POSTDOCTORAL RESEARCH DAY 2013

MARCH 22, 2013

<table>
<thead>
<tr>
<th>TIME</th>
<th>EVENT</th>
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<tbody>
<tr>
<td>8:30 – 9:30am</td>
<td>Registration for posters</td>
<td>Trivisible Room, RC2</td>
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<td>Poster judges check-In</td>
<td>2nd floor Atrium</td>
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<td></td>
<td>Continental breakfast</td>
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<td>9:30 – 10:45am</td>
<td>Poster Session 1</td>
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<td>10:45 – 12:00pm</td>
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<td>12:00 – 12:30pm</td>
<td>Tie-Breaker Judging</td>
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<td>Coffee Break</td>
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<td>12:30 – 1:00pm</td>
<td>Lunch (pizza)</td>
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<td>Take down posters</td>
<td>Hensel Phelps West</td>
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<td>1:00 – 2:00pm</td>
<td>Keynote: Peter S. Fiske, Ph.D.</td>
<td>Hensel Phelps West</td>
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<td>“Put your Science to WORK: Practical Career Strategies for Early-Career Scientists”</td>
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<td>2:00 – 2:15pm</td>
<td>Coffee Break</td>
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<td>Hensel Phelps West</td>
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<td>2:15 – 4:15pm</td>
<td>Workshop: Scott Morgan</td>
<td>Hensel Phelps West</td>
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<td>“Interview Skills”</td>
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<td>4:30 – 6:00pm</td>
<td>Reception and Awards</td>
<td>Trivisible Room, RC2</td>
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<td>2nd floor Atrium</td>
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Sponsor Tables open 8:30 am – 12 pm in RC2 2nd floor Atrium
Welcome

Postdoctoral Research Day 2013

Welcome to the fourth annual Postdoctoral Research Day at the University of Colorado Denver. Today we highlight the contributions of the over 300 postdocs at the University of Colorado Denver, and our affiliated institutions.

Postdoctoral fellows are a unique and integral component of any university community. Often viewed as the most enjoyable years in one’s career, the transition from “trainee” to “independent investigator” allows postdocs to focus their energies on their research programs without being overly burdened by academic requirements or faculty responsibilities. It is a time to explore new avenues, master new techniques, and think outside of the box. Their research contributions not only set in place their own career trajectories but often prove to be transformative to the host laboratory as well.

What better way to acknowledge the many contributions of our postdoctoral fellows than to spend this day showcasing their current research activities coupled with presentations that focus on career development. This morning’s poster sessions feature over 80 displays of the independent research being conducted by postdocs at both CU-Denver campuses and at National Jewish Health.

We are particularly pleased to have Dr. Peter Fiske as this year’s keynote speaker, who will discuss “Put Your Science to WORK: Practical Career Strategies for Early-Career Scientists.” Following Dr. Fiske’s presentation, Mr. Scott Morgan will hold an interactive workshop on developing interview skills. Everyone is invited to attend the closing reception and awards presentations. I hope you enjoy all aspects of the day and will take the opportunity to talk to the postdocs about their research and career aspirations.

My sincere thanks to all of you who have made this day possible – from the sponsors, and donors, to the faculty judges and Postdoctoral Association leadership, and especially to Valerie Saltou and the PDRD planning committee.

Best wishes for your continued success,

Barry D. Shur, Ph.D.
Dean, Graduate School
University of Colorado Denver
POSTDOCTORAL RESEARCH DAY 2013

Has been made possible by generous contributions from:

**UC Denver Administration and Affiliates**

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## PDRD2013 PLANNING COMMITTEE MEMBERS

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<th>Name</th>
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<tr>
<td>Jessica Finlay-Schultz</td>
<td>Postdoctoral Fellow, Dept. of Pathology, SOM</td>
<td>Dept. of Pathology, SOM</td>
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<td></td>
<td>Postdoctoral Advisory Committee member</td>
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<td>Treasurer, UCD Postdoctoral Association</td>
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<td>Isabelle Buard</td>
<td>Postdoctoral Fellow, Dept. of Psychiatry, SOM</td>
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<td>Diana Cittelly</td>
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<td>Lina Dimberg</td>
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<td>Darlynn Korns Johnson</td>
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<td>Adrienne Zweifel</td>
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<td>Valerie Saltou</td>
<td>Postdoctoral Office Director</td>
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<td>Pat Goggans</td>
<td>Graduate School Events Coordinator</td>
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<tr>
<td>Catherine Jankowski</td>
<td>Associate Professor, College of Nursing, Division of Aging and Senior Health</td>
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**SPECIAL THANKS** for their significant contributions to the success of the day go to:

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<tr>
<td>Bruce Mandt</td>
<td>Postdoctoral Fellow, Dept. of Psychology</td>
<td>College of Liberal Arts &amp; Sciences</td>
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<td>President, UCD Postdoctoral Association</td>
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<td>Postdoctoral Advisory Committee</td>
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<tr>
<td>Stephanie Schittone</td>
<td>Former Postdoctoral Fellow, Dept. of Pediatrics, SOM</td>
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UPCOMING POSTDOC EVENTS

NATIONAL POSTDOCTORAL ASSOCIATION

National Postdoc Appreciation Week starts September 16!
In September of 2010 the U.S. House of Representatives passed H.RES.1545, which officially recognizes National Postdoc Appreciation Week. National Postdoc Appreciation Week/Day (NPAW/NPAD) always starts the third Monday in September.

UCD POSTDOCTORAL ASSOCIATION

Postdoctoral Seminar Series
Monthly, every third Thursday, 11 am – noon
RC-1 North, room 6107 (Robertson Conference Room)

Upcoming Speakers:
Jessica Finlay-Schultz and Predrag Serbedzija
Thursday, April 18

Mariana Potcoava and Arockia Ranjitha Dhanasekaran
Thursday, May 16

UCD-PDA Executive Council meetings - All Postdocs are Welcome!
Monthly, every second Thursday at 4 pm
Academic Office Bldg. (A01), room 2101

Spring Postdoc Happy Hour - TBA

TRAINING AND CAREER DEVELOPMENT

Sponsored by the Training and Educational Development (TED) Committee

Training seminar
Wednesday, May 8, 3 pm
Hensel Phelps Auditorium West
"New Faculty Panel: Advice for Transition"

Mock Study Section
Wednesday, May 22, 1-4 pm
Academic Office Bldg. (A01), room 1601 (Dean’s Conference Room)
IMPROVING OBESITY CARE IN SCHOOL-BASED HEALTH CENTERS WITH WEB-BASED TRAINING FOR PROVIDERS

HL Aldrich, B Gance-Cleveland, S Schmiege, J Senecal, D Dandreaux, D Skiba
1College of Nursing, University of Colorado, Anschutz Medical Campus, Aurora, CO
2College of Nursing and Health Innovation, Arizona State University, Phoenix, AZ

The prevalence of overweight and obese children has risen dramatically, especially among underserved, minority youth. School-based health centers (SBHCs) provide primary care for many children who are at higher risk for developing obesity and its related chronic health conditions; and therefore are an avenue for addressing these health disparities. Though national experts have developed guidelines to assist providers in obesity treatment and prevention, research shows primary care providers face numerous barriers addressing weight management in children and publication of guidelines alone rarely changes practice. This study evaluated the effectiveness of a web-based training program with and without technology decision support to prompt SBHC providers in applying evidence-based obesity care. Providers (n=22) from SBHCs in 6 states (AZ, CO, NM, MI, NY, NC) completed The International Life Science Institute (ILSI) Research Foundation Assessment of Overweight in Children and Adolescents survey before and after web-based training. Results show after the training more providers (p<0.05) ask patients about their readiness to make weight management changes, as well as an improvement (p<0.05) in providers who address sedentary behaviors with patients. All providers surveyed follow guidelines using BMI percentile to assess excess weight in children and adolescents, sometimes in combination with other methods. More work is needed in some areas, such as history assessments and how to treat overweight youth who are not concerned about their weight. SBHC providers can improve obesity care in this high-risk population by implementing current evidence-based recommendations, and web-based training may be an approach to help providers better implement guidelines.

This project was supported by grant number 1R18HSO18646-01A from the Agency for Healthcare Research and Quality (AHRQ), an agency of the U.S. Department of Health and Human Services.

THE p53 TRANSCRIPTOME

MA Allen, JA Freeman, H Mellert, JM Espinosa, RD Dowell
1Computational Bioscience Program at the University of Colorado Anschutz Medical Campus,
2Molecular, Cellular and Developmental Biology at the University of Colorado-Boulder and HHMI,
3Molecular, Cellular and Developmental Biology and BioFrontiers at the University of Colorado-Boulder,

The guardian of the genome is a transcription factor p53 that can activate transcription of target genes in response to cellular stress. Thus, p53 plays a central role in the regulation of cellular processes and the suppression of cancer. Yet, despite the vast amount of research on p53, the precise transcriptional response to activating p53 is not understood. The traditional method of studying transcription examines steady state stable RNA levels (by sequencing or microarray) and is therefore unable to detect unstable transcription. Furthermore, the steady-state nature of the assay makes it impossible to distinguish genuine transcriptional response from changes in message stability. To overcome the limitations mentioned above, I have used Global Nuclear Run On-sequencing (GRO-seq) to directly measure changes in transcription genome-wide at early time points after p53 activation. My data contains several exciting results. Surprisingly, only one hour after p53 activation, transcription of several well-known target genes are up-regulated, something novel and undetectable by traditional methods. In addition, many previously unannotated transcripts are regulated by p53, including a intriguing percentage of potential p53 binding sites that are transcribed. These data demonstrate the potential of this approach to yield new insights into p53 and cancer regulation via p53. And open the field to new questions about the effect of transcription over a p53-binding site.
THE RAS/ERK/PI3K PATHWAYS DURING POSITIVE SELECTION OF PRIMARY B CELLS.
C Babolin, L Sinik-Teodorovic, R Torres and R Pelanda
Integrated Department of Immunology, University of Colorado Denver and National Jewish Health, Denver, CO.

Expression and signaling of the B cell antigen receptor (BCR) guide the differentiation and selection of immature B cells. BCR is also able to signal in the absence of ligand binding, a response called "tonic". We have found that tonic BCR signaling in immature B cells propagates via Erk and PI3K pathways that can be activated by Ras. These pathways promote the differentiation of non-autoreactive immature B cells and their selection into the peripheral B cell pool. Here, we propose that alterations of the Ras/Erk/PI3K pathways can modify the selection of immature B cells and the composition of the peripheral B cell pool, potentially increasing the frequency of autoreactive B cells. Using different transgenic mouse models in which the B cells display fixed receptor specificity, we investigate the BCR signaling pathways, Erk and PI3K, and the differentiation of immature B cells. We demonstrate that these pathways are less active in autoreactive cells than in non-autoreactive cells. Moreover, we show that activation of the tonic BCR signaling cascade within autoreactive immature B cells breaks central tolerance and leads to the differentiation into transitional B cells via the same Erk and PI3K pathways. Our findings support a model whereby arrest in differentiation and central B cell tolerance are caused by the absence of tonic BCR signaling rather than the presence of self-antigen-mediated signals.

AUTOIMMUNE RESPONSE TO ISLET AMYLOID POLYPEPTIDE IN NOD MICE
RL Baker, T Delong, G Barbour, B Bradley and K Haskins
Department of Immunology, University of Colorado School of Medicine and National Jewish Health, Denver, CO

CD4 T cells reactive to islet antigens are key players in the pathogenesis of type 1 diabetes (T1D). We recently reported islet amyloid polypeptide (IAPP) to be the target antigen for the diabetogenic CD4 T cell clone BDC-5.2.9 and identified KS20, a 20-mer peptide from IAPP, as a strong ligand for this T cell clone. Our goal in this study was to determine how CD4 T cells reactive to IAPP contribute to the pathogenesis of T1D. Using I-Ag7 tetramers loaded with a control peptide (CLIP), the KS20 peptide, or the BDC-2.5 mimotope peptide, we analyzed by flow cytometry the presence of tetramer-positive cells in different lymphoid organs and in the pancreas of NOD mice. Our results indicate that, in comparison to the CLIP control, a significant number of KS20 tetramer-positive cells are present in the pancreas of pre-diabetic mice and in even greater numbers in diabetic mice. To test whether IAPP-reactive cells were pathogenic, KS20 tetramer-positive cells were sorted from T cell lines previously isolated from spleen and lymph nodes of diabetic mice and were then cloned by limiting dilution. We isolated the BDC-5/S3.4 and BDC-9/S3 CD4 T cell clones which are both reactive to KS20 and secrete Th1 cytokines upon antigen stimulation. These clones also rapidly transfer diabetes, demonstrating that KS20-reactive T cells identified through tetramer analysis can be diabetogenic. This study indicates that IAPP-reactive T cells may play an important role in the development of T1D.
SURVIVAL OF MYCOBACTERIUM TUBERCULOSIS WITHIN ADIPOSE TISSUE - A RESERVOIR OF LATENT INFECTION?
IL Bartek and MI Voskuil

*Mycobacterium tuberculosis* (*Mtb*) is able to establish a latent infection for years to decades, after which it can reactivate to cause active infection. It is estimated that up to one third of the world’s population is latently infected. Where the bacteria survive within the host for years to decades is not fully known. Reactivation from latency primarily occurs in the upper lobes of the lung, but can occur anywhere in the body. This indicates that there is likely a tissue reservoir other than the presumed reservoir within the lung granuloma. Adipose tissue consists of between 15%-25% of human body mass, and is present throughout the body. This tissue has recently been implicated as a potential reservoir for latent *Mtb* infection. The 3T3-L1 fibroblast-like murine cell line is able to differentiate into mature adipocytes when given the proper stimuli. *Mtb* is able to infect and grow within non-differentiated 3T3-L1 cells. *Mtb* is also able to maintain an infection within differentiated 3T3-L1 cells for up to ten days without displaying bacterial growth. We wanted to determine whether adipose cells could support long-term infection with *Mtb*, and to analyze the transcriptional profile of *Mtb* within these cells. We differentiated 3T3-L1 cells into adipocytes, infected them with the H37Rv strain of *Mtb*, assayed bacterial survival over time, and analyzed transcriptional response. Transcriptional profiles indicate that many genes expressed during infection of non-differentiated 3T3-L1 cells are also expressed during our *in vitro* model of dormancy. RNA yields from differentiated 3T3-L1 cells are low, and optimization to obtain enough RNA for transcriptional analysis is ongoing. Bacilli are able to survive within both differentiated and non-differentiated 3T3-L1 cells short-term, and efforts are underway to analyze survival during long-term adipocyte infection.

AFFINITY THRESHOLD FOR THE THYMIC DEVELOPMENT OF INKT CELLS
R Bedel, T Mallevaey, J Hayward, AJ Clarke, MH Young, JL Matsuda, DI Godfrey, J Rossjohn, P Marrack and L Gapin

NKT TCRs can recognize both CD1d-restricted microbial and self-lipid antigens (Ags). Because of this inherent reactivity to “self”, it remains unclear whether NKT cells are subjected to negative selection during their development in the thymus. We have previously shown that the overall affinity of the NKT TCR for the CD1d-antigen complex is modulated by the TCRβ chain. We have now isolated and characterized autoreactive NKT TCRs that interact with CD1d molecules loaded with natural self-Ags. These autoreactive TCRs use unique sequences within the non-germline encoded CDR3β loop that promote self-association to CD1d, independently of the antigen presented. They also exhibit higher affinities for the CD1d-antigen complex than the affinities of NKT TCRs usually found within the natural repertoire. Consequently, using these autoreactive TCRs, we decided to assess the existence of a iNKT cells negative selection.

iNKT cells from a transgenic mice expressing one of these autoreactive TCRβ (2A3-D) chains were characterized by ELISA, flow cytometry analysis and High-Throughput Sequencing and provided us with a unique opportunity to assess the developmental outcome of NKT cell precursors expressing a high affinity autoreactive TCR. 2A3-D mice have a defect in iNKT cells numbers and percentage compared to C57BL/6. The iNKT cells present an immature phenotype and a limited capacity for cytokines secretion. The analysis of the Va repertoire through sequencing revealed a unique usage of the Va14 natural variants compared to wild type mice.

Our data suggest that while NKT cells can undergo negative selection in the thymus during their development, they can also escape the deletion process by altering the affinity of their TCR. These results demonstrate that the development of a functional NKT cell repertoire is under tight regulation, limited by the affinity of the TCR for the CD1d-self-antigen complex.
UPREGULATION OF RGK PROTEIN EXPRESSION IN AGING MOUSE SKELETAL MUSCLE
WA Sumner, D Begollari, CF Romberg, MP Scheele, RA Bannister
Department of Medicine-Cardiology Division, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

A component of muscle weakness in older individuals is directly attributable to compromised excitation-contraction (EC) coupling. In skeletal muscle, the L-type Ca2+ channel (CaV1.1) serves as the voltage sensor for EC coupling by triggering Ca2+ release from the sarcoplasmic reticulum (SR) in response to plasma membrane depolarization. Although it has been established that impaired EC coupling (termed “EC uncoupling” by O. Delbono and colleagues) in aged individuals is caused by a reduction in the number of L-type channels present in the plasma membrane, the molecular mechanisms responsible for transducing age-dependent cellular signals (e.g., oxidative stress) to decreased CaV1.1 membrane expression remain enigmatic. In this study, we have investigated a role for RGK (Rad, Rem, Rem2, Gem/Kir) family small G proteins in EC uncoupling because: 1) expression of endogenous Rad is enhanced in response to oxidative stress in skeletal muscle, and 2) overexpression of Rem in muscle cells mimics EC uncoupling. We have found that exogenous overexpression of Rad in cultured myotubes also mimics EC uncoupling. Moreover, immunoblotting of fast-twitch tibialis anterior muscle lysates revealed progressive age-dependent enhancement of Rad protein levels. Taken together, our observations raise the possibility that Rad acts as one molecular link between elevated oxidative stress and EC uncoupling in aging muscle.

