Welcome to the Twenty-first Annual Henry I. Russek Student Achievement Day!

Well, we made it through the toughest Boston winter! At times it was hard to remember why we loved New England but here we stand on the other side with the renewed hope for cherry blossoms and hydrangeas. 2015 was a year of many changes as we tried to recover from shrinking NIH dollars and the continued turmoil across the world. GMS garnered a BEST award to prepare our students for the next wave of new jobs that will face them upon graduation and BU dared to be great in its capitol campaign. All positive signs that the future will be bright and certainly illuminated by our outstanding young people that will take the baton and then hand it to their next generation. It is certainly hard to believe that 21 years have gone by since we started Achievement Day but the message is even more important now as we re-evaluate the role of science to our culture and the role of the mentor in the process of science for our own evolution and survival. Have fun today as this is why we all have chosen this wonderful profession where we can be part of a community of thinkers, doers, and dreamers.

I am always so proud of the members of our Division Awards Committee and the Program that they have helped me build: Drs. Marlene Oscar-Berman, Barbara Slack, Barbara Nikolajczyk, Jennie Luebke, Shoumita Dasgupta, Barbara Schreiber, Olga Gursky, Matt Jones, and Keith Tornheim. I would also like to thank all of our student volunteers and the faculty in each department who had to choose between excellent applicants while putting up with my persistence regarding community service. Finally, with sincere appreciation: Sara Johnson (Pharmacology), Wanda Roberts (Pharmacology), Dr. Theresa Davies in GMS, Daniel Madigan in Ed Media, Jerry Lavoie (GMS), Kayleigh Klegraefe (GMS), Sabita Bandyopadhyay (Russek lab), Dr. David Farb, Mrs. Elayne Russek and the generous support of the Russek Foundation.

Enjoy!

Shelley J. Russek

Excerpt from the Russek Lecture 1992 Journal of the American College of Cardiology (JACC) Must Cardiology Lose its Heart?

Delivered by Richard Gorlin, MD, FACC
New York, New York

As the Henry I. Russek Lecturer, I am mindful of the special honor of the invitation because Henry I. Russek was a personal friend as well as the paradigm of the medical humanitarian.

A Man of Science

I first met Henry back in the late 1950's while we were both interested in the actions of the nitrites on the ischemic heart. He believed in the widespread effects of the nitrite compounds in relieving angina pectoris and published some of the earliest reports showing an action of nitrites on both the exercise electrocardiogram and the inception of angina in patients with symptomatic coronary heart disease.

Henry I. Russek had a remarkable scientific prescience. As early as 1968 he described the synergistic effects of beta-adrenergic blocking agents and nitrites when given together. Moreover, in an era long before clinical trials, randomization, blinding and statistical analysis, he was one of the first to suggest that anticoagulant agents played a selective rather than a general role in the treatment of acute myocardial infarction. Without the biostatistical and study design tools we use today, he was able to determine that it was the patient at high risk for thrombosis who deserved the benefits and who could risk the hazards of anticoagulation. He identified the patients at risk as those with venous disease, arterial disease or congestive heart failure. Later in my own work, I cited this 1954 paper when discussing coronary heart disease.

A Man of Medicine

Perhaps more pertinent to this Henry I. Russek Lecture is what Henry was like as a man and physician. In the encomia for Henry provided by his family and by the College, one statement emerges repeatedly: he was a true physician-healer. Henry has been described as having a mind open to new science and new ideas. He was considered to be a student as well as educator and communicator. With his patients he was not only an astute diagnostician but a compassionate and active listener. Those who knew him well report that he knew how to heal the healer as well as the patient, and that he continually preached that humanism begins with humanizing the physician.

JACC Vol. 19, No.7
June 1992:635-640

DAVID LIVINGSTON

David Livingston is Deputy Director of the Dana-Farber/Harvard Cancer Center; Chief of the Charles A. Dana Division of Human Cancer Genetics, and the Emil Frei Professor of Genetics and Medicine at Harvard Medical School. From 1996 to 2000, he served as Chairman of the Executive Committee for Research at the Dana-Farber Cancer Institute, leading the senior faculty group that oversees all aspects of the Institute's research program. He reassumed that post in 2005 and has been in it since that time. Dr. Livingston has been a Harvard faculty member continuously since 1973. Dr. Livingston received an A.B. cum laude from Harvard College in 1961, an M.D., magna cum laude, from Tufts University School of Medicine in 1965, and served his internship and residency in internal medicine at the Peter Bent Brigham Hospital (now Brigham and Women's Hospital) in Boston. In 1967, he became a Research Associate at the National Cancer Institute (NCI) in molecular biology and biochemistry; he continued his work as a Research Fellow in Biological Chemistry at Harvard Medical School in 1969. Dr. Livingston returned to NCI in 1971 as a Senior Staff Fellow where he began his career in cancer research. He was recruited to Dana-Farber (then the Children's Cancer Research Foundation) in 1973. Dr. Livingston is an internationally recognized expert on genes that regulate cell growth in the body -- genes that, when they go awry, can lead to cancer. These genes are called oncogenes and tumor suppressor genes. Through his research, Dr. Livingston has uncovered detailed biochemical steps required to initiate and maintain the transformation of these cells into tumor cells. His focus is on the regulatory controls of signal transduction -- the smooth and coordinated flow of special chemical signals from the surrounding environment to the cell, where it is transduced into specific commands that tell cells whether or not to grow -- and their role in cancer development. In recent years, his work has centered on those key molecular steps that trigger the development of breast and ovarian cancer. Dr. Livingston is the recipient of numerous awards and honors. He has been elected to the Institute of Medicine of the National Academy of Sciences, the National Academy of Sciences, itself, and the American Academy of Arts and Sciences. He sits on multiple editorial boards, the science advisory boards of other research institutions, and is a member of the Association of American Physicians, the American Society for Clinical Investigation, the American Society for Microbiology, and the American Academy of Microbiology. He is also a Foreign Associate of the European Molecular Biology Organization and serves as Vice Chair of the Board of the Damon Runyan Cancer Research Foundation. Dr. Livingston has authored more than195 scientific publications.

Linkages Between Genome Disorder and Breast Cancer Development.

Approximately 3-4% of all women in Europe and America who develop breast cancer each year do so by inheriting a mutant copy of a gene that normally operates to suppress breast cancer development. Several such genes have been identified and are under active investigation. Among them, two, BRCA1 and 2, are the best studied and are, collectively, responsible for approximately 60% of these inherited cases. Germ line mutant BRCA1 and 2 genes also elicit high frequency ovarian cancer development.

Interestingly, much of this collection of inherited, breast cancer- suppressing genes operates to promote the maintenance of a stable and integral genome. Indeed, the majority of the genome integrity maintenance/ breast cancer suppressor genes in this group function in a coordinated way to support cellular responses to the development of double strand genomic breaks (DSB). More specifically, they do so by participating in the process that repairs these lesions in an error-free manor, so called homologous recombination (HR). Thus, insuring the proper repair of these DNA lesions, which develop normally in most, if not all, replicating cells, is a breast cancer- suppressing process.

Recently, evidence has emerged showing that at least some of these genes encode proteins that participate in cellular responses to yet other forms of genome damage, as well as in the processes which insure that breast epithelial cells maintain a standard, modal chromosome number. There is also evidence which suggests a role for some of these events in breast cancer suppression.

What has been particularly mysterious is why breast (and ovarian) cancer, in particular, as opposed to tumor development in other organs, is the prime outcome of BRCA1 - driven tumor development and the major clinical result of BRCA2 dysfunction. This subject will be discussed in some detail.

Retrieved on April 22, 2015, from:
http://www.thefutureofscience.org/speakerdetail/fifth-world-conference-on-the-future-of-science-the-dna-revolution-livingston-david-254

Student Achievement Day 2015

Program of Events:

Coffee and pastries available at 8:30 a.m.
Please pick up badges and abstract booklet at the front of the Heibert Lounge from our student hosts. Please put up your poster before 9:00 a.m.

9:00-9:30 a.m.
Welcoming addresses by Professor Shelley J. Russek (Vice-President, Russek Foundation), Professor Linda Hyman (Associate Provost, Division of Graduate Medical Sciences), Dr. Karen Antman (Dean of BUSM), and current President of the Graduate Medical Sciences Student Organization.

9:30-10:45 a.m.
Henry I. Russek Keynote Lecture "How Does BRCA1 Suppress the Development of Breast and Ovarian Cancer?" by Dr. David M. Livingston, Deputy Director, Dana-Farber Harvard Cancer Institute; Chief, Charles A. Dana Division of Human Cancer Genetics, and Emil Frei Professor of Genetics and Medicine, Harvard Medical School, Boston, Massachusetts.

10:45 a.m.-1:00 p.m.
Viewing of posters presented by graduate students enrolled in the Division of Graduate Medical Sciences (Buffet luncheon available at 11:30 a.m.)

Award winners (First, Second, and Third) please get your lunch and bring to the student lounge (14th floor L-Bldg). You will be having lunch with our Keynote Speaker & Visiting Professor Dr. David Livingston.

1:00 p.m.-2:15 p.m.
Slide presentations by the 2015 Henry I. Russek Student Achievement First Prize Recipients. (Each presentation is 10 min. with an additional 5 min. for questions. Student moderators will host the event.)

30 minute stretch and viewing of posters

2:45 p.m.-4:00 p.m.
Continued slide presentations by the 2015 Henry I. Russek Student Achievement First Prize Recipients.

4:00 p.m.
Award presentations by Shelley J. Russek, Russek Foundation.
Photo shoot of our wonderful award winners!

Oral Presentations

1:00-1:15 p.m. – Meredith Cler:
SPEECH SYNTHESIS VIA SURFACE ELECTROMYOGRAPHIC CONTROL: TRAINING EFFECTS.
(Graduate Program for Neuroscience, Advisor: C. Stepp)

1:15-1:30 p.m. – Fadie Coleman:
MACROPHAGE NECROPTOSIS AND PNEUMONIA CAUSED BY VIRULENT PNEUMOCOCCUS.
(Department of Microbiology, Advisor: J. Mizgerd)

1:30-1:45 p.m. – Dana Lau Corona:
EPIGENETIC ANALYSIS OF TEMPORAL CHANGES IN CONTINUOUS GROWTH HORMONE-RESPONSIVE SEX-BIASED GENES IN MALE MOUSE LIVER.
(Program in Genetics & Genomics, Advisor: D. Waxman)

1:45-2:00 p.m. – Madhurima Das:
STRUCTURAL STABILITY AND LOCAL DYNAMICS IN DISEASE-CAUSING MUTANTS OF HUMAN APOLIPOPROTEIN A-I.
(Department of Physiology & Biophysics, Advisor: O. Gursky)

2:00-2:15 p.m. – Jacquelyn Sikora Hanson:
THE TRANSTHYRETIN GENE VARIANT G6S MAY BE PROTECTIVE IN WILD-TYPE TRANSTHYRETIN AMYLOIDOSIS.
(Department of Pathology, Advisor: L. Connors)

30 min break to stretch and view posters

2:45-3:00 p.m. – Samantha Hiemer:
A YAP/TAZ-REGULATED MOLECULAR SIGNATURE IS ASSOCIATED WITH ORAL SQUAMOUS CELL CARCINOMA.
(Department of Biochemistry, Advisor: X. Varelas)

3:00-3:15 p.m. – Danielle Miller:

WHITE MATTER ABNORMALITIES ARE ASSOCIATED WITH CHRONIC POSTCONCUSSION SYMPTOMS IN BLAST-RELATED MILD TRAUMATIC BRAIN INJURY.

(Program in Behavioral Neuroscience, Advisor: M. Verfaellie)

3:15-3:30 p.m. – Kyle Trudeau:

MITOCHONDRIAL FRAGMENTATION IN RESPONSE TO NUTRIENT EXCESS REPRESENTS A COMPENSATORY ADAPTATION TO MAINTAIN HUMAN BETA-CELL FUNCTION.

(Program in Molecular & Translational Medicine, O. Shirihai)

3:30-3:45 p.m. – Megan Varnum:

THE ANTI-INFLAMMATORY GLYCOPROTEIN, CD200, RESTORES NEUROGENESIS AND ENHANCES AMYLOID PHAGOCYTOSIS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE.

(Department of Pharmacology & Experimental Therapeutics, Advisor: T. Ikezu)

3:45-4:00 p.m. – Chen-Yuan Yang:

HYDRODYNAMIC AND MORPHOLOGICAL CHANGES IN THE AQUEOUS HUMOR OUTFLOW PATHWAY AFTER Y27632 TREATMENT IN LASER-INDUCED HYPERTENSIVE MONKEY EYES.

(Department of Anatomy and Neurobiology, Advisor: H. Gong)

Recipients of the Henry I. Russek Student Achievement Awards 2015

First Prize

Meredith Cler – Graduate Program for Neuroscience
Advisor: C. Stepp

Fadie Coleman – Department of Microbiology
Advisor: J. Mizgerd

Dana Lau Corona – Program in Genetics and Genomics
Advisor: D. Waxman

Madhurima Das – Department of Physiology and Biophysics
Advisor: O. Gursky

Jacquelyn Sikora Hanson – Department of Pathology
Advisor: L. Connors

Samantha Hiemer – Department of Biochemistry
Advisor: X. Varelas

Danielle Miller – Program in Behavioral Neuroscience
Advisor: M. Verfaellie

Kyle Trudeau – Program in Molecular & Translational Medicine
Advisor: O.Shirihai

Megan Varnum – Department of Pharmacology & Experimental Therapeutics
Advisor: T. Ikezu

Chen-Yuan Yang – Department of Anatomy and Neurobiology
Advisor: H. Gong

Second Prize

Andrew Bogorad – Department of Physiology & Biophysics
Advisor: A. Marintchev

Joon Boon – Department of Pharmacology & Experimental Therapeutics
Advisor: B. Wolozin

William Bosl – Program in Behavioral Neuroscience
Advisor: K. Marcel

Kelsey Derricks† – Program in Molecular & Translational Medicine
Advisor: M. Nugent

Joseph Goodliffe† – Department of Anatomy and Neurobiology
Advisor: T. Haydar

Nadine Heyworth† – Department of Anatomy and Neurobiology
Advisor: D. Rosene

Caitlin Miller† – Department of Pathology
Advisor: S. Gummuluru

Akshaya Ramesh – Program in Genetics and Genomics
Advisor: T. Kepler

Liz Stanford† – Program in Molecular & Translational Medicine
Advisor: D. Sherr

Nicole Stauffer† – Department of Pathology
Advisor: J. Mizgerd

Madelane Teran – Department of Biochemistry
Advisor: M. Nugent

Greg Wasserman – Department of Microbiology
Advisor: M. Jones

Maya Woodbury – Graduate Program for Neuroscience
Advisor: T. Ikezu

† Shared Second Prize

Third Prize

Justin Berry – Department of Physiology & Biophysics
Advisor: C. Carter

Casey Carmichael – Department of Pharmacology & Experimental Therapeutics
Advisor: R. Wainford

Keri Dame – Program in Genetics and Genomics
Advisor: L. Ikonomu

Suzanne Kijewski – Department of Microbiology
Advisor: S. Gummuluru

Collette Laflamme†† – Program in Nutrition
Advisor: P. Pilch

Chendi Li – Department of Biochemistry
Advisor: S. Farmer

Kiana Mahdaviani†† – Program in Nutrition
Advisor: O. Shirihai

Arkadly Maksimovskiy – Program in Behavioral Neuroscience
Advisors: R. McGlinchey, C. Palumbo, M. Oscar-Berman

Dante Smith – Graduate Program for Neuroscience
Advisor: F. Guenther

†† Shared Third Prize

Department of Anatomy & Neurobiology

NOTE: Prize winners are noted in the following list of Abstracts by:
*1st Prize, **2nd Prize, ***3rd Prize
The accompanying number indicates each abstract's poster board.

Participants

Teresa Guillamon-Vivancos (59)
Joseph Goodliffe (**5)
Nadine Heyworth (**12)
Roman Loonis (72)
Philip Montenigro (40)
Mary Orczykowski (58)
Alexander Stankiewicz (45)
Chen-Yuan Yang (*)

*

Hydrodynamic and Morphological Changes in the Aqueous Humor Outflow Pathway After Y27632 Treatment in Laser-Induced Hypertensive Monkey Eyes

Yang, Chen-Yuan Charlie[1,2]; Chen, Chongsheng[2]; Toris, Carol B[3]; Gong, Haiyan[2,1]

1. Department of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA

2. Department of Ophthalmology, Boston University School of Medicine, Boston, MA

3. Department of Ophthalmology & Visual Sciences, Case Western Reserve University, Cleveland, OH

To investigate the effects of Y27632 on the hydrodynamic and morphological changes in laser-induced ocular hypertensive monkey eyes.

Argon laser photocoagulation burns were made to the trabecular meshwork (TM) of cynomolgus monkey eyes to induce chronic ocular hypertension. Eight laser-treated (Y27632: n=5, DPBS: n=3) and seven non-laser-treated normal eyes (Y27632: n=4, DPBS: n=3) were investigated. Eyes were enucleated and perfused at pre-mortem intraocular pressure (pneumatonometry) minus 7 mmHg to obtain outflow facility (C). A two-color fluorescent tracer method was used to label the active outflow areas before (green, 0.5μm, 0.002%) and after 50uM Y27632 or DPBS treatment (red, 0.2μm; 0.002%) followed by perfusion-fixation. All eyes were perfused with a fixed volume of tracers and Y27632/DPBS. After imaging the tracer distribution in the TM globally, the tissue was frontally sectioned for confocal, light, and electron microscopy.

The IOP was higher (35.0±14mmHg vs. 22±3mmHg, p=0.04) and the baseline C was lower (0.16±0.20 vs. 0.40±0.23 μl/min/mmHg, p=0.05) in laser-treated hypertensive eyes compared to normal eyes. After Y27632 treatment, C increased from baseline in normal but not hypertensive eyes; active outflow areas increased in normal eyes and non-lasered-regions of hypertensive eyes, but only minimal tracers were observed in lasered-regions of the hypertensive eyes. Morphologically, normal and non-lasered-regions of hypertensive eyes have open SC and TM. In contrast, lasered-regions of hypertensive eyes have compact TM and collapsed SC.

Y27632 increases C in normal eyes through increasing active outflow area of the TM, but appears less effective in lasered-induced ocular hypertensive eyes.

Support by NIH EY022634 and The Massachusetts Lions Eye Research Fund.

Contribution of Distinct Precursor Lineages to Neuronal Diversity in Layer 4 of the Mouse Barrel Cortex

Guillamon-Vivancos, Teresa; Medalla, Maria; Tyler, William; Haydar, Tarik and Luebke, Jennifer.

Department of Anatomy and Neurobiology.

The striking diversity of neurons in the mammalian neocortex is key for the specification of cortical areas, but how this diversity is achieved during development is still poorly understood. It was recently discovered that Radial Glial Cells (RGCs), the neural stem cells of the developing cortex, also generate intermediate progenitor cells (IPCs), which in turn are able to generate new neurons. Our overall hypothesis is that distinct IPCs are responsible for generation of neuronal diversity in the adult neocortex. Using a novel genetic fate mapping technique, our group has shown that neurons derived from different IPCs have distinct morphological and electrophysiological properties in layers 2/3 of the mouse frontal cortex.

