BOSTON UNIVERSITY

The 25th Annual Henry I Russek Student Achievement Day

"Genius is perseverance in disguise"

This day is in the name of Dr. Henry I. Russek, physician scientist, who recognized the importance of celebrating the accomplishments of young scientists as they become the next generation to cure disease and to enrich society by their own example.

Keynote Address/ Visiting Professor

Xiaowei Zhuang, Ph.D.

Investigator, Howard Hughes Medical Institute
David B. Arnold Professor of Science
Harvard University

"Illuminating Biology at the Nanoscale and Systems Scale by Imaging"

Hosted by the Russek Foundation
& BUSM Graduate Medical Sciences

26 April, Hiebert Lounge *10:00 a.m.-5:00 p.m.*

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

What a year! Life certainly has taken some challenging twists and turns. We have laughed our way through the political satires while our constitution faces its greatest test and have witnessed the pain that has come from the faces of families across the world as they try to rebuild their broken communities. This has not been an easy year for anyone who cares deeply about our humanity. But spring is finally here and with it comes renewed hope that we will find a way to be tolerant of each other and embrace our diversity as a strength and not as an excuse to drive out others who do not look or think the way we do.

BU students have demonstrated their compassion for others less fortunate and have been there with open arms and strong voices both here in Boston and around the globe as researchers and physician scientists. They have demonstrated that science has the power to open minds and restore sensibility in places where ignorance breeds fear and hatred. Our award winners are exemplary citizens of where we can all make a difference having balanced their lives between intensity at the lab bench and meaningful service to the community. However, all of our students are to be celebrated today because they represent the best of what we can do when the mind and heart are directed towards aspirational goals that are led by our dedicated and exceptional mentors.

I want to thank the members of our Division Awards Committee and the Program that they have helped me build (old members and new). A particular thank you to this year's members: Drs. Barbara Slack, Barbara Schreiber, Shoumita Dasgupta, Olga Gursky, Matt Jones, Anurag Singh, Rahm Gummuluru and Tarik Haydar. I would

also like to thank all of our student volunteers and the faculty in each department/program who had to choose between excellent applicants while putting up with my persistence regarding community service and the need to give out as many awards as possible! Finally, with sincere appreciation: My pharmacology crew! (Sara Johnson and Wanda Roberts), Associate Provost Dr. Linda Hyman, Dr. Theresa Davies for her endless energy, Mr. Jorge Fortin's generous help with the booklet, our precious David Keough in Ed Media for over twenty years of wonderful photos, and the helpful folks at GMS (Millie and Sherill) for all they do. A special thank you for their generous support through 25 years of this mission: Dr. David Farb and Mrs. Elayne Russek.

Enjoy!

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.

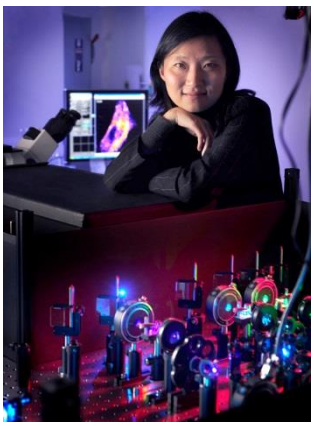
A MAN OF MEDICINE

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

Xiaowei Zhuang

Member, National Academy of Sciences / Professor of Science, Harvard University



Dr. Zhuang is a world-renowned pioneer and leader in super-resolution imaging, single-molecule imaging and genomic-scale imaging. The technologies developed by the Zhuang Research Lab have provided critical understanding of the molecular mechanisms of cellular function.

Zhuang pioneered the development of super-resolution microscopy through her invention of Stochastic Optical Reconstruction Microscopy (STORM), which was reported in 2006. The technology overcomes the diffraction limit of resolution of traditional light microscopes and is capable of creating images of cells and tissues with nanometer-scale resolution. It quickly became one of the most widely used super-resolution imaging methods. She followed this discovery with additional breakthroughs, including using STORM to achieve three-dimensional super-resolution imaging in 2008 and molecular-scale resolution imaging in 2012.

In addition to the development of the technology itself, Zhuang has employed STORM to make groundbreaking discoveries of novel cellular structures. She discovered the periodic membrane skeleton structure in neurons, provided new insights into the molecular organization of synapses in the brain, and revealed novel chromatin structures important for gene regulation.

Recently, she has taken molecular imaging to the genomic scale by inventing Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH), which has enabled the mapping of the spatial organization of the transcriptome and genome inside cells as well as the mapping of distinct cell types in complex tissues.

Collectively, Zhuang’s technologies and discoveries have served to transform biomedical research.

Retrieved on April 22, 2019, from: http://www.nasonline.org/programs/awards/2019-nas-awards/Zhuang.html

Student Achievement Day 2019

Program of Events:

Coffee and pastries available at 9:00 a.m. in the 14th floor Student Lounge. Please pick up badges in Hiebert lounge from student hosts.

9:00-10:00 a.m.
Poster Board set up.

10:00-10:30 a.m.
Welcoming addresses by Dr. Linda Hyman, Associate Provost of Graduate Medical Sciences and Dr. Shelley Russek, Vice-President of the Russek Foundation, Department of Pharmacology & Experimental Therapeutics and Biology. Keynote Speaker Introduction by Lucius K. Wilmerding (PhD student, Neuroscience)

10:30-11:30 a.m.
*Henry I. Russek Keynote Lecture by Dr. Xiaowei Zhuang, Investigator at Howard Hughes Medical Institute, Harvard University Professor of Science, "**Illuminating Biology at the Nanoscale and Systems Scale by Imaging**"*

11:30 a.m.-2:30 p.m.
***Poster Session:** Presented by graduate students enrolled in Graduate Medical Sciences (luncheon available at 12:30 p.m.). (Winners will present their posters from 11:30 - 12:30 p.m.)*

Award winners (First, Second, and Third and Moderators) please get your lunch at 12:30 and meet at the front of Hiebert Lounge. We will be going to room L804 for lunch with our Keynote Speaker & Visiting Professor Xiaowei Zhuang.

