Neurobiology of Disease

During adolescence, dysregulation of neurotransmitters could alter the maturation of brain circuits involved in reward, mood and cognitive behaviors. Caffeine is the most commonly consumed drug in the world. Despite several notable beneficial effects of acute caffeine that have been documented in adults, excessive caffeine consumption during adolescence has been shown to be associated with psychiatric disorder symptoms which include attention deficit hyperactivity disorder, anxiety, aggressiveness, sleep disturbances and addiction. In the central nervous system, caffeine inhibits mainly adenosine A1 and A2A receptors. However, prolonged exposure to chronic caffeine has been shown to mainly downregulate A1 receptor in several brain regions. Specifically, neonatal caffeine exposure for about 7 days increases mRNA levels of the A1 receptor while more prolonged administration during gestation and lactation promoted a decrease in adenosine A1 receptors in the whole brain of both dams and neonates. Thus, it is possible that many of the behavioral effects elicited by chronic caffeine are a result of reduced A1 receptor expression. In the present study, we examined the role of adenosine signaling in intermediate phenotypes associated with psychiatric disorders using a mouse model of chronic caffeine-induced adenosine dysregulation. Adolescent mice self-administered caffeine (1 mg/ml) for 115 days beginning at 4 weeks of age. We found that chronic caffeine-exposed mice showed reduced motivation to explore a novel environment compared to mice consuming water (control mice). Mice chronically consuming caffeine also had less motivation to walk on the accelerating rotorod compared to control mice. Caffeine-exposed mice buried less marbles in the marble-burying test, suggesting that chronic caffeine reduced attentive behaviors. Furthermore, caffeine consuming mice showed reduced motivation to escape the forced swim test suggesting the presence of depression-like behavior compared to control mice. In addition, caffeine consuming mice exhibited anxiety-like behavior as they spent significantly less time in the center zone of a novel open-field chamber and less time in the open arm of the elevated plus maze compared to control mice. In Pavlovian association experiments, mice chronically consuming caffeine exhibit increased impulsive behavior to obtain a hedonic reward (20% sucrose). Overall, these data indicate that chronic inhibition of adenosine receptors during adolescence may contribute to increased impulsivity as well as anxiety- and depressive-like behaviors in adult mice.
Title: A transgenic zebrafish model for monitoring glucocorticoid receptor activity

Authors: Randall G. Krug II, Tanya L. Poshusta, Kimberly J. Skuster, MaKayla R. Berg, Samantha L. Gardner, Karl J. Clark, Ph.D.

Advisors: Karl J. Clark, Ph.D., Stephen C. Ekker, Ph.D., John R. Henley, Ph.D., Rajiv Kumar, M.D., Susannah J. Tye, Ph.D.

Track: Neurobiology of Disease

Gene regulation resulting from glucocorticoid receptor and glucocorticoid response element sequence interactions is a hallmark feature of the vertebrate stress response. Imbalances in stress response signaling have been linked to socio-economically crippling neuropsychiatric disorders, and thus in vivo models are needed to help understand disease progression and management. Therefore, we developed a transgenic zebrafish reporter line with six glucocorticoid response element sequences used to promote expression of a short half-life green fluorescent protein (GFP) following glucocorticoid receptor activation. To characterize the ability of the reporter line to model glucocorticoid receptor signaling, transgenic larvae were either treated with exogenous glucocorticoid receptor ligands, or exposed to stressors including drugs of abuse or hyperosmotic conditions. The changes in GFP expression relative to control fish were assessed using both qRT-PCR and high-resolution imaging. Herein, we show that chronic and acute glucocorticoid treatment causes transgene activation in numerous tissues including the brain, and provides a single cell resolution in the effected regions. The specificity of these responses is demonstrated using the partial agonist mifepristone and mutation of the glucocorticoid receptor with transcription activator-like effector nucleases (TALENs). Importantly, the reporter line also modeled the dynamics of endogenous stress response signaling, including the increased production of the glucocorticoid cortisol following exposure to stress and fluctuations of basal cortisol concentrations with the circadian rhythm. Collectively, these results characterize our newly developed reporter line for elucidating modifiers of stress response signaling, which may provide insights to the neuronal mechanisms underlying neuropsychiatric disorders.
Title: A zebrafish model to characterize host stress response and gut microbiota interaction

Authors: Han B. Lee, Tanya L. Poshusta, Dakota C. Jacobs, Randall G. Krug II, Xianfeng Chen, Elizabeth van Tuinen, and Karl J. Clark, Ph.D.