The purpose of this study is to determine whether different IPCs contribute to neuronal diversity in other layers and areas of the brain. Using our genetic fate-mapping technique we were able to simultaneously label neurons originating from different lineages in the thalamic recipient layer 4 of the barrel cortex. The barrel cortex, where several morphological and physiological types of neurons have been described, emphasizes the principles of laminar and columnar organization of the neocortex. We studied whether neurons derived from distinct IPCs have different locations in the barrel field. We also used whole-cell patch clamp recordings to assess the electrophysiological properties of these neurons. During the recordings, neurons were filled and their morphology studied using high-resolution confocal microscopy. This study will contribute to a better understanding of how different streams of neurogenesis contribute to adult neuronal diversity and cortical organization.

ABSENCE OF DS-RELATED PRENATAL PHENOTYPES IN THE DP16 MOUSE MODEL OF DOWN SYNDROME

Goodliffe, Joseph; Haydar, Tarik

Anatomy & Neurobiology

Down syndrome (DS) is the most common genetic intellectual disorder and results in malformations of the central nervous system. Fetal studies have reported aberrations in brain developmental including microcephaly and altered cortical lamination. Several mouse models of DS have recapitulated these phenotypes and have established additional abnormalities in neurogenesis and neuronal differentiation. A recently developed model, the Dp(16)1Yey/+ (or Dp16), has the largest triplication of the human chromosome 21 (Hsa-21) homologous region to mouse chromosome 16 (Mmu16) and may better represent DS pathologies. To date, Dp16 studies have focused on adult behavioral, cerebellar and craniofacial abnormalities, all of which mirror other mouse models of DS. Here, we present the first comprehensive study on Dp16 prenatal brain development. Despite the presence of adult abnormalities, our study shows that all measured parameters of Dp16 forebrain development are unchanged from euploid mice. Specifically, several phenotypes previously reported in human fetal neocortex and in the developing forebrains of other mouse models such as microcephaly, altered cortical lamination and reduced neurogenesis are not present in Dp16. This striking absence of DS-related phenotypes confounds the use of this model for embryonic studies and highlights important differences in segmental models of trisomy 21. Data from this study isolate the temporal periods during which brain defects arise in DS and for the first time show that deficits in embryonic neurogenesis are not necessary for the manifestation of cognitive abnormalities in a mouse model of DS.

Work supported by NIH, NICHD/NIMH, RO1HD05780

CELLULAR IDENTIFICATION OF IMMATURE NEURONS IN THE MONKEY CORTEX

Heyworth, Nadine; Calderazzo, Samantha; Kyada, Margee; Rosene, Douglas

Department of Anatomy and Neurobiology

The continual proliferation of neurons in the adult brain is well established for the neurogenic regions of the subventricular zone (SVZ) and the subgranular zone (SGZ). Outside of these regions, immature neurons present in the cerebral cortex have generated speculation regarding ongoing proliferation and plasticity in additional areas of the adult brain. Doublecortin (DCX) is a microtubule-associated protein transiently expressed during the migration and maturation of neurons. The reported presence of DCX+ immature cortical neurons in layers II/III, highlights unresolved issues regarding these cells including the temporal origin and if they are adult-generated, the fate of the cells and whether they survive throughout the lifespan, and the cellular identity of these neurons as excitatory or inhibitory. Using a non-human primate model of normal aging, the goal of our study is to address these questions by examining DCX immature cortical neurons in the parahippocampal gyrus. We have found that DCX neurons in the cortex are not adult-generated and likely formed during development. The number of proliferating cells does not change significantly with age, but DCX neurons show an age-related decline. Finally, we found a population of DCX cells co-localized with Calretinin, but not with other calcium binding proteins or excitatory markers. These findings raise intriguing questions regarding the role of immature cortical neurons and connectivity within local circuitry and ongoing plasticity in the adult brain.

Transient Synchronizations and Cross-Frequency Interactions Between Prefrontal Cortex and Striatum Underlie Category Learning in the Macaque

Loonis, Roman[1,2]; Antzoulatos, Evan[2]; Brincat, Scott[2]; Miller, Earl[2].

[1] Department of Anatomy and Neurobiology, Boston University. [2] Department of Brain and Cognitive Sciences, Picower Institute, Massachusetts Institute of Technology.

Neural oscillations, arising when populations of neurons undergo coordinated changes in membrane potential, are thought to play a key role in the routing and encoding of information across cortex. In this study, we examined how oscillations were modulated by learning. Two rhesus macaques were trained on a category learning task, during which each animal learned to classify different dot patterns with an eye movement response. Previous studies had suggested that such learning involves the coordination of neural activity between prefrontal cortex and the caudate. We recorded local field potentials from these two structures, and focused our analyses on a period essential to learning: the feedback period. In this feedback period, the animal received juice reward and visual cues to indicate a correct or incorrect choice. During this task, we found that the caudate and prefrontal cortex synchronize for different trial outcomes at different frequency bands: beta band synchrony (12-30 Hz) for incorrect trials, and theta band synchrony (3-7 Hz) for correct trials. These synchronizations appeared to fluctuate over time in a structured way, suggesting the existence of some discrete computational process, and this structure dissipated over learning. Moreover, during learning, the phase of prefrontal theta signals modulated the amplitude of gamma (30-50 Hz) activity in the caudate. The presence of outcome-related synchrony, the prefrontal modulation of gamma amplitude in the caudate, and the temporal organization of the synchrony supports the hypothesis that prefrontal cortex serves an important role in training subcortical regions on category identification.

BETA-AMYLOID IN CHRONIC TRAUMATIC ENCEPHALOPATHY, ALZHEIMER'S DISEASE, AND NORMAL AGING: EVIDENCE FOR NON-OVERLAPPING ETIOLOGIES.

Montenigro, Philip; Alvarez, Victor; Tripodis, Yorghos; Stern, Robert; McKee, Ann; Stein, Thor

Department of Anatomy and Neurobiology

Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy that is associated with a history repetitive traumatic brain injury (RTBI). The goal of this study was to determine whether amyloid beta (Aβ) pathology in CTE differs from Alzheimer's disease (AD) and normal aging. 114 autopsy cases with both a history of RTBI and a neuropathological diagnosis of CTE were compared to 319 cases of neuropathologically diagnosed AD and to a large non-selected cohort of 2,332 normal aging cases. Our results demonstrated Ab deposition occurred in 43% of CTE cases. Compared to the normal aging cohort, Ab appeared at an earlier age and at an accelerated rate in CTE. The odds of developing neuritic Ab were 11.1 times higher in the CTE cohort (r^2=0.97, p=0.025), and a weighted two-sample chi-square test demonstrated that the distribution of Ab plaques by age in CTE was distinct from the distribution in normal aging ($\chi^2 = 21.4$, $p = 0.0015$). Age-adjusted multiple linear regression analysis demonstrated that the presence diffuse Aβ pathology predicted significantly greater CTE tau-pathological stage at the time of death (β=0.53, p=0.003) whereas the contribution of age on stage was negligible (β=0.026, p<0.001). Moreover, we hypothesized that more Ab pathology would accumulate in the cortical sulcus, where RTBI shear strains are highest, compared to the gyral crests. A significantly greater Aβ1-40 plaque burden was, in fact, found in the sulcus of CTE cases (t=2.21, p=0.029). These findings suggest that A β pathology in CTE is distinct from AD and normal aging.

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Effect of a Cell Therapy on Neuropathology Following Ischemic Damage in Rhesus Monkey Motor Cortex

Orczykowski, Mary; McBurnie, Megan; Mortazavi, Farzad; Rosene, Douglas; Moore, Tara

Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA

Ischemic strokes lead to a massive inflammatory response followed by macrophage infiltration causing oxidative stress and initiating apoptosis and a cascade of cell death. This study assessed a cell therapy using human-umbilical tissue-derived cells (hUTC) to enhance recovery in our non-human primate model of cortical ischemia. Monkeys were trained on a quantifiable battery of fine motor tasks. A reproducible ischemic lesion was made by electrophysiologically mapping the hand area of the primary motor cortex and disrupting cortical blood vessels in this area impairing only the contralateral hand. The following day, 10M cells/kg hUTC or vehicle was administered intravenously. Monkeys were retested on the fine motor tasks for 12-14 weeks starting two weeks after ischemia and were then perfused. There was significant recovery of fine motor function and strength in hUTC treated animals compared to controls. Quantitative immunohistochemistry using antibodies for LN-3 (pro-inflammatory microglial marker), GFAP (astrogliosis marker), and 4-HNE (oxidative stress marker) was used to assess the effects of therapy on the inflammation, astrogliosis, and oxidative stress. All subjects showed a pronounced inflammatory response as detected by LN-3+ and GFAP+ cells that was widespread throughout motor associated areas. Oxidative stress assessed by 4-HNE was pronounced in the perilesional gray and white matter as well as in motor associated areas. Results showed a significant increase in astrocytes (GFAP) in the perilesional gray matter in treated monkeys compared to controls ($p \leq 0.05$), which may be correlated with behavioral recovery suggesting a potential neurorestorative role.

[Supported by R21-NS081261 and ATRM Division of Johnson & Johnson]

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The Circadian Regulation of S- and G2/M-Phase of Adult Neurogenesis in Zebrafish

Stankiewicz, Alexander; Akle, Veronica; Yu, Lili; Zhdanova, Irina

Anatomy and Neurobiology

Adult neurogenesis in vertebrates includes the proliferation, maturation, migration and functional integration into existing neuronal circuitry in spatially restricted regions of the central nervous system. The cell cycle has been shown to oscillate as a function of circadian rhythms across a range of organisms, from one-cell cyanobacteria to cells throughout human tissues. However, the contribution of the clock to adult neurogenesis remains uncertain and studies in nocturnal vertebrates, mice and rats, provided conflicting results. Using the diurnal vertebrate, zebrafish, real-time RT-PCR (qPCR) analysis of cyclins D, E, A2 and B2 expression and complementary immunohistochemical analysis for cell cycle markers of S-phase (BrdU) and G2/M phase (pH3) and timed administration of a selective cyclin D kinase 4/6 inhibitor (PD 0332991), we have documented the presence of a robust daily rhythm of cell proliferation in adult brain. Furthermore, results indicate that an approximately 24-h period of the cell division cycle in neuroprogenitors is initiated on one night, with peak in S-phase over the day/night transition, and ends with G2/M by the end of the following night. These findings indicate that adult neurogenesis in zebrafish has a daily rhythm, with a cell cycle spanning over two consecutive nights.

Program in Behavioral Neuroscience

Participants

William Bosl (**4)
Arkadly Maksimovskiy (***20)
Danielle R. Miller (*)

*

White Matter Abnormalities are Associated with Chronic Postconcussion Symptoms in Blast-Related Mild Traumatic Brain Injury

Miller, Danielle R.; Hayes, Jasmeet P.; Lafleche, Ginette; Salat, David; & Verfaellie, Mieke

Behavioral Neuroscience

Due to the frequent use of improvised explosive devices in the Iraq and Afghanistan Wars, blast-related mTBI is common among Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF) service members. Recently, it has been suggested that the white matter changes associated with mTBI are reflected in behavioral outcomes such as postconcussion symptom (PCS) reporting. In this study, we examined whether blast-related mTBI is associated with diffuse white matter changes, and if these neural changes are associated with PCS. Ninety OEF/OIF Veterans underwent DTI and behavioral assessment. Veterans were assigned to one of three groups including a blast-exposed no-TBI group, a blast-related mTBI without loss of consciousness (LOC; mTBI-LOC) group, and a blast-related mTBI with LOC (mTBI+LOC) group. PCS were divided into three domains: physical, emotional, and cognitive. Results showed that participants in the mTBI+LOC group had more spatially heterogeneous white matter abnormalities than those in the no-TBI group. These white matter abnormalities were significantly associated with physical PCS severity even after accounting for posttraumatic stress disorder (PTSD) symptom severity, but not with cognitive or emotional PCS severity. A mediation analysis revealed that mTBI+LOC significantly influenced physical PCS severity through its effect on white matter integrity. PTSD symptom severity did not influence white matter abnormalities, but was highly associated with PCS severity across domains. These results support recent reports suggesting that white matter integrity is associated with PCS severity. Furthermore, these findings suggest that individuals with mTBI+LOC have more white matter abnormalities and in turn report more physical PCS.

EEG Screening for Autism and Epilepsy: A Measure of Epileptigenicity

Bosl, William

Loddenkemper, Tobias. Nelson, Charles

Behavioral Neuroscience

Background. Neurodevelopmental disorders (NDDs) are among the greatest threats to childhood health globally. Epilepsy and autism are two of the most prevalent and debilitating NDDs. Importantly, these co-occur in approximately 30% of children with a primary diagnosis of either disorder, suggesting a common brain pathology. Studies of NDDs have consistently shown that early intervention leads to better long-term outcomes. Yet, early intervention is predicated on early detection. In this paper, we demonstrate that methods from complex dynamical systems theory may be applied to analysis of short EEG measurements to detect subtle differences between typically developing children, and those with autism or absence epilepsy.

Methods. Data from 92 children collected from a research lab and the Epilepsy Clinic at Boston Children's Hospital were analyzed retrospectively. Nonlinear complexity measures were computed and machine learning algorithms were used in a cross-validation study to determine classification accuracy.

Results. Classification algorithms were able to distinguish 3 groups (absence, ASD, control) with nearly 100% accuracy. Important differences between epilepsy and autism groups may give insight into functional brain pathologies in these disorders.

Conclusions. Nonlinear (complex systems) analysis of EEG data may be useful as a much sought after and urgently needed biomarker for early detection, monitoring developmental trajectories, predicting severity and potentially guiding early treatment and interventions of emerging epilepsy or autism in children. The finding that autism values were intermediate between epilepsy cases and controls suggests a common pathological continuum that is measureable.

DISSOCIATING THE EFFECTS OF BINGE DRINKING AND HEAVY ALCOHOL CONSUMPTION ON GRAY AND WHITE MATTER BRAIN VOLUMES

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Behavioral Neuroscience Ph.D. Program

Background and Aims: Our aim was to examine whether binge drinking (BD), age of onset (early (EBD) vs. late (LBD)), or drinking quantity (heavy (H+) *vs*. light (H-)) accounts for alcohol-related brain changes. The following components were selected for these analyses: (1) global cerebral and cerebellar volumes, (2) the reward circuit (involved in top-down drinking urges), and (3) the insular cortex (involved in bottom-up salience processing).

Methods: Groups were characterized based on the NIH definition of BD (at least 4 drinks/ women and 5/men) and H+ (15 or more days/month). Groups consisted of Social Drinkers (N = 9), EBDH+ (N=18), EBDH- (N= 6), LBDH+ (N=12), and LBDH- (N= 9). Multilevel analyses were implemented by covarying for the effects of age and intracranial volume, and confirmed with larger group sizes (collapsed across non-significant group effects).

Results: Results for global brain measures indicated a marginally significant decrease in left cerebellar volume ($p < .07$), driven by heavy drinking. Neither binge onset nor drinking amount revealed any differences within the reward circuit. Right insular white matter volume was decreased as a result of binge drinking ($p < .05$), independently of other factors.

Conclusion: Results indicate an informative dissociation between cumulative drinking amount and drinking frequency. Heavy drinking over the course of a lifetime relates to decreased cerebellar gray matter, which has been linked to motor and cognitive dysfunction. Binge drinking, however, is related to decreased white matter adjacent to the right insular cortex, which may indicate a disruption of bottom-up salient signal processing.

Department of Biochemistry

Participants

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*

A YAP/TAZ-Regulated Molecular Signature is Associated with Oral Squamous Cell Carcinoma

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Oral squamous cell carcinoma (OSCC) is a prevalent form of cancer that develops from the epithelium of the oral cavity. OSCC is on the rise worldwide, and death rates associated with the disease are particularly high. Despite progress in understanding of the mutational and expression landscape associated with OSCC, advances in deciphering these alterations for the development of therapeutic strategies have been limited. Further insight into the molecular cues that contribute to OSCC is therefore required. Here we show that the transcriptional regulators YAP (*YAP1*) and TAZ (*WWTR1*), which are key effectors of the Hippo pathway, drive pro-tumorigenic signals in OSCC. Regions of pre-malignant oral tissues exhibit aberrant nuclear YAP accumulation, suggesting that dysregulated YAP activity contributes to the onset of OSCC. Supporting this premise, we determined that nuclear YAP and TAZ activity drives OSCC cell proliferation, survival, and migration *in vitro*, and is required for OSCC tumor growth and metastasis *in vivo*. Global gene expression profiles associated with YAP and TAZ knockdown revealed changes in the control of gene expression implicated in pro-tumorigenic signaling, including those required for cell cycle progression and survival. Notably, the transcriptional signature regulated by YAP and TAZ significantly correlates with gene expression changes occurring in human OSCCs identified by “The Cancer Genome Atlas” (TCGA), emphasizing a central role for YAP and TAZ in OSCC biology.

THE ROLE OF MYOCARDIN RELATED TRANSCRIPTION FACTOR A IN CONTROLLING THE COMMITMENT OF PROGENITORS TO ADIPOSE *VERSUS* OSTEOBLASTIC LINEAGE

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The differentiation of osteoblasts and bone marrow adipocytes are closely associated yet mutually exclusive processes that are essential for maintaining bone homeostasis. Investigating the underlying molecular mechanisms of the osteoblasto-adipogenic switch under osteoporotic conditions may eventually lead to the development of clinical therapeutic approaches for osteoporosis. Previous studies have shown that cellular morphological changes can affect the early commitment of pluripotent MSCs via modulation of Ras homolog gene family, member A (RhoA) activity. The RhoA pathway promotes actin polymerization to release globular actin (G-actin) bound myocardin-related transcription factors (MRTFs), which translocate to the nucleus and co-activate serum response factor (SRF) target gene expression.

Here we show that global MRTFA knockout mice (MRTFA KO) exhibited lower body weight, shorter femur and tibia lengths, and decreased trabecular bone volume. Furthermore, bone marrow MSCs isolated from MRTFA KO mice showed increased adipogenesis and compromised osteoblastogenesis as compared to WT mice. Treatment of WT bone marrow MSCs with the SRF inhibitor, CCG1423, mimicked these effects. Over-expression of MRTFA or SRF inhibited adipogenesis and enhanced osteoblastogenesis in C3H/10T1/2 cell lines, whereas over-expression of dominant-negative MRTFA or SRF variants had the opposite effects. In conclusion, our study identified MRTFA as a crucial regulator of skeletal homeostasis *via* regulating the balance between adipogenic and osteoblastogenic differentiation of the MSCs. Furthering our understanding of how the RhoA-actin-MRTFA-SRF circuit is involved in regulating the fate commitment of MSCs may ultimately lead to novel therapeutic strategies for treating osteoporosis and obesity.

GPS2 at the Crossroad of Lipid Metabolism and Inflammation in Adipose Tissue.