DIETARY FAT OXIDATION IN LEAN, OBESE AND REDUCED-OBESE MALE AND FEMALE ADULTS
Colorado Health and Wellness Center

While a defect in fat oxidation (FOx) due to altered oxidative capacity is thought to be a central mechanism leading to obesity, studies in human subjects have not consistently supported this view. We had previously demonstrated a reduction in dietary FOx in obese and moreso in reduced-obese rats as compared to lean rats. In an effort to see if this was also the case in humans, we measured 24hr total (room calorimeter) and dietary FOx (14C-oleate) in sedentary lean (n=10), obese (n=9) and reduced-obese (n=7) adults. Reduced-obese were thinner than obese (BMI=26.9±3.7 vs 33.6±2.5 kg/m² and %BF=30.0±6.1% vs 38.1±7.3%, p<0.05) but fatter than lean individuals (BMI=21.5±1.6 kg/m2 and %BF=22.5±6.2%, p<0.05). Although total fat oxidation was 28% lower in reduced-obese group compared to both lean and obese groups (p=0.06 and p=0.04, respectively), no difference was seen in the amount of meal fat oxidized (6.0±1.3g, 6.7±1.8g, 6.1±2.4g in lean, obese and reduced-obese respectively). A single bout of moderate-intensity exercise significantly increased both 24hr total and dietary FOx. In conclusion, obese individuals oxidize as much fat as lean individuals, which does not support the hypothesis of an altered muscle oxidative capacity in obese. FOx is however lower in reduced-obese, which could potentially contribute to weight regain. This reduction is not the result of a decrease in dietary FOx, suggesting a decrease in plasma free fatty acid oxidation and an impairment in adipose tissue metabolism in response to obesity and/or reduced-obesity. Moderate-intensity exercise may attenuate weight regain after weight loss. Further studies are needed to determine the respective role of adipose tissue and muscle in the altered fat metabolism developed in response to weight loss.
HUMAN RECOMBINANT ANTIBODIES DERIVED FROM MULTIPLE SCLEROSIS PATIENTS’ CEREBROSPINAL FLUID PLASMA CELLS BIND MYELIN-ASSOCIATED ANTIGENS IN THE DEVELOPING CENTRAL NERVOUS SYSTEM

KR Blauth, AM Ritchie, CR Reiter, JN Soltys, JL Bennett, and GP Owens
Department of Neurology, University of Colorado AMC, 12700 East 19th Avenue, Aurora, CO 80045

Multiple Sclerosis (MS) is a human central nervous system (CNS) demyelinating disease of unknown pathogenesis. Increased and persistent intrathecal IgG synthesis, and the resulting presence of oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) of MS patients indicate that oligoclonal IgG may be directed against disease-relevant antigens. It also may provide evidence for plasma cell involvement in MS pathology. Our laboratory has previously generated recombinant antibodies (rAbs) that faithfully reproduce the in vivo specificities of expanded patient CSF plasma cell clones. Here, we utilize immunohistochemistry (IHC) to analyze the pattern of reactivity of the rAbs in the developing mouse (postnatal days 5 – 17) via a spinal cord explant model. In addition, we have characterized the expression patterns of these rAbs throughout the adult cerebral cortex and cerebellum. We find that several of these rAbs bind antigen(s) expressed on myelin or myelinated axons in both the developing and adult mouse CNS. We conclude that the corresponding IgG Abs are directed against myelin-associated antigens, and therefore may play substantial roles in MS pathology. Next, we will use spinal cord explant culture assays and in vivo intracranial injections to investigate the ability of the rAbs to induce or enhance demyelination, attenuate remyelination, induce oligodendroglial cell death, and instigate axonal damage. Antigenic target identification will also be pursued, via IHC analysis of co-localization between rAbs and potential antigens.

CHARACTERIZING THE T CELL RECEPTOR REPertoire OF BERYLLIUM RESPONSIVE T CELLS

NA Bowerman, MT Falta, DG Mack, F Crawford, J Kappler, AP Fontenot
1Department of Medicine, University of Colorado Denver, Aurora, CO, 2Integrated Department of Immunology, National Jewish Health, Denver, CO

Multiple public T cell receptor (TCR) repertoires exist in CD4+ T cells derived from the lung of patients with chronic beryllium disease (CBD). Here, we characterize the TCR Vβ5 repertoire which involves expression of a conserved glutamine (Q)-containing motif that is essential for TCR recognition of HLA-DP2/peptide/Be2+. Using a panel of human TCR Vβ antibodies, we show that beryllium-responsive, Vβ5.1*CD4+ T cell expansions exist in all DP2+ CBD patients tested to date. Sequence analysis revealed oligoclonal expansions consisting of Vβ5+ TCR genes that express a conserved motif composed of identical lengths, conserved joining region expression (Jβ2.5/1.4), and homologous amino acid (A/G) residues surrounding an essential Q residue. The existence of a public Vβ5+ TCR repertoire suggests selection by an identical antigen in lung, which is likely composed of HLA-DP2, peptide, and Be2+. As expected, these Vβ5+ T cells recognize the same antigen, as revealed by positive staining of CBD T cell lines with a soluble tetrameric HLA-DP2/peptide/Be2+ molecule. This soluble MHCII tetramer specifically binds only Vβ5.1*CD4+ T cells and does not detect other beryllium-specific Vβ expansions in lung. Interestingly, the MHCII tetramer detects distinct T cell populations that express nearly identical Vβ5 chains. The role of the Vα chains in recognition of the beryllium antigen in these distinct tetramer positive populations is underway. The presence of beryllium-specific, Vβ5.1*CD4+ T cells in the lung of all DP2+ expressing CBD patients suggests that the MHCII tetramers can be used as an early method of detection of CBD development in beryllium-exposed workers.

Supported by NIH grants, 5T32HL007085-37 and R01-HL062410.
TRPM7 IS A CHANNEL-KINASE AND Mg\textsuperscript{2+}-SENSOR REGULATING NUTRITIONAL SIGNALING AND AUTOPHAGY

K Brandao, AL Perraud, C Schmitz
Department of Immunology, University of Colorado, National Jewish Health, Denver, CO

Mg\textsuperscript{2+} is the dominant divalent intracellular cation and is required for cell growth and proliferation. The adverse effects of Mg\textsuperscript{2+} deficiency on immunity and cell metabolism are well documented, but mechanistic insights into Mg\textsuperscript{2+} sensitive signal transduction are still lacking. TRPM7 and its close homolog TRPM6 are crucial regulators of Mg\textsuperscript{2+} homeostasis at the cellular and organ level. These two channels are the only known fusions between an ion channel and a cytoplasmic serine / threonine kinase domain. TRPM7 is permeant to both Mg\textsuperscript{2+} and Ca\textsuperscript{2+} and the channel is inhibited by intracellular Mg\textsuperscript{2+} and MgATP. Thus TRPM7 may act as a sensor of the nutritional status of the cell. Our lab previously found that deficiency of the channel kinase TRPM7 in DT 40 B-lymphocytes leads to growth arrest and cell death unless the growth media is supplemented with high concentrations of Mg\textsuperscript{2+} (5-10 mM). We found that under hypomagnesic conditions TRPM7 activates eukaryotic elongation factor-2 kinase (eEF2K), which is also a target of the master nutritional regulator kinase, mTOR. TRPM7 phosphorylates Ser77 of eEF2K, activating eEF2K to phosphorylate and inhibit the translational factor eEF2 and thus inhibiting protein synthesis and cell growth when Mg\textsuperscript{2+} levels are insufficient.

Here we examined whether TRPM7 could play a greater role in nutritional stress signaling beyond its influence on protein translation. Autophagy, the process by which cells recycle long lived proteins and organelles, also functions as a survival mechanism during states of cellular stress. We propose that TRPM7 could regulate autophagy signaling pathways through detection of Mg\textsuperscript{2+} and MgATP levels and phosphorylation of key substrates, and provide first indications that TRPM7 is involved in this central process of cellular maintenance.

ACTIVATED B CELLS INFILTRATE TUMORS OF NON-SMALL CELL LUNG CANCER PATIENTS

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\textsuperscript{1}Integrated Department of Immunology, University of Colorado-SOM and National Jewish Health, Denver, CO, \textsuperscript{2}Department of Oncology, National Jewish Health, Denver, CO

Lung cancer is the leading cause of cancer death in both men and women. Although novel therapies are emerging, we are interested in boosting tumor-specific immune responses in infiltrating lymphocytes. In a comprehensive flow cytometric analysis of the immune infiltrate in forty non-small cell lung cancer (NSCLC) patients, we found that the total number of infiltrating B cells (TIL-Bs) in the tumor versus the tumor-adjacent tissue was significantly increased compared to other immune subsets, specifically T cells. NSCLC TIL-Bs are activated (CD21lo and CD69\textsuperscript{+}), proliferate, and have a memory phenotype (CD27\textsuperscript{+}). Preliminary functional studies indicate that these cells generate activation cytokines (IFN-\gamma and TNF-\alpha), which suggest that the TIL-Bs have an effector phenotype that could aid in targeting the tumor. These cells secrete total IgG and may present antigen because of their juxtaposition to T cells at the site of the tumor. We are analyzing the surface expression of Fas-ligand and TRAIL on the TIL-Bs to determine if these cells are involved in the direct killing of tumor cells. Because we are interested in uncovering the unique potential of these TIL-Bs, we are collecting cells from tumor and tumor-adjacent tissue for gene-expression profiling. By comparing these two populations to normal lung B cells, we aim to discover a unique gene or pathway in the TIL-Bs that can be targeted for an immunotherapy clinical trial in lung cancer patients.

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AN MEG STUDY OF MOTOR-RELATED BETA OSCILLATIONS DURING IMITATION OF HAND MOVEMENTS
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Behavioral studies have shown that in the case of imitation of hand movements, the movement that is imitated naturally is that of the hand of the actor facing the hand used by the imitator. We investigated the motor-associated cortical oscillations relevant to this phenomenon. Neuromagnetic activity was recorded in 16 healthy adults during right hand imitation of finger-lifting movements from either a right hand projected on the left side of the screen (cross-body imitation) or a left hand on the right side (cross-hand imitation). Oscillatory changes within the beta frequency band (15-30 Hz) were calculated during and following movement, relative to the baseline activity. Decrease in beta activity (ERD, event-related desynchronization) was observed during the movement and imaged using a -500 to 500 msec time window (0: movement onset). Following the movement, an increase in beta-band activity (PMBR, post-movement beta rebound) was imaged using a 500-2000 ms time window. PMBR and ERD were compared at the sensor level between each hemisphere and for both hand imitation and subjected to statistical analysis using a dependant sample t-test. As expected for a right hand movement, we found relevant beta oscillations in both hemispheres during imitation of both hands. In the left hemisphere, PMBR was significantly stronger during imitation of the left hand (p=0.029) and ERD was more robust for right hand imitation (p=0.054). Indeed, when looking at left hand imitation, PMBR was bigger in the left hemisphere (p=0.015). Similarly, ERD in the left hemisphere was stronger then in the right during the right hand imitation condition (p=0.036). These results provide some physiological evidence of distinct brain activity associated with imitation of hand movements with distinct effects of mapping.

MY CHILD IS OVERWEIGHT?: HOW PARENTAL PERCEPTIONS DIFFER BY RACE/ETHNICITY
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Low socioeconomic status (SES) and minority populations are at increased risk for overweight (OW), and parents that are unaware of their child’s OW status are less likely to seek treatment. We examined the influence of parent’s race/ethnicity and SES on parental perceptions of child weight status and concern for the child becoming overweight (CONCERN) among a racially and socioeconomically diverse sample of parents and normal and OW children ages 7-12. BMI was assessed via anthropometrics and OW was defined as BMI ≥ 85% for age, sex, and height. Parental perceptions of OW were measured via the Child Feeding Questionnaire. Of the 312 subjects, 53% were male, 39% were white, 34% were African American (AA), and 27% were Hispanic (HA). HA had a higher prevalence of OW (p<0.05), and HA and AA parents report significantly more CONCERN than whites (p<.0001). In parents of OW children, more than two-thirds of parents in each racial/ethnic group incorrectly identified their child as normal weight. Among AA and HA parents, ~7% identified their OW child as being underweight. In whites, CONCERN was significantly correlated with parental ability to correctly identify weight status (p=0.0043). SES and child sex was not associated with CONCERN or parental ability to correctly identify weight status. This data suggests that among white parents only, concern for weight status was associated with correctly identifying child weight status.
IMPAIRED INHIBITORY CONTROL OF CORTICAL SYNCHRONIZATION IN FRAGILE X
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Epilepsy is a common comorbid condition associated with Fragile X Syndrome (FXS), a neurodevelopmental disorder characterized by severe cognitive impairments and intellectual disabilities. It is estimated that 20-25% of Fragile X patients will have recurring seizures at some point in their childhood (Musumeci et al., 1988) and 15% of them will be diagnosed with epilepsy (Berry-Kravis et al., 2010). The most common epilepsy diagnosis is benign focal epilepsy with centrotemporal (or “rolandic”) spikes, a type of seizure that is cortical in origin. Fmr1 knockout (KO) mouse models of FXS exhibit alterations in excitatory and inhibitory neurotransmission, but it is largely unknown how aberrant function of specific interneuronal subtypes contributes to deficits in cortical function. Here we show specific inhibitory circuit dysfunction in layer 2/3 of somatosensory cortex of Fmr1 KOs. Simultaneous recordings from pyramidal neurons reveal alterations in coordinated spike synchrony in response to the group I metabotropic glutamate receptor (mGluR) agonist DHPG. We further demonstrate reduced activation of somatostatin (SOM)-expressing low-threshold spiking (LTS) interneurons in response to DHPG in Fmr1 KOs, indicating a weakened LTS interneuron network. Additionally, we show an impairment of the endocannabinoid-mediated modulation of slow self-inhibition in cortical SOM-LTS interneurons, a mechanism that allows LTS interneurons control their own inhibitory properties and subsequently modulate the somatosensory cortex network activity. Together, these findings reveal a functional defect in a single subtype of cortical interneuron in Fmr1 KOs that largely affects cortical synchronization. Based on common abnormalities in EEGs and the high incidence of seizures, FXS and epilepsy may be thought of as “synchronization disorders”. These shared defects may be linked to altered activity of the cortical network in line with both the FXS phenotype and cortical seizure syndromes such as the benign focal epilepsies.

IMPAIRED FUNCTION OF CYTOTOXIC T LYMPHOCYTE ANTIGEN-4 IN THE LUNGS OF PATIENTS WITH CHRONIC BERYLLIUM DISEASE CONTRIBUTES TO PERSISTENT INFLAMMATION
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Chronic beryllium disease (CBD) is an occupational lung disorder characterized by granulomatous inflammation and the accumulation of beryllium-specific CD4+ T cells in the lung. These T cells express a differentiated effector memory phenotype and secrete IL-2, IFN-γ, and TNF-α upon in vitro activation. Beryllium-responsive CD4+ T cells in the lung are CD28 independent and have increased expression of the coinhibitory receptor, and program death 1, resulting in antigen-specific T cells that proliferate poorly yet retain the ability to express Th1-type cytokines. To further investigate the role of coinhibitory receptors in beryllium-induced disease, we examined the expression of cytotoxic T lymphocyte antigen-4 (CTLA-4) in blood and bronchoalveolar lavage fluid of CBD subjects. CTLA-4 expression was most elevated in blood cells inhibited beryllium-induced T cell proliferation while having no effect on the proliferative capacity of beryllium-specific CD4+ T cells in lung. Collectively, our findings suggest a dysfunctional CTLA-4 pathway in the lung and its potential contribution to the persistent inflammatory response that characterizes CBD.
TRAUMATIC BRAIN INJURY CAUSES PLATELET ADP AND AA RECEPTOR INHIBITION INDEPENDENT OF HEMORRHAGIC SHOCK IN HUMANS AND RATS

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Coagulopathy in traumatic brain injury (CTBI) is a well-established phenomenon, but its mechanism is poorly understood. We hypothesized that the platelet dysfunction of CTBI is an intrinsic effect of brain injury, distinct from the coagulopathy following hemorrhagic shock.

We first conducted an analysis of field blood from patients with isolated head injury. Thromboelastography (TEG) with platelet mapping was used to measure platelet function and the degree of inhibition of the ADP and arachidonic acid (AA) receptor pathways. Next, we studied the time course of platelet inhibition in a rat model of severe blunt TBI.