Advisors: Karl J. Clark, Ph.D., Stephen C. Ekker, Ph.D.

Track: Neurobiology of Disease

It is increasingly clear that gut microbiota interact with the neuroendocrine and immune system to modulate the host’s responses to environmental stressors. Disruption of gut microbiota results in altered anxious behaviors and brain chemistry in mice and zebrafish. When we derived germ-free (GF) larval zebrafish, the GF fish showed increased stress responsiveness compared to zebrafish with normal gut flora in hyperosmotic (100 mM NaCl) and physical (shaking) stress assays—demonstrating that the microbiota modulates host stress responses. It is largely unknown what host genes are the cognate partners of the gut microbiota responsible for these behavioral and physiological changes. We are developing a zebrafish model to characterize these host factors that modulate the gut microbiota with a focus on stress responses. We observed that 1h-acute stress did not significantly alter microbial compositions in wildtype zebrafish while microbial changes during the larval development were captured in 16S ribosomal RNA gene (rDNA) sequencing. We developed a novel prolonged-stress experiment in which larval zebrafish are subject to physical (shaking) stress overnight (10-14h) and shown to have a decreased innate immune response. We are using the system to investigate the effect of prolonged-stress on microbial compositions in zebrafish with the 16S rDNA sequencing. In addition, our data indicate that wildtype zebrafish in the Zebrafish Core Facility (ZCF) at the Mayo Clinic have a consistent microbial signature of selective commensal microbial strains in quantitative PCR (qPCR) assays. We are investigating the varying relative-compositions in the microbiota among wildtype and mutant fish strains (e.g. nod2 and il6) with qPCR. These genes are involved in inflammatory disorders (e.g. inflammatory bowel disorders) and upregulated pro-inflammatory responses. Comparing the microbial compositions of these mutant fish to those of wildtype fish under stress or non-stress conditions will increase our understanding on how host genes modulate the gut microbiota in the context of stress responses. Taken together, our zebrafish model might be useful to characterize the host factors that interact and alter the microbiota in zebrafish providing the microbiome field with unique insights difficult to be gained in other model systems.
Title: Identification of Alzheimer’s disease protective genetic variants in non-demented elderly individuals with APOE ε4/ε4 genotype

Authors: Lili N’Songo, and Minerva Carrasquillo, Yan Asmann, SaurabhBaheti, Gaihua Zhang, Thuy Nguyen, Richard Caselli, Neil R. Graff-Radford, Ronald C. Petersen, Guojun Bu, Ph.D., Nilufer Ertekin-Taner, M.D., Ph.D.

Advisors: Guojun Bu, Ph.D., Nilufer Ertekin-Taner, M.D., Ph.D.

Track: Neurobiology of Disease

Alzheimer’s disease (AD) is the most common type of dementia. AD can be divided into 2 different categories: early onset AD (EOAD) which accounts for <1% of all AD cases and is known to be caused by dominant mutations; and late onset AD (LOAD) resulting from complex interactions between environmental and genetic risk factors. Apolipoprotein (APOE) isoform ε4 is the most well established and strongest genetic risk factor for LOAD with an increased risk of ~4 fold for heterozygous carriers of this allele and up to ~30 fold for homozygote carriers. However, it does not account for all LOAD cases and is not alone sufficient to cause the disease. Our group has access to a unique longitudinally followed cohort of 24 APOE ε4/ε4 non-demented individuals aged ≥ 75 years old at last evaluation. The goal of this study is to identify genetic variants associated with protection from LOAD in those cognitively intact APOE ε4/ε4 elderly carriers.