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Department of Biochemistry

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Obesity- associated inflammation is widely recognized as a critical factor in the development of insulin resistance leading to type 2 diabetes (T2D) and other metabolic disorders. However, surprisingly, treatment with anti-inflammatory drugs have not proven successful in treating these metabolic syndromes and the critical question of whether inhibiting inflammation is a good approach in the attempt to treat insulin resistance remains unanswered. G-protein pathway suppressor 2 (GPS2) has recently emerged as an important, novel regulator of homeostasis and inflammatory responses in various metabolic organs, most importantly adipose tissue. Previous work from our lab and others describes GPS2 as a multifunctional protein. GPS2 is found to function in the cytosol, where it inhibits the stress kinase, JNK, activation by modulating ubiquitin signaling events downstream of the TNFα receptor, and in the nucleus, where it acts as a cofactor regulating gene expression by inhibiting transcription of pro-inflammatory targets and activating key mediators of the lipolysis pathway. Here, we present the characterization of the adipo-specific GPS2 knockout (AKO) mouse model confirming the critical role of GPS2 in regulating obesity-induced inflammation and lipid metabolism *in vivo.* We observe that the GPS2 AKO mice become more obese and inflamed than WT mice during high fat diet feeding yet are protected from developing insulin resistance. We hypothesize that GPS2 AKO mice are metabolically healthier than their wild type littermates because of increased adipose tissue lipid storage capacity resulting from increased lipogenesis and decreased lipolysis in the adipocytes. We also hypothesize that the increased stimulation of inflammatory responses in the adipose tissue has a positive and protective role against the development of insulin resistance. Thus, we propose to understand how GPS2 regulates lipid flux in adipose tissue by modulating key regulators of lipid metabolism and to elucidate the role of GPS2 in controlling macrophage infiltration with potential consequences for tissue remodeling and expansion. The GPS2 AKO mice represents a unique model to improve our comprehension of the interplays between inflammation and adipose tissue functionality in the development of insulin resistance and contribute to understand whether inhibiting inflammation in the context of obesity is a viable strategy to ameliorate metabolic functionality.

Integrated Omics of Influenza A Virus: Correlating Glycan Macro and Micro-Heterogeneity with Virus Evolution and Interactions with Host Immune System

Kshitij Khatri,

Mitchell R. White, Joshua A. Klein, Nancy Leymarie, Kevan L. Hartshorn, Joseph Zaia

Influenza A virus (IAV) fitness, under pressures from the host immune system, is regulated by accumulating mutations in hemagglutinin's (HA) genetic sequence, which disrupt existing consensus N-linked glycosylation sequons (NxS/T) or create new ones. Due to the challenges in studying glycosylation as a protein post-translational modification in general, most studies to date have relied on nucleotide sequencing experiments to reveal evolution of sequons as a surrogate for glycosylation, rather than direct structural analysis. Clearly, determination of site specific HA glycosylation would inform better understanding of evolution of IAV fitness.

A/Phil/2/82(H3N2), A/Phil/2/82/BS (H3N2) and A/PR/8/34 (H1N1) were analyzed in this pilot study. The HA from H3N2 strains were found to differ primarily in glycan macro-heterogeneity due to mutations accumulated by A/Phil/2/82/BS, disrupting two glycosylation sites. This loss of glycosylation also rendered A/Phil/2/82/BS resistant to surfactant protein-D (SP-D) binding. A/PR/8/34 was found to have a completely different glycosylation profile and was also found resistant to SP-D binding. Integrated omics generates complete phenotypic information on not only glycosylation but also on other post-translational modifications and genetic re-assortments leading to amino acid substitutions. This combination of information is crucial towards building structural information of biomolecules implicated in host-pathogen interactions. We combined the glycosylation profiles from LC-MS studies with bioassays for correlating changes in HA glycosylation with viral fitness and biological specificities. This workflow is now being applied to a larger set of evolutionarily important viral strains, to help understand the mechanisms of viral evolution and reveal crucial prophylactic and therapeutic targets on HA.

MYOCARDIN-RELATED TRANSCRIPTION FACTOR A REGULATES CONVERSION OF PROGENITORS TO BEIGE ADIPOCYTES

Li, Chendi; Farmer, Stephen; Matthew, Layne; McDonald, Meghan; Bian, Hejiao; Smith, Barbara.

Thermogenic brown adipose tissue generates heat via mitochondrial uncoupling protein-1 (UCP-1), increases whole-body energy expenditure and may protects against obesity and metabolic disorders. White adipocytes store excess energy in the form of triglycerides. UCP-1 positive adipocytes develop within white adipose tissue (beige or brite adipocytes) in response to cold exposure or beta3 adrenergic agonists. Signaling pathways that control beige adipocyte determination and formation are essentially unknown. Here, we identified a novel signaling pathway that regulates the lineage specification of beige adipocytes. Bone morphogenetic protein 7 (BMP7), a known brown adipogenesis inducer, suppresses Rho-GTPase kinase (ROCK) and depolymerizes F-actin (filamentous actin) into G-actin (globular actin) in mesenchymal stem cells. G-actin regulates myocardin-related transcription factor A (MRTFA) that co-transactivates serum response factor (SRF) and promotes smooth muscle cell differentiation in various organs. Subcutaneous white adipose tissue from *MRTFA*$^{-/-}$ mice had enhanced accumulation of UCP-1^{+} adipocytes and elevated levels of brown-selective proteins. Compared with wild type (WT) controls, *MRTFA*$^{-/-}$ mice exhibited improved metabolic profiles and were protected from diet-induced obesity and insulin resistance, suggesting that the beige adipocytes are physiologically functional. Compared to WT mice, stromal vascular cells from *MRTFA*$^{-/-}$ mice expressed higher levels of distinct beige progenitor markers and reduced levels of smooth muscle markers. Our studies demonstrate a novel ROCK-actin-MRTFA/SRF pathway that contributes to the development of beige adipocytes.

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SERUM AMYLOID A AND TOLL-LIKE RECEPTOR 2 ACTIVATION PROMOTE DE-DIFFERENTIATION OF VASCULAR SMOOTH MUSCLE CELLS.

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Smooth muscle cells (SMCs) regulate vascular tone, and during chronic inflammation associated with atherosclerosis, SMCs contribute to the disease process via de-differentiation from a contractile state. We study the impact of acute phase serum amyloid A (SAA), a cardiovascular risk marker that localizes to atherosclerotic plaques, on SMC function. The goal of this study was to define a role for SAA in SMC phenotypic expression. SMC marker expression was down-regulated by SAA, consistent with de-differentiation. Myocardin, a transcriptional co-activator of SMC marker gene expression, was down-regulated by SAA, and its overexpression rescued the SAA-mediated repression of the smooth muscle α-actin and smooth muscle 22 α (SM22) promoters. SAA-mediated down-regulation of SM22 promoter activity was also rescued by expression of the myocardin family members, myocardin related transcription factor (MRTF)-A and MRTF-B. It was reported that SAA is a ligand for the Toll-like receptor (TLR)2, which has been implicated in atherosclerosis. Interestingly, FSL-1 and Pam3CSK4, known TLR2 ligands, down-regulated SM22 promoter activity, and the effects were rescued with myocardin overexpression. Moreover, knockdown of TLR2 using siRNA rescued the de-differentiated phenotype induced by SAA. These data suggest that SAA and TLR2 activation promote SMC de-differentiation characteristic of atherosclerosis.

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SYNERGISTIC BINDING OF VEGF-A AND ITS RECEPTORS TO HEPARIN SELECTIVELY MODULATES COMPLEX AFFINITY

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Biochemistry Department

Angiogenesis is a highly regulated process orchestrated by the VEGF system. Heparin/heparan sulfate (HS) proteoglycans and neuropilin-1 (NRP-1) have been identified as co-receptors, yet the mechanisms of action have not been fully defined. In the present study we characterized molecular interactions between receptors and co-receptors, using surface plasmon resonance (SPR) as well as *in vitro* binding assays. We defined interactions and structural requirements for heparin/HS interactions with VEGF receptor-1 (VEGFR-1), NRP-1, and $VEGF_{165}$ in complex with VEGFR-2 and NRP-1. We demonstrate that the structural requirements are distinct for each interaction. We further show that $VEGF_{165}$, VEGFR-2 and monomeric NRP-1 bind weakly to heparin alone, yet show synergistic binding to heparin when presented together in various combinations. These data suggest that the presence of HS/heparin and NRP1 may dictate the specific receptor type activated by VEGF and ultimately determine the biological response of the VEGF system. The ability of co-receptors to fine-tune VEGF responsiveness suggests the possibility that VEGF-mediated angiogenesis can be selectively stimulated or inhibited by targeting HS/heparin and NRP-1.

Cell and Molecular Biology

Participants

Olga Novikov (73)

THE ROLE OF ENDOGENOUS AND ENVIRONMENTAL AHR LIGANDS IN MAMMARY EPITHELIAL CELL TUMOR GROWTH AND INVASION.

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Historically, AHR activation by environmental ligands was seen to facilitate mutations through CYP1 up-regulation. Our findings suggested that AHR activity drives cancer in the absence of environmental ligands. Our long-term goal is to identify endogenous ligands present in human mammary tumor cells, to determine how their production is controlled, and to assess how environmental AHR ligands alter this process. We hypothesize that metabolites produced by kynurenine (KYN) pathway of tryptophan metabolism drive AHR activity and enforce growth and/or invasion of malignant human mammary epithelial cells. As a corollary, we predict that environmental AHR ligands distort this signaling. We found that tryptophan metabolites kynurenine (KYN) and xanthurenic acid (XA), both detected in Hs578T cell lysates by LC/MS analysis, are potent AHR agonists. Hs578T cells highly express TDO, the rate-limiting enzyme in the kynurenine pathway. Knock-down of *Tdo* using *Tdo*-specific siRNA or short hairpin RNA results in reduction of *Cyp1B1,* a transcriptional target of the AHR, as well as reduced expression of invasion-related genes *MMP1* and *9,* reduced cell migration in a wound healing assay and reversion of malignant phenotype in Matrigel. We also found that AHR activity contributes to transcriptional regulation of *Tdo*. Our results support the hypothesis that AHR, hyper-activated by endogenous ligands produced via the KYN pathway, contributes to malignant phenotype in breast cancer. Moreover, our finding that the AHR transcriptionally regulates *Tdo* expression leads us to propose a mechanism via which environmental AHR ligands lead to increased production of endogenous AHR ligands by up-regulating *Tdo* and thereby enforce AhR-driven cell malignancy.

Program in Genetics and Genomics

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EPIGENETIC ANALYSIS OF TEMPORAL CHANGES IN CONTINUOUS GROWTH HORMONE-RESPONSIVE SEX-BIASED GENES IN MALE MOUSE LIVER

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Sex-dependent pituitary growth hormone (GH) secretory patterns determine the sex-biased expression of hundreds of genes in mouse and human liver, impacting sex differences in metabolism and liver disease. In males, GH is secreted in a highly pulsatile manner with virtually no plasma GH detectable between pulses. In females, GH secretion is more frequent, resulting in short GH-free periods and persistent activation of downstream GH signaling. Continuous GH infusion of intact male mice overrides the endogenous male, pulsatile plasma GH pattern. RNA sequencing of continuous GH-infused male mouse liver revealed time-dependent patterns of male-biased gene repression and female-biased gene de-repression. Global analysis of chromatin marks and DNase-I hypersensitivity sites (DHS) was used to cluster genes by their local chromatin environment. Sex-biased genes that responded early to continuous GH were enriched in active chromatin states, whereas late responsive genes were enriched in inactive chromatin states; thus, the basal chromatin environment dictates temporal patterns of responsiveness to continuous GH. Active chromatin marks associated with enhancers (H3-K27ac and H3-K4me1) showed time-dependent responsiveness to continuous GH that coincided with the associated changes in expression of several highly sex-biased genes. Further, continuous GH-stimulated decreases in H3-K27me3, preceded the de-repression of highly female-biased genes. Differential DHS analysis identified continuous GH-responsive sites, classifiable by their temporal patterns of response. Induced DHS were strongly associated with female-biased genes, and repressed DHS strongly associated with male-biased genes, indicating a functional role of these regulatory regions. Further integration of these GH-differential chromatin state maps with gene expression data will help elucidate global transcriptional and epigenetic networks that dictate sex-differential liver gene expression.

LUNG/THYROID CONVERSION OF MOUSE ESC-DERIVED ANTERIOR FOREGUT THROUGH TRANSIENT OVER EXPRESSION OF NKX2-1

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Genetics and Genomics

Lung/thyroid progenitors are derived from mouse embryonic stem cells (mESCs) through brief BMP4/TGFβ signaling inhibition (anteriorization) at the definitive endoderm stage leading to anterior foregut endoderm (AFE), followed by FGF2/BMP4 treatment. These progenitors are characterized by expression of Nkx21, a homeodomain transcription factor expressed in the developing lung, thyroid, and forebrain. To investigate if transient Nkx21 expression can increase the efficiency of Nkx21+ lung/thyroid progenitor specification, we utilized a mESC line double knockin GFPT/hCD4Foxa2 with a TetOn Nkx21 system. Activation of the Nkx2-1 transgene by addition of doxycycline for 24 hours specifically at the AFE stage induces and maintains high levels of endogenous Nkx21 and both lung and thyroid specific markers. Additionally, we sorted several AFE populations by hCD4Foxa2 expression and subsequently over-expressed Nkx2-1 for 24 hours. Differentiation propensity varied with Foxa2neg/low cells yielding increased thyroid progeny. To study mechanisms of this conversion and provide insights into the specification of the lung and thyroid domains, we are integrating Nkx2-1 ChIP-Seq and RNA-Seq data sets acquired from relevant stages during lung/thyroid directed differentiation to identify potential binding targets of Nkx2-1 and changes in global gene expression. The results demonstrate Nkx21 can act as a stagespecific inductive signal during directed differentiation and exemplify the potential of a more efficient system for deriving and studying Nkx21+ lung/thyroid progenitors.

RNASEQ AND INTEGRATIVE NETWORK ANALYSIS REVEALS MIR-424 AS A DRIVER OF MIGRATION IN NEVER SMOKER LUNG ADENOCARCINOMA

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There is a growing population of lung cancer patients worldwide who have never smoked. We hypothesize that lung adenocarcinoma (ADC) in never smokers (NS) compared to ever smokers (S) arises through distinct molecular processes that may be leveraged to identify personalized lung cancer treatment options.

Matched pairs of ADC tumor and adjacent-normal tissue (T/N) were obtained from NS (n=14) and S (n=17). RNA was isolated for RNA sequencing. Differential expression analysis yielded 120 large RNA, and 15 miRNA whose expressions were uniquely altered in NS tumors. Specifically, miR-424 was up-regulated in NS tumor, inversely associated with the expression of many predicted targets, and has been described as an oncomiR in other cancers. Strikingly, the functional knockdown of potential oncomiR miR-424 in NS cell lines caused reduced migration by a scratch assay, while no significant change was observed in S cell lines.

Our data reveals a unique large and small RNA signature in the tumors of never smokers compared to their smoking counterparts. Network analysis revealed miR-424 as a regulator of migration in NS cell lines. Further characterizing these discoveries will ultimately lead the way for targeted therapies designed for lung ADC in never smokers.

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MACAQUE IMMUNOGENETICS THROUGH *DE-NOVO* GENOME ASSEMBLIES

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High-throughput sequencing of the Immunoglobulin (Ig) repertoire from HIV-infected humans and immunized Rhesus macaques has led to important insights into vaccine candidates against HIV. Further elucidation of the antibody response in macaques is limited due to the incompleteness of the whole genome sequence (WGS) and the inherent difficulty of obtaining Ig sequences due to its complex and repetitive nature. To address this issue, we are generating an immunologically detailed, WGS of the macaque. We are using Illumina TruSeq to generate long reads, which we have assembled *de-novo* into 130,130 contigs with 26,559 contigs representing 50% of the WGS. In addition, we performed a bait-and-sequence strategy using human Ig probes to capture and sequence Ig from nine macaques. We have completed assembly and annotation of the Ig loci from nine macaques and have identified several Ig genes that have not been reported so far. We noted that the macaque Ig genes are highly polymorphic and identified key differences between human and macaque Ig genes. These differences are extremely important in the light of eliciting broadly neutralizing antibodies in these animal models. To obtain all the Ig genes into one contig, we are currently combining TruSeq long read data with the short read baited Ig data to generate complete macaque Ig loci. This information is essential to generate a complete map of allelic diversity of the Ig loci. This new and improved Ig database will improve macaque Ig repertoire analysis and aid in design and interpretation of vaccine studies.

DISSECTING THE SMAD4 METASTASIS SUPPRESSOR TO IDENTIFY NOVEL PROGNOSTIC BIOMARKERS AND THERAPEUTIC TARGETS IN COLON CANCER

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Colon cancer is the second most lethal cancer in men and women in the United States and metastasis is the primary cause of mortality. Hence, there is an urgent need to elucidate the molecular mechanisms underlying metastasis in order to develop prognostic markers and identify druggable targets. In colon cancer, *SMAD4* is mutated frequently (10-20%) and its low expression level is associated with poor prognosis, presence of metastases, and chemoresistance. Previously, we have shown that loss of SMAD4 in colon cancer is associated with increased cell migration and resistance to 5'-fluorouracil, the common chemotherapeutic agent. We also found that SMAD4 can interact with and inhibit HIF1a, leading to reduced expression of HIF1a targets, VEGF (angiogenesis) and GLUT1 (glucose uptake). Based on these studies, we hypothesized that SMAD4 forms a metastasis suppressor complex to inhibit colon cancer progression. We have generated cell lines overexpressing FLAG- and HA-tagged SMAD4 proteins and are in the process of identifying the components in the SMAD4 metastasis suppressor complex using co-immunoprecipitation and mass spectrometry. The functional roles of these SMAD4 interacting partners in regulating metastasis will be characterized in experimental models of cancer progression and their contribution to human colon cancer assessed through *in silico* analysis of gene expression profiles and examination of clinical specimens at different stages of disease progression. We suggest that these genes may serve as biomarkers in predicting disease progression and therapeutic targets to treat metastatic colon cancer.

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Participants

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*

MACROPHAGE NECROPTOSIS AND PNEUMONIA CAUSED BY VIRULENT PNEUMOCOCCUS

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RATIONALE: Pneumococcal disease progression is complex, resulting from intersections of bacterial virulence and host immunity. Knowing that macrophage NF-kappaB is critical to anti-pneumococcal defense, we explore the effect of high/low NF-kappaB activation on macrophage responsiveness and disease severity during pneumonia. We hypothesized that low macrophage NF-kappaB activation and increased pneumococcal virulence represent an altered macrophage response that contributes to the development of severe pneumonia. METHODS: Macrophage responses were ascertained by comparing differences in cell morphology and reactive oxygen species (ROS) production using confocal microscopy. Virulence during pneumonia was measured by lung CFU following intratracheal infection in mice. RESULTS: Cells stimulated with the low NF-kappaB activator, demonstrated cell swelling and rapid ROS production compared to cells stimulated with the high NF-kappaB activator. Necrostatin-1, (RIPK1-inhibitor), completely prevented these effects of the low NF-kappaB activator, supporting a necroptotic pathway. During co-stimulation (high and low combined), the high activator prevented the morphological changes caused by the low NF-kappaB activator, suggesting the high NF-kappaB activator elicited a protective response against the deleterious effects of the low NF-kappaB activator. Co-infection mouse studies with high and low NF-kappaB activating isolates showed the presence of the high activator enhanced host defense and elimination of the low NF-kappaB activator from the lungs. Interestingly, the high NF-kappaB activator was incapable of enhancing host defense against the low NF-kappaB activator in the lung of mice with a macrophage NF-kappaB RelA deficiency. CONCLUSION: Virulent pneumococci avoid activating NF-kappaB and induce macrophage necroptosis to facilitate survival in the lung and exacerbate pneumonia.