Severe TBI patients showed a significant increase in ADP receptor inhibition in their immediate post-injury sample, compared to both mild TBI patients and healthy controls (p<0.0001). Median ADP receptor inhibition was 95.0% (IQR 61.5-98.6%) in the severe TBI cohort, compared to 56.0% (IQR 35-74.6%) in mild TBI and 15.4% (IQR 12.7-30.5%) in controls. No patient had significant hypotension or acidosis. In rats with TBI, ADP receptor inhibition peaked at 77.6% ± 6.7% versus 39.0% ± 5.3% for uninjured controls (p<0.0001; n=45). Parallel trends were noted in AA receptor inhibition in both humans and rats.

Platelet ADP and AA receptor inhibition is a prominent early feature of CTBI in humans and rats and is linked to severity of brain injury and to poor outcomes in patients with isolated head trauma. This phenomenon is observed in the absence of hemorrhagic shock or multi-system injury. Thus, TBI alone is shown to be sufficient to induce a profound platelet dysfunction equivalent to the use of clopidogrel and aspirin.

POST-TRANSCRIPTIONAL REGULATION FOR NEURAL PROGENITOR MAINTENANCE

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Neural stem cells (NSCs) use distinct self-renewal programs at different stages but the switchers that allow cells to pass from one state to another are undefined. mLIn41 is a novel RNA regulator and an E3 ubiquitin ligase. We found that mLIn41 is highly expressed in neural progenitor cells and its expression declines during neural differentiation. Loss of mLIn41 function in mice causes reduced proliferation and premature differentiation of embryonic neural progenitor cells. Mechanistically, mLIn41 directly binds and ubiquitinates miRNA generating complex, and represses the expression of pro-differentiation miRNAs including let-7 family.

In addition, mLIn41 deficient neural progenitors exhibit hyposensitivity for Fibroblast Growth Factor (FGF) signaling. We show that mLIn41 promotes FGF signaling by directly binding to and enhancing the stability of SHCBP1, and that SHCBP1 is an important component of FGF signaling in neural progenitor cells. Thus, we identify mLIn41 as a post-transcriptional regulator contributing to the switching of NSC from progenitor state to differentiation state. It does so through coordinating miRNA expression with FGF signaling. We are using mouse genetic and genome wide approaches to dissect out the regulatory network involving additional RNA binding proteins and non-coding RNAs in the control NSC properties during brain formation.
METABOLIC PHENOTYPES OF MOUSE PLASMA TO IDENTIFY MARKERS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE
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Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the United States with a higher incidence seen in cigarette smokers. As such, we applied mouse models of air control, smoked, or treated mice to mimic COPD. Plasma samples from mice (DBA2/J) were obtained through collaboration with Dr. Irina Petrache at Indiana University. Mice were exposed to cigarette smoke or air control conditions for four or six months and then treated with two sphingosine-1 phosphate (S1P) analogs. Plasma samples underwent liquid-liquid extraction (LLE) followed by solid-phase extraction to obtain 4 fractions enriched for aqueous, fatty acid, neutral, or phospholipid species. Mass spectrometry-based metabolomics was then applied to the mouse plasma samples to identify biomarkers for COPD.

Data analysis resulted in approximately 5800 compounds, with a few hundred of these compounds exhibiting significant differences (p < 0.05). Of those metabolites, approximately 85% were tentatively identified with compound names (30%), or molecular formulas (55%). Eleven candidate biomarkers were found in the six month study of air control versus continue smoking, while 32 candidate biomarkers were found in the six month study of treated versus untreated smoked mice. Metabolites were tentatively identified as amino acids, glycerophospholipids, glycerolipids and sphingolipids. Because similar and identical biomarkers were discovered during a human metabolomics study, it appears that these compounds play a critical role in smoking-induced COPD.

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TAp63α PROMOTES THE TRANSITION OF HAIR FOLLICLE STEM CELLS TO INTERFOLLICULAR KERATINOCYTES
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p63 is an essential transcription factor expressed as isoforms that either contain (TA) or lack (∆N) a transactivation domain. The ∆Np63 isoforms are constitutively expressed in the basal layer of the epidermis, and have been shown to orchestrate the stratification and differentiation of simple epithelia to stratified epithelia. This contrasts with the TAp63 isoforms, which are not required for epidermal development, but are instead induced upon wounding. In order to understand the role of TAp63 during the wound response, we have generated an inducible mouse model that expresses the TAp63α isoform in the epidermis when crossed to a K5 activator and treated with doxycycline.

Unexpectedly, continual treatment with doxycycline from birth resulted in the failure of TAp63α-expressing mice to develop a coat of hair. Histological analyses of TAp63α-expressing skin revealed a progressive transformation of hair follicles into cyst-like structures within the dermal compartment. Due to the dramatic hair phenotype in the TAp63α-expressing mice, we examined hair follicle differentiation via immunofluorescence using the hair follicle markers and found that the remaining abnormal follicles were fully differentiated despite their hair shafts never protruding through the epidermis. To determine if the ectopic expression of TAp63α had an effect on the interfollicular epidermis, we examined the differentiation marker K1 via immunofluorescence and found no differences between treated and untreated mice. Surprisingly however, we did find that K1 was ectopically expressed in the follicular cysts, suggesting a transdifferentiation of follicular keratinocyte stem cells into interfollicular epidermal keratinocytes. In conclusion, our data suggest that TAp63α alters the cell fate of hair follicle keratinocytes, and provide a mechanism by which hair follicle stem cells convert to interfollicular keratinocytes to accelerate wound healing.
TARGETING ANDROGEN RECEPTOR IN HER2-DRIVEN BREAST CANCER
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The androgen receptor (AR) is expressed in approximately 60% of Her2+ breast cancers. Activated AR elicits transcriptional upregulation of Her3, which can heterodimerize with Her2. Her3 is essential for growth of Her2+ tumors, and has been implicated in therapeutic resistance to tamoxifen, paclitaxel and trastuzumab. Enzalutamide (ENZ) is a new anti-androgen that impairs nuclear entry of ligand-bound AR, binds to AR with higher affinity than bicalutamide, and was recently approved for treatment of castrate-resistant prostate cancer. We hypothesized that ENZ will enhance the efficacy of trastuzumab in Her2+ breast cancer cell lines by inhibiting Her3 expression. The androgen dihydrotestosterone (DHT) induced an increase in total Her3 and phospho-Her3 in some Her2+ breast cancer cell lines and this effect was inhibited by the addition of ENZ. Interestingly, ENZ inhibited proliferation in MDA-MB-453 and SUM185PE cells (Her2 overexpression without amplification) as well as or better than trastuzumab, whereas trastuzumab showed a greater inhibitory effect than ENZ in SKBR3 cells (Her2 amplified). The combination of ENZ and trastuzumab inhibited proliferation more effectively than either agent alone in multiple Her2+ cell lines. In previously-generated trastuzumab-resistant lines, ENZ combined with trastuzumab significantly inhibited proliferation. Our results suggest that ENZ may serve as an effective therapeutic in Her2+ breast cancers when combined with Her2-directed therapies such as trastuzumab, pertuzumab, or T-DM1. Furthermore, in tumors resistant to Her2-directed therapy, ENZ may be useful alone or in combination with anti-Her3 therapy. Targeting AR with ENZ in patients with Her2+ disease may result in therapeutic benefit and warrants clinical investigation.

ACQUIRED RESISTANCE TO ROS1 INHIBITION IN NON-SMALL CELL LUNG CANCER
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Genetic rearrangements involving the ROS1 receptor tyrosine kinase have recently been discovered in non-small cell lung cancer (NSCLC). These rearrangements result in the expression of constitutively active fusion proteins that function as ‘oncogenic drivers’ by activating growth and survival signaling pathways. As a consequence of this, NSCLC patients whose tumors are positive for ROS1 rearrangement often show dramatic responses to treatment with crizotinib, an FDA approved drug that has activity against ROS1. Unfortunately, despite initial efficacy, acquired resistance to kinase-inhibitor drugs invariably arises in cancer patients. In this study, we sought to discover mechanisms of resistance to ROS1 inhibition in ROS1 rearrangement-positive NSCLC. To accomplish this, we obtained samples from a ROS1 rearrangement-positive patient who became resistant to crizotinib. In addition, we created a ROS1 inhibition-resistant derivative of the ROS1 inhibition-sensitive NSCLC cell line HCC78, which we termed TAER. We found that neither ROS1 kinase domain mutation nor ROS1 fusion copy number gain (two common mechanisms of resistance for other targeted therapies) occurred in the patient sample or the TAER cells. However, in the TAER cells, activation of growth and survival signaling switched from being ROS1-dependent to being EGFR-dependent. As a consequence of this, concurrent inhibition of ROS1 and EGFR was able to inhibit proliferation in this cell line. In conclusion, EGFR pathway activation may play a role in resistance to ROS1 inhibition in ROS1 rearrangement-positive NSCLC, and therefore a combination therapy strategy with ROS1 and EGFR inhibitors may be effective at combating or preventing resistance in the clinic.
COMPUTATIONAL ANALYSES OF PROTEIN PROFILES GENERATED USING REVERSE PHASE PROTEIN ARRAY
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Down syndrome (DS), due to an extra copy of human chromosome 21 (HSA21), is the most common genetic cause of intellectual disability. For analysis of learning and memory deficits, mouse is a valuable model system. Determining differences in protein profiles between mouse models of DS and their littermate controls can pave the way for developing therapeutics that correct protein activities, and learning deficits in DS. The reverse phase protein array (RPPA) is an antibody-based technique developed to measure protein levels simultaneously in a large number of biological samples in a quantitative manner. Using RPPA, levels of ~100 proteins were measured in nuclear, cytosolic and membrane fractions from cortex, hippocampus and cerebellum of mouse models of DS and controls. This high-throughput technology generated enormous amounts of data for which computational and statistical programs were developed to expedite analyses of i) preliminary quality control; ii) normalization; iii) correlations among protein levels; iv) calculation of mean differences between genotypes using a three-level mixed-effects model; and v) graphical display of results of all the computational analyses. Patterns of protein levels and their changes in mouse models of DS provide a novel view of the complex molecular dynamics exhibited in the brain. Loss of correlations among proteins in the brains of DS mice was observed. Thus the computational tools that were developed in this lab accelerated RPPA data analysis and facilitated interpretation of the results. These tools are generally applicable and RPPA datasets continue to provide opportunities for development of new computational tools.

USE OF A GENOME-WIDE LOSS-OF-FUNCTION SCREEN TO IDENTIFY NOVEL MECHANISMS OF RESISTANCE TO TRAIL INDUCED APOPTOSIS
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A biological approach to combat cancer is to define and specifically target signaling pathways involved in tumor progression and metastasis. One such target is the TRAIL signaling pathway. TRAIL kills tumor cells selectively without inducing apoptosis in normal tissue. Recombinant TRAIL and agonistic antibodies against its receptors are in early-phase clinical trials as an anti-cancer treatment in breast cancer. However, since cancer cells must evade tumor surveillance, it is not surprising that many tumors are TRAIL resistant. Our goal is to gain an increased understanding of how cells can become resistant to TRAIL and how this resistance can be predicted and circumvented, ultimately leading to more efficient TRAIL based therapy. To this end, we utilized two cell lines, BJAB, a lymphoma cell line, and MDAMB231, a breast cancer cell line, both of which were made resistant to TRAIL as a result of long-term exposure to the drug. Using a genome-wide loss-of function shRNA screen we have identified several putative novel TRAIL resistance genes. When comparing RNA expression in our resistant cell lines to their wild type sensitive counterpart using microarray analysis, we found that in both cell line systems, many differentially expressed genes converged on the RAS/MAPK pathway. Interestingly, resistance genes identified in the shRNA screens were related to the Ras/MAPK pathway as well. Studies of how of our identified targets and the Ras/MAPK pathway participate in mediating TRAIL resistance and influencing metastasis are currently underway.
EFFECTS OF LOW LEVEL INFLAMMATION ON BETA CELL COUPLING IN ISOLATED MOUSE ISLETS
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Type 2 diabetes is characterized by progressive degeneration and eventual death of β-cells, the building blocks of pancreatic islets. Obesity is one major risk factor for disease development and is associated with low level systemic inflammation, which has recently been implicated as a possible link between excess adipose tissue and disease development1. Previous studies in isolated islets have shown that low levels of cytokines impair normal insulin secretion in response to glucose challenge2 and disrupt calcium signaling within the islet3. We hypothesize that cytokines affect β-cell insulin secretion and calcium signaling in part through changes in cell coupling. To test this hypothesis, mouse islets were isolated and incubated with a cytokine cocktail containing 0, 10, 100, or 1000x dilutions of tumor necrosis factor-α (10ng/ml), interleukin-1β (5ng/ml) and interferon-γ (100ng/ml) for 24 hours. Cell viability, glucose induced insulin secretion, calcium signaling and cell coupling were assessed with respect to an untreated control. Cell viability and glucose induced insulin secretion decreased with increasing cytokine concentration. Calcium transients became increasingly uncoordinated across the islet with increasing cytokine concentration. This disruption in calcium signaling supports the hypothesis that cell coupling is altered with cytokine treatment. To further test this hypothesis, changes in cell coupling are directly quantified through fluorescence recovery after photobleaching (FRAP). Future studies will probe the mechanism of cell uncoupling through chemical regulation of upstream signaling factors which regulate cell coupling. Potential targets for future investigations include regulation of cell coupling by cAMP and the involvement of nitric oxide in initiating cell uncoupling.

ROLE OF MICRORNA-141 IN HORMONE-DEPENDENT BREAST CANCER
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MicroRNAs (miRNAs) are involved in many aspects of cancer biology, including tumor progression, chemoresistance, and metastasis. In breast cancer specifically, miRNAs are more abundant in well differentiated cancer cells, and many are downregulated or lost in more dedifferentiated cancer cells. Luminal breast cancers are estrogen receptor (ER) and progesterone receptor (PR) positive. These cancers are hormone dependent and require estrogens for growth; the role of progesterone is less clear. Progesterone is known to rapidly downregulate the expression of several miRNAs in breast cancer cell lines. This includes miR-141, a member of the miR-200 family, of which miR-200c is a well-known tumor suppressor. Interestingly, of the miR-200 family, only miR-141 was found to be significantly hormone regulated in breast cancer cells, and is also reported to be downregulated in stem-like cells. Therefore, we hypothesize that loss of miR-141 plays a role in triggering expansion of the stem-like population in breast tumors.

Preliminary studies of stable inhibition of miR-141 showed significant upregulation of the basal marker CK5/6 with progestin treatment. To investigate the role of miR-141 in hormone dependent breast cancer, we have measured levels of miR-141 and family in multiple luminal and basal breast cancer cell lines and in CK5/6+ and CK5/6− cells. We have also created cells with stable knockdown and overexpression of miR-141 to measure mRNA and protein levels of basal and luminal markers. We have validated targets using luciferase reporter assays and miR-141 mimics. Finally, we have measured miR-141 levels in fractionated stem-like and non-stem-like populations and show that miR-141 specifically downregulated in the stem-like fraction. These data support a role for miR-141 in potentiating the transition from luminal cancer cells to stem-like cells.
OVARIAN HORMONE SUPPRESSION IN PREMENOPAUSAL WOMEN REDUCES FAT-FREE MASS AND RESTING AND 24-HOUR ENERGY EXPENDITURE

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Evidence from animal studies suggests that estradiol (E₂) deficiency disrupts energy balance and causes excess weight gain, but the effects of sex hormone suppression in women have not been well studied. Purpose: To compare the effects of ovarian hormone suppression with add-back of placebo (PL) or E₂ in premenopausal women on body composition, resting energy expenditure (REE), and 24-h energy expenditure (EE). Methods: Forty-five premenopausal women underwent 5 months of gonadotropin releasing hormone agonist therapy (GnRH₆G, monthly injections of leuprolide acetate 3.75mg) to suppress ovarian hormones, with add-back of transdermal E₂ (0.075mg/d, GnRH₆G+E₂, n=21) or placebo patch (GnRH₆G+PL, n=24). At study entry (mean±SD): age 35±8yr; fat mass (FM) 25.9±11.5kg; fat-free mass (FFM) 46.7±5.9kg. Results (mean [95% CI]): GnRH₆G+PL decreased FFM (-0.5kg [-1.0, -0.1]) with no change in FM (-0.2kg [-1.0, 0.7]) or body weight (-0.7kg [-1.6, 0.2]). E₂ prevented the decrease in FFM (0.5 [-0.1, 1.1]), but FM (0.8 [0.1, 1.5]) and body weight (1.3 [0.4, 2.1]) increased. REE decreased in response to GnRH₆G+PL (-57.3kcal/d [-103, -11.9]); this was attenuated by E₂ (-4.2 [-38.5, 30.2]). GnRH₆G+PL also decreased 24-h EE (-128kcal/d [-214, -41.8]), which was reduced (non-significant) by E₂ replacement (-100 [-163, -37.8]). Conclusions: Ovarian hormone suppression led to decreases in FFM, REE, and 24-h EE. E₂ add-back therapy had a favorable effect on some, but not all, bioenergetic changes resulting from chronic suppression of ovarian hormones. Loss of ovarian function at menopause may influence metabolism in a way that accelerates loss of FFM and gain of FM, and reduces energy expenditure.