Whole exome sequencing was performed on genomic DNA samples using the Sure Select V4 + UTR Exome Capture kit on the Illumina HiSeq platform. After quality control analysis, coding variants were prioritized by association p-value for Fisher’s exact test against Exome Variant Server Caucasian controls. Variants with a p-value > 0.05 were excluded. The variants which were selected for follow-up genotyping by Sequenom in an AD case-control cohort, met the following criteria: 1) Variants significantly enriched in our cohort (p-values < 3.3x10^-5 after Bonferroni correction), as well as any other variants in those same genes with a SIFT or Polyphen prediction score of possibly damaging or damaging, 2) Variants with a suggestive enrichment in our cohort (p-values < 1x10^-3) and a SIFT or Polyphen prediction score of possibly damaging or damaging, 3) Variants within candidate AD risk gene.

After statistical analysis, several variants showed a significant association for enrichment in APOE ε4/ε4 non-demented elderly cohort compared to the general population.

Whole exome sequencing of elderly, non-demented subjects with APOE ε4/ε4 genotypes can identify coding variants that may be protective against AD. The study of the functional effect of those variants would allow us to have a better understanding of the molecular mechanism of AD and to identify possible biomarkers and therapeutic targets.
Title: **RdRP-mediated pan-viral protection via stable activation of innate immunity**

Authors: Meghan M. Painter, M.S., and Moses Rodriguez, M.D.

Advisor: Moses Rodriguez, M.D.

Track: Neurobiology of Disease

All RNA viruses except retroviruses encode RNA-dependent RNA polymerases (RdRPs), which produce viral double-stranded RNA (dsRNA) that can trigger acute innate immune activation through sensors such as toll-like receptor 3 (TLR3), retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated gene 5 (MDA5). Mammalian genomes lack RdRPs, and RNA viruses sequester them tightly within specialized membranous compartments. This concentrates the viral synthetic apparatus, but could also be a means of immune sensor avoidance. Here, we show that unsequestered picornavirus RdRP expression in transgenic mice profoundly reconfigures innate immunity. RdRP-expressing mouse tissues have high, stable (life-long) upregulation of many antiviral sensors/effectors (mRNAs elevated up to 300-fold), including prominent interferon (IFN) stimulated genes (ISGs). This augmented ISG network is considerably broader than that induced by viral infection itself. Equally remarkably, RdRP mice are developmentally and phenotypically indistinguishable from wild-type (WT) mice, with normal longevity. The mice display marked resistance to viral diseases that is independent of the adaptive immune system; RdRP-Rag1-/- mice displayed the same gene expression profiles and immunity to lethal viral challenge. Additional mouse genetic studies further elucidated that the mechanism is MDA5- and MAVS-dependent, and also IFNoβR-dependent despite normal circulating type I IFN levels. This antiviral phenotype can be reproduced in human monocyte-macrophage lineage cells, where RdRP expression stably elevated antiviral gene mRNAs up to > 550-fold in a pattern virtually identical to RdRP mouse tissues, and HIV-1 infection was blocked. The mechanism requires catalytically-active RdRP, is unaffected by pervasive RdRP mRNA alteration, and involves templating on host RNA. Together these data reveal that unsequestered RdRP expression can produce highly amplified, broadly antiviral, and sustained mammalian innate immune system activation that it is stable and well-tolerated throughout life.
Title: **Critical role for protease activated receptor 2-driven interleukin-6 signaling in astrogliosis**

Authors: M. Radulovic, H. Yoon, J. WU, I.A. Scarisbrick, Ph.D.