IMMUNOGENOMICS OF THE EGYPTIAN FRUIT BAT, AN IMPORTANT VIRAL RESERVOIR

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The Egyptian fruit bat (Rousettus aegyptiacus) is the suspected reservoir host for Marburg virus, and there is mounting evidence for the long-term circulation and evolution of the virus in these bats. Currently, there is no available reference genome for the Rousettus bat, limiting the ability to study this virus in its natural host. The lack of genomic data for this bat also prevents detailed study of the molecular mechanisms and genetic changes that allow bats of this species to coexist with Marburg and other highly pathogenic viruses. To address this need, we are constructing a high quality, annotated genome of R. aegyptiacus with a hybrid assembly approach. Using a combination of short and long read data, we have produced a draft genome of 99,254 scaffolds with 11,314 scaffolds representing 50% of the assembly. We are testing a few hybrid assembly pipelines to improve our current assembly. For automated annotation of the whole genome, we are using ab initio software trained with paired-end RNA-Seq data from ten bat tissues. We are simultaneously annotating key immune loci in both innate and adaptive immune systems. Thus far, we have found evidence for seven families of immunoglobulin heavy chain variable genes, and an expanded family of Type I Interferons, an important component of innate antiviral immunity. These reference annotations will open a new suite of tools in bat immunology and will be valuable for assessing how the Rousettus bat hosts a virus that is deadly to humans without experiencing any major pathology.

HOST IMMUNE RESPONSES TO *NEISSERIA GONORRHOEAE* INFECTION IN VIVO USING A DEPO-PROVERA-PRIMED HUMANIZED MOUSE-MODEL OF GONORRHEA

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BACKGROUND: A major roadblock to developing novel therapeutics for *Neisseria gonorrhoea* infection is its host specificity. There may be an effect of the estrous cycle of which there is little research. The aim of this study was to evaluate the effect of estradiol or progesterone treatment on cytokine production and transcription during Ng infection *in vivo*, using either wild-type mice or mice expressing human CEACAMs (a human specific gonococcal adhesin).

METHODS: Mice were infected in the context of either β-estradiol to mimic estrous, or progesterone to mimic diestrus. Serum, upper and lower genital-tract tissues were collected at 6h and 18h post infection. Whole tissue lysates were analyzed for cytokine and chemokine levels by multiplex immuno-assay while mRNA expression was analyzed by microarray.

RESULTS: Pro-inflammatory cytokines were significantly higher in infected tissue 6 hours post infection regardless of hormone treatment as compared to mock infection. In addition, both CLC and CXC cytokines showed increased levels at the same time point. Tissue from diestrus mice had significantly higher cytokine and chemokine levels than from estrous mice. Transcriptional analysis reinforced these observations with inflammatory gene sets being enriched in infected diestrus mice as compared to infected estrous mice. Expression of hCEACAMs enhanced this pattern of increased immune activation in diestrus mice compared to wild type mice. These results suggest that infection of diestrus phase mice with *Neisseria gonorrhoeae* induces a greater immune response, characterized by increased transcription and expression of cytokines and other canonical immune pathways, than similarly infected estrous mice.

EFFECTS OF DIFFERENTIAL CELL SIGNALING IN HIV TRANSCRIPTION

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Department of Microbiology

Objective: After cessation of antiviral treatments, HIV viral levels rebound due to the existence of latent reservoirs. The elucidation of signals that lead to latency is necessary for more thorough treatment of HIV/AIDS patients. We propose a model system by which signals from CD3 and CD28, receptors necessary for T cell activation, can be manipulated at the time of infection of CD4+ T cells. This model allows us to alter the balance between productive infection and the establishment of latent reservoirs.

Methods: Lentiviral vectors were used to transduce Jurkat and primary T cells with engineered Her2 receptors, which act as surrogates for the T cell receptor upon engagement with the Her2 ligand. These receptors include intracellular CD28 and CD3-zeta signaling domains. Mutations in the Her2 receptor alter its affinity for the Her2 ligand, creating a tunable system. Flow cytometry was used to isolate transduced cells. Expression of HIV was measured via luciferase. Integration of HIV was measured via nested Alu RT-PCR.

Results: Receptors were successfully transduced into CD4+ T cells. Preliminary data indicates that Her2 activation can lead to as much as an 18 fold increase in HIV expression. Increased receptor affinity for Her2 is also correlated with increased HIV expression.

Conclusions: Adjusting the intensity of cellular signals at the time of infection allows us to control the robustness of HIV transcription and the course of the viral infection.

MECHANISTIC DIFFERENCES IN INTERACTIONS OF HIV -1 AND HIV -2 WITH DENDRITIC CELLS

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HIV-2 infection has been restricted predominantly to West Africa; in contrast HIV-1 has spread rapidly and accounts for 95% of all HIV infections globally. The mechanistic basis for viral control and slower progression to AIDS in HIV-2$^+$ individuals remains unclear. We hypothesized that reduced interaction of HIV-2 with CD169, the primary HIV-1 attachment factor on myeloid dendritic cells (DCs) that targets captured virus particles to the trans infection pathway, plays an important role in its restricted pathogenesis. We constructed an HIV-2 proviral plasmid that encodes GFP between MA and CA and flanked by protease cleavage sites (HIV-2-iGFP), such that infectious virions contain GFP, similar to the previously described HIV-1-iGFP proviral clone (*J. Virol. 2007, 81:12596*). We have previously shown that HIV-1 evades cell-intrinsic restrictions in DCs by exploiting CD169 for enhanced transfer to CD4$^+$ T lymphocytes. Interestingly, there was a 3-fold reduction in capture of HIV-2 compared to HIV-1 by THP1/CD169 cells. Similar reduction in CD169 specific capture is seen with mature DCs, though both HIV-1 and HIV-2 virions were localized following capture within a CD81$^+$ compartment. Although HIV-1 and HIV-2 are equally infectious for CD4$^+$ T cells, there was a 7-fold decrease in HIV-2 trans infection of CD4$^+$ T cells compared to HIV-1 suggesting that HIV-2 might interact with additional receptors, which inhibit access to the trans infection pathway. We conclude that a reduced interaction of HIV-2 with CD169 inhibits access of HIV-2 to the DC-mediated trans infection pathway and might result in attenuated dissemination in vivo.

The Essential Role of Macrophages in Vaccine Induced Immune Responses upon Immunization with TLR-Based Adjuvants.

Munir Mosaheb, Paola Massari, Lee Wetzler

Vaccines are vital in the fight against infectious diseases and adjuvant immune enhancement is essential for their efficacy. The majority of adjuvants are derived from microbial pathogens and work via recognition by pattern recognition receptors. However, most adjuvants have been used with little understanding of their mechanism. Herein, we have investigated the role of individual antigen-presenting cell types, e.g. B cells, macrophages and dendritic cells in vaccine adjuvant activity in vivo using PorB, a TLR2 adjuvant, derived from Neisseria meningitidis, along with a variety of TLR-dependent and independent adjuvants. We found that MyD88 is required in B-cells, macrophages and DC for optimal adjuvant activity of PorB along with the TLR dependent adjuvants, CpG and MPLA, to induce high levels of antigen specific IgG. However, the humoral response was affected to the greatest extent in mice with macrophage specific MyD88 deletion (Mac-MyD88-/- mice). We demonstrated that the Mac-MyD88-/- mice have a decrease in germinal center formation in the spleens and draining lymph nodes when immunized with TLR-dependent adjuvant. In contrast, these mice form normal germinal centers with the use of TLR-independent adjuvants. Intact MyD88 signaling in macrophages is also crucial for the vaccine induction of Th2/Th1 cytokines when immunized with TLR-based adjuvants. Our findings, thus far, reveal a here-to-for unrecognized importance of macrophages in general, as well as intact in vivo MyD88 signaling in these cells, to allow for a robust vaccine induced immune responses. These insights will aid in vaccine development by allowing more intelligent and judicious use of adjuvants.

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THE CELLULAR STRESS RESPONSE AS AN ANTIVIRAL MECHANISM DURING EBOLA VIRUS INFECTION

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Replication of the highly pathogenic Ebola virus (EBOV) takes place in the cytoplasm of infected cells, making the virus prone to cellular antiviral responses. One such mechanism includes the formation of stress granules (SGs), which leads to the aggregation of stalled translation pre-initiation complexes and culminates in global translational arrest. Many viruses disrupt SG formation to avoid the detrimental effects of stalled translation. Therefore, we examined the stress response during EBOV infection. SGs were not observed at any time point during EBOV infection. When infected cells were treated with sodium arsenite (Ars) to induce stress, SGs formation mirrored the SG formation observed in Ars-treated, mock-infected cells. However, at late time points SGs were reduced. Individual EBOV proteins were then tested for their ability to block SG formation in Ars-treated cells. VP35, a known inhibitor of the IFN response, was able to disrupt SG formation at high concentrations. High levels of VP35 also disrupted nucleoprotein derived viral inclusions and the aggregation of proteins associated with neurodegenerative diseases. These data suggest that VP35 may interfere with the nucleation of self-aggregating proteins. Staining of newly synthesized proteins during EBOV infection demonstrated that these nascent proteins localize to viral inclusions in the absence of stress, but cannot do so under Ars treatment. This indicates that although VP35 is able to disrupt SG formation induced by Ars, viral replication is still sensitive to cellular stress. Therefore, we are currently exploring the possibility of a therapeutic intervention aimed at inducing the stress response in infected cells.

THE ROLE OF ANTI-V3 LOOP ANTIBODIES ON THE EMERGENCE OF CXCR4 TROPIC VIRUS IN HIV -1B

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Department of Microbiology

Background: Entry of HIV-1 into host cells is mediated by binding of HIV envelope (env) to CD4 and a co-receptor, CCR5 (R5) and/or CXCR4 (X4). While R5 viruses predominately initiate infection, in 15-50% of individuals, X4 viruses evolve during the chronic stage of infection. The mechanism for this late emergence remains unclear. Co-receptor usage is determined by the env V3 loop. Infected individuals develop antibodies against this region. As a result, the virus shields and/or changes the V3 loop. We hypothesize that neutralization escape from anti-V3 loop neutralizing antibodies (Nabs) engenders co-receptor switching.

Methods: We have examined a cohort of HIV-1B infected treatment naive subjects, and have identified individuals who harbored dual-mixed (DM) populations. X4 and R5 envs are inserted into an NL4-3 backbone and the resulting viruses are tested against autologous and heterologous plasma to determine neutralization sensitivity.

Results: We cloned the X4 and R5 env from 2 DM subjects and found that the X4 as compared to co-circulating R5 variants are significantly less neutralization sensitive to autologous contemporaneous plasma. The X4 as compared to the R5 envs were also less sensitive to heterologous pooled plasma from individuals with strictly R5 virus population (pooled R5 plasma).

Conclusions: These findings suggest that X4 viruses are neutralization escape variants. Further elucidation of the biological mechanisms for co-receptor switching will aid in developing better prediction tools.

A Systematic Assessment of Errors in Immunoglobulin Repertoire Sequencing

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Immunoglobulins (Ig) are the genes which code for antibodies - a primary effector of the adaptive immune system. Igs are highly diverse due to multiple recombination events and somatic hypermutation (SHM). In addition, billions of Ig-expressing B cells are present in the blood at any given time. Sequencing and analysis of these genes has proven very difficult, slowing pipelines for both basic and translational research alike.

We assessed the methodological challenges of Ig-repertoire sequencing (Ig-Seq) by 1.Determining the reproducibility of repertoire data and 2.Testing computational approaches to accurately quantify these data. Blood samples were taken from two volunteers and processed for comparison of differences introduced during sample collection, standard processing, Illumina MiSeq sequencing and analysis.

We first used Cloanalyst, which partitions raw sequences into inferred lineages. We determined that there are clonal differences from samples taken at the same time, and that biological replicates should be an integral part of Ig-Seq to capture this diversity. For a quantitative analysis, we utilized RNA-labeling as a gold standard for comparison. Biased amplification significantly distorts read output, but can be mitigated by statistical lineage analysis. Lineage analysis combined with phylogenetics can also stratify methods-induced mutations from true SHM. These results indicate that standard sample processing may bias Ig-Seq, and these biases can be resolved through lineage analysis and phylogenetic approaches.

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ROLE OF THE piRNA BINDING PROTEIN MIWI2 DURING BACTERIAL PNEUMONIA

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Multiple nodes of gene regulatory mechanisms delicately balance efficient innate immune responses in the lung during bacterial pneumonia. While pro-inflammatory cytokine elaboration is required for bacterial clearance, excessive inflammation can be injurious to host tissue. Results from a recent microarray performed on sorted epithelial cells from infected mice revealed that the mRNA encoding a Piwi-interacting RNA (piRNA)-binding protein, Miwi2, was significantly induced during pneumonia. In contrast to microRNAs, which are abundant in diverse tissue and cell types, piRNAs and their associated protein(s) are understood to be restricted to germ cells, where they guard against genomic instability by repressing retrotransposable elements. Given these unanticipated findings, we sought to explore biological roles for Miwi2 in somatic cells by conducting loss-of-function studies both *in vitro* using shRNA knockdown and *in vivo* using a Miwi2 knockout model (Miwi2$^{-/-}$). In cell lines, Miwi2 knockdown significantly altered the expression of select innate immune cytokines. During pneumococcal pneumonia *in vivo*, Miwi2$^{-/-}$ mice exhibited an enhanced host defense, as evidenced by increased neutrophil emigration and reduced *S. pneumoniae* burdens. Furthermore, we will present evidence for Miwi2-dependent gene expression in lung epithelial cells during infection, delineate their role in directing inflammatory responses, and propose a molecular model by which Miwi2 functions. In sum, little is known about how the biology of inflammation and Miwi2 biology intersect, however our preliminary data provide compelling evidence that Miwi2 does in fact shape innate immunity and host defense. These studies will define new molecular, cellular and physiological roles of Miwi2, and forward the innovative concept that Piwi clade proteins function outside of germ cells during inflammatory stress.

Molecular and Translational Medicine

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Mitochondrial Fragmentation in Response to Nutrient Excess Represents a Compensatory Adaptation to Maintain Human Beta-Cell Function

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Molecular and Translational Medicine

We have previously reported that high glucose and fatty acids, termed glucolipotoxicity (GLT), causes mitochondrial fragmentation and is concurrent with beta-cell dysfunction in cultured insulinoma (INS1) cells and mouse islets. However, it remains unclear if GLT causes mitochondrial fragmentation in human islets and whether this fragmentation plays a compensatory or pathological role during GLT-induced beta-cell dysfunction. To address if GLT alters mitochondrial shape in human beta-cells, islets were cultured in media with normal nutrients (5mM glucose, 10% BSA) or media with high glucose (11.2 mM) and fatty acids (1mM oleate and palmitate) for 4 days. Assessing mitochondrial morphology and membrane potential (MMP) by confocal imaging revealed that GLT significantly fragments mitochondria and increases MMP heterogeneity in human beta-cells. Concomitantly, glucose-stimulated insulin secretion (GSIS) was inhibited. Genetically inducing mitochondrial fragmentation by decreasing levels of mitochondrial fusion protein, Mfn2, in INS1 cells protected from GLT-induced cell death. Conversely, preventing GLT-induced fragmentation by expressing dominant-negative construct of mitochondrial fission protein, DRP1 (DRP1-DN), further disrupts MMP and causes significant accumulation of depolarized mitochondria in human beta-cells. Moreover, GLT-induced inhibition of GSIS is further decreased in human islets expressing DRP1-DN. In conclusion, mitochondrial fragmentation in response to GLT represents a compensatory adaptation to GLT insult, which works to preserve mitochondrial and beta-cell function.

COMPARATIVE ANALYSIS OF HISTOLOGICAL AND TRANSCRIPTOMIC FETAL BRAIN ABNORMALITIES IN THREE MOUSE MODELS OF DOWN SYNDROME

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Molecular and Translational Medicine

Down syndrome (DS), a genetic condition leading to intellectual disability, is characterized by triplication of human chromosome 21 (HSA21). Abnormal brain growth and reduced neurogenesis during fetal development are hallmarks of DS. Here, we directly compare prenatal brain phenotypes in three cytogenetically distinct mouse models of DS: Ts1Cje, Ts65Dn and Ts1Yey. Using immunohistochemistry and confocal/brightfield imaging, we analyzed proliferation and neurogenesis in the ventral and dorsal germinal zones and measured pallial thickness and brain size at embryonic day (E)15.5. We also utilized microarray technology to analyze telencephalic gene expression at E15.5 in these mice. Results from Ts1Cje show no differences in gross brain measurements or pallial thickness, but show an increased number of proliferating cells in subsections of the ventral germinal zone compared to euploids (n=3-4 per genotype; $p<0.05$). In Ts65Dn, results show a decrease in the thickness of the neocortical intermediate zone (78.0%±2.4% of euploids; n=3 per genotype; $p<0.05$) and in the percent of proliferating cells within the neocortical ventricular/subventricular zones (n=3 per genotype; $p<0.001$) compared to euploids. In Ts1Yey, results do not show appreciable neurogenesis, gross brain measurement, or pallial thickness defects (n=10-11 per genotype). However, microarray data show significant changes in differentially expressed genes in all three mouse models. Ts1Cje and Ts65Dn demonstrate larger genome-wide effects than Ts1Yey, yet all models show dysregulation in brain development and cognition-associated pathways. Taken together, our data show varying degrees of gene expression and brain developmental phenotypes in these three mouse models of DS.

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Pluripotent Stem Cell Modeling of Airway Epithelial Development

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Molecular and Translational Medicine

Many chronic lung diseases, including COPD, involve perturbations of airway epithelial cell fate. Novel model systems able to discern the mechanisms regulating airway epithelial cell fate are therefore desperately needed. One pathway of interest is the Wnt signaling pathway due to prior evidence that stage-dependent oscillations in canonical Wnt signaling are critical for lung development. To study the developmental stage-specific effects and downstream consequences of this signaling, we have employed a novel in vitro model system to derive lung progenitors from human pluripotent stem cells (hPSCs).

We differentiated hPSCs to NKX2-1+ primordial lung progenitors at an efficiency of 10-50% by Day 15. We found that NKX2-1+ cells at this stage express multiple ligands, receptors, and targets of the Wnt signaling pathway. Modulation of Wnt signaling at this stage led to rapid changes within the NKX2-1+ population consistent with altered airway patterning. Specifically, reduced Wnt signaling resulted in increased expression of markers of proximal lung lineages in the NKX2-1+ population. In contrast, sustained Wnt agonism was linked to inhibition of proximal cell marker expression.

Our findings suggest that sustained canonical Wnt signaling inhibits the proximal airway program in developing human lung progenitors. Ongoing studies seek to understand the mechanisms driving this inhibition, with the goal of elucidating the key signals that regulate airway cell fate in development and chronic lung disease.