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DECRYPTING A GENE EXPRESSION SIGNATURE INDICATIVE OF LONG-TERM PROGNOSIS FOR PEDIATRIC EPENDYMOMA PATIENTS

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Ependymomas (EPNs) are the third most common type of pediatric brain tumors, with the majority of incidents occurring in infants and young children. Prognosis remains relatively poor with a 5-year progression-free survival rate of roughly 50% and late relapses are not uncommon. Until now, no prognostic factors have been able to objectively predict a patient’s outcome during early stages of disease.

Using a local brain tumor microarray dataset comprised of patient gene expression profiles, demographics, and survival metrics, we have developed a gene expression signature indicative of long-term prognosis of pediatric EPN patients. Cox Proportional Regression Analysis with continuous expression was used to determine genes that have a significant p-value (p-value ≤ 0.05) correlating gene expression with patient survival. Regression modeling defined signature coefficients to quantitate the patient’s risk of a disease-related event.

Analysis of 34 patient samples looking at a subset of 127 genes identified 2 genes that in aggregate are indicative of patient survival. Patients with good prognosis have high expression of RIPK1 and DOCK1, genes associated with inflammation and cell death, suggesting that the immunologic response to the tumor may influence outcome. Conversely, patients with poor prognosis have low levels of these genes. Classification of patients using our signature reveals significant separation of survival curves (p-value = 0.0003). We will next determine if this signature can be validated in an independent dataset. The results highlight the potential use of gene expression data as prognostic tools. Future work will apply our methodology to define genome-wide gene expression profiles for a multitude of pediatric brain tumors.

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MAPPING THE LONG RANGE CHROMOSOMAL INTERACTIONS OF COMPLEMENT RECEPTOR 2
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Systemic lupus erythematosus (SLE) disease susceptibility is influenced by both genetic and environmental factors. The complement system plays a key role in disease pathogenesis with complement activation resulting in tissue damage, and deficiencies in early pathway components being strongly associated with disease development. Previous studies have identified complement receptor 2 (CR2/CD21) to be a candidate gene in the NZM2410 mouse model of lupus, and a common CR2 haplotype was identified in Caucasian and Chinese populations that increased risk of disease. A recent genetic association study identified a single nucleotide polymorphism (SNP), rs1876453, in the first intron of CR2 that was associated with decreased risk of developing SLE. The encyclopedia of DNA elements (ENCODE) indicated that the SNP is located in a transcription factor hotspot that includes the binding region of CCCTC-binding factor (CTCF). CTCF, also known as the master organizer of the genome, is the only known insulator in vertebrates and can mediate long-range effects on gene regulation/transcription and RNA splicing. Binding of CTCF to the region encompassing the SNP of interest was confirmed in vitro by electrophoretic mobility shift (EMSA) and supershift assays, and in vivo by chromatin immunoprecipitation (ChIP). Because CTCF is known to mediate long-range chromosomal interactions, we utilized chromosome conformation capture (3C) to determine interaction frequencies of CR2 intron 1 with the surrounding region. This is the first report exploring the chromosomal interactions of CR2 and provides a possible mechanism by which a non-coding, intronic SNP could be the causal variant responsible for decreased risk of developing SLE.

INTRACELLULAR HEPATITIS C VIRUS RNA AND EXTRACELLULAR CORE PROTEIN DIFFERENTIALLY REGULATE TYPE I AND III IFN SIGNALING WITHIN HUMAN LIVER SINUSOIDAL ENDOTHELIAL CELLS
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Liver sinusoidal endothelial cells (LSECs) are a unique organ-resident, non-myeloid cell population with diverse functions. LSECs express receptors that capture HCV particles, and show enhanced activation in livers of HCV-infected patients compared to non-infected individuals. Our goal was to determine the effect of intracellular sensing of the HCV-RNA PAMP (pathogen-associated molecular pattern) versus extracellular HCV-core protein on IFN signaling in human LSECs.

Flow cytometry was used to phenotype primary human LSECs from non-HCV infected livers. LSECs were transfected with the HCV PAMP and qRT-PCR was performed after 8 hrs. As a comparison, LSECs were co-cultured with HCV-core protein or TLR4 ligand. ELISAs were used to measure Type III IFNs in supernatants. Reactive oxygen species (ROS) was assessed by a fluoro-based FACS assay. Intracellular HCV-RNA induces significant upregulation of IL-28A (p=0.012), IL-28B (p=0.018) and IL-29 (p=0.001) Type III IFN genes after 8 hrs. Type III IFNs were increased in the supernatants of PAMP-treated LSECs. Co-culture of LSECs with HCV-core protein induced upregulation of Type I IFNs, but not IL-28B nor IL-29. In contrast, co-culture with LPS (TLR4 ligand) induced significant upregulation of IL-6 and TNF-α, but not IFNs. Furthermore, HCV-core significantly induces CXCL10 and ROS, including 12-fold transcriptional upregulation of NOXO1.

Type III IFNs are the predominant IFNs produced by LSECs in response to HCV infection, whereas Type I IFNs, CXCL10, and ROS are induced by HCV-core protein. These results provide novel insights into the potential effector mechanisms by which LSECs contribute to anti-HCV immunity within the hepatic compartment.
STOCHASTIC VARIATION IN AUTOPHAGY DETERMINES APOPTOTIC RESPONSE VIA DEGRADATION OF FAP-1

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Macroautophagy regulates apoptosis; however, the direction of this regulation is context dependent for reasons that are poorly defined. We demonstrate that stochastic differences in basal autophagic flux in individual cells determine which cells live or die in response to death receptor activation. Surprisingly, autophagy can either contribute to cell death or to cell survival depending on the death receptor ligand (Fas ligand or TRAIL) and the mode of apoptosis used by the cell (Type I or Type II). Pro-apoptotic autophagy in Fas ligand-induced apoptosis depends on the Fas inhibitor Fap1, which is degraded through autophagy via p62. These results illustrate that steady state differences in autophagy in a population determine cell fate decisions in a stimulus- and cell type-specific manner and define a molecular switch responsible for positive and negative cell fate regulation by autophagy.

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IDENTIFYING NOVEL SUBSTRATES OF THE TYROSINE PHOSPHATASE ACTIVITY OF EYA, A PROTUMORGENIC AND PROMETASTATIC TRANSCRIPTIONAL CO-FACTOR, IN BREAST CANCER

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Breast cancer is the second leading cause of cancer-related deaths among women. The current 5-year survival rate upon localized breast cancer is an impressive 98%, but if the tumor has metastasized at the time of diagnosis, the survival rate drops to a staggering 20%. Despite the clinical importance of metastasis, much remains to be understood regarding the mechanisms by which metastasis occurs. Eya is a unique transcriptional co-factor that contains intrinsic tyrosine phosphatase activity and promotes transformation, migration, invasion, and metastasis of breast cancer cells. Although it is known that the phosphatase activity of Eya is specifically required for its protumorigenic and prometastatic effects, further understanding of how the phosphatase activity of Eya promotes breast cancer has been limited by the fact that only one in vivo substrate of Eya has been identified thus far. Therefore, we took an unbiased proteomics approach to identify novel substrates of Eya in MDA-MB-231 breast cancer cells. GST-pull down experiments were performed using phosphatase “trapping” mutants of Eya (which can bind endogenous substrates but not release them) followed by mass spectrometry to identify interacting proteins. This analysis identified approximately 30 putative substrates of Eya including cytoplasmic and nuclear factors involved in cell migration (IQGAP1), replication (MCM7), metabolism (6-phosphofructokinase), and transcription (ZBTB1). Studies are currently underway to determine whether these hits are indeed substrates of the Eya phosphatase and future work will investigate the role of confirmed substrates in mediating the protumorigenic and prometastatic effects of Eya in breast cancer.
ELECTRICAL ACTIVITY IN MUSCLE SHAPES, THE SYNAPTIC LANDSCAPE
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Diseases of the neuromuscular junctions (NMJ) weaken, destabilize and denervate the synapse leading to myopathy, and in some cases to paralysis and death. Proper configuration and positioning of the synapse is imperative for neuromuscular function. Prior to nerve-muscle contact, synaptic assembly occurs in the muscle giving rise to regionalized pre-patterned clusters of acetylcholine receptors (AChR). During innervation, AChR clusters centralized with nerve synaptic components to form the NMJ. However, little is known about the muscle-dependent mechanisms of AChR cluster regionalization, centralization or elimination. Our data demonstrates that the synaptic landscape in embryonic muscle is dynamic and malleable. In addition, absence of muscle contractility disrupts regionalization while depolarization induces centralization and elimination. We show that internal calcium release channels, ryanodine receptor type 1 (RyR1) and type 3 (RyR3), have opposing roles in formation of the synaptic landscape. RyR1 activity is necessary for regionalized patterning while RyR3 activity is sufficient to eliminate pre-patterned aneuronal clusters. In the mouse model for muscular dystrophy, synapses are irregular shaped and muscles are weakened. We show that inhibition of ryanodine receptors in these adult muscles rescues the synaptic irregularities and strengthen dystrophic muscles.

EXTRACELLULAR MATRIX INTERACTIONS MODULATE OLIGODENDROCYTE MIGRATION AND DIFFERENTIATION
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Following central nervous system demyelination, remyelination is often incomplete with fewer oligodendrocyte progenitor cells (OPCs) found in the center of lesions, and many OPCs failing to differentiate into mature myelinating oligodendrocytes. OPC migration and differentiation is modulated by short and long-range soluble guidance factors as well as contact with the surrounding extracellular matrix (ECM). For example, semaphorins 3A and 3F are expressed in active demyelinating lesions, and in demyelination/remyelination studies in animal models, semaphorins influence OPC migration into lesions, affecting the rate at which remyelination occurs. Aberrant expression of ECM ligands occurs in and around MS lesions, but the impact of these ECM changes on OPC responses to guidance molecules is unknown. Previous in vitro studies of OPC migration in response to semaphorins have yielded inconsistent results, but these studies were performed on a variety of ECM substrates, i.e., collagen, poly-D-lysine. We systematically studied OPC migration and differentiation in response to chemotropic molecules on several different ECM substrates, to better understand the influence of ECM interactions on OPC migration and differentiation. Our live imaging experiments suggest that ECM substrate can regulate both the direction and rate of OPC migration. Semaphorin 3A is repulsive to OPCs on laminin 1, while on fibronectin OPC migration remains uniform in the presence of semaphorin 3A. Migration on laminin 1 is mediated by β1 integrins, while OPC migration on fibronectin requires αV integrins. Thus, signaling through the semaphorin-neuropilin-plexin receptor complex may be mediated by interactions with the β1 integrin-signaling pathway. Understanding the regulation of OPC migration and differentiation has important implications for the treatment of demyelinating diseases such as Multiple Sclerosis.

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CYTOSKELETAL REGULATION DOMINATES PROTEOMIC CHANGES ASSOCIATED WITH HIBERNATION IN 13-LINED GROUND SQUIRRELS
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13-lined ground squirrels are obligate hibernators that transition from summer homeothermy to winter heterothermy – wherein they exploit episodic torpor bouts. To explore the elements of winter neuroprotection, we applied 2D gel electrophoresis coupled with LC-MS/MS to brain extracts of squirrels in two summer, four winter and fall transition states. Only 88 protein spots differed significantly among groups. The abundance pattern of the brain proteome was reciprocal between cold-torpid and euthermic animals. The brain proteome of fall warm-, but not cold-torpid squirrels strongly resembled the homeotherms, indicating that the changes observed in torpid hibernators are defined by body temperature, not torpor per se. Metabolic enzymes were largely unchanged despite varied metabolic activity across annual and torpor-arousal cycles. Proteins elevated in summer and aroused winter squirrels represented pathway enrichments in neural growth/differentiation and synaptic transmission. Differential cytoskeletal regulation across torpor-arousal cycles is revealed by changes in microtubule dis/assembly regulating protein abundance (STMN1, DPYSL2), suggesting a mechanism for cytoskeletal stabilization during torpor and rapid reorganization on return to euthermoy.

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FUNCTION AND FATE OF INSULIN REACTIVE B CELLS IN TYPE 1 DIABETES
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Nearly 70% of newly produced B cells express autoreactive antigen receptors and must be silenced to prevent autoimmunity. Failure of silencing mechanisms is apparent in type 1 diabetes (T1D), where islet antigen specific B cells appear critical for disease. Evidence for a B cell role in T1D includes success of B cell depleting anti-CD20 therapy, which delays T1D progression in both NOD mice and new onset patients. Demonstrating the importance of specificity, NOD mice whose B cell repertoire is biased toward insulin reactivity show increased disease development while bias away from insulin reactivity prevents disease. Finally, though not required for illness, high affinity insulin autoantibodies are often the first harbingers of T1D. B cell cytokine production and auto-antigen presentation to self-reactive T cells are likely important in pathogenesis.

To further dissect the role of B cells in T1D we have examined the status of insulin specific B cells (IBC) in diabetes prone (NOD) and resistant (C57BL/6) mice. In C57BL/6, most IBCs are eliminated by central tolerance. Cells that reach the periphery appear largely anergic. They do not change in character or frequency with age. In contrast, NOD mice IBCs that reach the periphery have higher affinity for insulin, and these cells increase in frequency with age. They show signs of activation prior to appearance of insulin autoantibodies and overt disease. Preliminary data suggests these activated IBC can present antigen effectively to insulin-specific T cells and may be capable of disease transfer. We hypothesize that these cells may function as T1D initiators.
MECHANISMS OF FEEDFORWARD MOTOR CONTROL IN THE CEREBELLUM
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The cerebellum is a brain region with well-characterized anatomy, incorporating sensory inputs and generating precise motor outputs in real time. Theoretical models suggest that the cerebellar cortex and nuclei support rapid movements through feedforward control even in the absence of sensory feedback. It has been proposed that such feedforward computations require information about outgoing motor commands to generate the correct output. Therefore, the cerebellum is predicted to receive a copy of the motor output. Interestingly, collaterals of nuclear output neurons terminate in the cerebellar cortex, forming the nucleocortical pathway, and provide a copy of the motor command sent to the red nucleus and the ventrolateral thalamus. These collaterals are a source of motor input to the cerebellum but the significance is unknown. We hypothesize that this projection modifies incoming sensory input to the cerebellum. For example, nucleocortical axons could terminate onto feedforward inhibitory interneurons that converge with sensory inputs. To begin testing this hypothesis, we have implemented modern tracing techniques to better visualize these projections and determine which cells they contact. Using injections of retrograde tracer, we have labeled the somata of the nuclei and their collateral terminals making up the nucleocortical pathway. This technique enables us to characterize the targets and morphology of the nucleocortical terminals. Combining this with fluorescent antibodies and transgenic mice, we have identified cells in the cerebellar cortex adjacent to the nucleocortical terminals. Our data suggest that these cells are different subsets of inhibitory Golgi cells. These results are the first to define the detailed circuit organization of an identified motor pathway into the mammalian cerebellum, providing a mechanism to modify sensory input through inhibitory interneurons and supporting precise movement.

DURING HIBERNATION PROTECTION FROM WARM ISCHEMIA AFTER PROLONGED COLD ISCHEMIA IS MEDIATED BY XIAP AND PBAD
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Delayed graft function (DGF) is primarily caused by cold ischemia (CI) and warm reperfusion (WR). The 13-lined ground squirrel (GS) undergoes winter hibernation, when its core body temperature falls to 4°C for 6-18 days. Since hibernation is a normal part of GS life-cycle, we hypothesized that during hibernation GS are protected from apoptosis due to WR after CI. Kidneys of C57BL6 mice and hibernating GSs were exposed to CI in UW solution for 72hrs. Apoptotic renal tubular epithelial cells (RTECs) were scored by a pathologist. Immunoblots were performed to determine protein expression. Protein expression knockdown studies were performed using SI RNA approach. RTECs apoptosis was significantly increased in mouse vs. GS kidneys subjected to ex vivo CI. Anti-apoptotic XIAP, pAkt and pBAD were significantly more in hibernating GS kidneys compared to mouse kidneys. We sought to determine mechanism of protection of GS RTECs from 2 apoptotic stimuli: cisplatin (an agent known to cause apoptosis) and WR after CI. Cisplatin treated and WR subjected GS RTECs had significantly less apoptosis, no cleaved-caspase3 (CC3), increased XIAP, pAkt and pBAD vs. mouse RTECs. To demonstrate that XIAP and pAkt are required for protection against apoptosis, GS RTECs were treated with siRNA to reduce XIAP and pAkt expression. Treated cells had significantly increased apoptosis and CC3. Conclusion: We have shown for the first time that protection of GS RTECs from apoptotic stimuli such as WR after CI and cisplatin exposure is mediated by up-regulation of XIAP and pBAD. Application of these findings to human donor preservation may help prevent RTECs apoptosis and DGF.
SAXAGLIPTIN RESTORES VASCULAR MITOCHONDRIAL EXERCISE RESPONSE IN DIABETES
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Exercise decreases cardiovascular disease (CVD) risk and all-cause mortality. We and others have defined that exercise stimulates mitochondrial biogenesis via upstream signaling through endothelial nitric oxide synthase (eNOS), sirtuins (SIRTs), and/or PPARγ co-activator alpha (PGC1-α). Further, this response is absent in diabetes and hypertension. We tested the hypothesis that pharmacological restoration of vascular signaling with the dipeptidyl peptidase-4 (DPP-4) inhibitor saxagliptin will improve vascular mitochondrial adaptation to exercise through activation of eNOS, SIRTs, and/or PGC1-α. We examined the impact of an 8 day treadmill exercise intervention in the Goto-Kakizaki (GK) rat, a model of lean, type 2 diabetes (T2DM), and Wistar control rats with or without saxagliptin. Aortas were probed for mitochondrial complexes I-V, as well upstream signaling molecules. In Wistar rats, the expression of mitochondrial complexes III, IV, and V, along with SIRT3 (p<0.05), eNOS, and PGC1-α, increased with exercise. Conversely, in GK animals, exercise decreased complexes I, III, and IV, a decrease in the expression of COX IV (p<0.05) and AMPK, and no effect on eNOS or SIRT3. In exercised GK rats treated with saxagliptin, the expression of all mitochondrial complexes increased, complex IV significantly (p<0.05). Significant increases (p<0.05) were also observed in cytochrome c, eNOS and nNOS, PGC1-α, and UCP3 protein content in GK rats treated with both exercise and saxagliptin. In summary, our data suggest that saxagliptin restores vascular mitochondrial adaptation to exercise in a rodent model of diabetes. These data are proof of concept of a targetable mitochondrial defect in the diabetic vasculature.