2:30-4:00 p.m.
***Oral Session:** Slide presentations by the Henry I. Russek Student Achievement First Prize Recipients. (Each presentation is 7 min. with an additional 3 min. for questions.)*

3:40 p.m. Award presentations by Shelley J. Russek, Russek Foundation, and photos of our award winners!

Oral Presentations

Student Presentations (2:30 p.m. - 3:40 p.m.)

2:30-2:40 p.m.
Stefanie Chan: THE ROLE OF GPS2 IN THE PI3K/AKT PATHWAY IN BREAST CANCER. (Genetics & Genomics, Advisor: V. Perissi)

2:40-2:50 p.m.
William Mau: VISUALIZATION AND MODULATION OF ENSEMBLES IN THE HIPPOCAMPUS AND AMYGDALA DURING FEAR REINSTATEMENT. (Neuroscience, Advisors: H. Eichenbaum & S. Ramirez)

2:50-3:00 p.m.
Molly Braun: DIFFERENT POPULATIONS OF THE RESPIRATORY SYNCYTIAL VIRUS TRANSCRIPTION ELONGATION FACTOR, M2-1, DURING INFECTION. (Microbiology, Advisor: R. Fearns)

3:00-3:10 p.m.
Brandon Maziuk: DEREGULATION OF RNA BINDING PROTEIN FUNCTION AND mRNA PROCESSING IS A MAJOR PATHOLOGICAL FEATURE OF TAUOPATHY. (Pharmacology & Experimental Therapeutics, Advisor: B. Wolozin)

3:10-3:20 p.m.
David Swain: DENSITY AND VOLUME OF GIANT VACUOLES WITH AND WITHOUT PORES IN THE INNER WALL ENDOTHELIUM OF SCHLEMM'S CANAL WITH INCREASING PRESSURES. (Anatomy & Neurobiology, Advisor: H. Gong)

3:20-3:30 p.m.
Mehraj Awal: THE BREAKDOWN OF NEURAL FUNCTION UNDER VOLATILE ANESTHESIA: IN VIVO, MULTINEURONAL IMAGING IN C. ELEGANS. (Physiology & Biophysics, Advisor: C. Gabel)

3:30-3:40 p.m.
Elena Stanpouloglou: THE LATS1/2 KINASES ARE ESSENTIAL REGULATORS OF T CELL DEVELOPMENT AND ACT AS SUPPRESSORS OF LYMHPOMAGENESIS. (Biochemistry, Advisor: X. Varelas)

Recipients of the Henry I. Russek Student Achievement Awards 2019

First Place

Mehraj Awal
Physiology and Biophysics
Advisor: C. Gabel

Molly Braun
Microbiology
Advisor: R. Fearns

Stefanie Chan
Genetics and Genomics
Advisor: V. Perissi

William Mau
Graduate Program for Neuroscience
Advisors: E. Eichenbaum & S. Ramirez

Brandon Maziuk
Pharmacology &
Experimental Therapeutics
Advisor: B. Wolozin

Rekha Raghunathan
Molecular Medicine
Advisor: J. Zaia

Eleni Stampouloglou
Biochemistry
Advisor: X. Varelas

David Swain
Anatomy & Neurobiology
Advisor: H. Gong

Second Prize

Richard Giadone
Molecular Medicine
Advisor: G. Murphy

Julia Hicks-Berthet
Biochemistry
Advisor: X. Varelas

Whitney Manhart
Microbiology
Advisors: E. Muhlberger and G. Mosotslavsky

Emily Mason-Osaan
Pharmacology & Experimental
Therapeutics
Advisor: R. Flynn

Katelyn Trecartin
Anatomy & Neurobiology
Advisor: D. Rosene

Angela Urdaneta
Physiology & Biophysics
Advisor: D. Atkinson

Ellen Witkowski
Graduate Program for Neuroscience
Advbisor: I. Davidson

Third Place

Deborah Chang
Biochemistry
Advisor: J. Zaia

Kathryn Kern
Anatomy & Neurobiology
Advisor: K. Schon

Sanghee Lim
Pharmacology & Experimental Therapeutics
Advisor: N. Ganem

Christina Lisk
Molecular Medicine
Advisor: L. Wetzler

Sarah Nodder
Microbiology
Advisor: R. Gummulury

Elizabeth Spencer
Graduate Program for Neuroscience
Advisor: M. Kramer

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

Razan Alotaibi (2)
Katharine Babcock (3)
Karen Bottenfield (8)
Chris Brooks (10)
Bryant Chang (13)
Renee DeVivo (17)
Kathryn Kern (31***)
Chelsey LeBlang (37)
Yee Fun Lee (38)
Yashar Rahimpour (59)
David Swain (*)
Katelyn Trecartin (67**)
Ajay Uprety (68)
Lauren Zajac (76)
Zannan Zhang (77)

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CARDIORESPIRATORY FITNESS, HIPPOCAMPAL VOLUME, ENTORHINAL CORTICAL THICKNESS AND MEMORY IN HEALTHY ELDERLY HUMANS

R. Alotaibi, R. Nauer, M. Dunne

It is well-established that physical exercise and cardiorespiratory fitness are beneficial to brain health. Cardiorespiratory fitness (CRF) can attenuate both the neurobiological and cognitive consequences of age-related decline. Although rodent research has provided strong evidence that exercise positively affects the neuronal system involved in learning and memory, human research has mostly focused on the effect of exercise and CRF on executive function, rather than their hippocampal and entorhinal cortex-dependent memory function. In a cross-sectional study using established behavioral tasks known to recruit the hippocampus and entorhinal cortex (EC) in structural MRI of healthy older adults, we tested the following hypotheses: (1) CRF levels are positively associated with hippocampus-dependent memory task performance; (2) CRF levels are positively associated with the structure of the hippocampus and EC; and (3) the volume of hippocampus and thickness of EC are associated with behavioral task performance. Healthy older adult (age 55-85 years) underwent a standard graded submaximal treadmill test to determine cardio-respiratory fitness (Modified-Balke protocol, VO_2max). FreeSurfer MRI analytic software was used to calculate cortical thickness and volume of T1-weighted MR images. Greater aerobic fitness was associated with greater volumes in the left and right hippocampus, but not with EC thickness or behavioral task performance. Our results indicate that higher fitness level is positively associated with hippocampal volume and may be protective against loss of hippocampal volume with aging. These data extend prior work on the cerebral effects of aerobic exercise and fitness to the medial temporal lobe in healthy older adults thus providing compelling evidence for a relationship between aerobic fitness and structure of the medial temporal lobe memory system.