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Track: Neurobiology of Disease

Reactive astrogliosis is a key component of central nervous system (CNS) injury and disease. Whereas early stages of astrogliosis appear beneficial and initiate wound healing, chronically reactive astrocytes contribute to glial scar formation hindering neural regeneration. Despite its central importance, the underlying molecular mechanisms of astrogliosis are incompletely understood. We recently demonstrated that neurosin (kallikrein 6), a CNS endogenous secreted serine protease is induced in reactive astrocytes and exhibits prolonged expression in astroglial scar tissue in cases of human spinal cord trauma, multiple sclerosis and glioblastoma multiforme in addition to animal models of these disorders. In the current study, we address the hypothesis that neurosin participates in astrogliosis by proteolytic cleavage and activation of a G-protein coupled receptor, protease activated receptor 2 (PAR2). First, we show that like neurosin, PAR2 is elevated at sites of CNS injury, including contusion compression injury of the murine spinal cord. Furthermore, we show that the ability of recombinant neurosin to drive key hallmarks of astrogliosis, including a rapid transformation of primary murine astrocytes from an epithelioid to a stellate morphology in vitro, secretion of the pro-inflammatory cytokine interleukin 6 (IL-6) and increased expression of glial fibrillary acidic protein (GFAP) are significantly reduced in astrocytes derived from PAR2 knockout mice. Neurosin also promoted increased levels of a signal transducer and activator of transcription (STAT3). Notably, genetic deletion of PAR2 or pharmacologic blockade of STAT3 signaling using Stattic, blocked neurosin-induced IL-6 secretion in primary astrocytes. Moreover, genetic deletion of PAR2 was associated with reductions in molecular signatures of astrogliosis in the case of murine SCI, including decreases in markers of astrogial markers such as GFAP and in Th1 pro-inflammatory cytokines, including IL-6. Together, these results indicate that the neurosin-PAR2 signaling axis plays essential roles in promoting astrogliosis and therefore should be investigated as a potential therapeutic target to modulate the astroglial scar in cases of CNS injury and disease.
Microinfarcts are a common pathology identified at autopsy and are associated with cognitive impairment and dementia. They are not visible on conventional MRI; therefore, their relationships to brain atrophy in vivo are unknown. Our objective was to investigate the pattern of brain atrophy rates in patients with microinfarcts compared to patients without microinfarcts. We retrospectively identified cases (n=15) with one or more microinfarcts at autopsy and controls with no microinfarcts (n=15) matched on age, sex, APOE4 status, MMSE score, and pathologic diagnosis of Alzheimer’s disease (AD) to the cases. All subjects had serial MRI up to 3.5 years before death. Rates of whole brain atrophy and ventricular expansion were calculated using the boundary shift integral method. Voxel-wise gray matter atrophy rates were determined using Tensor Based Morphometry-Symmetric Diffeomorphic Image Normalization. Group differences were displayed using two-sided t-tests in SPM5 thresholded at p<0.001, with adjustment for Braak neurofibrillary tangle stage. Rate of whole brain atrophy was higher (p=0.05) in cases with microinfarcts compared to controls with no microinfarcts at autopsy. Rate of ventricular volume increase showed a similar trend (p=0.37) in cases with microinfarcts. Voxel-wise analysis revealed greater rates of atrophy in bilateral precuneus, primary motor and somatosensory cortices in cases with microinfarcts compared to controls with no microinfarcts. Microinfarcts are associated with an increased rate of brain atrophy independent of AD pathology. The increased gray matter loss occurs at the border zones of the major vascular territories, which are areas that are susceptible to ischemia.
Title: Histological Characterization of a Mid-Cervical Contusion Injury in Rats

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Advisors: Carlos B. Mantilla, M.D., Ph.D. and Gary C. Sieck, Ph.D.