COUNSELOR EXPERIENCES FACILITATING SOCIAL INCLUSION OF CHILDREN WITH DISABILITIES IN AN OVERNIGHT SUMMER CAMP
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A program evaluation was conducted of a program designed to include children with disabilities in a residential summer camp. The Summer Camp Inclusion Program (SCIP) served children ages 8-16 who carried diagnoses of developmental, physical, and/or cognitive disabilities, and who lived and participated in activities with typically functioning same-age peers over 4 and 8 week camp sessions. The purpose of the program evaluation was to provide the camp’s leadership with information about SCIP that would be helpful in understanding how the program was being implemented and identifying its strengths and areas in need of improvement. The evaluation also sought to determine the reactions to SCIP of various stakeholders, including camp staff members. Methods used included both written questionnaires and oral interviews conducted with 58 camp staff members. Both qualitative and quantitative data were gathered and analyzed. Results of the program evaluation indicated that camp staff members perceived the program to be effective at meeting the identified social and adaptive goals for program participant growth. Camp staff members also observed the program to have inadvertent positive effects on participants’ peers, including increased sensitivity to difference, sense of responsibility, and patience. As a result of their involvement with inclusion, staff members reported both added stress and added reward to their job roles, and described personal gains including new skills, character growth, and increased job satisfaction. Findings suggest that camp-based social inclusion of children with disabilities is not only feasible and beneficial to those children, but also has benefits for typically developing peers and the facilitating staff members.
ROLE OF HYPOXIA-INDUCIBLE FACTORS IN MODULATING VIRUS-SPECIFIC T CELL RESPONSES

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CD8$^+$ T cells are exposed to many environmental factors, including changes in oxygen levels, as they migrate from the circulation and accumulate in primary lymphoid organs during infection. In response to decreased oxygen availability, inflammation, and antigen stimulation, T cells activate hypoxic signaling pathways, including stabilization of hypoxia inducible factors (HIFs). This has the potential to influence the magnitude and functionality of the pathogen-specific response. T cell-specific deletion of HIF-1$\alpha$ enhances cytokine production, proliferation, and is protective against bacterial sepsis, implicating an inhibitory role for HIFs in T cells. However, the role of HIFs in regulating virus-specific CD8$^+$ T cell responses is not well characterized. Previous studies examining CD8$^+$ T cell-specific transcriptional changes after Lymphocytic Choriomeningitis Virus (LCMV) infection showed that decreased expression of HIF-1$\alpha$ and HIF-2$\alpha$ mRNA correlated with enhanced effector function. This led us to examine the role of HIFs in modulating primary and secondary CD8$^+$ T cell responses to LCMV. We found that during primary LCMV infection, expression of both HIF-1$\alpha$ and HIF-2$\alpha$ was upregulated in virus-specific T cells. Additionally, chemical stabilization of HIFs moderately impaired primary and secondary LCMV-specific T cell responses. However, T cell-specific deletion of HIF-1$\alpha$ only slightly enhanced primary and impaired secondary T cell responses. These results suggest that while HIFs are likely involved in modulating virus-specific T cell responses, HIF-2$\alpha$ may have a more prominent role. However, HIF-1$\alpha$ does appear to be important for survival and/or proper function of virus-specific memory T cells, suggesting that the timing and extent of HIF stabilization is likely to have considerable influence on the magnitude and efficacy of virus-specific T cell responses.

INTERACTIONS BETWEEN PRMD1A AND INTEGRINS DURING NEURAL CREST DIFFERENTIATION

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Migration of cranial neural crest cells (CNCCs) into the pharyngeal arches and subsequent interaction with the surrounding environment is necessary for the formation of the adult craniofacial skeleton. prdm1a is a zinc-finger containing transcription factor that is expressed in the posterior pharyngeal arches of the zebrafish (Danio rerio). Loss of prdm1a function results in a loss of posterior arch derived cartilage including ceratobranchials 2-5. To date, little is known about signaling downstream of prdm1a in NCCs and craniofacial development. We are taking a molecular based approach to identify downstream targets for Prdm1 in craniofacial development. Previously published microarray data from our lab show that itga5 is downregulated in prdm1a (nrd) mutants and analysis of potential enhancer regions surrounding itga5 reveals that there is a potential prdm1a binding site and preliminary data show an interaction by ChiP. Our ChiP-seq data shows that the binding partner of itga5 in mouse and human, itgb1b, is also a potential direct downstream target of prdm1a. Currently we are working towards understanding whether prdm1a is acting as an activator, repressor, or both in this gene regulatory network during differentiation of NCCs into craniofacial skeletal derivatives.
CD146-MEDIATED ENDOTHELIAL CELL INTEGRITY IS LOST DURING CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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The adhesion molecule CD146 is predominantly expressed at endothelial cell junctions, where it mediates cell-cell interactions and contributes to vascular integrity. When cleaved from its intracellular and transmembrane domains, a soluble form (sCD146) consisting of only the extracellular domain circulates in the bloodstream. Because of the importance of CD146 in endothelial barrier integrity, we investigated the role of CD146 in the pathogenesis of cigarette smoke-induced emphysema in humans and in an animal model.

We found that lung tissues from smokers exhibiting COPD (a condition including emphysema and chronic bronchitis) and from rats exposed to second hand smoke had significantly decreased levels of CD146. At the same time, both smokers with COPD and experimentally smoked rats had increased levels of sCD146 in their plasma and bronchoalveolar lavage fluid (BALF). Increased plasma levels of sCD146 correlated with the presence of anti-endothelial cell antibodies, both being potentially useful biomarkers for COPD. In CD146 knockout (KO) mice, distinct perivascular edema was seen in the lungs together with an influx of both inflammatory cells and protein, as measured in BALF.

Our findings in vivo were supplemented with in vitro studies in rat pulmonary micro- and macrovascular endothelial cells, where treatment with cigarette smoke extract or CD146 silencing decreased CD146 protein expression. This decrease was accompanied by increased endothelial monolayer permeability as well as an enhanced macrophage infiltration.

This is the first study to provide evidence that the loss of membrane-bound CD146 on endothelial cells diminishes pulmonary endothelial integrity, suggesting an involvement of CD146 in the pathogenesis of emphysema.

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CAESARIAN SECTION IS ASSOCIATED WITH INFANT FAT MASS AT 4-6 MONTHS OF AGE

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Infants delivered by caesarian section (CS) are twice as likely to develop pediatric obesity at age 3; however the biological mechanisms underlying these observations remain uncertain. Postulating that CS exerts influence on infant body composition by altering the timing and acquisition of gut microflora, we hypothesized mode of delivery (MOD) would be associated with infant fat mass (FM) postnatally. Infant body composition was assessed using displacement plethysmography (PEAPOD). Linear regression models were used to test MOD-FM associations at birth (n=544) and 4-6 months (n=213) in mother-child pairs currently enrolled in the Healthy Start study. At birth, the proportion of CS (n=113) and vaginal deliveries (n=431) was 20.7% and 79.2%, respectively. Among infants measured at 4-6 months, the proportion of CS deliveries (n=44) and vaginal deliveries (n=169) was similar to those at birth (20.6% and 79.3%, respectively). Our uncorrected analyses showed that infant FM did not differ according to MOD at birth (p=0.4) however MOD was significantly associated with infant fat mass at 4-6 month of age (p=0.04). Even after correction for infant sex, gestational age at birth, and maternal pre-pregnant BMI, CS was still associated with greater infant fat mass at 4-6 months of age (2 gr; 95% CI 0.3gr to 4.7gr; p=0.03) relative to vaginal deliveries (VD). In conclusion, CS may be associated with infant adiposity at 4-6 months of age whereas this association is not present at birth. Taken together, these results suggest that MOD may result in early changes to pediatric body composition, in part, via a potential mechanism that alters the timing and acquisition of neonatal gut microflora.
Y Liu, EA Jasion, JW Daily, R Nash, E Batte, P Arangua, FG La Rosa, PD Maroni, MS Lucia, AV Bokhoven, HT Sullivan, C Jones, K Lux, E Crawford, PN Werahera

Current TRUS-guided prostate biopsies are taken randomly and subjected to serious sampling errors often failing to diagnose >50% of clinically significant prostate cancer (PCa). Fluorescence spectroscopy guided prostate biopsies can focus on the abnormal tissue enabling precise targeting of the disease. First clinical trial results show a feasibility of a 14G optical biopsy needle adjutant with fluorescence spectroscopy for real-time in vivo PCa diagnosis.

Optical biopsy needle has an optical sensor which consists of eight 100µm fibers for tissue excitation and a single 200µm fiber to collect fluorescence spectra. Fluorometers include a spectrometer and two low-power LED light sources at wavelengths of 290 and 340nm. Spectral data acquisition takes approximately 0.5 second for each biopsy location. The in vivo optical biopsy were performed during radical prostatectomy surgery as an open procedure when the prostate is exposed but the blood vessels to the gland were not yet severed. Prostate biopsies were obtained after optical spectra have been collected from each biopsy location. Each biopsy core was histopathologically classified as benign or malignant and correlated with their spectra. Linear support vector machine (SVM) and leave-one-out cross validation method were tested for their ability to classify benign vs. malignant prostatic tissue.

Based on 13 patients data, SVM analysis provided 85% sensitivity, 92% specificity, 61% positive predictive value, and 98% negative predictive value for in vivo and 84%, 95%, 77% and 97%, respectively, for ex vivo benign vs. malignant prostatic tissue classification.

Clinical application of our optical biopsy needle can minimize false negative biopsies and eliminate a large proportion of repeat biopsies by targeting areas positive for PCa.

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Elevated expression of erbB3 rendered erbB2-overexpressing breast cancer cells resistant to paclitaxel. It is unclear whether an erbB3-targeted therapy may abrogate erbB2-mediated paclitaxel resistance. Here, we study the antitumor activity of an anti-erbB3 antibody MM-121 in combination with paclitaxel against erbB2-overexpressing breast cancer.

Cell growth assays were used to determine cell viability. Apoptosis was measured by ELISA. Western blot analyses were performed to assess the expression and activation of proteins. The tumor xenografts were established by inoculation of BT474-HR20 cells into nude mice. Immunohistochemistry was carried out to study the combinatorial effects on tumor cell proliferation and induction of apoptosis in vivo.

MM-121 significantly facilitated paclitaxel-mediated cytotoxicity in SKBR3.B3.1 and SKBR3.B3.2 cells and Herceptin resistant cell line BT474-HR20 which also showed resistance to paclitaxel. It specifically downregulated Survivin associated with inactivation of erbB2, erbB3, and Akt. MM-121 enhances paclitaxel-induced apoptosis. Furthermore, either MM-121 or low-dose paclitaxel had no effect on tumor growth, their combinations significantly inhibited tumor growth, reduced the cells with positive staining for Ki-67 and Survivin, and increased the cells with cleaved caspase-3 in vivo.

The combinations of MM-121 and paclitaxel not only inhibit tumor cell proliferation, but also promote erbB2-overexpressing breast cancer cells undergoing apoptosis via downregulation of Survivin in vitro and in vivo, suggesting that inactivation of erbB3 with MM-121 enhances paclitaxel-mediated antitumor activity against erbB2-overexpressing breast cancers. Our data supports further exploration of the combinatorial regimens consisting of MM-121 and paclitaxel in breast cancer patients with erbB2-overexpressing tumors, particularly those resistant to paclitaxel.
DECIPHERING THE ROLE OF REACTIVE OXYGEN SPECIES IN MITOCHONDRIAL DNA TRANSCRIPTION AND PROTECTION OF THE MITOCHONDRIAL GENOME

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The mitochondrial genome (mtDNA) encodes for 13 essential protein components of the oxidative phosphorylation pathway that produces the bulk of the energy consumed by the eukaryotic cell. Transcription of mtDNA is initiated by mitochondrial transcription factor A (TFAM), and in addition to its role as a transcription factor, TFAM is the primary protective agent of mtDNA. TFAM is a member of the high mobility group (HMG) box family of proteins that bind to the DNA minor groove and bend DNA to function properly. Recent work has shown that reactive oxygen species (ROS) can alter the role and cellular location of several HMG box proteins. mtDNA is constantly bombarded by ROS that can potentially damage the genome and alter TFAM function. We therefore investigated how ROS affects the TFAM/mtDNA relationship. We have previously shown that TFAM preferentially bends promoter DNA over genomic DNA, and this bending mechanism is key for both initiating DNA transcription and protection of mtDNA. In this study, we found that when TFAM is exposed to ROS, TFAM loses the ability to preferentially distort promoter DNA over genomic DNA, and that ROS treated TFAM has diminished transcriptional capabilities using FRET and in vitro mtDNA transcription assays. On the other hand, we found that ROS treated TFAM retains the ability to bend and protect genomic DNA using DNA compaction assays. These findings potentially reveal a novel mitochondrial signalling mechanism using ROS to regulate mitochondrial DNA transcription.

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INDIVIDUAL DIFFERENCES IN ACUTE COCAINE-INDUCED LOCOMOTOR ACTIVITY AND THE DEVELOPMENT OF CONDITIONED DOPAMINE RESPONSES TO A NATURAL REWARD

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Individuals vary in their initial response to drugs of abuse and in particular, initial responses to cocaine have been found to predict later development of cocaine use disorders. Outbred Sprague-Dawley rats also differ in their initial response to cocaine and we have developed a model that distinguishes rats based on the magnitude of their acute cocaine-induced locomotor activity (i.e., low or high cocaine responders; LCRs or HCRs, respectively). Interestingly, while acute cocaine inhibits the dopamine (DA) transporter to a greater extent and causes larger increases in extracellular DA in HCRs, LCRs more readily develop cocaine conditioned place preference and display greater sensitivity to the interoceptive effects of cocaine. These findings suggest that although the initial pharmacological effects of cocaine are smaller in LCRs, these animals are more sensitive to cocaine’s conditioned effects. However, a direct assessment of the development of conditioned DA responses in LCRs and HCRs has not been conducted. Thus, this study utilized *in vivo* fast-scan cyclic voltammetry to investigate the development of conditioned phasic DA responses to sucrose-reward within the nucleus accumbens core of rats classified as LCRs and HCRs. Although phasic DA events were detected in response to both conditioned and unconditioned stimulus presentation, preliminary analysis did not reveal differences between LCRs and HCRs. However, these findings are consistent with the hypothesis that LCR/HCR differences are specific to the effects of cocaine and not reward in general. Furthermore, this study sets the stage for future investigation into the development of conditioned DA responses to cocaine reward in LCRs and HCRs, which could provide a potential mechanism to help explain previously observed LCR/HCR differences in the rewarding effects of cocaine.

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ROLE OF HISTONE DEACETYLASE 9 (HDAC9) IN GNRH NEURON BIOLOGY

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SC-29425.