3

EMBEDDING BRAINS TO EDUCATE MINDS

K. Babcock, E. Kong[2], and A. Zumwalt[1]

[1]Department of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA
[2]Education Division, Museum of Science, Boston, MA

Human brains are a powerful tool for teaching neuroscience at all educational levels as their strong emotional salience facilitates a memorable, and thus more effective, learning experience. Unfortunately, these post-mortem tissues are delicate and typically preserved in chemicals that require handling with gloves in well-ventilated areas, making their use in educational settings cumbersome. Graduate students in our program currently give brain demonstrations at the Museum of Science, Boston, but the tissues can only be handled by demonstrators due to their fragility. Plastination is a well-known alternative to this conundrum, however it is expensive, often causes tissue shrinkage, and is still vulnerable to wear and tear. A new technique embedding brain tissue in silicone gel has recently shown great promise as a cost-effective way to produce specimens that are structurally preserved, safe to handle, and durable for long-term use. The main objective of this project is to create brain specimens that people can hold and interact with in a way that is safe for both them and the tissues. These specimens will be used in several ways, from supplementing neuroanatomy lessons in medical and graduate school courses, to providing brain demonstrations at different outreach events in the community. By doing so, brains that were donated for educational purposes will be made accessible to wider audiences while having their delicate structures protected and thus their educational shelf lives extended.

8

EVALUATION OF GLIAL GROWTH FACTOR 2 TO ENHANCE RECOVERY OF FINE MOTOR FUNCTION AFTER CORTICAL INJURY IN MONKEYS

K. Bottenfield; A**.** Caggiano,; J. Iaci; T. Moore; D. Rosene,;
Department of Anatomy and Neurobiology

Neuregulins are growth factors involved in facilitating the survival and function of neurons, proliferation of oligodendrocytes, and suppression of inflammatory cytokines following cortical injury. Glial Growth Factor 2 (GGF2) is a variant of the neuregulin-1 gene and in studies with rodent models of stroke it has shown potential as a treatment for enhancing endogenous mechanisms to reduce cortical damage and promote plasticity. To further explore the efficacy of GGF2, we are using our non-human primate model of cortical injury and will determine the rate and pattern of recovery in monkeys receiving GGF2. Monkeys were pre-trained on our task of fine motor function of the hand and then underwent surgery to produce focal, cortical damage to the hand representation of the primary motor cortex. IV administration of GGF2 began 24 hours after surgery and continued for a period of 7 days. This was followed by 21 days of sub-cutaneous administration of GGF2. Monkeys were then re-tested on the motor task for 12 weeks. Preliminary analyses suggest a positive effect of GGF2 on the recovery of fine motor function of the hand when compared to monkeys that received a vehicle-control. The brain tissue of these monkeys will undergo histological and immunohistochemical analyses to identify the effects of GGF2 in tissue surrounding the brain regions of the cortical injury. The results of this study will help to determine the extent to which GGF2 enhances recovery of function and provide insight to the potential effects it may have on the enhancement of cortical plasticity.

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ACTIGRAPHICALLY CHARACTERIZED INFRADIAN REST-ACTIVITY RHYTHMS IN PARKINSON'S DISEASE

C. Brooks; J. Bhangu; M.K. Erb; R. Koehler; F. Mortazavi; K. Thomas

Department of Anatomy and Neurobiology

Accelerometry monitoring (i.e. actigraphy) is well-suited to capturing the regular 24-hour oscillation in human activity across the day, the cumulative effect of our circadian rhythm and behavior. However, rest-activity rhythms and whether between-day differences are part of a regular infradian (i.e. < 24 hr) rhythms has not been well characterized in PD.
6 PD participants (H&Y Stage 1 – 3) wore a Philips Actiwatch Spectrum PRO continuously for one week (32Hz). Rest-activity rhythms were quantified by fitting an oscillating cosinor model with a 24 hour period to each participant-day of activity data. ANOVAs were used to examine between-day differences across the week for the three cosinor parameters – MESOR, Amplitude, and Acrophase, which represent the mean, mean range, and relative timing of activity during a day, respectively.
We observed an infradian oscillation in activity with its peak on Friday/Saturday and its nadir on Tuesday/Wednesday. The parameters did not significantly vary across the week (MESOR: $p = 0.59$, Amplitude: $p = 0.80$, Acrophase: $p = 0.89$). The largest between-day contrasts were generally antipodal: Sunday/Wednesday for MESOR ($p = 0.10$), Saturday/Tuesday for Amplitude ($p = 0.30$), and Saturday/Wednesday for Acrophase ($p = 0.30$). There was pronounced inter-individual variability in MESOR ($p < 0.01$) and Amplitude ($p < 0.01$), but not Acrophase ($p = 0.07$).
Actigraphically measured rest-activity rhythms in PD vary by day, by individual, and demonstrate infradian effects. Replication is needed in larger cohorts to better characterize the degree to which inter-individual and inter-daily variability interact to produce the observed infradian rhythms.