Track: Neurobiology of Disease

Damage to the spinal cord is a coalesced result of the primary insult to the cord and the secondary injury cascade that follows the impact. In order to identify therapeutic targets for spinal cord injuries (SCI), we must be able to use an injury model that closely represents the damage caused in the human spinal cord. Mid-cervical contusion injury is one of the most common types of SCI and can lead to respiratory impairment via a complete or partial paralysis of the diaphragm muscle. Thus, it is important to characterize the injury and the extent of loss of the phrenic motor neuron (PhMN) pool that compromise respiratory function. In this study, we assessed the degree of damage following a 100kD unilateral C4 contusion injury in rats. We hypothesized that the extent of injury as measured by the cavity volume and loss of PhMNs will be maximal and stable by 7 days post-injury. However, tissue scarring should increase progressively past 7 days post-injury to 14 days post-injury. Intra-pleural injections of Alexa 488-conjugated cholera toxin subunit B were employed to label the PhMNs in the spinal cord. Immunohistochemical analysis by Wisteria Floribunda Agglutinin that labels perineural net formation was used to assess the extent of tissue scarring at the injury site. Quantitative confocal microscopy was used to quantify volume of the cavity formed following the injury and compare loss of PhMNs ipsilateral and contralateral to the injury. Our results show that there is no difference between the amount of tissue scarring and cyst size at 7 and 14 days post-injury. The PhMN counts are comparable at both time points, although there is a significant decrease in PhMN counts on the ipsilateral side of the injury. Our results confirm that cyst formation and loss in PhMN have plateaued by 7 days post-injury and tissue scarring is also comparable at both 7 and 14 days post-injury. Collectively, these findings illustrate the loss in tissue integrity and formation of an inhibitory environment at the injury site post contusion injury. These results also corroborate that the C4 unilateral contusion model is a useful tool in the assessment of effective therapeutic strategies for SCI, although, an earlier intervention might be needed to assess its effectiveness on PhMN loss.
Title: A duality of roles for perforin in CD8+ T cell- glioma interactions: contributions to cytotoxicity and altered vascular permeability

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Track: Neurobiology of Disease

Glioblastoma multiforme (GBM) is a highly aggressive malignancy of the central nervous system that exhibits extensive vascularization and a high degree of invasiveness into the surrounding brain parenchyma. Recent evidence in both animal model systems and clinical trials have demonstrated the viability of immunotherapeutic strategies at selectively targeting tumor cells, with the generation of tumor antigen-specific CD8+ T cells strongly correlating to reductions in tumor burden. We have utilized the GL261 glioma model in immune-competent C57BL/6 mice to demonstrate the efficacy of a novel picornavirus vaccination approach. Treatment of established gliomas with a picornavirus engineered to express tumor-specific antigens delayed tumor progression and extended survival, outcomes which were accompanied by increased tumor-specific CD8+ T cell responses in the brain. Importantly, both intracranial and peripheral vaccination routes were effective at promoting anti-tumor CD8+ T cell responses. Perforin, an immune effector molecule, is well characterized for its role in CD8+ T cell-mediated cytotoxicity. Our data demonstrate that, in addition to this role in direct tumor cell lysis, perforin also contributes to alterations in tumor vascular permeability. Untreated perforin deficient mice bearing GL261 gliomas demonstrated no difference in tumor burden compared to C57BL/6 controls. However, the heterogeneity of tumor vascular permeability in mice lacking perforin was significantly reduced. As such, we propose that perforin serves dual functions in CD8+ T cell-glioma interactions, contributing to both direct killing of tumor cells and immune-mediated alterations to tumor vasculature.
CypA (encoded by the gene \textit{PPIA}) is a peptidyl-prolyl isomerase that binds lentiviral capsids. It catalyzes the cis/trans isomerization of the G89-P90 peptide bond in the HIV-1 capsid protein. CypA has distinctive and at times opposite context- and species-specific dependent effects. The protein promotes HIV-1 infectivity in most human cells but contrasting effects occur with simian lentiviruses and/or simian cells. While multiple possibilities have been proposed for the role of CypA in the viral life cycle, a unifying mechanistic explanation remains elusive. It has been hypothesized that this peptidyl-prolyl isomerase protects HIV-1 from human cell restriction by competing with binding of the restriction factor Trim5α to capsid, shielding the virus from nucleic acid sensors, or directing it to a Nup358-dependent nuclear import pathway. Recent evidence suggests that another peptidyl-prolyl isomerase CypB (encoded by the gene \textit{PPIB}), may oppose CypA effects in human cells. To investigate the roles of these proteins in the HIV-1 life cycle, we sought to create single (PPIB) and double (PPIA + PPIB) gene knockouts in a human CD4+ T cell line (Jurkat).