Microarray analysis of GT1-7(differentiated) as compared to NLT(undifferentiated) GnRH neuronal
cell lines revealed an upregulation of class-IIaHDAC9. Increases in HDAC9 by microarray(6-fold), RT-PCR(2.9-fold),
Immunoblot(10-fold) and specific-deacetylase activity in GT1-7cells suggested a role of that HDAC9 in GnRH neurons. Over-expression of
hHDAC9 in NLT GnRH neuronal cells protected cells from serum withdrawal induced apoptosis as assessed by cleaved-caspase3 (0.6vs.1.2fold in controls) and by Hoechst staining of condensed nuclei (0.5 fold), confirming a pro-survival role for HDAC9. Silencing of HDAC9 (by 80%) in GT1-7cells, did not alter caspase-3 cleavage, suggesting a redundancy with other Class-II HDACs (4,5 and/or 7).To ask the role of the domains and nuclear versus cytoplasmic
location to mediate HDAC9’s pro-survival effects, WT, N- and C-terminal HDAC9-mutants were tested. Immunocytochemistry demonstrated WT-hHDAC9 protein was expressed preferentially in the nucleus(N>C), whereas HDAC-N was only nuclear and HDAC9-C was exclusively cytoplasmic. In response to growth factor withdrawal induced apoptosis, both WT and C-terminal, but not N-terminal HDAC9 mutants showed decreased caspase3/7
luminescence as compared to vector (0.8-fold, p=0.05), suggesting that nuclear localization may not be critical for this cell specific effect. To ask if loss of HDAC9 had adverse effects on
GnRH neuron development, GnRH neuron numbers were counted in brains of WT and HDAC9-null mice (E11-E16 based on crown-rump length). A 37% decrease in GnRH neurons was observed in HDAC9-null (210±82, n=5) compared to WT (335±87, n=5) embryonic brains. Thus our data supports the role of HDAC9 to promote neuron survival across GnRH neuronal development via a unique cytoplasmic rather than nuclear site of action.

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PATIENT-LEVEL MEDICATION REGIMEN COMPLEXITY IN HIV-POSITIVE PATIENT POPULATIONS

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Medication effectiveness relies on medication adherence, yet medication regimens can become complex leading to decreased adherence. Regimens differ in complexity based on medication count, various dosage forms, dosing frequencies and additional directions. A gap exists relating to adherence and the corresponding patient-level medication complexity index as the entire medication regimen has not been considered. We derived the patient-level Medication Regimen Complexity Index (MRCI) for HIV-positive cohorts to identify key medication regimen complexity impact factors. This index considers both prescribed medications and over-the-counter medications. Sample cohorts of adult HIV-positive patients in active care in 2011-2012 with a qualifying medical diagnosis and prescribed an HIV-specific antiretroviral were randomly selected from electronic medical records at the Universities of Colorado and San Diego clinics. HIV-specific antiretrovirals provided only 21% of the total patient-level MRCI score. The non disease-specific prescriptions represented the largest portion of the score and the dosing frequency was the score driver in all medication types. Individual MRCI scores ranged from 2-67.5. Medication count, comorbidity count, and the Charlson comorbidity index were the distinguishing factors between highest and lowest medication regimen complexity patients. The two most influential factors of the MRCI patient-level scores were the non disease-specific prescribed medications and dosing frequency component. Thus, although medication regimen complexity tools for specific antiretroviral therapy regimens exist, HIV clinics should consider the entire patient-level medication regimen. A broader defined regimen allows for greater opportunities to decrease complexity and improve adherence. MRCI scores recognized high versus low complexity scores allowing identification of patients that could benefit from additional medication management.
COMPLEMENT RECEPTOR TYPE 2: THE LINK BETWEEN INNATE AND ADAPTIVE IMMUNE RESPONSES IN HEAD INJURY?
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The complement system represents one of the first cascades of neuroinflammation activated after head injury and can largely contribute to the development of secondary brain injury. Although the role of adaptive immunity in the central nervous system stays controversial, several studies could reveal an involvement of lymphocytes in tissue damage post-injury. Complement receptor 2 (CR2) mainly expressed by B-cells is known to provide a bridge between complement activation and humoral immune responses. In the present study, we hypothesized that CR2−/− mice would be protected from complement-mediated secondary neuropathology after closed head injury.

C57BL/6 wild-type and CR2−/− mice were subjected to experimental closed head injury, using a standardized weight-drop device. Outcome parameters consisted of neurological scoring, Western blot analysis of complement expression in brain homogenates, and histological assessment of intracerebral complement deposition, astrogliosis and neuronal cell death.

At one and four hours after trauma, brain-injured CR2−/− mice showed a significantly improved neurological outcome, compared to wild-type mice. Western blot and immunohistochemical analysis of brain tissue revealed markedly attenuated C3 levels in the injured hemispheres of CR2−/− mice. Intracerebral accumulation of GFAP-positive astrocytes and CD11b-positive microglia was reduced in head-injured CR2−/− mice, compared to wild-type littermates. CR2−/− mice showed a clearly decreased extent of neuronal cell death within 7 days post-trauma, as determined by TUNEL histochemistry.

These data emphasize a crucial role of CR2 in promoting neuroinflammation, secondary neurodegeneration and adverse outcome after head injury. Targeting complement activation on the level of CR2 might represent a promising approach for immunomodulation in posttraumatic patients.

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MICRORNA-MEDIATED six1a/b REGULATION DURING ZEBRAFISH MYOGENESIS
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Re-activation of developmental pathways is a well-recognized tumor-promoting mechanism. Pro-tumorigenic functions of the transcription factor SIX1 have been demonstrated in several cancers, including rhabdomyosarcoma, which arises in muscle precursors. SIX1 overexpression is observed in tumors originating from >10 different tissues and during embryogenesis SIX1 functions in the formation of muscle and kidney. Throughout development, microRNAs (miRs) have been shown to coordinate complex temporal and tissue-specific patterns of protein expression. miRs also act as tumor suppressors and have been shown specifically to regulate SIX1 expression in a kidney cancer model. In zebrafish, there are two SIX1 homologs, six1a and six1b, with very little known about six1a function. We have performed genetic studies to further elucidate the function of six1 paralogs and the miR-mediated regulation of six1 in normal myogenesis.

Morpholino-mediated knockdown of six1a leads to similar phenotypes as observed with six1b knockdown, including disrupted somite morphology, disarranged muscle fiber arrangement, and increased cell death. Further, six1a/six1b double morphants display enhanced phenotypes, indicating redundant functions. Utilizing prediction algorithms, we have identified three miRs predicted to target zebrafish six1a/b, including miR-30a. six1a/b expression is strongest in the somites at 24 hours post fertilization (hpf) and decreases by 48 hpf, while miR-30a displays a reciprocal expression pattern, consistent with miR-30a mediated regulation of six1a/b. Overexpression of RNA duplexes representing the 22-nucleotide mature miR sequence indicate that increased miR-30a leads to decreased six1a/b mRNA and protein levels. Importantly, abnormal somite morphology and increased cell death are observed, phenocopying six1a/b inhibition.

Altogether, these data suggest that six1a/b are controlled by miRs during zebrafish myogenesis, and provide a framework to examine whether miR dysregulation leads to SIX1 upregulation in rhabdomyosarcoma. Elucidating miR-mediated SIX1 regulation may provide insight into potential therapeutic interventions.
Luminal breast cancer metastasis is hormone-dependent, and progesterone activates dormant disease

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Luminal, estrogen (ER) and/or progesterone (PR) receptor-positive breast cancers are the most common subtype and contribute to deaths due to metastases. However, little is known about the role of estrogens (E) or progesterone (P) in breast cancer metastasis. To this end, we have generated a metastasis model in ovariectomized immune-compromised mice using human Luminal breast cancer cells injected directly into the arterial circulation. Tumor cells were tagged with luciferase and ZsGreen to quantify whole-body and organ-specific metastatic burden in vivo, and mice were untreated (control), or supplemented with physiological concentrations of E or E+P. We also tested ER–PR– breast cancer cells. Due to extensive metastases, survival of mice injected with ER–PR– cells was brief regardless of hormone treatment. On the other hand Luminal ER+PR+ cells failed to produce metastases in the absence of hormones. However, E or E+P promoted their metastasis and the major sites were analogous to those seen clinically, including bone, brain and lungs. Despite lack of metastases in the non-hormone controls, the Luminal tumor cells remained in mice however, apparently in a dormant state. When control mice were switched to hormones, metastases were rapidly activated. Hormones also promoted induction of an ER–PR– cell subpopulation which would be resistant endocrine therapies. These cells, which we call Luminobasal, also proliferate slowly, likely making them resistant to chemotherapies. In sum, we demonstrate that E and P promote Luminal breast cancer metastasis, may reactivate dormant disease at metastatic sites, and stimulate expression of an ER–PR– metastatic tumor-cell subpopulation that would be resistant to hormone therapies.

LYSOPHOSPHATIDIC ACID SIGNALING VIA LPA₅ INHIBITS CD8⁺ T CELL ACTIVATION AND CONTROL OF TUMOR PROGRESSION

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Lyso phosphatidic acid (LPA) is a phospholipid that induces a diversity of biological and pathophysiological effects by binding extracellular G-protein coupled receptors (designated LPA₁₋₄). LPA is well-associated with cancer, as a number of different malignancies produce elevated levels of LPA (e.g. ovarian, myeloma), and the increased concentration has been shown to promote tumor progression by enhancing metastasis, viability, angiogenesis, and therapeutic resistance. In contrast, the presence of tumor-infiltrating CD8⁺ T cells (TIL) have been associated with prolonged patient survival. However, multiple inhibitory mechanisms within the tumor microenvironment exist that impede TIL-mediated tumor rejection, such as inhibitory receptor signaling and low affinity antigen receptors. Although CD8⁺ T lymphocytes express LPA receptors, the effect of LPA signaling on the CD8⁺ T cell immune response has not been thoroughly studied. Here we show that LPA suppresses CD8⁺ T cell receptor signaling via LPA₅, and this leads to reduced antigen-specific activation and proliferation. Through use of the OT-I TCR transgenic mouse model, we find that LPA signaling more potently inhibits activation to a lower affinity antigen, suggesting increased suppression of TIL function. Finally, we show that transfer of LPA₅-deficient, but not wild type, tumor-specific CD8⁺ T cells significantly abates progression of established melanoma tumors. This indicates that increased production of LPA by the tumor serves as a mechanism to suppress adaptive immunity, highlighting a potential target to improve the immune response to cancer.
POST-TRANSLATIONAL MODIFICATION AND REGULATION OF GLUTAMATE CYSTEINE LIGASE BY S-Glutathionylation

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The glutathione (GSH) antioxidant defense system plays a critical role in maintaining cellular redox homeostasis and counteracting deleterious effects of oxidative stress. GSH can also be utilized in disulfide exchange reactions resulting in formation of mixed protein-glutathione disulfides and S-glutathionylation of proteins is gaining recognition as an important signal transduction mechanism during oxidative stress. The first and rate-limiting step in GSH biosynthesis is catalyzed by glutamate cysteine ligase (GCL), a heterodimeric holoenzyme composed of a catalytic (GCLC) and modulatory (GCLM) subunit. While cellular GCL activity is highly sensitive to relative GCL subunit expression levels, we and others have demonstrated that post-translational modifications of the GCL subunits may play a major role in the acute regulation of GCL activity. In this study, purified recombinant proteins were utilized to demonstrate that GCLC and GCLM are both direct targets for reversible S-glutathionylation in vitro. S-Glutathionylation increased GCLC activity ~2-fold, yet had little effect on GCL holoenzyme activity. While S-glutathionylation prevented GCL holoenzyme formation and activity, it did not dissociate the GCL holoenzyme complex. The masking of relevant cysteine residues may account for these apparent discrepancies as prior formation of GCL holoenzyme significantly reduced S-glutathionylation of both GCL subunits. Mass spectrometry analysis identified multiple residues on both subunits that are modified and may be functionally relevant based on in silico molecular modeling. These findings demonstrate that GCLC and GCL holoenzyme formation and activity can be regulated via post-translational S-glutathionylation of the GCL subunits in vitro. This novel post-translational regulation of GCL activity could significantly affect cellular redox homeostasis and signal transduction during periods of oxidative stress.

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RAMAN SIGNATURES ANALYSIS OF OVARIAN CANCER CELL LINES

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Ovarian and breast cancers are the leading cancers among women. Histopathology and laparotomy/frozen sections analysis are often subjective interpretations and time consuming. Many diagnosis protocols can be simplified if specific information is obtained directly from the cells alone. Raman spectroscopy provides chemical-specific information in a label-free minimally invasive measurement.

In this work, cancer cells nuclei Raman spectra, Linear Discriminant Analysis (PCA-LDA) and Naive-Bayes classifier (NBC) with 10-fold cross-validation algorithms were applied to measure and analyze cancer cell lines, CAOV3, OV429, A2780, HEY, & MCF7 according to their spectral variables in the range 600 – 1800 cm⁻¹. Our goal was to find specific markers, related to nucleic acids, proteins, lipids, and carbohydrates signatures, for delineation of ovarian and breast cancer cells.

Specific Raman vibrational frequencies were found among these cancer cell lines particularly at 783 (O-P-O, DNA), 810 (O-P-O, RNA), 935 (carbohydrates), 1093 (DNA/RNA) 1003 (phenylalanine), 1093 (PO₄ DNA/RNA), 1228 (Amide III, lipids), 1424 (CH deformation of DNA/RNA, lipids), 1448 (CH₂ deformation, DNA/RNA, proteins, lipids), 1552 (Amide II), 1670 (Amide I, C=C stretching) cm⁻¹. The PCA-LDA and NBC identify the cell type with 78%-100% sensitivity, 95%-100% specificity, and 87%-100% precision. The test errors for both methods were about 7.14%.

Based on our results, we conclude that DNA may be a good marker to target ovarian and cancer cell lines. Raman spectroscopy together with PCA-LDA and NBC is capable to discriminate the cell lines based on their DNA spectral signatures.
MUTATION IN SPLICEOSOMAL PROTEIN SF3B1 INDUCES ERYTHROID MATURATION ARREST
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Myelodysplastic Syndrome (MDS) is a group of clonal hematopoietic disorders characterized by ineffective production of blood cells by the bone marrow and a propensity to transform to acute leukemia. Over 14,000 new cases of MDS are reported each year in the US. Recently, whole genome sequencing (WGS) of MDS patient samples revealed recurrent mutations in proteins of the splicing machinery. RNA splicing is the molecular process by which introns are removed from pre-mRNA in the nucleus. SF3B1 is the most commonly mutated spliceosomal protein in MDS. SF3B1 is a 155 kilodalton (kD) protein that forms part of the U2 spliceosomal complex. A non-synonymous mutation (K700E; lysine to glutamic acid at the 700th amino acid) is the most common SF3B1 mutation in MDS. As MDS patients with SF3B1 mutation have profound anemia, we hypothesized that expression of SF3B1-K700E in normal hematopoietic precursors will result in maturation arrest of the erythroid lineage. Wild-type (SF3B1-WT) and mutant (SF3B1-K700E) were expressed in human CD34+ cells by means of retroviral vectors. Erythroid maturation was then effected by culture in two phases: A 7 day culture in hematopoietic cytokines IL3, Stem Cell Factor, FLT3 ligand and IL6 and a subsequent 10-14 day culture in Erythropoietin (EPO). Cells were assayed every 3 days for expression of erythroid maturation markers CD71, CD105 and CD235A. At days 7 to 10 of erythroid induction, cells expressing SF3B1-K700E were found to express significantly lower levels of CD71 and CD105 when compared to SF3B1-WT expressing controls. Studies defining the molecular mechanisms of SF3B1-K700E in this maturation arrest are ongoing.

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HUMAN APOLIPOPROTEIN A-1 DECREASES PLATELET ACTIVATION RESPONSES
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Increased levels of high density lipoprotein have been associated with decreased occurrence of thromboembolism. Apolipoprotein A-1 (apoA-1), the active component of HDL, but its effects have never been investigated. We explored the effects of ApoA-1 on platelet activation responses using functional assays and murine induced-thrombosis models.

All experiments were performed in samples from healthy volunteers at a concentration of 300 µg/mL of apoA1. Thromboelastography (TEG) was carried out in whole blood. Light transmission platelet aggregometry was conducted using apheresis platelet concentrates. Platelet activation were measured by flow cytometry. Murine thrombosis models included ferric chloride (FeCl3)-induced carotid artery thrombosis and collagen/epinephrine-induced pulmonary embolism (PE).

Collagen induced platelet aggregation of 23.9 +/- 5.8% in apoA1 treated platelets and 80.7 +/- 3.3% in controls (p<0.001, t-test).

ApoA1 induced decreased strength and increased lysis at 30 minutes (4.9 +/- 0.3 kd/cm² and 11.8 +/- 3.1%), compared to controls (7.9 kd/cm² and 0.8 +/- 0.6 %, p<0.01) employing kaolin-TEG.

P-selectin surface expression was inhibited by apoA1 compared to controls (10.4 +/- 1.1 MFI vs. 17 +/- 2.3 MFI, p<0.01).

Injection of collagen and epinephrine induced rapid fatality (presumably by PE) at 3.3 +/- 1 min in controls (n=5), which was abrogated in apoA1 treated mice such that none died at 30 min (n=5, p< 0.001).

Lastly, application of 6% FeCl3 to the murine carotid artery induced initial occlusion within 5.7 +/- 0.7 min in controls treated with vehicle (n=5) vs. 7.7 +/- 1.8 min in apoA1 treated mice (n=5, p<0.05).