VEGF-ENDOTHELIAL CELL PROCESSING IS MODULATED BY EXTRACELLULAR MATRIX STIFFNESS

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Vascular endothelial growth factor A (VEGF) drives endothelial cell maintenance and angiogenesis. Endothelial cell behavior is altered by cell contact surface stiffness, suggesting that VEGF activity might also be influenced by cell-substrate mechanics. We studied VEGF binding, internalization, and signaling as a function of substrate stiffness using endothelial cells cultured on Fibronectin linked polyacrylamide gels. To facilitate VEGF binding to the endothelial ECM, we pre-treated ECM with heparin, exposing a cryptic VEGF-Fibronectin binding site. Cells on the softest substrates were least able to respond to heparin, but they internalized the most VEGF after heparin treatment and demonstrated increased VEGF signaling. Attenuating matrix binding decreased cell internalization of VEGF similarly regardless of stiffness. β1 integrin, which connects endothelial cells to fibronectin, modulates changes in VEGF uptake due to stiffness. β1 integrin levels were similar regardless of stiffness, yet cells on hard surfaces exhibited a greater decrease in internalization than cells on softer matrices after β1 integrin inhibition. Stiff matrices facilitate the unfolding of fibronectin protein, which can reduce the binding capacity of β1 integrin. Thus a greater proportion of activated β1 integrin may be targeted in the stiff condition as compared to the soft. These findings present new targets for drug development and for the creation of new regenerative medicine parameters. Supported by BrightFocus grant (M2012014).

Role of Neutralizing and Non-neutralizing Antibodies in Protecting Infants Exposed to HIV-1C through Breastfeeding

Ghulam-Smith, Melissa; Sagar, Manish

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HIV-1 can be transferred from mother-to-infant in utero, intrapartum, or post-partum through breastfeeding. In the absence of antiretrovirals, breastfeeding introduces a 15-20% risk of transmission. The inefficiency in this mode of transmission may be due to the presence of neutralizing and/or non-neutralizing antibodies against specific epitopes on HIV-1 strains passed from mother to infant. We have obtained mother-infant samples and are studying the ability of antibodies found in infant plasma to neutralize replication competent recombinant viruses, which incorporate HIV-1 envelopes of the viruses circulating in the corresponding mother. Neutralization assays in the presence of cell-free virus are compared between infant groups. We hypothesized that infants exposed to HIV-1 though breastfeeding who did not acquire infection may have higher titers of neutralizing and/or non-neutralizing antibodies against their mothers' viruses. Thus far, comparing mean IC50s ($p = 0.5$) and area under the inhibition curve (AUC) ($p = 0.84$) of six matched pairs, there does not appear to be a significant difference in the neutralizing antibody titers of infants that acquired infection compared to those that did not. We will continue processing and analyzing the remainder of our samples ($n = 21$) to obtain sufficient power in comparing the two groups. We also plan to compare the ability of non-neutralizing antibodies that eliminate virus through antibody dependent cellular cytotoxicity (ADCC) in plasma of both infected and uninfected infants. This study will aid in attaining a better understanding of the immune correlates of protection in infants exposed to HIV-1C through breastfeeding.

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NEISSERIA GONORRHOEAE INDUCES LOCALIZATION OF THE INHIBITOR OF APOPTOSIS PROTEIN cIAP2 TO EXOSOMES

Goodmon, Kathleen; Massari, Paola; Genco, Caroline

The family of inhibitors of apoptosis proteins (IAPs) has been implicated in the establishment of microbial infection in host target cells and in cancer progression, due to their role in cell death and inflammation. It has been reported that survivin, an IAP family member, has both an intracellular and extracellular role in cancer cells by inhibiting apoptosis while promoting proliferative and metastatic potential in neighboring cells when secreted in exosomes. Exosomes are small lipid vesicles released from various cell types and deliver messages in the form of protein and mRNA to neighboring cells near and far. We have previously established that *Neisseria gonorrhoeae* protects against staurosporine-induced apoptosis in transformed human endocervical epithelial cells (End/E6E7 cells). The ability of *N. gonorrhoeae* to inhibit apoptosis correlates with the upregulation of cIAP2. In this study, we have further characterized the role of cIAP2 in the host inflammatory response to gonococcal infection. In End/E6E7 cells, we demonstrate that gonococcal infection induces a significant increase followed by a loss of intracellular cIAP2, yet a stable increase in extracellular cIAP2. Notably, extracellular cIAP2 is located in exosomes released after *N.gonorrhoeae* infection. Furthermore, we demonstrate that cIAP2 has a role in the response to gonococcal infection as it is required for cytokine production and protection from a caspase independent cell death. Collectively, our studies reveal significant alterations in exosome production and cIAP2 expression following gonococcal infection. Such changes may affect both immune signaling and cell death of infected epithelial cells and potentially, uninfected neighboring cells.

Characterization of Pluripotent Stem Cell Derived Type II Alveolar Cells

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Molecular and Translational Medicine

RATIONALE: Alveolar type II cell (AEC2) dysfunction has been implicated as a primary cause of pathogenesis in many poorly understood lung diseases that lack effective therapeutics. Childhood interstitial lung disease (chILD), is a group of monogenic diseases of the AEC2, which can be caused by autosomal dominant mutations in the surfactant protein C (SFTPC) gene. These mutations may result in protein misfolding and aggregation, impaired lipid metabolism, ER stress, and ultimately apoptosis in AEC2s. AEC2s are inaccessible to study in the developing human embryo and difficult to study in infants/children. They proliferate poorly and rapidly transdifferentiate into other cell types when isolated and cultured. Generating AEC2s de novo using induced pluripotent stem cell (iPSCs) technology would provide novel opportunities to study diseases of the alveolar epithelium, including SFTPC mutations.

METHODS AND RESULTS: We targeted a fluorescent reporter (GFP) into the endogenous SFTPC locus of a human iPSC, We employed a directed differentiation approach that recapitulates the key developmental milestones of the developing embryo to generate lung progenitors from these iPSC. Briefly, iPSC are sequentially differentiated into definitive endoderm, anterior foregut endoderm, immature lung progenitors, and finally mature lung lineages using specific combinations of exongenous signals suggested from the study of the embrygonesis. On day 32, a population of SFTPC-GFP+ cells emerges (1.21%), and when sorted to purity and analyzed by qRT-PCR, they express several AEC2-specific genes at levels similar to or higher than primary adult lung tissue. A 3D culture approach resulted in growth of a 3D epithelium and an increase in the percentage SFTPC-GFP+ cells by day 46 (15.8%). We also targeted tdTomato into the SFTPC locus of an iPSC line with an NKX2.1-GFP reporter, resulting in a dual reporter, with putative AEC2s expressing both NKX2.1-GFP and SFTPC-tdTomato.

CONCLUSIONS: We demonstrate a working reporter iPSC line that will facilitate identification, quantification and characterization of pure populations of iPSC-derived SFTPC -expressing cells. We describe the efficient derivation of an SFTPC+ population with a gene expression profile reminiscent of AEC2s. We will use this model to target GFP/SFTPC fusion cDNA into the endogenous SFTPC locus of chILD patient-derived iPSCs, providing a novel in-vitro platform for disease modeling and visualization of protein mistrafficking.

Development of an In Vitro Model to Study the Impact of Estradiol on Gonococcal Infection

Essence Maston, Robin Ingalls, MD

African-American women have significantly higher rates of gonorrhea compared to other racial and gender groups. Data from the breast cancer literature suggests African-American women also have higher circulating levels of estradiol compared to Caucasian women. We asked whether estradiol might alter the innate immune response to Neisseria gonorrhoeae to account for some of the racial disparity. Because many cell lines are poorly characterized in terms of estradiol responsiveness, we first needed to develop a reliable in vitro model system. Ishikawa (IK) cells, derived from an endometrial tumor, were incubated with estradiol to mimic ovulation, and estrogen receptor (ER)-α expression was determined. IK cells were then infected with gonorrhea in the presence or absence of exogenous estradiol to determine the effect on ERα expression as well as secretion of inflammatory cytokines. We observed that pretreatment with estradiol combined with gonococcal infection altered ERα expression in IK cells at both the level of protein and gene transcription. Induction of IL-8 was altered in the presence of estradiol compared to no hormone in IK cells. This was also true when comparing treatment of cells with live and dead bacteria, suggesting that bacterial driven invasion was not required to elicit an inflammatory response. In summary, IK cells express estrogen receptors and are responsive to changes in estradiol concentrations. These data demonstrate that IK cells are a useful model for hormonal influences on host pathogen interactions and support our original hypothesis that the estradiol alters the innate immune response to gonococcal infection.

PULMONARY MAST CELLS ARE REQUIRED FOR EARLY LIFE ALLERGEN-INDUCED NEUROPLASTICITY BY RELEASING NEUROTROPHIN 4(NT4)

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Asthma is a chronic inflammatory disease of the airway with a hallmark of hyper-reactive airway smooth muscle (ASM). In the United States alone, about 9 million children have asthma. Asthma in children is linked with exposure to pollutants, allergens or respiratory viral infection. Often, childhood asthma persists into adulthood. Our recent study in a mouse model of neonatal asthma demonstrates that early-life allergen exposure increases ASM innervation by up regulating levels of neurotrophin 4 (NT4). Neurotrophins are trophic factors for neurons that support axon outgrowth, survival and differentiation. We found that the hyper innervation of ASM is causally linked to persistent airway hyper-reactivity into adulthood. However, how allergen exposure increases NT4 is unknown. Here we show that repetitive allergen exposure in early life triggers expansion of the mast cell pool and is required for NT4 release through degranulation, thereby elevating the NT4 levels. We observed that the expression pattern of NT4 in the lung is similar between mouse, nonhuman primate and human. In addition, adoptive transfer of mast cell deficient pups (*Kit* $^{W\text{-}sh}$) with pulmonary mast cells elevates the ASM innervation after allergen exposure. Collectively, our data suggest that mast cells are a source of NT4 mediated ASM innervation on early life allergen exposure. This role of mast cells may be evolutionally conserved to connect early life environmental insults with long-term airway dysfunction in human. Our research findings suggest that blockade of mast cell degranulation may be a preventative strategy for young children at high risk of asthma.

The Role of the Aryl Hydrocarbon Receptor in the Development of Cells with Molecular and Functional Characteristics of Breast Cancer Stem Cells

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Molecular and Translational Medicine

Self-renewing, chemoresistant cancer stem cells (CSCs) are believed to contribute significantly to cancer metastasis and patient relapse. Therefore, the identification of signaling pathways that regulate CSC development and/or function is an important step towards understanding why patients relapse, and towards development of novel therapeutics that specifically target CSC vulnerabilities. Recent paradigm-shifting studies indicate a role for the aryl hydrocarbon receptor (AHR), an environmental carcinogen receptor implicated in cancer initiation, in normal tissue-specific stem cell self-renewal. These studies inspired the hypothesis that the AHR plays a role in CSC development. To test this hypothesis, AHR activity in Hs578T triple negative and SUM149 inflammatory breast cancer cells were modulated with AHR ligands, shRNA, or AHR-specific inhibitors whereupon phenotypic, genomic, and functional CSC characteristics were evaluated. ANOVAs were used to evaluate significance. The results demonstrate that: 1) ALDHhigh cells express elevated levels of Ahr and the AHR-driven gene, Cyp1b1, 2) AHR knockdown reduces ALDH activity, 3) AHR hyper-activation significantly increases ALDH1 activity, expression of stem cell- and invasion/migration-associated genes, and accelerates cell migration, 4) a highly significant correlation between Ahr or Cyp1b1 expression (as a surrogate marker for AHR activity) and expression of the CSC- and invasion/migration-associated gene sets was seen with genomic data obtained from 79 human breast cancer cell lines and over 1850 primary human breast cancers, 5) the AHR interacts directly with Sox2 and Runx1, and AHR ligands increase this interaction, 6) AHR knockdown inhibits tumorsphere formation in low adherence conditions, 7) AHR inhibition blocks the rapid migration of ALDHhigh cells and reduces ALDHhigh cell chemoresistance, and 8) AHR knockdown inhibits tumor growth and reduces tumor Aldh1a1, Sox2, and Cyp1b1 expression in orthotopic xenografts. These data suggest that the AHR plays an important role in development of CSCs in a large fraction of human breast cancers and that environmental AHR ligands may exacerbate breast cancer by enhancing expression of CSC-like properties.

METRONOMIC CYCLOPHOSPHAMIDE-ACTIVATED ANTI-TUMOR IMMUNE RESPONSES: DOSE AND SCHEDULE DEPENDENCE IN MOUSE MODELS

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Metronomic cyclophosphamide (CPA) treatment activates robust anti-tumor immunity and induces regression of implanted brain tumors in mice when administered on an intermittent, every 6-day schedule (CPA/6d), but not on a daily low-dose or a maximum-tolerated dose schedule. Here we found that metronomic CPA treatments spaced longer than 6-days were associated with un-sustained natural killer cell responses, leading to rapid tumor growth rebound. Increasing the CPA dose prolonged the tumor regression period on the every 9-day schedule, but natural killer cell activation was markedly decreased. Thus, sustained immune and anti-tumor responses were only achieved on the CPA/6d schedule. Furthermore, CPA/6d treatment eradicated GL261 tumors implanted in immunocompetent C57BL/6 mice by activating anti-tumor CD8-T cell responses and immune memory. In contrast, CPA/6d only induced minor and moderate immune responses in LLC and B16F10 tumors implanted in C57BL/6 mice, respectively. RNA-seq profiles reveal that untreated GL261 tumors showed a higher level of immune activity than untreated LLC and B16F10 tumors. Moreover, CPA-treated LLC tumors were associated with inhibited VEGFA-targeted genes, down-regulated cell adhesion and transendothelial migration genes, and up-regulated drug metabolism pathways. In B16F10 tumors, CPA activated genes in chemokine signaling and antigen processing and presentation pathways, but no NK cell and T cell effector pathways were activated. Together, these studies elucidate the dose, schedule, adaptive immune and tumor model-dependence of CPA-induced anti-tumor immune responses, giving new insight into the molecular signaling events underlying the deficiencies in immune responses seen in intermittent metronomic CPA-unresponsive tumor models.

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SPEECH SYNTHESIS VIA SURFACE ELECTROMYOGRAPHIC CONTROL: TRAINING EFFECTS

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Graduate Program for Neuroscience

Individuals who use augmentative and alternative communication (AAC) devices due to motor impairment require two complementary systems to communicate: an interface that produces speech (e.g. a grid of letters on a computer screen) and an input modality with which to select targets (e.g. a head-tracker or sip-and-puff system). This study evaluated a novel AAC interface using both typical computer mouse input and a cursor control system that is available to users with severe paralysis. The novel AAC interface allows users to have direct control over their synthesized speech by enabling them to select sounds (or phonemes) rather than letters. The cursor control system translates surface electromyography (sEMG) of spared musculature into cursor movements, and was designed for individuals with spared facial muscle control as in users with high spinal cord injuries. An evaluation of the novel AAC interface was completed by ten healthy participants over eight training sessions. Participants produced speech with the phonemic AAC interface using both a typical mouse and the sEMG cursor control system, in order to capture how participants were learning to use the interface itself (assessed with the typical mouse control) and learning to use the sEMG cursor control and the interface. Results showed that the communication rates achieved with a typical mouse stabilized after six sessions, while users continued to improve with the facial sEMG cursor control system throughout all eight sessions. Both the novel phonemic interface and the sEMG-controlled cursor show promise for future communication applications.

HIGH TRAIT IMPULSIVITY PREDICTS FOOD ADDICTION-LIKE BEHAVIOR IN THE RAT

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Graduate Program for Neuroscience and Department of Pharmacology

Background: Impulsivity is a behavioral trait frequently seen not only in drug-addicted individuals but also in individuals who pathologically overeat. However, whether impulsivity predates the development of uncontrollable feeding is unknown. In this study, we hypothesized that a high impulsivity trait precedes and confers vulnerability for food addiction-like behavior. ***Methods:*** For this purpose, we trained ad libitum-fed male Wistar rats in a differential reinforcement of low rates of responding (DRL) task to select Low- and High-impulsive rats. Then, we allowed Low- and High-impulsive rats to self-administer a highly palatable diet (Palatable group) or a regular chow diet (Chow group) in 1-h daily sessions, under fixed ratio (FR) 1, FR3, FR5, and under a progressive ratio (PR) schedules of reinforcement. In addition, we tested the compulsiveness for food in Low- and High-impulsive rats by measuring the food eaten in the aversive, open compartment of a light/dark conflict test. Finally, we measured the expression of the transcription factor DeltaFosB in the shell and the core of the nucleus accumbens, which is a marker for neuroadaptive changes following addictive drug exposure. ***Results:*** The data we obtained demonstrate that impulsivity is a trait that predicts the development of food addiction-like behaviors, including: (i) excessive intake, (ii) heightened motivation for food, and (iii) compulsive-like eating, when rats are given access to highly palatable food. In addition, we show that the food addiction phenotype in high impulsive subjects is characterized by an increased expression of the transcription factor DeltaFosB in the nucleus accumbens shell. These results reveal that impulsivity confers an increased propensity to develop uncontrollable overeating of palatable food.

EFFECTS OF COCAINE ON VISUAL PROCESSING IN ZEBRAFISH

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Graduate Program for Neuroscience

Department of Anatomy and Neurobiology

Biomolecular Pharmacology Training Program

To determine whether prenatal or acute cocaine exposure interferes with visual processing we studied neuronal responses to whole-field stimuli in two visually responsive brain regions of larval zebrafish expressing the calcium indicator GCaMP-HS. We found that both red light (LF) and dark (DF) flashes elicited similar responses in the optic tectum neuropil (TOn), while dorsal telencephalon (dTe) responded only to LF. Acute cocaine (0.5 μM) reduced neuronal responses to LF in both regions but did not affect responses to DF. Repeated stimulus presentation led to habituation of dTe neurons to LF. Acute cocaine prevented habituation. TOn neurons habituated to DF, but not LF, and DF habituation was not modified by cocaine. Remarkably, prenatal cocaine exposure prevented the effects of acute cocaine on LF response and habituation at 7 days post fertilization in both TOn and dTe, but did not affect DF responses. The characteristic effects of acute cocaine on LF responses in both TOn and dTe were absent in transgenic zebrafish lacking the dopamine transporter, the canonical target of cocaine. Here we discovered that, in spite of similar neural responses to LF and DF in the optic tectum (superior colliculus in mammals), responses to LF are more complex, involving the dorsal telencephalon (homologous to mammalian cortex), and more vulnerable to the effects of cocaine. These effects of cocaine may be mediated by the dopamine system. We demonstrated that acute cocaine exposure affects visual processing differentially by region, and that prenatal cocaine exposure modifies zebrafish visual processing in a stimulus-dependent manner.

CENTRAL AMYGDALA PACAP IN THE BEHAVIORAL STRESS RESPONSE

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Anxiety-related disorders are the most common forms of mental disorders; characterized by feelings of excessive worry in the absence of specific external stimuli, they are accompanied by physical, affective and behavioral symptoms.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38-amino acid peptide located in the hypothalamus and in various extra-hypothalamic regions including the central nucleus of the amygdala (CeA). PACAP and PAC1 receptor (PAC1R) have been proposed to play a key role in mediating the behavioral and endocrine responses to stress; however, few studies have examined the involvement of the extrahypothalamic PACAP system in the modulation of stress. Our aim was to elucidate the role of the PACAP/PAC1 system of the CeA in the context of anxiety.

We first assessed the effects of PACAP microinfused into the CeA of male rats in the elevated plus maze (EPM) and the acoustic startle response (ASR), behavioral tests sensitive to states of anxiety and fear. We then assessed the effects of the PACAP antagonist, PACAP(6-38), on ASR and sensitization by footshock of ASR. We then used restraint stress to determine whether the endogenous PACAP-PAC1 system is recruited by acute stressors.