We utilized TAL effector nucleases (TALENs), which have emerged as an important gene-editing tool over the last few years. They are designable site-specific nucleases engineered from fusions of FokI endonucleases to modularly assembled transcription activator-like effectors from \textit{Xanthomonas} spp. When the two Fok1 nucleases dimerize upon DNA binding, it induces a double stranded break in the DNA. This is repaired by the error prone non-homologous end joining pathway that often leads to small insertions or deletions at the breakage site, causing a frame shift and thus a gene knockout. We aimed to take advantage of this technology to target \textit{PPIB}.

We designed and constructed two TALEN pairs targeting PPIB exons 1 and 2, respectively, using the Golden Gate assembly method. Diploid parental Jurkat cells and an existing PPIA -/- Jurkat cell line were transfected with TALEN pair plasmids. Targeted cells were single cell-cloned by limiting dilution. Strikingly, screening by western blotting revealed that 10 out of 24 clones were PPIB -/- after exon 1 targeting and 5 out of 28 after exon 2 targeting. These clones were further confirmed as \textit{PPIB} -/- by sequencing of targeted chromosomal regions in the spacer region between TALEN pair binding sites, which revealed diverse bi-allelic disruptions. We are currently characterizing the phenotypes of these cells with respect to the HIV-1 life cycle and re-expressing CypB in the knock out cells to see if this will restore the wild type phenotype.
Autosomal recessive polycystic kidney disease (ARPKD) is an inheritable cystic disease, for which there are currently no effective therapies. Increased levels of 3’-5’-cyclic adenosine monophosphate (cAMP) are found in the kidney and liver in various animal models of polycystic kidney disease (PKD). Intriguingly, reduced levels of cAMP in kidneys by genetic elimination of arginine vasopressin (AVP) or with AVP antagonists inhibits cystogenesis. Cardiac B-type natriuretic peptide (BNP) is a guanylyl cyclase A (GC-A) agonist, central in the control of intravascular volume and arterial pressure. Additionally, BNP inhibits fibroblast proliferation and counteracts the activation of renin-angiotensin-aldosterone system/AVP release. These characteristics, together with its blood pressure-lowering propensity, make BNP a promising therapeutic for preventing the progression of PKD. In this study, we interrogated the therapeutic effect of single dose AAV mediated long-term BNP overexpression in the rat model of ARPKD (PCK). We employed adeno-associated virus 9 (AAV9) under a CMV promoter to achieve BNP overexpression in age matched PCK female rats, treated at 3 days of age by intraparenteral injection. Diastolic blood pressure at three months was significantly lower with treatment, (treated 71.24 +/-9.94mmHg, control 87.61 +/-4.83mmHg). Furthermore, echocardiography data demonstrated treated PCK rats were significantly protected from a decrease in cardiac output (EF teich, treated 74.2 +/-2.96, control 68.0 +/-2.97), and thickened intraventricular septum (IVSd, treated 1.32mm +/-0.04, control 1.68mm +/-0.34). Urinalysis showed a significantly reduced 24 hour urine volume (treated 8.80 mL/24hr +/-2.69, untreated 13.2 mL/24hr +/-1.15), improved urine concentrations of sodium (treated 130.3mmol/L +/-38.7, untreated 