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PREVALENCE, ETIOLOGY, AND TREATMENT OF SLEEP DISORDERS IN AUTISM SPECTRUM DISORDER

B. Chang; M. Bauman

Department of Anatomy and Neurobiology

Autism Spectrum Disorder is a range of neurodevelopmental disorders that typical manifest as social deficits, delayed or impaired communication skills, and repetitive behaviors in day-to-day life. Patients with Autism Spectrum Disorder (ASD) often present with other concurrent clinical disorders. Sleep disorders (SD) and sleep issues are highly prevalent in ASD children and rank as one of the most common concurrent clinical disorders. Prevalence rates vary widely, ranging from 40 to 80 percent, as compared with that of typically developing children in which prevalence rates are approximately 30 percent. Sleep problems can have an impact on daytime health and may result in neurocognitive dysfunction and behavioral disruptions. A cyclical pattern arises: individuals with autism are observed to have sleep difficulties, which may exacerbate autistic traits, which can in turn further worsen their quality of sleep.
Current models and theories on the relationship between ASD and SD suggest that the underlying etiology of autism itself may contribute to sleep troubles, and might even have wide-reaching impacts on other unrelated aspects of ASD. Gastrointestinal, otolaryngologic, and psychiatric comorbidities are observed in autism and may affect sleep in these patients, but the mechanism by which this occurs is unclear. There are

many treatments for sleep troubles in ASD such as melatonin and behavioral interventions, with varying success. Much work is required to understand the underlying mechanism between both autism and sleep disorders. Future studies should also incorporate robust data-collection instruments such as polysomnography to validate findings.

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DIFFERENTIATING BETWEEN HEALTHY CONTROL PARTICIPANTS AND THOSE WITH MILD COGNITIVE IMPAIRMENT USING VOLUMETRIC MRI DATA

R. DeVivo; M. Alosco; A. Cervantes-Arslanian; R. Killiany; J. Mez,; A. Mian; E. Steinberg; R. Stern; L. Zajac

Department of ANATOMY AND NEUROBIOLOGY

Objective: To determine whether volumetric measures of the hippocampus, entorhinal cortex, and other cortical measures can differentiate between cognitively normal individuals and subjects with mild cognitive impairment (MCI). **Method:** Magnetic resonance imaging (MRI) data from 46 cognitively normal subjects and 50 subjects with MCI as part of the Boston University Alzheimer's Disease Center (BU – ADC) research registry and the Alzheimer's Disease Neuroimaging Initiative (ADNI) were used in this cross-sectional study. Cortical, subcortical, and hippocampal subfield volumes were generated from each subject's MRI data using FreeSurfer version 6.0. Nominal logistic regression models containing these variables were used to identify subjects as control or MCI. **Results:** A model containing regions of interest (superior temporal cortex, caudal anterior cingulate, pars opercularis, subiculum, precentral cortex, caudal middle frontal cortex, rostral middle frontal cortex, pars orbitalis, middle temporal cortex, insula, banks of the superior temporal sulcus, parasubiculum, paracentral lobule) fit the data best (R^2 = 0.7310, whole model test chi square = 97.16, $p < 0.0001$). **Conclusions:** MRI data correctly classified most subjects using measures of selected medial temporal lobe structures in combination with those from other cortical areas yielding an overall classification accuracy of 93.75%. These findings support the notion that while volumes of medial temporal lobe regions differ between cognitively normal and MCI subjects, differences that can be used to distinguish between these two populations are present elsewhere in the brain.

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HIPPOCAMPAL SUBFIELD VOLUMES, CARDIORESPIRATORY FITNESS, AND PATTERN SEPARATION TASK PERFORMANCE IN OLDER ADULTS

K. Kern.[1]; R. Nauer, [1, 2, 3]; K.Schon, [2, 3]

[1]Department of Anatomy and Neurobiology, Boston University School of Medicine; [2]Department of Psychological and Brain Sciences, Boston University; [3]Center for Memory and Brain, Boston University

Cognitive aging is associated with reduced integrity of the hippocampal memory system. Accurate episodic memory formation requires the computational process of pattern separation (PS), or the disambiguation of similar stimuli during encoding. PS relies on the unique anatomical and physiological properties of the dentate gyrus (DG), the primary neurogenic subfield of the hippocampus. Functionally, adult-born neurons play a critical role in PS. Rodent models have shown that adult hippocampal neurogenesis (AHN) is downregulated in aging, but upregulated following exercise. Although AHN cannot be measured directly in humans *in vivo*, behavioral and neuroimaging studies have demonstrated that greater fitness is associated

with better PS task performance in young adults and greater hippocampal volume in older adults. However, the relationship between PS task performance, DG volume, and fitness in older adults remains unclear. To this point, we used high-resolution structural MRI and a behavioral PS task to test the hypothesis that DG volume and fitness would positively predict PS task performance in older adults. 24 participants underwent a treadmill test to determine cardiorespiratory fitness and an MRI scan to measure hippocampal subfield volumes. We demonstrate that right and left DG body volume significantly predicts performance in the task condition placing the highest taxation on PS. However, there was no relationship between fitness and PS. These findings provide further evidence for a specific role of the DG in PS in humans, while also suggesting that further research is necessary to elucidate the role of fitness as a modulator of the hippocampal memory system.

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MODULATION OF THE INFLAMMATORY RESPONSE BY THE RNA-BINDING PROTEIN TIA1 IN THE P301S MOUSE MODEL OF TAUOPATHY

C. LeBlang[1*], B. Wolozin[2], J. Luebke[1]

[1]Department of Anatomy & Neurobiology
[2]Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine

In the healthy brain, RNA-binding protein TIA1 regulates the immune response through the sequestration of TNFα and COX2 mRNA into normal stress granules. We have recently shown that TIA1 regulates tau pathophysiology in part through binding of phospho-tau oligomers into pathological stress granules. Haplo-insufficiency of TIA1 in the P301S mouse model of tauopathy reduced the accumulation of tau oligomers, and development of downstream deficits. The role of TIA1 in the immune response led us to hypothesize that TIA1 reduction impacts the immune system of the P301S mouse. We characterized the inflammatory response in P301S vs. WT mice that were TIA1+/+, TIA1+/- or TIA1 -/- at both 9 months and 5 months. TIA1 knockout was associated with a striking increase in inflammation at 9 months. Immunohistochemistry and 3D cell counting techniques were used to assess microglial morphology and MHCII presentation in the dentate gyrus. At 9 months, microglial reactivity was significantly greater in all P301S groups compared to WT. There was also a significantly greater number of reactive microglia in the P301S TIA1 -/- compared to P301S TIA+/+ and P301S TIA+/- which did not significantly differ. The inflammatory response was not observed in the WT TIA1 -/- group, indicating that the phenotype seen in the P301S TIA-/- group requires the presence of tau. The inflammatory response was not observed at 5 months of age in any group. These results suggest an important role of TIA1 as a regulator of neuro-inflammation in the context of tauopathy.