Infusion of PACAP into the CeA significantly reduced percent open arm time in the EPM without effecting motor activity, as evidenced by entries into the closed arms. Infusion of PACAP into the CeA also increased acoustic startle reactivity. The antagonist, PACAP(6-38), had no effect on ASR, but blocked the sensitization of ASR by footshock. Finally, we observed that an acute, 1h exposure to restraint produced a rapid elevation of PACAP and PAC1R expression in the CeA, but not the basolateral amygdala or the bed nucleus of the stria terminalis.

These data prove an anxiogenic role for the PACAP/PAC1 system of the CeA and suggest that hyperactivity of this system may underlie the anxiogenic effects of stress.

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THE DENSITY OF GALECTIN-3 CELLS IS INCREASED WITH AGE AND COGNITIVE DECLINE

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Graduate Program for Neuroscience/Anatomy and Neurobiology

A premier feature of aging is a decline in cognitive function that occurs at different ages and progresses at different rates depending on the individual. Most associated with cognitive decline are changes in white matter volume and a loss of myelinated fibers and an increase in morphological deficits associated with myelin. Additionally, microglia, the resident brain macrophage, become chronically pro-inflammatory (M1) activated with age. White matter M1 activation is also associated with cognitive decline. With increased myelin debris and chronic microglial M1 activation, it is expected that phagocytosis is also increased with age and cognitive decline. Galectin-3 (Gal-3, also known as MAC-2) is a lectin selectively upregulated in phagocytic macrophages and Gal-3 knockout mice have reduced phagocytic ability. In the current study, brains from young (6-10 years old) and old (20-30 years old) rhesus macaques were immunostained with Gal-3. The density of cells containing immunostaining positive for Gal 3 in four white matter regions was increased in old animals compared to young animals. Additionally, Gal 3 cell density was increased in old cognitively impaired animals compared to old cognitively unimpaired animals. These data suggest an involvement of phagocytic cells in both aging and cognitive decline.

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EFFECTS OF ATTENTION ON EVOKED POTENTIALS FOR BRAIN-COMPUTER INTERFACE CONTROL

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Graduate Program for Neuroscience

Many proposed EEG-based brain-computer interfaces (BCIs) make use of visual stimuli to elicit steady-state visual evoked potentials (SSVEP). By focusing their visual attention to a flashing light of a specific frequency, a participant's visual cortex activity entrains to that frequency. This oscillatory activity can be detected via EEG and translated to a computer input. However, such a control scheme can be ineffective if a user has no motor control over their eyes and cannot selectively attend to one of a number of flashing stimuli using their peripheral vision (covert attention). Tactile-based methods, such as somatosensory steady-state evoked potentials (SSSEP), are a potentially attractive alternative in these scenarios. Attention to tactile stimulation does not require muscle activation in the way visual stimulation does. Here, we compare the neural signals elicited by covert attention to multiple visual stimuli and covert attention to multiple tactile stimuli in naïve BCI users, with the goal of evaluating the feasibility of event potential-based control of an EEG BCI. Although both SSSEP and SSVEP signals could be detected above chance levels, our initial results suggest that the signal-to-noise ratio of SSVEP signals is larger than that obtained using SSSEP.

PATHOLOGICAL CHANGES IN RNA BINDING PROTEINS IN PARKINSON'S DISEASE

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The translational response to stress is mediated in part by aggregation of RNA binding proteins (RBPs) to form stress granules (SGs). An overactive SG pathway has been proposed as a novel therapeutic target in the field of neurodegeneration. Previous work in Alzheimer's diseased (AD) brains demonstrates co-localization of Tau pathology with the RBP TIA-1, highlighting a significant increase in SGs in aged AD brains (1). In the current study, we explored the response of RBPs to the pathophysiology of Parkinson's diseased (PD) and PD spectrum brain samples. Cortical tissue from PD brain showed striking loss of nuclear TIA-1, with a corresponding increase in cytoplasmic TIA-1. SGs were unexpectedly absent. The only other RBP for which we observed disease-linked correlations, was HuD (Elav4), which is a RBP that is genetically associated with PD.

We hypothesized that low levels of SGs in PD may result from either a compensatory increase in autophagy, which might occur in PD, or interference in SG formation mediated by α-synuclein (αSyn). To explore these questions, BEM17 neuroblastoma cells were transfected with fluorescently labeled TIA-1 ± αsyn, and treated ± rapamycin. Activating autophagy decreased SG number and size. Interestingly, over-expressing WT αsyn appeared to increase SG number and size while A53T αsyn did not have this effect. We are currently testing the effects of exposure to recombinant oligomerized syn. This data suggests that the pathophysiology of PD is associated with dysfunction of the SG pathway, and could provide novel insights into mechanisms of degeneration in PD.

miR-155 is Essential For Inflammation-Induced Hippocampal Neurogenic Dysfunction

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Peripheral and central nervous system (CNS) inflammation leads to aberrations in developmental and postnatal neurogenesis, yet little is known about the mechanism linking inflammation to neurogenic abnormalities. Specific microRNAs (miRNAs) regulate peripheral and CNS inflammatory responses. *MicroRNA-155* (*miR-155*) is the most significantly up-regulated miRNA in primary murine microglia stimulated with lipopolysaccharide (LPS), a pro-inflammatory Toll-Like Receptor 4 ligand. Here we demonstrate that miR-155 is essential for robust *IL6* gene induction in microglia under LPS stimulation *in vitro*. LPS-stimulated microglia enhance astrogliogenesis of co-cultured neural stem cells, whereas blockade of *IL6* or genetic ablation of microglial *miR-155* restores neural differentiation. *miR-155* knockout mice show reversal of LPS-induced neurogenic deficits and microglial activation *in vivo*. Moreover, mice with transgenic elevated expression of miR-155 in nestin-positive neural and hematopoietic stem cells, including microglia, show increased cell proliferation and ectopically localized doublecortin-positive immature neurons and radial glia in the hippocampal dentate gyrus granular cell layer. In addition, miR-155 elevation leads to increased microglial numbers and amoeboid morphology in the dentate gyrus. Our study demonstrates that miR-155 is essential for inflammation-induced neurogenic deficits via microglial activation and induction of IL6, and is sufficient for disrupting normal hippocampal development.

Graduate Program in Nutrition & Metabolism

Participants

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Chronic Exposure to Excess Nutrients Left-Shifts the Concentration Dependence of Glucose-Stimulated Insulin Secretion in Pancreatic Beta-Cells

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Hyperinsulinemia is elevated plasma insulin at basal glucose. Impaired glucose tolerance is associated with hyperinsulinemia, though the exact cause and effect relationship remains poorly defined. We tested the hypothesis that hyperinsulinemia can result from an intrinsic response of the beta-cell to chronic exposure to excess nutrients, involving a shift in the concentration dependency of glucose-stimulated insulin secretion (GSIS). INS-1 (832/13) cells were cultured in either a physiological (4 mM) or high (11 mM) glucose concentration with or without concomitant exposure to oleate. Isolated rat islets were also cultured with or without oleate. A clear hypersensitivity to sub-maximal glucose concentrations was evident in INS-1 cells cultured in excess nutrients such that the 25% of maximal ($S_{0.25}$) GSIS was significantly reduced in cells cultured in 11 mM glucose ($S_{0.25}$ = 3.5 mM) and 4 mM glucose with oleate ($S_{0.25}$ = 4.5 mM) compared to 4 mM glucose alone ($S_{0.25}$ = 5.7 mM). The magnitude of the left shift was linearly correlated with intracellular lipid stores in INS-1 cells (r^2 = 0.97). We observed no significant differences in the dose responses for glucose stimulation of respiration, NAD(P)H autofluoresence or Ca^{2+} responses between left and right-shifted β-cells. However, a left-shift in the sensitivity of exocytosis to Ca^{2+} was documented in INS-1 cells cultured in 11 versus 4 mM glucose ($S_{0.25}$ = 1.1 and 1.7 μM, respectively). Our results suggest the sensitivity of exocytosis to triggering is modulated by a lipid component, the levels of which are influenced by the culture nutrient environment.

ADIPOREDOXIN; AN UPSTREAM REGULATOR OF ER REDOX HOMEOSTASIS AND OXIDATIVE FOLDING

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Adiporedoxin (Adrx), is a protein highly enriched in adipocytes which regulates protein secretion in *in vitro* and *in vivo* models. In adipocytes, Adrx regulates adipokines, leptin, adiponectin, and collagen for example, and dysfunction of this secretion can significantly contribute to the metabolic syndrome. While enriched in adipose tissue, Adrx is also found in pancreatic islets, similarly affecting insulin secretion. Proper redox homeostasis in the ER is essential for oxidative protein folding of disulfide bonds and protein secretion. Adrx contains a –CXXC- active site motif characteristic of the thioredoxin super-family, typically involved in disulfide bond formation. As this sequence suggests, Adrx oscillates between a reduced and oxidized form through the aforementioned –CXXC- active site and becomes oxidized to a greater or lesser extent in response to the redox environment of the ER. Consequently, knocking down Adrx impairs the re-oxidation of PDI, indicating an overlapping function with known regulators of ER redox function, namely endoplasmic reticulum oxidoreductase, Ero1a, and the peroxiredoxin Prdx4. Although Adrx does not look like a classical peroxiredoxin, overexpression of Adrx in beta cells decreases overall ROS in the cell and protects against induced cellular oxidative stress. Adrx is upregulated by HIF1a in response to an increase in ROS during both hypoxia and menadione treatment. By over expressing Adrx in adipocytes, we were able to protect the ER from oxidative stress and rescues adipokine secretion. Our results support the hypothesis that Adrx is an important mediator of the redox state and oxidative folding in the adipocyte and islet ER.

PERI-DROPLET MITOCHONDRIA IN BROWN ADIPOCYTES FORM AN EXCLUSIVE SUBPOPULATION OF MITOCHONDRIA

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Nutrition and Metabolism Program

Mitochondria play essential roles in brown adipose tissue differentiation and function. We have recently shown that mitochondrial dynamics is a physiological regulator of adrenergically-induced changes in energy expenditure. Moreover, mitochondrial architecture can represent an adaptation of mitochondria to respire according to the bioenergetic needs of the cell. Mitochondria are involved in the great variety of other important cellular functions; however, interactions of all those roles and cellular function remain to be understood.

Mitochondria in brown adipocyte are divided to two different populations, mitochondria that are found with submicron proximity to the lipid droplet or Peri-droplet (PD) mitochondria, and mitochondria that are located at least 5µm away from the vicinity of a lipid droplet or Cytoplasmic (C)mitochondria. Labeling mitochondria with TMRE and PAGFP showed that PD-mitochondria are more elongated as compared to C- mitochondria (~40% of PD-mitochondria vs. ~10% of cytoplasmic (C) mitochondria are 5-10µm). In addition, using TMRE and NADH auto fluorescent, we found that PD- mitochondria in brown adipocytes but not in other lipid-containing cells are in different energetic state than C-mitochondria; they also have higher membrane potential and NADH content. Mitochondrial dynamic is also slower in PD-mitochondria compared to C-mitochondria, but they have higher protein imports and protein content supporting by their higher Tomm20 content.

These differences may reflect the different roles of those mitochondria in differentiation and stimulation of the brown adipocytes. More investigations are going on to reveal further metabolic and functional differences of those mitochondria in the brown adipocyte.

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VITAMIN STATUS AND SEVERITY OF PNEUMONIA IN ECUADORIAN CHILDREN

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Background: Adequate vitamin D status may play a role in preventing acute lower respiratory infections (ALRI).

Objective: To determine if vitamin D status is associated with a reduction in time to recovery from severe pneumonia in Ecuadorian children.

Design: We conducted a secondary analysis of the Ecuadorian Pneumonia And Zinc supplementation trial (EcuaPAZ),which was a randomized, placebo-controlled trial of zinc as an adjunct to the treatment of children under 5 years who were hospitalized with pneumonia in Quito, Ecuador. Serum 25-hydroxyvitamin D (25(OH)D) concentration was measured at baseline by an automated enzyme immunoassay. The definition of severe pneumonia included the presence of cough and/or chest wall indrawing, tachypnea (>50 breaths/min in children 2-12 mo, >40 breaths/min in children 12-59 mo), and hypoxemia (SpO_2, <90%). Vitamin D deficiency is defined as serum 25(OH)D levels ≤20 ng/mL.

Results: 352 children, hospitalized with severe pneumonia, had 25(OH)D concentrations measured. Of these, 170 (48%) were vitamin D deficient. The mean time to remission of three respiratory signs did not differ by vitamin D status (mean ± S.E.: Deficient, 89.5 hr ± 5.6, Sufficient 99.1 hr ± 5.7, p=0.2335). Similarly, time to resolution of the individual signs of chest wall indrawing, tachypnea, and hypoxemia did not differ between the two groups (p=0.2969, p=0.4221, p=0.1287, respectively). A Kaplan Meier analysis of time to remission of three respiratory signs did not differ by vitamin D status (p=0.2145).

Conclusions: Overall, the vitamin D status was not associated with time to remission of respiratory signs in this population.

Program in Oral Biology

Participants

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SEX-LINKED SKELETAL PHENOTYPE OF LYSYL OXIDASE LIKE-1 MUTANT MICE

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Lysyl oxidases are required for collagen and elastin cross-linking and extracellular matrix maturation including in bone. The lysyl oxidase family consists of lysyl oxidase (LOX) and 4 isoforms (LOXL1-4). Here we investigate whether deletion of LOXL1, which has been linked primarily to elastin maturation, leads to skeletal abnormalities. Left femurs (n=8), L5 vertebrae (n=8) and tibiae (n=8) were analyzed by micro-computed tomography (μCT) in 13-week old wild type (WT) and *Loxl1* -/- male and female mice. Right femurs (n=8) were subjected to histochemical/histology analyses of osteoclasts and growth plates. Sera from all mice were analyzed for bone turnover markers. Results indicate significant deterioration of trabecular bone structure in long bones and vertebrae from female-, but not from male, mutant mice compared with WT. Decreases in BV/TV, Conn.D, and trabecular thickness and number in the femoral distal metaphysis were observed in female, but not male, mutant mice. Trabecular spacing was increased significantly in femurs of female mutant mice. Findings were similar in trabecular bones from L5 vertebrae of female mutant mice. Secretion of tartrate resistant acid phosphatase (TRAP) was increased by active osteoclasts at the trabecular bone surface in female mutant mice compared with WT female, consistent with increased serum Rankl and decreased Opg levels. Chondrocyte columns were disorganized in both female and male *Loxl1* -/- mice, but to a greater extent in females. Data indicate that *Loxl1* -/- mutant mice develop appendicular and axial skeletal phenotypes characterized by decreased bone volume fraction and compromised trabecular microstructure, predominantly in females.

Department of Pathology & Laboratory Medicine

Participants

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The Transthyretin Gene Variant G6S may be Protective in Wild-Type Transthyretin Amyloidosis

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Department of Pathology and Laboratory Medicine

Abstract

Mutation of the transthyretin (TTR) gene causes a hereditary condition (ATTRmut) that destabilizes the normal TTR tetramer, leading to aggregation and amyloid formation in heart and nerves. In contrast, wild-type transthyretin amyloidosis (ATTRwt) is a sporadic and fatal congestive heart disease resulting from myocardial amyloid deposits of unmutated TTR. Why the wild-type protein causes disease is poorly understood. We hypothesized that genetic variation may impact ATTRwt and analyzed 4 non-coding regions and all 4 exons of the TTR gene; we Sanger-sequenced these regions in 108 Caucasian males with ATTRwt cardiomyopathy and 118 healthy controls. One previously reported coding polymorphism was identified, rs1800458, which corresponds to the G6S TTR protein variant, long thought to be non-pathologic. The variant allele frequency was 0.04 in subjects and 0.08 in controls, yielding an odds ratio <1, suggesting G6S is protective against ATTRwt. The G6S heterozygote occurrence was 7% in subjects and 9% in controls, with G6S homozygotes only occurring in the control group at 3%. Further, the control group was out of Hardy-Weinberg equilibrium only for G6S. These findings prompted us to further investigate G6S TTR as a potential ATTRwt modifier. Using circular dichroism (CD) analysis and aggregation assays, we characterized the biophysical and biochemical properties of G6S. Preliminary results suggest G6S structure is highly stable and similar to wild-type. Interactions between G6S and less stable, amyloid-forming TTR variants are varied; L55P exerts a destabilizing effect on G6S, while G6S stabilizes V122I. These data suggest that G6S TTR may ameliorate development of ATTRwt amyloidosis.

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IKAROS REGULATES THE FOXO1-DRIVEN PROGRAM IN NAÏVE PERIPHERAL CD4 T CELLS

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A mature CD4 T cell is the result of a progression of developmental events. These begin early at the hematopoietic stem cell stage and continue into polarization of T helper (Th) cell subsets. Transcription factors dictate each of these processes. Ikaros is one such protein, which is expressed at high levels in immature and mature T cells. Lack of Ikaros reduces the ability of cells to commit to the lymphoid lineage, resulting in reduced numbers of early thymic T cell progenitors and mature T cells. In CD4 T cells, a lack of Ikaros affects cell proliferation, development, cytokine expression profiles and T cell anergy. However, little is known about a role for Ikaros in the naïve T cell. In order for the naïve T cell pool to be maintained and ready to respond swiftly to antigenic encounter, mechanisms are in place to ensure their survival and proper homing. The transcription factor Foxo1 regulates these processes through its direct role in activating expression of the genes encoding IL7Rα and CD62L. Foxo1 also regulates development of the inducible subset of regulatory T cells (iTreg) and it functions to suppress effector phenotypes in these cells. Despite these important roles, little is known about how the Foxo1 gene itself is transcriptionally regulated. Using Ikaros knockout and conditional knockout mouse models, our studies reveal that Ikaros is required for survival and homing of naïve T cells, as well as iTreg development, and provide evidence that it does so through maintaining levels of Foxo1 gene expression.

DEVELOPMENT OF A NOVEL TDP-43 GRANULE INHIBITOR: PUTATIVE MECHANISM OF ITS ACTION

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The RNA binding protein TDP-43 accumulates in the cytoplasm of brain and spinal cord from ALS and FTLD patients. Mutations in its gene TARDBP increase formation of intracellular TDP-43 inclusions in disease. Under stressed conditions TDP-43 is often hyper phosphorylated and forms granules that act very similar to stress granule (SG) proteins in cells and in the ALS/FTLD brain and spinal cord. Recent studies show TDP-43 inclusions localize to SGs in neurodegeneration, but the mechanism and the role of TDP-43 in pathogenesis are poorly understood. To investigate the pathophysiology of TDP-43 granule formation and potential therapeutic targets, we screened a chemical library of 75,000 compounds using PC12 cells that inducibly express wild-type human TDP-43-EGFP. 22 compounds dose-dependently decreased the arsenite-induced TDP-43 granules without significant cytotoxicity. The immunoprecipitation of TDP-43-EGFP followed by mass spectrometry revealed a candidate compound C8j reduced the phosphorylation at novel, previously uncharacterized T103/S104 amino acid residues of TDP-43 under arsenite stress. The phospho-mimetic mutation of the sites induced the spontaneous intracellular TDP-43 granules, but the phospho-null mutation did not reduce the formation of the TDP-43 granules under stressed condition. These results suggest the phosphorylation at T103/S104 is sufficient, but not essential to induce the TDP-43 granules. We tested the direct inhibition of 383 kinases by C8j, but it did not affect the kinase activities. These results suggest the compound is acting upstream of kinases to reduce the phosphorylated TDP-43. We anticipate this study will help to elucidate the biological pathways regulating TDP-43 aggregation and toxicity.