85mmol/L +/-19.7) and potassium (treated 395.8mmol/L +/-41.1, untreated 282.1mmol/L +/-75.3) in treated PCK. When compared to untreated animals, the estimated glomerular filtration rate was significantly higher in vector-treated rats. Kidney injury molecule-1 expression detected in urine was also significantly lower in treated animals (treated 21.2 +/-6.18ug/24hr, untreated 28.5 +/-0.81ug/24hr), compared to controls. Histologically, BNP overexpression preserved glomerular architecture, retarded basement membrane thickening, and glomerular sclerosis; however no notable effect was observed on renal cystogenesis. Real-time RT-PCR analysis demonstrated BNP treatment significantly reduced (1.8 fold) hepatic expression of the fibrosis-associated gene fibronectin. These observations suggest that single dose, long term BNP overexpression can preserve the renal urine-concentrating ability, protect renal function and architecture, as well as establish significant cardiac protection. Importantly, these effects occurred despite progressive renal cystogenesis. The present study demonstrates the efficacy of AAV vector-mediated BNP gene therapy in protecting cardio-renal organ integrity, and provides a novel opportunity for the treatment of ARPKD-associated renal damage, secondary to progressive renal cystogenesis.
Aging and obesity are linked to an increased risk of type 2 diabetes (T2D), a metabolic disorder characterized by relative insulin insufficiency that results in hyperglycemia. Increased β-cell proliferation would benefit patients with T2D. We have previously shown that the use of mouse INS2 or ELS internal promoter restricts the expression of the transgene to the β-cells or the acinar cells, respectively, following systemic AAV delivery. When the β-cell-targeted vector system was used to over-express SV40 large T antigen, a 10-fold increase in β-cell proliferation was observed in aged mice (control, 0.4%; SV40T, 3.9%). In this study, we investigated the potential of glucokinase (GCK) and betatrophin (BTRP) to induce β-cell proliferation. GCK is a hexokinase that catalyzes the phosphorylation of glucose in the first step of glycolysis. Unlike the other hexokinases, GCK is not inhibited by its product and is used for glucose-sensing by the β-cells. When GCK was over-expressed in β-cells of young 6-week old mice, a 2-fold increase in β-cell proliferation was observed (control, 2.3%; GCK, 5.3%). This correlated with enhanced β-cell mass and enhanced glucose clearance. When aged mice were treated with the same GCK vector, we observed a 6-fold increase in β-cell proliferation (control, 0.8%; GCK, 4.2%) and enhanced glucose clearance. When aged, high-fat-diet mice were treated with the GCK vector, a 3-fold increase in β-cell proliferation (control, 1.5%; GCK, 4.4%), but no notable effect on glucose clearance, was observed. BTRP was recently identified as a liver- and fat-secreted hormone that induces β-cell replication. When the acinar-cell-targeted AAV vector was used to over-express BTRP in aged mice, β-cell proliferation increased by 2-fold (control, 0.8%; BTRP 2.0%) without notable effects on glucose tolerance. These data indicate the utility of GCK for enhance β-cell proliferation in young, aged and obese mice.
Title: Rapid measles virus intercellular transport in the airway epithelium: roles of the matrix protein