Supported by NIH AG050471

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ASTROCYTIC CONTRIBUTIONS TO DISRUPTED SLOW OSCILLATIONS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Y.F Lee[1,2], S. Hou[1], A. Synder[1], B. Bacskai[1], K. Kastanenka[1]

[1]Department of Neurology, MassGeneral Institute of Neurodegenerative Diseases, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, United States of America.
[2]Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, United States of America.

Alzheimer's disease (AD) is the leading cause of dementia. In addition to memory disruptions, Alzheimer's patients experience sleep disturbances and exhibit slow oscillations of low amplitude. The downregulation of slow wave power was recapitulated in transgenic mouse models of amyloidosis. However, the mechanisms underlying slow wave disruptions in AD remain unknown. Reports of neuronal hyperactivity have been made. Yet, what is the role of astrocytes, in disruptions of slow oscillations? Astrocytes are known to support neuronal circuit function. Therefore, disruptions in astrocyte activity might contribute to circuit dysfunction and result in slow wave aberrations in AD. Here we investigated astrocytic contributions to slow wave disruptions in cortices of APP mice. First, we used genetically encoded calcium indicators to monitor whether astrocytic calcium transients are uncoupled from slow waves in APP mice. Fourier Transform analysis showed that some GCaMP6f- and YC 3.6-targeted astrocytes exhibited Ca^{2+} transients at the frequency of slow waves (< 1Hz). However, astrocytes in APP animals did not exhibit calcium transients at that frequency, suggesting that astrocytic calcium transients were uncoupled in APP mice. Second, we used optogenetics to stimulate the cortical astrocytes at 0.6Hz to restore their activity to normal. Our results showed that light activation of ChR2-expressing astrocytes at the frequency of slow waves was able to restore the power of slow waves in APP mice. These results indicate that astrocytes participate in synchronizing cortical slow oscillations, and suggest a novel target to restore slow wave power in APP mice, with translational potential to treat AD.

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MAJOR WHITE MATTER PATHWAYS ALTERATIONS IN VETERANS WITH GULF WAR ILLNESS (GWI) AS ASSESSED USING MRI DIFFUSION METRICS

Y. Ramiphour, MS, B.B Koo, Ph.D., J. Ajama, D. Little, Ph.D., L. Steele, Ph.D., R. Killiany, Ph.D., and K. Sullivan, Ph.D.

Department of Anatomy and Neurobiology, Boston University School of Public Health and Baylor Medical College

Introduction: Approximately one third of 1991 Gulf War veterans suffer from a chronic disorder called GWI, characterized by symptoms including cognitive dysfunction, debilitating fatigue, joint and muscle pain, skin rashes, and gastrointestinal problems. Meta-analyses of cognitive data from these veterans has shown significant decrements in visuospatial skills, executive function, attention, and learning and memory. MRI of veterans with GWI, found reduced white matter (WM) volumes in the brain. To gain better understanding of the microstructural changes in the WM, we assessed major WM pathways using diffusion-weighted MRI (DWI). **Participants and Methods:** Data were obtained from 60 1991 Gulf War veterans (12 healthy, 48 GWI symptoms) as part of the Boston University Gulf War Illness Consortium (GWIC). We used Freesurfer v6 TRActs-Constrained-by-UnderLying-Anatomy (TRACULA) to reconstruct 18 major pathways in the brain of each subject. A single high-resolution structural scan and a High Angular Resolution Diffusion-weighted Imaging (HARDI) scan was input into TRACULA. For each pathway, we obtained measures of average Fractional Anisotropy (FA), Radial Diffusivity (RD) and Mean Diffusivity (MD). Neurite Orientation Dispersion and Density Imaging (NODDI) analysis was run on the data from each subject to view microstructural changes *in vivo.* NODDI outputs include: Intra-cellular volume, extra-cellular volume, and orientation dispersion. **Results:** Independent samples student's t-tests identified differences between groups. These analyses revealed the microstructural integrity of the Superior Longitudinal Fasciculus (SLF) was compromised in veterans with GWI. **Conclusions:** These results add to the evidence indicating that alterations to the WM of the brain is one key element of GWI.

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DENSITY AND VOLUME OF GIANT VACUOLES WITH AND WITHOUT PORES IN THE INNER WALL ENDOTHELIUM OF SCHLEMM'S CANAL WITH INCREASING PRESSURES

D. Swain; T.D Le; B. Fernandes; H. Yamada; G. Lamaj; S. Yasmin; I. Dasgupta; H. Gong

Purpose: To investigate changes in density and volume of giant vacuoles (GV) with and without pores with increasing perfusion pressure and whether GV volume with pores is larger than that without pores in the inner wall endothelium of Schlemm's canal (SC) using serial block-face scanning electron microscopy (SBF-SEM) and 3D reconstruction. **Methods:** Six normal human donor eyes were perfused at three different pressures (7, 15, and 30 mmHg, 2 eyes each pressure). Two small radial wedges of trabecular meshwork including SC were dissected from each eye and processed for SBF-SEM. More than 1000 serial images were analyzed per wedge to identify GV and pores. GV were traced, reconstructed in 3D, and volumes with or without pores were determined. Statistical analysis was performed using R. **Results:** Density of GV with pores was higher in 15mmHg and lower in 30mmHg compared to 7mmHg. The percentage of GV with pores significantly increased in 15mmHg ($P<0.01$) and 30mmHg ($P=0.018$), compared to 7mmHg. GV volume was significantly increased in 15 and 30 mmHg ($P<0.05$), compared to 7mmHg. The volume of GV with pores was significantly larger than GV without pores at 15mmHg ($P<0.01$). GV with pores at 15mmHg were larger than those at 7mmHg ($P<0.05$).
Conclusions: SBF-SEM provided an accurate method to quantify the GV density, volume, and percentage of GV with pores. GV volume was significantly increased at higher pressure. GV with pores were significantly larger than GV without pores only in 15mmHg, not in 7 and 30mmHg, suggesting other factors than GV size may influences pore formation.