We have shown that exogenous apoA1 inhibited human platelet activation responses, and protects mice from arterial and venous thrombosis.
**T-BET: A KEY PLAYER IN A NOVEL TYPE OF B CELL ACTIVATION ESSENTIAL FOR EFFECTIVE VIRAL CLEARANCE**

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IgG2a is known to be the most efficient antibody isotype for viral clearance. Here we demonstrate a novel pathway of B cell activation, leading to IgG2a production, and involving synergistic stimulation via B cell antigen receptors, TLR7 and interferon receptors. This leads to induction of T-bet expression in B cells, driving their differentiation into age-associated B cells (ABCs). T-bet positive ABCs appear during anti-viral responses and produce high titers of anti-viral IgG2a antibodies which are critical for efficient viral clearance. The results thus demonstrate a previously unknown role for T-bet expression in B cells during viral infections. Moreover the appearance of ABCs during anti-viral responses and during autoimmunity suggests a possible link between these two processes.

**THYROID HORMONE RESPONSIVE PROTEIN (SPOT14) DIRECTLY INCREASES DE NOVO FATTY ACID SYNTHESIS**

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Departments of \(^1\)Pathology and \(^2\)Pharmacology

The de novo fatty acid synthesis pathway plays a pivotal role in obesity and multiple forms of cancer, and activity of the de novo fatty acid synthesis pathway has been linked to a poorer outcome for patients with most cancers. The de novo fatty acid synthesis pathway also contributes prominently to the triglyceride fat (TAG) component of milk during lactation via synthesis of medium chain fatty acids (MCFAs). Effector proteins present specifically in mammary epithelial cells (MECs) modify the de novo pathway output such that the distribution of FASN products is converted from 16 carbon fatty acids to MCFAs containing 8-14 acyl carbons. The Spot14 genomic knockout mice exhibit a lactation defect. Analysis of Spot14 null milk, whole mammary glands, and adipose depleted MECs revealed significant decreases specifically in the de novo synthesized MCFAs. Loss of Spot14 did not result in decreased expression of fatty acid metabolism genes, enzyme protein levels, or in a variety of intermediate metabolites that support de novo MCFA synthesis. The MEC specific enzyme required for synthesis of MFCAs, thioesterase 2, was present at equivalent levels in Spot14 null and control MECs. Together, these results suggested that the activity of FASN is deficient in MECs from Spot14 knockout mice during lactation. Spot14 protein co-migrated with native enzyme complexes of FASN in non-denaturing gels, implying that Spot14 might associate with FASN to thereby alter enzyme activity for the synthesis of MCFAs. To test this possibility, a novel FASN activity assay was designed to sensitively quantify FASN products and to discriminate those products by acyl chain length. The new in vitro FASN assay revealed that Spot14 increased FASN kinetics 1.6-fold by lowering the Km for Malonyl-CoA resulting in increased amount of MCFAs synthesized in the reaction. Given that inhibitors FASN often result in whole body complications, we posit that understanding the role of FASN effector proteins might expand the repertoire of drug-based therapies.
CHARACTERIZATION OF SEIZURES AND EFFECTS OF FLUPIRTINE IN AN ANIMAL MODEL OF NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY
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Hypoxia-ischemia is a common cause of seizures in full-term infants. Current antiepileptic drugs, that were developed using adult animal models, are not fully effective in treating neonatal seizures. The Rice-Vannucci model is commonly used to study acute and chronic pathologies caused by neonatal hypoxia-ischemia. However, the occurrence of seizures in this model has not been well characterized. In the current study, we have characterized hypoxia-ischemia-induced neonatal seizures and evaluated the effects of flupirtine, a potassium channel opener, on acute seizures. To induce hypoxia-ischemia, the right carotid artery of 7-day-old rats was ligated and exposed to hypoxia for 2 hours. To characterize seizures, pups were video-EEG monitored during hypoxia and at various time points after hypoxia-ischemia. Pups were treated with flupirtine or vehicle prior to hypoxia or 5 minutes after the occurrence of first electroclinical seizure during hypoxia. Analysis of video-EEG recordings revealed that during hypoxia, all pups exhibit multiple electroclinical seizures. All of them have seizures during the reperfusion period and 40% of them have seizures 24 hours after hypoxia-ischemia. However, seizures were not observed in any of these rats 48 or 72 hours after hypoxia-ischemia. In a separate study, rats treated with 50mg/kg flupirtine prior to hypoxia did not develop seizures, whereas all vehicle treated rats developed seizures upon exposure to hypoxia (p<0.005). Additionally, 25mg/kg flupirtine given 5 minutes after the first electroclinical seizure reduced the number and duration of subsequent seizures during hypoxia. Our study suggests that the Rice-Vannucci model is a valid model to test the efficacy of drugs to treat neonatal seizures. Further, our initial results suggest that flupirtine effectively treats hypoxia-ischemia-induced acute neonatal seizures.

EXAGGERATED CARDIAC CONTRACTILE DEPRESSION IN AGING ENDOTOXEMIC MICE IS ASSOCIATED WITH ENHANCED INFLAMMATORY RESPONSES
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Understanding of the impact of endotoxemia and inflammatory response on vital organ function is helpful for improving post-surgery outcomes in the elderly. It is unclear whether aging heart is more vulnerable to endotoxemic cardiac depression and whether augmented inflammatory response is responsible for age-dependent vulnerability if it does.

Adult (4-6 months) and aging (18-20 months) C57BL/6 mice were treated with a low dose of endotoxin (0.5 mg/kg, iv) for 6 h. Left ventricle (LV) function was assessed using a microcatheter. Chemokines (MCP-1, KC and MIP-1) and cytokines (TNF-α, IL-1β and IL-6) in plasma and the myocardium, as well as cardiac troponin I in plasma were analyzed by ELISA. Neutrophils and mononuclear cells infiltration in the myocardium were examined using immunofluorescence staining.

Aging endotoxemic mice exhibited worse LV function (cardiac output 1.5±2.3 ml/min vs 5.6±3.6 ml/min in adult mice). In addition, cardiac troponin I levels in plasma were higher in aging endotoxemic mice. Aging endotoxemic mice had markedly higher levels of MCP-1 and KC, but lower levels of MIP-1 in both plasma and myocardium in comparison with adult endotoxemic mice. The exaggerated cardiac contractile depression in aging mice is associated with greater densities of neutrophils and mononuclear cells in the myocardium, and higher levels of TNF-α, IL-1β and IL-6 in the circulation and myocardium.

Endotoxemia causes exaggerated cardiac contractile depression in aging mice. The increased vulnerability to endotoxemic cardiac depression in aging mice is associated with enhanced systemic and cardiac inflammatory responses. Our findings suggest that special attention is needed to suppress the inflammatory responses and to protect the heart in the elderly with endotoxemia.
PITHELIAL-MESENCHYMAL SIGNALING INTERACTIONS COORDINATES OUTGROWTH AND PATTERNING OF THE MOUSE LIMB

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The development of the limb relies on epithelial-mesenchymal interactions to coordinate patterning and outgrowth. Previous studies in chick and mouse support a model in which a signaling cascade beginning with Fgf10 in the flank mesenchyme activates Wnt/b-catenin signaling in the overlying ectoderm. The activation of the canonical Wnt pathway subsequently stimulates FGF induction that directs AER formation and limb outgrowth. We show that pan-ectodermal stabilization of b-catenin is sufficient to cause growth expansion of limb tissue into the inter-limb flank, resulting in a transient uni-limb phenotype. This phenotype is accompanied by an early and substantial up-regulation of Fgf8 expression in the ectoderm of the inter-limb flank. We are now extending these studies to test specific predictions raised by the linear model Fgf10 – Wnt/b-catenin – Fgf8 – AER by manipulation of Fgf8, Fgf10, and β-catenin levels in the mouse embryo. In this context, we have recently developed new Fgf8b lox-stop-lox alleles that can be used to test how alterations in Fgf8 expression impact limb patterning. Together, our results demonstrate that a fundamental balance of ectodermal β-catenin and Fgf signaling is necessary for proper limb development and reveal unexpected interactions between these pathways in limb patterning.

THE ANIONIC SURFACTANT LIPID, PALMITOYLOLEOYL-PHOSPHATIDYLGLYCEROL (POPG), SUPPRESSES DER P 2-STIMULATED INFLAMMATORY RESPONSES IN LUNG EPITHELIAL CELLS AND MACROPHAGES

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Der p 2 is the major allergen from house dust mite (HDM). More than 80% of HDM allergic patients have high Der p 2-specific IgE reactivity. Previous studies showed that the lung surfactant lipid, POPG, inhibits LPS-induced inflammatory responses by mouse and human macrophages. This study reports the protective effects of POPG on Der p 2-induced allergic responses in human bronchial epithelial cells (BEAS2B) and mouse alveolar macrophages (RAW264.7). Methods: Recombinant Der p 2 (rDer p 2) was produced in Pichia pastoris. The structure and IgE reactivity were confirmed by Circular Dichroism (CD) and rDer p 2 inhibition of serum-IgE binding to natural Der p 2. Pro-inflammatory responses were determined using BEAS2B and RAW 264.7 incubated with rDer p 2, Pam3cys, or LPS in the presence, or absence of surfactant phospholipids. Results: rDer p 2 stimulated IL-8 secretion from BEAS2B cells and TNFα secretion from RAW 264.7 cells. POPG suppressed TNFα secretion with IC₅₀ of 11.77 μg/ml and suppressed IL-8 secretion from BEAS 2 B cell with IC₅₀ of 14.69 μg/ml. A negatively charged phospholipid, palmitoyloleoyl-phosphatidic acid, which has a structure similar to POPG, was ineffective as an inhibitor of IL-8 and TNFα secretion. The site of action of POPG is upstream of JNK and p38 MAP Kinase and appears to be at the level of cell surface receptors for Der p 2. Conclusions: The Der p 2-TLR2 activated proinflammatory responses of macrophages and epithelial cells occur via JNK and p38 MAPK signaling pathways which can be inhibited by the lung surfactant lipid POPG.

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TOWARD THE SECOND GENERATION OF LIGHT INDUCIBLE DIMERIZERS; OPTIMIZING PHOTO RESPONSES IN THE CRY2-CIB1 MODULE VIA DIRECTED EVOLUTION

A Taslimi, J Vrana and C Tucker

Optogenetic tools offer high level of spatial and temporal control over biological processes. One class of these tools includes light dependent protein interaction modules that allow for the control of protein-protein interactions with light. This class includes a couple systems for light inducible dimerization, one of which is the CRY2 (Cryptochrome)-CIB1 (cryptochrome-interacting basic-helix-loop-helix) module. CRY2p undergoes a blue light dependent conformational change upon which it rapidly associates with CIB1 and over the course of approximately 15 minutes completely dissociates. While CRY2-CIB1 module is useful for many applications it could be potentially optimized and further engineered. Using directed evolution and screening via the yeast two-hybrid assay we have identified CRY2 variants with altered dark reversion rate and variants with blue shifted absorption spectra.

SIX1 REGULATES THE PATTERNING OF MAXILLARY NEURAL CREST CELLS.

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Endothelin-1 (Edn1)-induced signaling of the endothelin-A receptor (Ednra) is crucial for dorsal-ventral (D-V) patterning of cranial neural crest cell (CNCC) within the mandibular pharyngeal arch. Targeted deletion of Edn1 or Ednra in mice causes perinatal lethality due to severe craniofacial birth defects that include homeotic transformation of mandibular arch-derived structures into more maxillary-like structures. CNCCs express Ednra whereas Edn1 is derived from the overlying ectoderm, core mesoderm and pouch endoderm. To define pathways that establish a more dorsal/proximal or more ventral/distal fate in the first arch, we created transgenic mice containing a silent Edn1 expression cassette (CBA-Edn1). Overexpression of Edn1 in CNCCs (CBA-Edn1;Wnt1-Cre) caused a homeotic transformation of maxillary structures into more mandibular-like structures. This transformation was preceded by proximal expansion of distal genes (Dlx3, Dlx5, Hand2) and disruption of proximal genes (Dlx2, Twist1, Pou3f3, Six1, Eya1) in the first arch. We focused on Six1, as plays roles in vertebrate development, with mutations in Six1 and/or its partner Eya1 are found in patients with branchio-oto-renal syndrome, an autosomal-dominant developmental disorder characterized by hearing loss, branchial arch defects and various renal anomalies. We found that in Six1-null mouse embryos, mutants had a partial transformation of maxilla into a more proximal mandible structure. In addition, Dlx3 expression expanded and Twist1 expression was reduced in the first arch of mutant embryos. Deletion of one allele of Ednra rescued this transformation (Six1-/-;Ednra-/-) in 1/3 of mutant embryos. Together, our results show that the proximal first pharyngeal arch is competent to form either a mandible or a maxilla and inductive/repressive signals are responsible to drive CNCCs into either fate.

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ROLE OF MEMO1 IN CRANIOFACIAL DEVELOPMENT

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Development of the cranium and craniofacial structures is a tightly regulated developmental process, involving interactions between multiple embryonic tissues and requiring precise regulation of cellular growth, proliferation, death and migration. Due to this highly orchestrated series of events, small perturbations in this process can result in a range of birth defects affecting these tissues, evident by the high incidence of birth defects involving the head (~75% of all birth defects), most common of which includes clefting of orofacial tissues (~1-600 to 1-1,000 live births). These birth defects will impart a significant decrease in the quality of life of those afflicted and present a significant economic burden associated with corrective operations. Thus, one goal of current research efforts is to precisely define genes controlling these cellular processes during normal craniofacial development, in turn providing clarity in understanding how their disruption would impact human development. To this end, through a recessive ENU-based mutagenesis screen, we have identified Mediator of ErbB2-driven cell motility 1 (Memo1) as a novel regulator of multiple aspects of craniofacial development. Memo1nu/nu mutants display defects in components of the skull-base, including the presphenoid, basisphenoid, basioccipital, and hyoid bones as well as disruption of the palatine shelf and consequently clefting of the secondary palate. Interestingly, most of these structures are derived from the cranial neural crest cells (CNCC), a multi-potent early embryonic stem-cell population that when disrupted causes a plethora of craniofacial disorders. Thus, Memo1 controls important aspects of craniofacial development, likely mediated to some extent through CNCCs, although its mechanistic role in this process is largely unknown. Given the Memo1-null is early embryonic lethal, our ENU-allele provides a unique model to begin to decipher Memo1’s role in craniofacial development.

DELETERIOUS CROSS TALK BETWEEN ESTROGEN AND THYROID SIGNALING ENHANCES STEM CELL POPULATION IN LUMINAL BREAST CANCERS

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Luminal breast cancers (BC) contain heterogeneous cell subpopulations, including non-hormone receptor expressing stem cells that are typically resistant to chemotherapy. Regulatory promoters that enrich stem cells are not well understood, although the transition is believed to be plastic and bidirectional. Up to two-thirds of women with newly diagnosed BC have clinical/subclinical thyroid disease, as compared to 10% of the general population. One in five women with BC have taken thyroid hormone replacement therapy (THRT) long term, prior to BC development. While this co-morbidity has been observed for over a century, causality has not been established. To explore the effects of THRT on BC patients, we studied clinical, histologic and outcomes data from >800 archival node negative (Stage I) cases. THRT was independently associated with shortened disease free and overall outcomes in estrogen receptor (ER)+, but not ER- patients. We hypothesized that estrogen (E2) and thyroid hormone (TH) interact to promote BC and tumor aggression. In preclinical studies, we have shown that ER+ but not ER- breast cancer cells in culture, treated with TH ± E2, show higher rates of proliferation, have altered expression of cell-cycle regulatory genes (E2F1/cyclins (A/B/D1/E)), increase their capacity to form mammospheres and show expansion of the CD24+/CD44+ stem-like/basaloid cell subpopulation by flow cytometry. These stem-like cells were also less responsive to chemotherapy. Coadministration of the partial ER antagonist tamoxifen enhanced, whereas a complete antagonist (Fulvestrant) (ICI182780) blocked these interactions, indicating that ER signaling is required for these interactions. Additionally, we have shown that the BMI-1 gene in the c-MYC signaling pathway, is altered by these interactions.
NOVEL FUNCTIONS OF THE HOMEOPROTEIN SIX2 IN MEDIATING ANCHORAGE INDEPENDENCE AND METASTASIS IN BREAST CANCER

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Homeobox genes encode for transcription factors that are master regulators of embryogenesis, and their misexpression has been implicated in multiple cancers. The role of the homeoprotein Six2 in developing kidney has been well demonstrated; however, its role in cancer progression is largely unknown. Here, we demonstrate, for the first time, that Six2 is causally involved in mammary tumor progression. When Six2 is knocked down (KD) in the 66cl4 mammary carcinoma cells, lung metastasis is significantly decreased compared to control KD; however, Six2 KD conferred no significant effect on primary tumor growth or on tumor-associated lymphangiogenesis, in contrast to its closely related family member, Six1, which has been implicated in all the aforementioned properties. Expression of Six2 in the 4TO7 cell line (syngeneic with 66cl4, but expresses low levels of Six2) led to changes in cell morphology, increased growth in soft agar, increased resistance to anoikis, and significantly enhanced lung metastasis in Balb/c mice. To determine the mechanism by which Six2 mediates metastasis, microarray analysis was performed; genes have been implicated in lung metastasis (MMP, ANGPTL4, VCAM1) are significantly up-regulated in Six2 expressing 4TO7 cells; while the epithelial marker, E-Cadherin, is dramatically decreased. Finally, analysis of SIX2 expression from public microarray datasets indicates that SIX2 is increased in breast cancers compared to normal breast tissue. In addition, high expression of SIX2 correlates with poor prognosis in 1881 human breast tumors examined using the Gene Expression-Based Outcome for Breast Cancer Online database, and upon further investigation we found that SIX2 expression is particularly associated with poor prognosis in luminal A and ER-positive breast tumors. Together, our studies define a novel role of Six2 in breast cancer metastasis.