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Advisor: Roberto Cattaneo, Ph.D.

Track: Virology and Gene Therapy- Molecular Virology

Measles Virus (MeV) is a member of the Paramyxoviridae family and part of the Morbillivirus genus in the order Mononegavirales. Though replication of MeV in activated immune cells accounts for pathogenesis, MeV is eventually transferred to epithelial cells by circulating infected immune cells. Once in the epithelia, this cell-associated virus can infect neighboring cells rapidly and discreetly without activation of the immune system through a mechanism that still remains unclear. Towards understanding this process, we use well-differentiated primary human airway epithelia grown at the liquid-air interface. In these epithelial sheets, we have observed the formation of large, infectious centers in the absence of cell fusion, while epithelial function remains intact. We also observed that the viral assembly organizer matrix protein co-localizes with actin rings on the apical side of columnar epithelial cells. Matrix accumulates selectively at infected-to-uninfected cell borders and we have documented that cytoplasmic green fluorescent protein flows from infected to uninfected cells at apical locations by video microscopy. We hypothesize that the MeV fusion apparatus opens pores at the adherens junction location of the epithelial receptor nectin-4. The cytoskeleton anchored to the adherens and tight junctions may constrain these pores, making them into “canals” that allow the flow of viral components through epithelia without full assembly of mature viral particles, but rather through the rapid transport of the viral nucleocapsid through the virus-induced canals and along the actin cytoskeleton. The matrix protein of MeV is known to interact with the fusion apparatus, ribonucleocapsid, and the actin cytoskeleton. We thus posit that the matrix protein takes advantage of this interaction with the actin cytoskeleton to facilitate transport of viral components through the canals. To test this hypothesis, we have generated a set of recombinant viruses with mutations in the matrix gene. These mutations are known to interfere with actin binding, membrane association, or fusion apparatus binding. By using the structure of a related paramyxovirus matrix protein, we have identified key, conserved residues that could interfere with these interactions. We now plan to compare the spread of these viruses to wild-type MeV in our human airway epithelium model. Preliminary results showing matrix accumulation with the apical-belt support our working model. Intercellular transport of viral components without particle budding could occur during infections of epithelia with other enveloped viruses.
There are 60 serotypes of human adenoviruses (Ads), but nearly all human studies have used only one: Ad5. To broaden the Ad pallet, we identified low seroprevalence species C Ad6 and species D Ad26 as lead therapeutics. To better understand their differing biologies, we conducted next generation mRNA sequencing of viral and host cell transcripts after infection. Both viruses orchestrated early and late viral mRNA expression with many similarities, but a number of differences occurred in early genes. There were striking differences in E3 gene activation by the two viruses suggesting different immunevasion strategies. Ad6 and Ad26 induced differential expression of 277 host mRNAs in common, but 1030 and 59 host mRNAs were unique, respectively. These data demonstrate both shared and distinct viral activation and manipulation of host responses. Understanding these differences will enable better engineering of therapeutic vectors as well as contributes to the understanding of basic adenoviral biology.
Blood brain barrier (BBB) disruption is a common feature of numerous neurological conditions, including multiple sclerosis (MS), cerebral malaria (CM), and viral hemorrhagic fevers (VHFs). CD8 T cells have been implicated in promoting BBB in CM and VHFs. A pore forming molecule perforin, which normally assists with CD8 T cell and NK cell killing, is required for BBB disruption to occur. Given the implied role of CD8 T cells and perforin in VHF and CM related pathogenesis, we conducted a literature search for notable mutations in the human perforin (Prf1) gene. Although necessary for controlling viral infection, perforin, in its fully active state, could be potentially devastating to the virus or parasite infected host. We identified over 100 reported human perforin mutations in the literature; suggesting diversity in perforin function may occur for beneficial cytotoxic killing to control pathogens, yet have tempered onset pathologic BBB disruption and mortality in a human population. We put forward that a reduced amount of perforin activity would reduce the amount of BBB disruption observed in our mouse model. To test this hypothesis, perforin −/−, perforin +/− and perforin +/+ C57Bl/6 were induced to undergo the peptide induced fatal syndrome (PIFS) model; a model designed by our lab to study pathologic BBB disruption. Perforin knock out mice do not have BBB disruption. Perforin competent mice do have BBB disruption and behavioral deficit. Mice heterozygous for perforin have an intermediate amount of BBB disruption, as measured by MRI. Future studies will test the specific mutations of the perforin gene and determine the extent by which mice with reduced perforin activity can control CNS virus infection.
Title: Monocytes from CD38−/− mice have a defective migratory response to pristane-induced inflammation

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A subset of inflammatory monocytes expressing CCL2 and high levels of the surface marker Ly6C is recruited to the peritoneum in response to MCP-1 and is a major source of IFN-I in pristane-treated mice. In the present study, the role of CD38 in the early phase of the pristane-induced lupus disease was examined. Two weeks following pristane injection, the total number of peritoneal cells in pristane-treated CD38−/− mice was slightly lower than in wild-type mice but much higher than in untreated mice, indicating that pristane could induce an inflammatory response in CD38−/− mice. The effect of CD38 deficiency on the recruitment of Ly6C hi monocytes (CD11b+Ly6C hiLy6G−) also was examined. Peritoneal exudates from pristane-treated CD38−/− mice contained less Ly6C hi monocytes in comparison with pristane-treated B6 controls. Peritoneal neutrophils were largely unaffected. Because Ly6C hi monocytes are a major source of IFN-I production in the inflamed peritoneum of pristane-treated mice, these results are consistent with the low IFN-I expression in peritoneal exudates cells from CD38−/− mice. These findings suggest an important role for CD38 in the response of monocytes to pristane and their recruitment to the primary site of inflammation that is thought to trigger lupus onset in this experimental model of SLE.