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T CELLS SELECTIVELY INFILTRATE THE WHITE MATTER OF THE AGING MONKEY

K. Trecartin, D. Rosene, PhD, E. Shobin,

Department of Anatomy and Neurobiology

Even in the absence of neurodegenerative diseases like Alzheimer's (AD), normal brain aging in humans and monkeys is associated with declines in learning, memory and executive function. In normal aging monkeys and humans free of AD, studies show that neuronal loss does not underlie these cognitive impairments. Instead, loss of white matter volume and accumulation of myelin pathology underlies cognitive decline. Myelin homeostasis depends on many factors including the maturation of oligodendrocyte precursor cells, production of myelin scaffolding proteins, and clearance of debris from myelin turnover. Microglia play a pivotal role in these processes and deficits in phagocytosis of myelin debris impair oligodendrocyte differentiation and remyelination. We have found that in the white matter of the aging monkey brain, microglia take on disproportionately reactive and phagocytic phenotypes, become laden with lipofuscin, and both are associated with cognitive decline. This led us to hypothesize that microglia in the aged brain signal trafficking of adaptive immune system T-cells into regions of the brain parenchyma where they may be myelin reactive and exacerbate myelin pathology. The brains of 34 cognitively assessed male and female rhesus monkeys, 5-30 years old, were analyzed to quantify T-cells using immunohistochemistry for anti-CD3 (present on all T-cells). It was found that T-cells increase with age in in the parenchyma ($p\leq0.005$) and the perivascular space around blood vessels ($p\leq0.05$) of the cingulum bundle as well as in the corpus callosum parenchyma ($p\leq0.05$) and perivascular space ($p\leq0.005$). This is the first report that T-cells enter the healthy aging monkey brain.

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THE EFFECTS OF CHRONIC CURCUMIN TREATMENT IN A NON-HUMAN PRIMATE MODEL OF AGING

A. Uprety; B. Bowley; S. Calderazzo; T. Moore; M. Moss; D. Rosene; E. Shobin; P. Shultz

Studies of both humans and non-human primates have demonstrated that aging is characterized by a decline in cognition starting as early as midlife. While the cause of age-related cognitive decline is unclear, there is evidence for age-related pathology in the white matter associated with an increase in inflammation. Nutraceuticals like Curcumin (CUR), have been shown to produce significant anti-inflammatory and antioxidative effects in humans and rodents. Using the rhesus monkey, we assessed the efficacy of dietary supplementation of CUR as an intervention to reduce the aging effects on cognitive function and inflammation. Daily oral doses (500mg) of CUR or a vehicle control were given to 17 monkeys over an 18-month period during which they completed tasks of visual recognition memory, spatial working memory, and reversal learning. To date, we have demonstrated that CUR treated monkeys evidenced enhanced performance on spatial working memory and reversal tasks compared with monkeys treated with vehicle control. No differences were seen between groups on recognition memory or object discrimination tasks. In addition, we have data demonstrating that CUR treatment significantly alters the morphology and antigen presentation of microglia. Specifically, there is a reduction in LN3+ expression in the frontal white matter and corpus callosum of CUR treated monkeys. Additionally, microglia in the cingulate cortex (BA 25) of CUR subjects had longer distal processes with increased branching characteristic of a 'surveying' state morphology. Together these findings are consistent with the anti-inflammatory properties of CUR and its potential for reducing age-related cognitive decline.

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ACTIVITY STRENGTH IN OPTIC FLOW-SENSITIVE CORTICAL REGIONS DURING A VISUAL PATH INTEGRATION TASK PREDICTS PERFORMANCE IN AGED ADULTS

L. Zajac; R. Killiany

Department of Anatomy & Neurobiology

Spatial navigation declines in normal aging and age-related disease, and knowledge of the mechanisms underlying this decline is limited. Self-motion perception is one factor potentially involved in age-related decline in this ability. When moving forward through one's environment, a pattern of outward, radial motion is experienced on the retina that is called optic flow (OF). This is an important source of self-motion information and several cortical areas are sensitive to it. In 51 adults (29 young, 22 aged), we defined 11 OF-sensitive cortical areas using functional magnetic resonance imaging (fMRI). Brain activity was measured with fMRI during a visual path integration (VPI) task filmed in a Boston neighborhood in first-person perspective; participants used OF to track their position relative to their starting location during several short paths. Activity strength within 5 OF-sensitive regions during the VPI task was inversely associated with radial motion sensitivity measured outside of the scanner; we focused our analyses on these areas. Significant age x activity interactions on VPI accuracy were found for activity strength in LMT+, LpVIP, and RpVIP during VPI. Positive correlations between VPI accuracy and activity strength in these regions during VPI were significant in aged adults. These relationships were not found for activity in primary auditory cortex (a negative control), or primary visual cortex (not OF-sensitive). This provides novel evidence that activity strength within OF-sensitive regions implicated in self-motion perception is

one factor associated with accuracy on a VPI task, and potentially in spatial navigation ability, in cognitively normal aged adults.