REGULATION OF MAMMARY TUMOR GROWTH AND FATTY ACID SYNTHESIS BY SPOT14

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Elevated fatty acid synthesis is a distinguishing feature of many solid tumors thought to be acquired during tumor progression, as rapidly proliferating cancer cells have a heightened requirement for membrane phospholipid precursors. In the mammary epithelium, elevated fatty acid synthesis is associated with lactogenic differentiation, and with malignant transformation, suggesting that the timing and cellular context of fatty acid synthesis activity strongly dictates its effect. Spot14 (S14), which is encoded by the THRSP gene, regulates fatty acid synthesis in the liver, adipose, and lactating mammary gland, and has been linked to breast cancer cell growth, differentiation, and to the outcome of patients with invasive disease. To determine the effect of S14 overexpression on mammary tumorigenesis, we crossed transgenic mice expressing S14 in the mammary epithelium to MMTV-Neu mice, and we also crossed mice S14-null mice with MMTV-PyMT mice. We analyzed tumor latency, cell proliferation, and fatty acid contents, and tumor gene expression profiles. Here, we show that elevated S14 shortens Neu-induced tumor latency and stimulates fatty acid synthesis and proliferation, but reduces tumor metastasis. In PyMT transgenic mice, S14 loss delays solid tumor formation and decreases cell proliferation. Our in-depth analysis of mouse and human tumors suggests that high S14 expression is associated with features of mammary lobular epithelial differentiation and predicts a relatively favorable prognosis for breast cancer patients. This study describes a role for S14 in regulating mammary tumor formation, fatty acid synthesis, and growth in vivo. We suggest that S14 is highly expressed in well-differentiated mammary epithelial cells, and S14-mediated fatty acid synthesis promotes the expansion of this population in the presence of oncogenic stimuli.
GAMMA-BAND DEFICITS DURING LANGUAGE PROCESSING IN AUTISM
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Deficits involving phonology are observed in a significant subset of children with autism and have been observed in unaffected first-degree relatives. Despite this, no neuroimaging studies have investigated phonological processing in individuals with autism. Magnetoencephalography (MEG) was utilized to investigate the neurobiology of phonological processing in adults with autism as well as parents of children with autism.

Thirteen adults with autism, sixteen parents of a child with autism, and seventeen controls performed a phonological priming task while undergoing whole-cortex MEG. The task consisted of four prime-target word conditions including homophones (e.g., PAUSE-paws), pseudohomophones (e.g., JURM-germ), unprimed (e.g., CHECK-slang), and word/nonword pairs (e.g., FISH-nath). Primes were presented below perceptual threshold (i.e., 30ms), and subjects performed a lexical decision task (i.e., is it a word or a nonword?) on all targets.

In our pseudohomophone condition placing heavier demands on phonological decoding skills, adults with autism exhibited reduced evoked gamma activity relative to both controls and parents in the left SMG, reduced evoked gamma activity relative to controls in the left STG, and reduced induced gamma activity relative to parents in the left STG and SMG. Reductions in evoked gamma were also observed in adults with autism for unprimed relative to primed stimuli in the left SMG relative to controls, but in the right STG relative to parents.

Abnormalities involving gamma-band activity, a candidate endophenotype in autism, were observed during phonological processing. These findings suggest a possible neurobiological substrate of phonological impairments in individuals with autism that has potential translational significance for language interventions in autism. Preliminary evidence for alternate neural strategies during phonological processing in parents of children of autism was also found.

DUAL MTOR AND HSP90 TARGETED THERAPY IN PANCREAS CANCER
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Pancreatic cancer remains a devastating disease with minimal therapeutic options. This is in part due to the complex genetics associated with this disease. A combination of in vitro cell line proliferation and clonogenic assays along with cell line-based orthotopic xenografts with a mTOR and HSP90 inhibitor provide initial evidence of the synergistic activity of dual mTOR and HSP90 inhibition on tumor growth inhibition. This occurs in part through the induction of tumor cell apoptosis and the inhibition of angiogenesis. The patient-derived xenograft (PD-Xeno) model represents a better predictive model of chemotherapy induced tumor growth inhibitory effects than cell line-based xenografts which show the strong synergistic effects of dual mTOR and HSP90 inhibition on tumor growth inhibition.
NEGATIVE AFFECT INFLUENCES FRONTO-LIMBIC BRAIN ACTIVITY DURING DECISION-MAKING IN SUBSTANCE DEPENDENCE

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Chronic negative emotional states are hypothesized to play a role in substance dependence by perpetuating poor decision-making leading to repeated drug use. The mesolimbic reward system is crucial for the positive reinforcing effects of drugs, but few studies have examined neural correlates underlying negative emotional states that may influence poor decision-making in substance users. We hypothesized that compared to controls, substance dependent individuals (SDI) would show decreased activity in fronto-limbic brain regions and this would correlate with negative affect and performance on a decision-making task.

37 SDI (18M/19F) and 43 controls (23M/20F) performed a decision-making task during fMRI scanning and completed the Positive and Negative Affect Scales. Brain activity was compared across group in four fronto-limbic regions: orbitofrontal cortex [OFC], anterior cingulate [ACC], insula, and striatum. fMRI signal in each region was correlated with negative affect and performance.

Compared to controls, SDI showed lower activity in the ACC, OFC, insula, and striatum during decision-making (p<0.01). SDI had greater negative affect (p<0.001) and worse decision-making performance (p=0.01) than controls. Across all participants, lower brain activity correlated with higher negative affect in ACC, insula, and striatum (p<0.03), but not in OFC. Striatal brain activity negatively correlated with task performance (p<0.04) and was driven primarily by SDI.

Compared to controls, SDI show lower fronto-limbic activity, greater negative affect, and poorer performance on a decision-making task. These results suggest that fronto-limbic pathways are involved in negative emotional states associated with poor decisions in SDI. The finding that lower performance correlated with higher striatal activity was unexpected. Given the role of striatum in habit-formation, this may reflect perseveration on outcomes in SDI.

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DOES BREASTMILK COMPOSITION DIFFER BY MATERNAL PHENOTYPE? PRELIMINARY INSIGHTS FROM AN ONGOING LONGITUDINAL COHORT

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The Milk and Infant Growth (Mig) Study is a contemporary longitudinal cohort designed to investigate differences in bioactive components of human milk (HM) among normal weight (NW), overweight/obese (OW/Ob) gestational diabetics, and type 2 diabetic mothers over the first 4 months of lactation. Whether differences in HM composition impact or, are related to infant weight and body composition during this critical window of growth will also be determined.

To date, 19 NW and 15 OW/Ob women have been recruited. 11 infants have completed the study. Preliminary analyses have been performed on fasted HM samples collected at 2-weeks from 17 participants (10 NW; 7 OW/Ob). In these women, there was a trend for higher glucose (18.5±5.1 vs. 23.3±6.8 mg/dL, p=0.17) and higher insulin (10.7±4.1 vs 16.6±9.1 IU/dL, p=0.06) in HM from OW/Ob mothers. HM Triglyceride and Ghrelin concentrations did not differ between the groups.

Recruitment is ongoing and future analyses will investigate differences in breast milk caloric and fatty acid composition, and markers of inflammation and oxidative stress between groups.

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ANTI-CITRULLINATED PROTEIN ANTIBODIES ARE ASSOCIATED WITH RHEUMATOID ARTHRITIS-RELATED CHARACTERISTICS IN FIRST-DEGREE RELATIVES WITHOUT RHEUMATOID ARTHRITIS

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Examine anti-citrullinated protein antibodies (ACPA) reactivity and determine associations between ACPA and other rheumatoid arthritis (RA)-related autoantibodies and clinically-assessed swollen or tender joints in first-degree relatives (FDRs) without 1987 and 2010 American College of Rheumatology classified RA.

A bead-based assay measured 16 separate ACPA in sera from 111 FDRs (Ab+) who were positive on at least one visit for any of 5 RA-related autoantibodies (RF, anti-CCP2, and RF isotypes), and 99 FDRs (Ab-) who were never autoantibody positive. Cut-offs for positivity for each ACPA were determined using receiver operating characteristic curves of data from 200 RA cases and 98 blood-bank controls, wherein positivity for ≥ 9 ACPA had 92% specificity and 62% sensitivity for RA. In FDRs, we assessed ACPA reactivity and examined associations between ACPA (number positive and positivity for ≥ 9 ACPA) and RA-related characteristics.

Four of 7 anti-CCP2 positive and 8% of anti-CCP2 negative FDRs were positive for ≥ 9 ACPA. After adjusting for age, gender, ethnicity and pack-years of smoking, increasing number of ACPA was directly associated with having ≥ 1 tender joint on exam (OR=1.19, 95% CI 1.03-1.37), with the greatest risk seen in FDRs positive for ≥ 9 ACPA (OR=5.15, 95% CI 1.27-20.88).

RA-free FDRs demonstrate reactivity to multiple ACPA, even in those negative for rheumatoid factor and anti-CCP2, and increasing ACPA may be associated with signs of joint inflammation. Prospective evaluation of the relationship between these findings and progression of classifiable RA is warranted.

TLR4 REGULATED MYOCARDIAL INFLAMMATORY RESPONSE TO MEDIATES MYOCARDIAL INJURY AFTER GLOBAL ISCHEMIA AND REPERFUSION: AUGMENTATION OF THE RESPONSE BY AGING

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The myocardial inflammatory response augments ischemia/reperfusion (I/R) injury and TLR4 plays an important role in the regulation of myocardial inflammatory response. We test the hypothesis that myocardial tissue TLR4 mediates myocardial mononuclear cell infiltration and myocardial injury causes by cold I/R through up-regulation of MCP-1 production.

Syngeneic heterotopic heart transplant was performed in mice for global I/R. Donor hearts were subjected to 4 hours cold ischemia followed by 4 hours reperfusion. TLR4 mutant and MCP-1 KO hearts were transplant to wild type mice (WT), also transplant old WT mice heart to adult to determine effects of aging on cold I/R injury. Myocardial injury was evaluated by plasma cardiac troponin-I levels. MCP-1 level and monocytes infiltration were assessed by ELISA and immunofluorescent staining respectively. Statistical analysis was performed using the student’s T-test with a significance P<0.05.

Following global I/R the wild type hearts exhibit marked increase in mononuclear cells infiltration and its attenuated in TLR4 mutant P<0.05. Plasma and myocardial MCP-1 production is abrogated in TLR4 mutant and further reduce is present in MCP-1 KO P<0.05. cTn-I level significantly reduced in the mutant type, P<0.05. Furthermore monocytes infiltration, cTn-I and MCP-1 levels were increased more in old mice after global I/R compared with adult mice P<0.05.

These findings demonstrate that myocardial tissue TLR4 plays a major role in mediated myocardial mononuclear cell infiltration, MCP-1 production and cardiac injury following global I/R with further inflammatory response in aging heart. Suppression of myocardial TLR4 signaling and/or neutralization of MCP-1 would protect the myocardial against global I/R injury associated with open-heart surgery and heart transplant.
MUTATIONS IN HCFC1 CAUSE AN X-LINKED COBALAMIN DISORDER (cblX) WITH A SEVERE NEUROLOGICAL PHENOTYPE
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We used whole exome sequencing to discover the genetic basis of a novel, X-linked, combined methylmalonic acidemia and hyperhomocysteinemia, designated cblX. Five missense mutations in HCFC1, a global transcriptional coregulator, were identified in 14/18 subjects with severe neurological but mild biochemical manifestations, most of whom were previously diagnosed with MMACHC deficiency (cblC). The observed mutations alter three highly conserved amino acids in the first two motifs of HCFC1 Kelch domain. Consensus HCFC1 binding sites were identified in several genes involved in cobalamin and succinyl-CoA metabolism, connecting HCFC1 and binding partner, THAP11, in the transcriptional control of critical enzymes of intermediary metabolism and explaining the diminution of MMACHC mRNA observed after knock-down of HCFC1 using RNAi. This disorder highlights a novel disease mechanism by which mutations in a transcriptional coregulator causes dysregulation of downstream effectors, including MMACHC, leading to a complex clinical phenotype.

A BIOMIMETIC POLYMER FOR GUIDED NERVE REGENERATION
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Nerve function recovery is a major technical challenge in the rehabilitation of patients suffering from severe neuropathies such as spinal cord injuries. Facilitating functional recovery requires the creation of a growth-permissive environment that directs the extension and myelination of surviving neurons. To this end, an electrospun nanofiber scaffold composed of arginine-glycine-aspartate-modified poly(serinol hexamethylene urethane)-blend-poly-ε-caprolactone (RGD-PSHU-PCL) has been employed. Initial studies investigated the cytotoxicity of PSHU in PC12 cell culture followed by functional examination of electrospun scaffolds for cell viability, proliferation, differentiation and neurite outgrowth. MTT proliferation assays indicated no cytotoxic effects of polymer extracts on cultured cells as compared to laminin-coated surfaces. Functional testing revealed RGD-PSHU surfaces to be comparable to the positive control, laminin-coated surface, in neurite outgrowth studies with average neurite lengths of 84.6μm (laminin), 218.2μm (RGD-PSHU), 570.2μm (laminin+NGF) and 958.2μm (RGD-PSHU+NGF) after two weeks on homogeneously modified surfaces, and 554.8μm (non-aligned nanofiber scaffolds) and 1512.3μm (uniaxially aligned nanofiber scaffolds) for RGD-PSHU-PCL + NGF scaffolds after one week. We modified PSHU with the tripeptide, RGD, which provided chemical and physical cues to PC12 cell proliferation and differentiation. We expect that RGD-PSHU-PCL to be electrospun into highly aligned nanofiber scaffolds capable of directing and promoting neurite outgrowth in 3-D cell cultures utilizing PC12.
MITOCHONDRIAL APOPTOTIC ACTIVITY OF P53 CONTRIBUTES TO NEURON APOPTOSIS UPON REOVIRUS-INFECTION

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Reovirus infection is a well-characterized experimental system for studies of viral pathogenesis within the central nervous system (CNS). The tumor suppressor p53 plays a critical role in the determination of cell fate following a variety of cellular insults, including virus infection. It has been reported that p53 and mitochondria-mediated pathways play an important regulatory role in avian reovirus-induced apoptosis in BHK-21 cells and that stabilization and activation of p53 enhanced reovirus oncolysis. However, the role of P53 and mitochondria-mediated pathways in neuronal apoptosis occurring in reovirus-infected brains is still unknown. In this preliminary study, we demonstrate that p53 is upregulated in reovirus-infected brain tissue and accumulates in mitochondria. Further, specific inhibition of mitochondrial p53 translocation, by Pifithrin μ, reduced caspase 3 activity and alleviated tissue injury induced in ex vivo brain slice cultures following reovirus infection. Pifithrin μ also significantly improved the survival rate of mice that had been infected with reovirus by intracerebral inoculation. Overall, our preliminary studies imply that mitochondrial apoptotic activity of p53 contributes to neuron apoptosis upon reovirus-infection.

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Dr. Fiske is President and CEO of PAX Mixer, Inc., a new company created to commercialize high-efficiency mixing technologies developed by parent company, PAX Scientific. Prior to joining PAX Mixer, Inc., Fiske was co-founder and VP for Business Development and Sales of RAPT Industries, in Fremont, CA. Prior to starting RAPT, Fiske led a research team in condensed matter physics at Lawrence Livermore National Laboratory, developing new experimental diagnostic techniques for high-speed non-steady state phenomena and also utilized 2- and 3D hydrodynamic models to simulate shock phenomena in solids and fluids.

Dr. Fiske is also a nationally-recognized author and lecturer on the subject of leadership and career development for young scientists and engineers. He is the author of To Boldly Go: A Practical Career Guide for Scientists (AGU Press, 1996). A new edition, Put Your Science to Work was published in December of 2000. From 1996 to 2000 he wrote the career advice column Tooling Up. He presently writes the monthly on-line column “Opportunities” for the American Association for the Advancement of Science and, with fellow scientist/entrepreneur Dr. Geoff Davis, keeps an active dialog with the science community through his blog Engineering Scientists.

A native of Bethesda, Maryland, Fiske received his A.B. (Magna Cum Laude) in Geological and Geophysical Sciences and a Certificate of Accreditation in Civil Engineering from Princeton University in 1988. He was subsequently awarded an NSF Graduate Fellowship and received his Ph.D. in Geological and Environmental Sciences from Stanford University in 1993. He received his MBA from U.C. Berkeley’s Haas School of Business in the Spring of 2002.

Scott Morgan

Scott Morgan has been teaching communication skills to scientists for almost 20 years. His clients include the National Institutes of Health, the Mayo Clinic, Merck, NASA, EPA, City of Hope Cancer Center, Mount Sinai and several universities: UNC Chapel Hill, Cornell, Maryland, Ohio State, Minnesota, Duke, Nebraska and NC State University, Texas A&M and the Harvard Medical School.

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