Cardiotoxicity and Internalization of Pre-Fibrillar Transthyretin Oligomers and Attenuation by Doxycycline

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Department of Pathology & Laboratory Medicine

Transthyretin (TTR)-associated amyloidoses are diseases wherein wild-type or mutant TTR forms amyloid fibrils that infiltrate multiple organs. Wild-type TTR amyloidosis, ATTRwt, is a sporadic disease characterized by deposits that occur mainly in the heart. Alternatively, >100 TTR mutants cause inherited forms, ATTRm, frequently featuring cardiac amyloid deposits.

The goals of this research were to create a cell-based model of ATTR amyloidosis, to define the mechanism of cardiac TTR-associated amyloid at the cellular level, and to study doxycycline as an agent that could interrupt the amyloid process. We hypothesized that TTR oligomers were cardiotoxic and played a role in the mechanism of ATTR amyloidosis, and that cytotoxicity could be inhibited by doxycycline. Focusing on TTR proteins associated with cardiac amyloidosis, we developed a thermal denaturation method for creating TTR oligomers that allowed us to study the direct effect of oligomers on cells.

We tested the effect of TTR oligomers on rat and human cardiac cells by measuring cell viability and stress response (through live protease activity and qPCR). TTR-L55P oligomers elicited a cytotoxic effect; fluorescent microscopy indicated cellular uptake of the oligomers and continued intra-cellular aggregation. Cytotoxicity was blocked when TTR was heated in the presence of doxycycline; the drug appeared to dissociate TTR aggregates or stabilize the monomeric forms. These data provide evidence that TTR oligomers are cardiotoxic, possibly due to cellular internalization and progressive intracellular aggregation. Furthermore, our results support the use of doxycycline as a therapeutic in ATTR to target these amyloidogenic oligomers.

THE ROLE OF VIRAL PROTEIN R IN HIV-1 INFECTION OF DENDRITIC CELLS

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Viral protein R (Vpr) is an accessory protein encoded by HIV-1 that remains associated with the virus core at early times post-infection. Though Vpr expression is critical to the ability of HIV-1 to infect both macrophages and dendritic cells (DCs), the mechanism of Vpr-mediated enhancement of infection has remained unclear. In these studies, I examined the role of Vpr in mediating HIV-1 infection of DCs. I have determined that there is an early block to infection of Vpr deficient (ΔVpr) HIV-1. Using quantitative real time PCR, I demonstrated that the deficiency in HIVΔVpr replication is not attributed to reduced levels of reverse transcription or efficiency of integration of the viral cDNA into the host genome, suggesting that block to HIVΔVpr infection occurs at a post-integration step. Interestingly, infections of DCs in the presence of viral protease inhibitor (Indinavir) that restricts replication to a single cycle revealed a reproducible 2-3 fold decrease in the number of DCs expressing HIV-1 $p24^{gag}$ (capsid), suggesting a deficiency in viral gene expression. To further define the mechanistic basis for Vpr-mediated enhancement of virus replication, I have made a number of mutations in various functional regions of Vpr. Infections with viruses containing Vpr mutations (Q65R or H71R) that disrupt interactions of Vpr with DNA damage repair protein complex (SLX4com) and prevent Vpr-mediated dysregulated activation of the DNA damage response pathway show decreased infection of DCs, thus phenocopying ΔVpr infections. These results suggest that induction of Vpr-mediated DNA damage response is critical to infection of DCs.

MOLECULAR IMPACT OF ELECTRONIC CIGARETTE EXPOSURE ON AIRWAY EPITHELIUM

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Electronic cigarettes (ECIGs) are battery powered electronic nicotine delivery systems. Since ECIG aerosols should not contain high levels of the toxic chemicals present in tobacco smoke, ECIGs are thought to be a safer alternative to traditional tobacco cigarette (TCIG) smoking. We aimed to determine the effects of ECIG exposure on human bronchial epithelial cells *in vitro*. Human bronchial epithelial cells (HBECs) grown in an Air Liquid Interface (ALI) were exposed to direct ECIG vapor or TCIG smoke, while p53/KRAS mutant HBECs were exposed to ECIG or TCIG conditioned media (CM). The impact of these exposures on airway epithelium was evaluated using PCR, 8-Isoprostane enzyme immunoassay, growth in soft agar, and Affymetrix gene expression microarrays. ECIGs induce expression of genes involved in both oxidative and xenobiotic stress pathways. The gene expression pattern in airway epithelium after ECIG CM exposure with high nicotine was similar to the effect of TCIG CM. Cytochrome P450 genes including CYP1A1 and CYP1B1 were significantly increased after both TCIG and ECIG exposure. PRDX1 and NQO1, oxidative stress genes, were also significantly increased after TCIG and ECIG exposure. In general, nicotine had a synergistic effect with ECIG flavoring in inducing these genes. The production of reactive oxygen species increased after both TCIG and ECIG exposure and demonstrated a dose response to ECIGs. Furthermore, high dose ECIG CM induced anchorage independent cell growth in the p53/KRAS mutant HBECs. These results indicate that ECIG vapor, similar to TCIG smoke, may induce significant cellular stress and molecular alterations within airway epithelium.

NEISSERIA GONORRHOEAE MODULATES IMMUNE CELL SURVIVAL THROUGH NON-CANONICAL PYROPTOSIS

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Cell death is a common mechanism for maintaining immune cell homeostasis and is induced in response to both endogenous and exogenous stimuli. Classically, cell death occurs through either apoptosis or necrosis. However, recent studies have defined additional cell death pathways including pyroptosis and pyronecrosis. In macrophages exogenous stimulation with some Gram-negative bacteria has been demonstrated to induce pyroptosis. Due to inflammasome activation and the spilling of intracellular components, pyroptosis is highly pro-inflammatory. Recent studies have reported that the Gram-negative pathogen *Neisseria gonorrhoeae* induces pyronecrotic cell death in monocytic cells. In contrast, other immune cells, such as phagocytic neutrophils, are resistant to cell death following stimulation with *N. gonorrhoeae*. We have previously shown *N. gonorrhoeae* inhibits apoptosis in human endocervical cells. In this study we have further characterized the cell death pathways induced by *N. gonorrhoeae* in THP-1 like-macrophages. We demonstrate that stimulation of macrophage-like cells with *N. gonorrhoeae* induces cell death in a lytic manner. *N. gonorrhoeae* stimulation did not activate caspase-3, but did activate immune caspases: caspase-1, caspase-4, caspase-5. Inhibition of caspase-4, decreased cell death induced by *N.* gonorrhoeae. We conclude that *N. gonorrhoeae* stimulates cell death in human macrophages by non-canonical pyroptosis and postulate that this contributes to both bacterial persistence and the induction of inflammatory pathways.

RESPIRATORY EXPOSURES TO PNEUMOCOCCUS ESTABLISH LOCALIZED MEMORY CAPABLE OF HETEROTYPIC LUNG PROTECTION

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Pneumonia is a persistent and pervasive public health burden, often caused by diverse serotypes of *Streptococcus pneumoniae.* We hypothesize that repeated respiratory infections establish capsule-independent adaptive immunity that protects against subsequent unrelated pneumococcal pneumonia. We have established a mouse model in which mice are infected with diverse pneumococci in the respiratory tract, given time to recover, and then challenged with a mismatched virulent serotype 3 (Sp3) in the lung, which they cannot typically eliminate. Prior exposures to unrelated pneumococci proved protective, with multi-log reductions in lung CFU in mice previously exposed to unrelated pneumococci, compared to vehicle controls. RNAseq analysis of lungs 24h after Sp3 infection revealed an enrichment of lymphocyte-related pathways due to prior pneumococcal exposures, including immunoglobulin and other B cell-related genes (suggesting a local production of antibodies) as well as T helper cell signatures (particularly relating to Th17 cells). Prior to final infection, there were no differences in numbers of B cells or T cells in the lung, and no inflammation in H&E sections. However, *ex vivo* stimulation of $CD4^+$ T cells revealed such lungs to contain more IL-17A producers, suggesting resident memory. We conclude that serial respiratory infections establish lung resident memory Th17 and B cells that cooperate to protect against pneumonia.

NEW INSIGHTS INTO NONCANONICAL SIGNALING AND ACTIVATION OF NOTCH IN T-CELLS

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Notch is an essential transcriptional regulator in most cells and especially in T-cells, where its deregulation during development leads to leukemia. Moreover, Notch remains important throughout the life of T-cells, regulating events such as proliferation and differentiation. Despite its importance, the exact function of Notch in peripheral T-cells is poorly understood. Until recently, it was thought that Notch solely operates as a nuclear regulator of transcription via its interaction with the DNA-binding protein RBP-Jkappa (RBPJ). However, using $RBPJ^{-/-}$ mice, our data indicate that canonical signaling through RBPJ is dispensable for proliferation in peripheral T-cells. These findings suggest that Notch utilizes non-canonical RBPJ-independent pathways, which are increasingly becoming the focus of Notch signaling. Furthermore, our data indicate that Notch functions very early upon T-cell receptor (TCR) stimulation without the need for Notch ligands, as shown by western blots and proliferation experiments. Additional evidence suggests that canonical Notch signaling is in fact down-regulated 48h post TCR-stimulation as shown by the decrease of the Notch activation complex member MAML, active Notch levels in the nucleus, and message of Notch target genes. Surprisingly, we also showed that 48h post activation T-cells themselves can express the Notch ligand Jagged-2. This may indicate that Jagged-2 facilitates a cis-induced inhibition of Notch instead of activation. Overall, we show that in T-cells Notch utilizes additional RBPJ-independent pathways early upon TCR stimulation, while shutting down the canonical machinery in the nucleus. This insight may reveal new therapeutic approaches in the treatment of otherwise therapy resistant Notch-induced leukemias.

IGPR-1 IS A NOVEL ADHESION MOLECULE INVOLVED IN COLORECTAL TUMOR GROWTH

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Colorectal cancer (CRC) is among the most prevalent and lethal cancers in the United States. The mechanisms by which tumor cells sense their microenvironment have profound importance in driving the progression of malignancy and evasion from treatment. Specialized microenvironment-sensing cell surface receptors such as cell adhesion molecules allow tumor cells to survey and respond to their microenvironment. We have recently identified a novel cell adhesion molecule named immunoglobulin-containing and proline-rich receptor 1 (IGPR-1) that is normally expressed in both endothelial and epithelial human cell types; however, its potential role in human malignancy remains unknown. To investigate the role IGPR-1 plays in CRC tumor growth, we overexpressed IGPR-1 in human HT29 and HCT116 colon adenocarcinoma cells and examined the effect of IGPR-1 on tumor growth and the mechanisms involved in a cell culture system. The data demonstrate that overexpression of IGPR-1 enhances CRC cell proliferation and survival in vitro. Furthermore, we demonstrate that the extracellular domain of IGPR-1 is required for its ability to support tumor growth. While deletion of the extracellular domain of IGPR-1 impaired its ability to promote tumor cell survival, stimulation of a chimeric IGPR-1 molecule containing the extracellular domain of CSF-1 receptor promoted tumor cell survival. Additionally, the presence of serine 186 and 220 in the cytoplasmic domain is important for IGPR-1 activity in tumor cells. This work identifies IGPR-1 as an important protein in the regulation of CRC cell growth and survival, and this makes it a possible therapeutic target in the clinical management of CRC.

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THE ANTI-INFLAMMATORY GLYCOPROTEIN, CD200, RESTORES NEUROGENESIS AND ENHANCES AMYLOID PHAGOCYTOSIS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive decline of cognitive function and memory formation. It is distinguished by the build-up of amyloid-β peptide (Aβ) into plaques and intracellular tau accumulation in the brain. Cluster of Differentiation-200 (CD200) is an anti-inflammatory glycoprotein expressed in neurons, T cells, and B cells, and its receptor is expressed on glia. Both AD patients and mouse models show an age-related or Aβ-induced reduction in CD200. The goal of this study was to determine if neuronal CD200 expression restores hippocampal neurogenesis and reduces Aβ in the Tg2576 APP mutant mouse model (APP) of AD. APP and wild-type mice were intracranially injected at 6 months of age with a tetracycline-inducible adeno-associated virus expressing CD200 into the CA1 region of the hippocampus, and then sacrificed at 12 months of age. CD200 expression restored neural progenitor cell proliferation and differentiation in the subgranular and granular cell layers of the dentate gyrus and reduced diffuse but not thioflavin-S+ plaques in the hippocampus. This restoration in neurogenesis was associated with an increase in anti-inflammatory marker chitinase-3-like-3 (YM1). In vitro studies demonstrated that CD200-stimulated microglia increased neural differentiation of neural stem cells and enhanced axon elongation and dendrite number. CD200 stimulation also enhanced the survival of microglia and the amount of Aβ they phagocytosed in vitro. These data indicate that CD200 is a promising molecule to enhance microglia-mediated Aβ clearance and neural differentiation and has potential as a therapeutic for AD.

ASSOCIATION OF TAU WITH TIA1 REGULATES STRESS GRANULE BIOLOGY AND TAU PATHOPHYSIOLOGY

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Increasing evidence links neurological disease processes to dysfunction of RNA binding proteins (RBPs). RBPs contain prion-like, poly-glycine rich domains that facilitate a process of regulated protein aggregation required for normal RBP function. In neurons, RBPs mediate all facets of RNA metabolism including splicing, dendritic transport, local translation at synapses, and nucleation of stress granules (SGs). Disease-linked mutations in RBPs increase the tendency of these proteins to aggregate leading to the formation of pathological SGs. SG proteins, including T-cell intracellular antigen 1 (TIA1), co-localize with neuropathology in brain tissue of subjects with Alzheimer's Disease (AD), frontotemporal dementia (FTD), and Amytrophic Lateral Sclerosis (ALS).

Here, we report a novel role for tau in SG biology and show that the interaction of tau with TIA1 promotes tau pathology and neurodegeneration. Tau co-immunoprecipitates with TIA1, accelerates SG formation, and reduces the movement of TIA1 granules. Genetic deletion of tau inhibits SG formation, reduces cytoplasmic translocation of TIA1, and abrogates the binding of TIA1 to various proteins in its core proteome. TIA1 also regulates tau pathophysiology. Overexpressing TIA1 induces tau misfolding and phosphorylation, increases the level of insoluble tau bound to TIA1, and stimulates neurodegeneration. Live-cell imaging studies indicate that TIA1 stabilizes tau in granules and prolongs its half-life. This system can be modulated by translational signaling, as treatment with puromycin or salubrinal potentiates tau granule formation and neurodegeneration, while treatment with cycloheximide attenuates the neurotoxicity. Further, genetic knockout of TIA1 rescues neurotoxicity caused by the disease-linked P301L tau mutation. These results point to translational control as a novel tau regulatory pathway and highlight new therapeutic strategies for the treatment of tauopathies such as AD.

POST-TRANSCRIPTIONAL REGULATION OF A-SYNUCLEIN BY LRRK2 THROUGH INTERACTIONS WITH MICRORNAS IN NEURODEGENERATIVE DISEASES

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Abstract: In this study, we hypothesize that the expression and toxicity of α-synuclein are regulated by LRRK2 post-transcriptionally through interactions with microRNAs. Our preliminary data shows that co-expression of α-synuclein with either long or short 3' untranslated region (UTR) and wild-type LRRK2 in HEK293FT cells has a significant increase in α-synuclein expression relative to without LRRK2 as measured by florescence level of the GFP-tagged α-synuclein. In addition, our data shows that in rat primary culture cortical neurons, with co-transfection of LRRK2 and α-synuclein with either long or short 3'UTR, there is a distinct difference in the accumulation and localization of α-synuclein in neurons as well as the morphology of neurons. In the presence of LRRK2, there is a higher level of α-synuclein protein expression in conditions with short 3'UTR compare to long 3'UTR. And in conditions with the short 3'UTR, neurons demonstrate a broader distribution of α-synuclein, along with a greater number of α-synuclein containing puncta along the processes. This data suggests a differential effect of LRRK2 on α-synuclein with either long or short 3'UTR's expression and localization. To further investigate the underlying mechanisms, we looked into the microRNAs which bind to the 3'UTR of α-synuclein transcripts. Mir-7 can bind to the short 3'UTR and both mir-7 and mir-153 can bind to the long 3'UTR of α-synuclein transcript. We performed site-directed mutagenesis where we mutated the binding site for mir-7 on the short 3'UTR and the binding site for mir-7 or mir-153 on the long 3'UTR, so these miRs can no longer bind. Our preliminary data shows that co-expression of α-synuclein with mutated miRs binding site with WT LRRK2 in HEK293FT cells, the effects of WT LRRK2 in increasing the α-synuclein expression were abolished when the binding site of mir-7 in both short and long 3'UTR has been mutated. Whereas, when binding site of mir-153 in the long 3'UTR was mutated, it significantly diminished the effect of WT LRRK2 in increasing the expression of α-synuclein, but it did not lead to as large a decrease in LRRK2's effect as in mutation in mir-7's binding site did. This data suggests that LRRK2 interacts with the microRNA pathway in regulating α-synuclein transcription and thus its expression level.

CENTRAL GAI$_2$ PROTEINS FACILITATE PVN PARVOCELLULAR NEURONAL ACTIVATION DURING CHRONIC ELEVATED SODIUM INTAKE TO MAINTAIN SODIUM HOMEOSTASIS

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Brain Gαi_2 protein-mediated signal transduction acts as a mechanism to potentiate central sympathoinhibition to maintain fluid and electrolyte homeostasis in settings of chronic elevated sodium intake. The current study determined whether brain Gαi_2 proteins impact the pattern of neuronal activation evoked during high-salt intake to maintain sodium homeostasis. Male Sprague-Dawley rats received an ICV miniosmotic-pump infusion of a scrambled (SCR) or Gαi_2 oligodeoxynucleotide (ODN; 25µg/day) and were maintained on a low (LS; 0.03% NaCl), normal (NS; 0.4%), or high salt (HS; 8%) diet for 7 days. 24-hour metabolic balance measurements were recorded on day 5 and rats were sacrificed on day 7 for FosB/ΔFosB IHC to examine dietary sodium evoked neuronal activation. Following 5-d of a HS, but not LS or NS diet, ICV Gαi_2 ODN infusion reduced 24-h total sodium excretion compared to SCR ODN-infused rats (Na^+ excretion [mEq/24-h] SCR HS: 27.0±1.2 *vs*. Gαi_2 HS: 19.6±3.3, $P<0.05$). Chronic HS, but not LS, diet evoked a significant increase in total PVN FosB in SCR infused rats that was not observed in Gαi_2 infused rats (Δ from NS [$FosB^+$ cells] SCR: 11±3 vs. Gαi_2: 1±2, $P<0.05$). Specifically, Gαi_2 ODN infusion prevented increases in PVN dorsal, ventrolateral, and lateral parvocellular nuclei that were observed in SCR infused rats ($P<0.05$). Gαi_2 ODN infusion had no effect on sodium-evoked increases in FosB in PVN magnocellular nuclei, the SON, and MnPO. These data highlight Gαi_2 protein signal transduction as a novel CNS mechanism acting to influence neuronal activation, likely at the level of PVN parvocellular neurons, upon chronic HS challenge in a salt-resistant phenotype.