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USING NEURITE ORIENTATION DISPERSION AND DENSITY IMAGING (NODDI) AND TRACTS CONSTRAINED BY UNDERLYING ANATOMY (TRACULA) TO DIFFERENTIATE BETWEEN SUBJECTS ALONG THE ALZHEIMER'S DISEASE CONTINUUM

Z. Zhang; M. Alosco (Ph.D.); R. Killiany (Ph.D.); E. Steinberg (M.N); R. Stern (Ph.D.)

Department of Anatomy & Neurobiology

Objective: To assess the involvement of changes in known white matter pathways in cognitive decline across the spectrum from healthy aging to Alzheimer's disease.
Method: MPRAGE-T1, multi-b shell diffusion and High Angular Resolution Diffusion Imaging scans were obtained from 28 participants in the Health Outreach Program for the Elderly at the Boston University Alzheimer's Disease Center. Scans were processed with Freesurfer and the Neurite Orientation Density and Dispersion Imaging toolkit. White matter pathways were generated using the Freesurfer Tracts Constrained by Underlying Anatomy tool. This resulted in an Orientation Dispersion Index (ODI) and Fractional Anisotropy (FA) values for 18 established white matter pathways. Ordinal logistic regression and multiple regression statistical models were used to assess relationships between the measures of pathway integrity (FA and ODI) and 1) Informant reported Cognitive Change Index (ICCI) 2) self-reported Cognitive Change Index (CCI), and Clinical Dementia Rating (CDR) values.
Results: Measures of white matter pathway integrity predicted ICCI and CDR well in statistical models. In particular, FA and ODI values of the bilateral superior longitudinal fasciculi, inferior longitudinal fasciculi, and the cingulum bundle tracts all showed significant prediction of ICCI and CDR. None of the pathway FA or ODI values were related to CCI.
Conclusions: These findings suggest that the integrity of several known white matter pathways is compromised by the etiology responsible for Alzheimer's disease. Further, these changes appear to be more apparent to trained clinicians and study partners than they are to the individuals themselves.

Program in Behavioral Neuroscience

Participants

Clara Zundel (78)

78

OBJECTIVE BIOMARKERS OF GULF WAR ILLNESS: WHITE MATTER MICROSTRUCTURAL INTEGRITY, COGNITION AND BLOOD MARKERS IN GULF WAR VETERANS

C. Zundel; M. Abreu; J. Ajama; T. Heeren; P. Janulewicz-Lloyd; R. Killiany,; N. Klimas; B.B. Koo; M. Krengel,; D. Little; E. Sisson; L. Steele; R. Toomey; K. Sullivan;

Program in Behavioral Neuroscience

Identifying objective biomarkers of Gulf War Illness (GWI) is one focus of the Boston GWI Consortium. GWI symptoms include fatigue, pain and cognitive/mood problems. Prior imaging and cognitive studies of GW veterans have found reduced brain white matter (WM) volumes and cognitive decrements in GWI veterans. The current study correlates fatigue, sleep, pain, and cognitive outcomes with WM microstructural integrity in veterans with GWI. Assessments included a full cognitive battery, Pittsburgh Sleep Quality Index, multi-dimensional fatigue scale, McGill pain scale, as well as brain imaging of the major WM pathways using diffusion-weighted MRI (DWI) and blood glutamate and phosphate levels. Participants included 72 GW veterans (57 GWI cases, 15 healthy controls). Cases and controls did not differ by age, sex or education (mean age 50yrs; mean education 15yrs). For each WM pathway, we obtained measures of average Fractional Anisotropy (FA), Axial Diffusivity (AD) and Mean Diffusivity (MD) using Freesurfer software. ANCOVA volumetric comparisons showed significantly lower pars-triangularis WM and superior longitudinal fasciculus (SLF) volumes in GWI cases vs. controls (all $p<0.05$). WM microstructural integrity decrements in GWI cases were found in SLF-parietal and SLF-temporal endings, inferior longitudinal fasciculus, corpus callosum and anterior thalamic radiations ($p<0.05$). WM changes were significantly correlated with poorer cognitive performance (COWAT and D-KEFS Stroop) as well as reduced sleep quality, increased fatigue and pain severity, and higher glutamate and phosphate levels in GWI cases (all $p<0.05$). Results show that WM changes are an integral part of GWI pathobiology and behavioral outcomes that should be further validated.

Department of Biochemistry

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

Deborah Chang (14***)
Julia Hicks-Berthet (28**)
Joseph Kern (32)
Nathan Kingston (33)
Matthew Lawton (36)
Alex Luebbers (43)
Adeline Matschulat (46)
Eleni Stampouloglou (*)

14***

MEASURING STATISTICAL SIMILARITY OF GLYCOSYLATION BETWEEN INFLUENZA A VIRUS VARIANTS

D. Chang, (1); W. Hackett ([2]); J. Klein ([2]); J. Zaia,[1], [2])

Center for Biomedical Mass Spectrometry, Department of Biochemistry

Bioinformatics Program, Boston University

Influenza A virus (IAV) presents a significant human health burden. Antigenicity of the IAV envelope protein hemagglutinin (HA) depends on protein sequence and glycosylation state. For rigorous quantification of antigenicity, glycosylation similarity of HA from different states must be measured. Currently, there are no well-tested methods for comparing glycosylation similarity among virus samples. Here, we present a method for quantification of statistically rigorous similarity between two related virus strains that considers the presence and abundance of glycopeptide glycoforms. We compared intact glycopeptides from alpha1-acid-glycoprotein (AGP) and HA from the strain A/Philippines/2/1982 (Phil82) to glycopeptides from the same samples with terminal galactose residues removed using beta-galactosidase (denoted AGPgal and Phil82gal, respectively). The glycosylated sample pairs were analyzed by reversed-phase LC-MS/MS. Site-specific glycopeptide identifications were made using GlycReSoft, and glycosylation similarities were calculated using a modified Tanimoto coefficient. AGP has complex glycans; therefore, removing terminal galactose should modify all of its glycoforms. Indeed, we confirm that the Tanimoto coefficient comparing AGP and AGPgal is 0.00. Phil82, however, has three sites that are primarily high-mannose, which are unaffected by beta-galactosidase. The Tanimoto coefficient comparing Phil82 and Phil82gal was found to be 0.49, reflecting the expected similarity between the two groups. We tested four replicates for each sample to assess the reproducibility of each method. These data collected represent a proof-of-principle that the Tanimoto coefficient is an appropriate method for assessing glycosylation similarity. Statistically rigorous comparisons of HA glycosylation will inform an understanding of how glycosylation affects antigenicity, benefiting the field of vaccine design.