INVESTIGATING THE ROLE OF SMARCAL1 IN THE ALTERNATIVE LENGTHENING OF TELOMERES PATHWAY

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The alternative lengthening of telomeres (ALT) pathway is one of two known telomere maintenance mechanisms that cancer cells must employ to continually proliferate. The ALT pathway relies on homologous recombination between two telomeric sequences to promote telomere elongation, however, the exact mechanism and regulators of this pathway remain elusive. ALT is present in cancers across many tissue types, but the prevalence of ALT can be much higher within certain subsets of cancer, such as osteosarcomas (59%) and astrocytomas (88%). These subsets of cancers have a very poor prognosis. Therefore, dissecting the mechanisms regulating ALT activity may allow us to design novel therapeutic approaches in the treatment of these aggressive cancers.

Homologous recombination between the telomeres in ALT cells increases the mutation frequency of the telomeric sequence itself. Consequently, ALT telomeres contain an increase in variant repeats leading to a disruption in the binding of SHELTERIN –a six protein subunit complex that binds and protects telomeres. SHELTERIN loss leads to the formation of stalled replication forks specifically at the telomere. Recently, the reannealing helicase SMARCAL1was demonstrated to catalyze chromatin remodeling at sites of replication stress to promote replication restart. Given the prevalence of replication stress at the telomeric DNA in ALT cells, we hypothesized that SMARCAL1 functioned to ensure telomere stability in ALT. To support this, we demonstrate that SMARCAL1 binds telomeres exclusively in ALT positive cancer cells, and we have demonstrated that loss of SMARCAL1 leads to replication fork collapse, double strand breaks, and ultimately significant defects in ALT activity.

CASEIN KINASE- 1 EPSILON DELETION ENHANCES OPIOID REWARD AND IS ASSOCIATED WITH INCREASED STRIATAL OPRM1 AND NPAS4 EXPRESSION

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Recent genetic and pharmacological studies indicate that Casein kinase-1 epsilon (Csnk1e) contributes to the behavioral properties of multiple abused substances. Pharmacological inhibition of Csnk1e enhanced the locomotor stimulant properties of the selective mu-opioid receptor agonist fentanyl, indicating a negative regulatory role in drug-induced behaviors. Here, we tested the hypothesis that Csnk1e negatively regulates fentanyl reward using the conditioned place preference (CPP, 0-0.2 mg/kg fentanyl) assay in Csnk1e knockout (KO) and wild-type (WT) mice. KOs showed a leftward shift in the inverted u-shaped curve for opioid reward compared to WTs, exhibiting significantly enhanced reward at lower doses (0.05 mg/kg) and decreased reward at higher doses (0.2 mg/kg). No significant differences were observed in fentanyl analgesia in the 52.5°C hot plate assay (0-0.4 mg/kg), suggesting a neural mechanism selective for dopaminergic reward circuitry. To generate novel hypotheses regarding the molecular mechanisms that mediate enhanced opioid reward in Csnk1e KOs, we used transcriptome analysis via mRNA sequencing of striatum from naïve KO and WT mice to identify the transcription factor Npas4 as the top differentially expressed gene (2.246 fold-change; $p=4.96 \times 10^{-36}$), supporting a previous study demonstrating Npas4 transcript covariance with morphine reward (Piechota et al., 2010). Importantly, expression of Oprm1 (mu- opioid receptor) was significantly higher in KOs compared to WTs. We conclude that Csnk1e KO mice show enhanced opioid reward, possibly via a mechanism involving Npas4-mediated increases in mu-opioid receptor expression.

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DENDRITIC REMODELING BY THE ANGELMAN SYNDROME AND AUTISM PROTEIN E6AP

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Following growth and extensive branching of dendrites, structural maturation of neurons is characterized by a pruning process in which the extra, non-functional dendritic branches are actively removed. The timing of pruning and selection of specific dendrites determines the wiring of neural circuits and, ultimately, brain function. Angelman Syndrome (AS) and some autism spectrum disorders (ASD) are caused by genetic disruption of E6AP, an E3 ligase that targets multiple proteins for ubiquitination and proteasome-mediated degradation. However, the neuronal function of E6AP remains largely unknown. Using cultured rat hippocampal neurons, we find that expression of E6AP leads to a reduction in dendritic branches via dendritic pruning. We show that E6AP causes activation of the caspase proteases, a pathway known for local neurite degeneration and spine pruning. E6AP targets the endogenous inhibitor of apoptosis XIAP for ubiquitination and degradation, therefore decreasing the inhibition of caspases and inducing the pruning process. Furthermore, we provide live imaging data suggesting that pruning occurs by distal fragmenting and shrinking of dendrites, eventually leading to the removal of entire dendritic segments. These findings provide novel mechanistic insight into our understanding of the physiological function of E6AP and the pathogenesis of Angelman syndrome and ASDs. Additionally, this study provides the first *in vitro* model of dendritic pruning in mammalian neurons.

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SDE2, A NOVEL PROTEIN IN TELOMERE MAINTENANCE

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Telomeres are repetitive DNA sequences at the ends of chromosomes that protect the genome from DNA degradation and chromosomal fusions. Defects in telomere maintenance lead to genomic instability and consequently, promote cellular transformation. Therefore, defining the mechanisms regulating telomere maintenance is essential to our understanding of cancer. To tease out of the details of this process, identifying evolutionally and functionally conserved proteins that bind to telomeres is essential. Recently, a novel protein named SDE2 (silencing defective 2) has been discovered to play an important role in telomeric silencing and genomic stability in fission yeast Schizosaccharomyces pombe. In yeast, SDE2 demonstrated a genetic interaction with the telomere regulators Taz2 (assembles telomeric heterochromatin and part of DNA damage response), Pof3 (maintains telomere length), and Ccq1 (recruits telomerase). Deletion of SDE2 was seen to increase mitotic mini-chromosome loss in yeast, abnormal spore formation and defects in cellular proliferation. C1ORF55 is the human homolog of SDE2; however, C1ORF55 function in mammalian cells remains uncharacterized. Therefore, the goal of our studies is to define the role of C1ORF55/SDE2 in telomere maintenance.

NEURONAL ADAPTOR PROTEIN MINT2/APBA2 ASSOCIATED IN AUTISM SPECTRUM DISORDERS (ASDS)

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Autism Spectrum Disorders (ASDs) comprise a heterogeneous group of neurodevelopmental disorders characterized by a complex genetic etiology. Chromosome 15q11-q13 has been identified as a strong candidate region for autism susceptibility. *MINT2*, located on Chromosome 15q11-q13, encodes for a neuronal adaptor protein and has been implicated as an important mediator of ASDs. Seven nonsynonymous coding variants have been identified in the *MINT2* gene associated with autism. Also, several studies have independently identified copy number variants (CNVs) in the *MINT2* gene in autism. However, the functional consequences of these Mint2 mutations and CNVs in neuronal development and physiology have yet to be examined. **We hypothesize that the Mint2 plays an important role in neuronal development and function**. Here, we study the role of human Mint2 mutations and CNVs and their role in the pathogenesis of ASDs. This study will provide much needed research that validates a role for Mint2 in the pathology of ASD. The validation and mechanistic role of new genes associated with ASD will directly lead to a better understanding of the molecular pathways critical for diagnosis of ASD and may improve determination of prognosis and lay the foundation for novel therapeutic approaches.

IMPAIRED REGULATION OF THE RENAL SODIUM CHLORIDE COTRANSPORTER (NCC) IN ANIMAL MODELS OF SALT-SENSITIVE HYPERTENSION

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Aim: These studies test the hypothesis that excess norepinephrine (NE) and a high salt intake impairs renal NCC regulation evoking salt-sensitive hypertension.

Methods: Sprague-Dawley (SD) rats receiving a s.c. saline or NE (600ng/min) infusion were fed a 0.4% (NS) or 8% NaCl (HS) diet for 14 days. Naïve Dahl Salt-Resistant (DSR) and Dahl Salt-Sensitive (DSS) rats were placed on a NS or HS diet for 21 days. Immunoblotting for NCC, STE-20 Alanine/Proline kinase (SPAK), and oxidative stress response-1 (OxSR1) was performed on kidney cortex tissue and normalized to β-actin (N=5/6).

Results: Salt-resistant phenotypes (SD & DSR) show dietary sodium evoked suppression of NCC. Salt-sensitive phenotypes fail to suppress NCC expression on a high salt diet. Dietary sodium evoked suppression of SPAK was observed in salt-resistant phenotypes and this was prevented in salt-sensitive phenotypes. A high salt diet did not alter OxSR1 expression but, salt-resistant phenotypes have lower endogenous levels of OxSR1 than salt-sensitive phenotypes.

Table 1:

	SD s.c. Saline		SD s.c. NE		DSR		DSS	
	NS	HS	NS	HS	NS	HS	NS	HS
MAP (mmHg)	124±2	124±1	129±3	131±2	138±6	166±6 *	138±6	166±6 *
Plasma NE (nmol/L)	n.d	n.d.	48±6	29±4	47±6	76±4 τ	47±6	76±4 τ
NCC (ODU/ mm^2)	1.7±0.4	1.1±0.3	1.2±0.3	0.5±0.01	2.4±0.75	2.9±0.4 τ	2.4±0.75	2.9±0.4 τ
SPAK (ODU/ mm^2)	5.95±0.1	0.8±0.3*	2.42±1	0.7±0.2*	4.2±1.6	4.17±0.6	4.2±1.6	4.17±0.6
OxSR1 (ODU/ mm^2)	6.7±1.8	3.9±1.6	2.2±0.6	1±0.13	11.2±4.5	11.4±2.7	11.2±4.5	11.4±2.7

Significance symbols:*$p<0.05$ vs. resp. NS gp; τ$p<0.05$ vs. resp. control gp; ≠$p<0.05$ vs. resp. control + HS gp

Conclusion: These data support an interaction of increased dietary salt intake and excess NE in the dysregulation of the NCC to drive the development of salt-sensitive hypertension. Our findings suggest that NCC dysregulation may depend on impaired upstream dietary sodium evoked suppression of SPAK and an underlying phenotypic difference in endogenous OxSR1 levels.

HNRNPH1 IS A QUANTITATIVE TRAIT GENE FOR METHAMPHETAMINE SENSITIVITY

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Sensitivity to the locomotor stimulant effects of amphetamines is a heritable trait in mice that may aid in our understanding of the genetic and neurobiological basis of neuropsychiatric disorders involving perturbations in dopaminergic transmission. We previously used short-term selected mouse lines derived from a C57BL/6J (B6) x DBA/2J (D2)-F_2 cross to identify a quantitative trait locus on chromosome 11 that was causally associated with reduced methamphetamine-induced locomotor activity (D2 < B6). We have since replicated this QTL in a standard B6 x D2-F_2 cross and used phenotypic analysis of interval specific congenic lines containing various D2-derived segments of chromosome 11 on an isogenic B6 background to uncover a 206 Kb critical interval containing only two protein-coding genes, *Rufy1* and *Hnrnph1*, both *necessary* for reduced MA sensitivity. Here, we used transcription activator-like effector nucleases (TALENs) to induce small deletions in the first coding exon of *Rufy1* or *Hnrnph1*. Phenotypic analysis of replicate lines heterozygous for the *Hnrnph1* deletion recapitulated the congenic phenotype, thus identifying a quantitative trait gene. Transcriptome analysis of B6.D2 congenic (chr.11: 50-60 Mb) striatal tissue followed by pathway analysis revealed perturbations in "glutamate receptor signaling" and "GalphaQ signaling", and identified "Cellular development, nervous system development and function, behavior" as the top network. We hypothesize that *Hnrnph1* regulates neurodevelopment of the mesocorticolimbic circuitry, thereby affecting both dopaminergic neuron development and glutamate signaling, and hence the stimulant response to amphetamines. These results will likely have widespread implications for understanding the genetic and neurobiological bases of disorders comprising perturbations in dopamine neurotransmission, including addiction, schizophrenia, ADHD, OCD, and Parkinson's disease.

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STRUCTURAL STABILITY AND LOCAL DYNAMICS IN DISEASE-CAUSING MUTANTS OF HUMAN APOLIPOPROTEIN A-I

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High-density lipoproteins (HDL) and their major protein, apoA-I, remove excess cell cholesterol and protect against atherosclerosis. Plasma levels of apoA-I and HDL correlate negatively to risk of atherosclerosis. However, some mutations, including those causing human familial amyloidosis (AApoAI), are not pro-atherogenic despite reduced levels of apoA-I and HDL. To address this paradox and elucidate molecular basis for protein misfolding in AApoAI, we combined structural, stability, and lipid-binding studies of amyloidogenic mutants (G26R, W50R, F71Y, L170P) and non-amyloidogenic mutant that causes aberrant HDL metabolism (L159R). Hydrogen-deuterium exchange mass spectrometry was used to analyze local backbone dynamics.

The results revealed that each mutation causes distinct changes in the protein conformation and lipid interactions. Although mutational effects on the protein and lipoprotein stability varied, all mutants showed decreased protection in residues 14-22, which we predicted to trigger protein misfolding. The non-amyloidogenic L159R mutant showed rapid deuteration near mutation site indicating helical unfolding, which helps explain protein cleavage at this site in plasma. Such cleavage removes one helix from the 4-helix bundle, thereby destabilizing remaining 3 helices and augmenting protein degradation. Interestingly, the rank order of the mutation-induced changes is different in the N-terminal helix bundle, central linker, and C-terminal tail, providing unique insights into dynamic interactions among these regions. Our results suggest that the fate of apoA-I in vivo depends upon balance between its misfolding, proteolysis, and protective protein-lipid interactions. We propose that reduced protection of the major amyloidogenic segment combined with sufficient lifetime of the native 4-helix bundle structure facilitates its aggregation.

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ELUCIDATION OF ROD PHOTORECEPTOR DARK ADAPTION MECHANISMS

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Purpose: Exposure of retinal rods to light leads to photoisomerization of the rhodopsin chromophore, resulting in activation of the visual transduction cascade. This activation is terminated by the phosphorylation of a cluster of serine and threonine residues located at the carboxyl terminus of rhodopsin and by subsequent binding of visual arrestin. Conventionally, it is thought that recovery of sensitivity following bleaching requires, among other factors, dephosphorylation of rhodopsin and unbinding of arrestin. The purpose of my Ph.D. studies has been to test this view by determining the extent to which rhodopsin dephosphorylation affects rhodopsin regeneration and recovery of dark-adapted sensitivity.

Results: Photoactivation (bleaching) of 50% of the rhodopsin in retinal rods isolated from the retinal pigment epithelium resulted in persistent desensitization as well as persistent phosphorylation at all 6 phosphorylation sites on rhodopsin. Little dephosphorylation of rhodopsin was observed in darkness during three hours subsequent to bleaching or following total pigment regeneration. Surprisingly, near complete recovery of sensitivity was observed in spite of persistent phosphorylation in a large fraction of the regenerated visual pigment.

Conclusions: Our results demonstrate that, despite the presence of substantial amounts of regenerated phosphorylated rhodopsin, the flash sensitivity recovers substantially to a level within 2-fold of the dark-adapted value. Dim flash kinetics were fully restored, indicating that the basal PDE and cyclase activity were at dark-adapted rates. These results are consistent with a model in which phosphorylated rhodopsin has a reduced efficiency of transducin activation, leading to diminish visual sensitivity at absolute threshold.

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A MECHANISM FOR EIF2(αP) COMPETITIVE INHIBITION OF EIF2B

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The eukaryotic initiation factor eIF2 is a heterotrimeric G-protein that along with GTP and Met-tRNAi forms the active ternary complex, which is required for every round of protein translation. eIF2B is the GEF that recycles eIF2 from its inactive, GDP-bound form to its active, GTP-bound form. In response to a variety of cellular stresses, phosphorylation of a single serine in the N-terminal domain of the α-subunit of eIF2 converts it from a substrate of eIF2B to a competitive inhibitor, preventing further nucleotide exchange by eIF2B and thus causing the subsequent global slowing of protein translation. It has long been assumed that the mechanism of inhibition is due simply to the "direct effect" of the phosphate group on the interaction between eIF2α and eIF2B. Instead, we propose a further "indirect effect," wherein the C-terminal domain of eIF2α competes with eIF2B for binding to the N-terminal domain, effectively serving an autoinhibitory role. Phosphorylation results in the disruption of this intradomain interaction, relieving competition by the C-terminal domain of eIF2α and allowing for the observed tighter, nonproductive binding by eIF2B.

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Structural Studies of ATP Binding Cassette Transporter A1 and its Lipid Acceptor Apolipoprotein A-I

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Plasma high-density lipoprotein (HDL) levels are inversely related to the risk of cardiovascular disease. ATP-binding cassette transporter A1 (ABCA1) plays an important role in HDL formation. In peripheral cells, ABCA1 mediates the efflux of cellular cholesterol and phospholipids to plasma lipid-poor apolipoproteinA-I (apoA-I). Mutations in the ABCA1 and apoA-I genes can lead to severe HDL deficiency which may exacerbate cholesterol deposition in peripheral tissues and increase susceptibility for atherosclerosis. Thus, it is of vital importance to understand how ABCA1 and apoA-I functions in the initial step of HDL formation. However, despite intensive studies over years, the mechanism of the function of ABCA1 still remains unclear. The objective of the proposed study is to elucidate this mechanism from a structural perspective. We aim to achieve this goal by pursuing the following two specific aims: 1. to characterize the interactions between ABCA1 and its lipid acceptor apoA-I; 2. to determine the molecular structure of ABCA1. These studies will provide us detailed structural and functional information on ABCA1, thereby significantly enhancing our understanding of the process of HDL formation. Furthermore, this knowledge may facilitate the development of molecularly based strategies to control cardiovascular disease related to dysfunctions of lipid metabolism.

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Expression and Purification of the IcmSW Chaperone Complex from a Type IVb Secretion System

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Legionella pneumophila, a gram negative facultative intracellular bacteria, is the causative agent of Legionaires' disease. In order to survive in human alveolar cells the bacterium assembles a Type IVb secretion system that is used to transport over 300 effectors into the host cell. These effectors modulate the host cell's processes allowing the pathogen to evade normal cellular defense systems. Two proteins, IcmS and IcmW, form a chaperone complex that deliver effectors to the Type IVb secretion system translocase, a protein complex that spans the bacteria inner and outer membranes and possibly the host endosomal membrane. The purpose of this study is to develop an expression and purification scheme for isolating a stable, soluble IcmSW complex in order to structurally characterize this chaperone assembly. An expression system was designed using pMal and custom-designed pMal derivatives that allowed for the co-expression of both MBP-IcmW and MBP-IcmS fusion proteins. This co-expression resulted in robust soluble protein expression for both fusions. To further stabilize the chaperone complex, we have generated soluble DotL protein constructs that bind to the chaperone complex. These constructs are derived from the C-terminal, IcmSW-binding region in the DotL coupling protein. Purification of these proteins using a combination of Ni-NTA and amylose purification columns coupled with limited digestion by chymotrypsin-linked beads resulted in complexes around 90Kd. This chaperone-effector complex runs primarily as a single peak in size exclusion chromatography and a single band in native gels indicating that it may be a suitable candidate for crystallography.

Notes