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YAP AND TAZ-REGULATED CONTROL OF GOBLET CELL FATE IN THE AIRWAY EPITHELIUM

J. Hicks-Berthet; A. Federico; X. Ai; S. Monti; and X. Varelas

Department of Biochemistry

The epithelium lining the conducting airways of the lung serves essential roles in host defense against inhaled irritants. Efficient airway function requires balanced numbers of ciliated and mucus-secreting goblet cells that are maintained in homeostasis and replenished following injury. Disproportional numbers of these specialized cell types is a hallmark of numerous airway diseases, such as asthma, chronic obstructive pulmonary disease (COPD), and cancer. The Hippo pathway effectors Yap and Taz have emerged as essential regulators of lung epithelial cell fate. We have begun to dissect the roles of these transcriptional regulators in adult lung epithelial cell sub-types, with our recent observations indicating key roles for Yap/Taz in restricting the differentiation of mucus secreting goblet cells. Conditional deletion of Yap and Taz in the lung epithelium of adult mice results in severe goblet cell hyperplasia resembling asthmatic models. Furthermore, we have found that dysregulation of Yap and Taz is sufficient to drive goblet cell fate in the absence of inflammatory inputs. Through global gene expression analyses of Yap/Taz-deleted primary airway epithelial cells we have identified a transcriptional profile regulated by Yap/Taz that we are currently investigating to understand how goblet cell fate differentiation is controlled. Our observations highlight the essential role of Yap/Taz in controlling lung epithelial cell fate and offer molecular insight into mechanisms by which cell fate may become dysregulated in the context of airway diseases such as asthma.

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THE HIPPO PATHWAY KINASES LATS1/2 CONTROL MAMMARY EPITHELIAL CELL FATE AND TUMORIGENESIS

J. Kern,[1]; A. Tilston-Lunel; A. Federico; E. Stampouloglou[1]; S. Monti[2]; X. Varelas,[1]

Department of Biochemistry,
Department of Biostatistics,

Breast cancer is the most prevalent cancer among women worldwide. The basal-like subtype of breast cancer is associated with a particularly poor prognosis, yet the molecular mechanisms leading to the origin and pathogenesis of this disease are inadequately understood. The Hippo signaling pathway, an important regulator of cell fate across numerous tissues, has been implicated in breast cancer pathology. Central to Hippo signaling are the transcriptional regulators YAP and TAZ (YAP/TAZ), which drive transcriptional events that are required for basal stem cell traits. YAP/TAZ activity is restricted through the tumor suppressor kinases LATS1 and LATS2 (LATS1/2). In this study, we aimed to elucidate the role of LATS1/2 in the maintenance of mammary epithelial cell fate and tumorigenesis. We found that in the homeostatic mammary gland, luminal epithelial cells exhibit active LATS1/2, with restricted YAP/TAZ activity. Using genetic mouse models, we show that conditional deletion of LATS1/2 in luminal cells leads to the adoption of a bi-lineage state exhibiting both luminal and basal markers, accompanied by high nuclear YAP/TAZ and rapid proliferation. Transcriptional and flow cytometric analysis of LATS1/2-null cells demonstrate that these cells adopt basal-like traits and transcriptional signatures correlative with human basal-like breast cancer. Furthermore, LATS1/2-null cells are capable of forming tumors when

injected subcutaneously into immunodeficient mice. Collectively, this study demonstrates a role for LATS1/2 in the maintenance of luminal fate in the mammary epithelium, and suggests that dysregulation of Hippo signaling in this system drives cell plasticity that may contribute to the initiation of basal-like breast cancers.

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ROLES FOR HIPPO PATHWAY EFFECTORS TAZ AND YAP IN FIBROBLAST FATE DURING PULMONARY FIBROSIS

N. Kingston[1], M. Laynes[1], D. Tschumperlin[2], and X. Varelas[1]

[1] Department of Biochemistry
[2] Department of Department of Physiology & Biomedical Engineering, Mayo Clinic, Rochester, Minnesota

Idiopathic pulmonary fibrosis (IPF) is a fibrotic disease that is driven by a feed-forward loop, in which pathological matrix stiffness is both the cause and result of fibroblast activation. While fibroblasts are the major effector cells in the pathology of pulmonary fibrosis, they are a very heterogeneous cell population whose origin and differentiation patterns are not well understood. Major effectors of the response to matrix stiffness are the transcriptional regulators Taz and Yap, which are key effectors of the Hippo signaling pathway. Taz and Yap are activated by a stiff microenvironment, and they induce transcription of target genes implicated in fibrosis as well as genes associated with more progenitor-like cells. We have demonstrated an association of aberrant nuclear Taz/Yap localization in human IPF patients and the importance of Taz/Yap in mouse models of fibrosis. Further we have shown increased Taz/Yap nuclear levels in specific fibroblast populations in a bleomycin-induced mouse model of pulmonary fibrosis. We propose to gain insight into the origin of the different fibroblast populations that arise and expand during fibrosis, and the importance of Taz/Yap in these populations. We hypothesize that Taz/Yap play a key role in promoting the early proliferation and differentiation of pro-fibrotic cells during the development of pulmonary fibrosis. To test this hypothesis, we have deleted Taz/Yap in distinct lung fibroblast populations, examining the consequences on fibroblast cell fate and fibrotic phenotypes. We hope that knowledge into the early events contributing to fibrotic onset will guide therapeutic strategies for treatment of this devastating disease.

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PHOSPHOPROTEOMIC PROFILING OF CAR T CELLS

M. Lawton; J. Cho; A. Emili; W. Wong

Department of Biochemistry

Chimeric antigen receptor (CAR) T-cells are a booming new technology that hold a lot of promise in cancer therapy. A CAR is an artificially designed receptor that is transfected into T-cells that use signaling proteins from the endogenous TCR pathway to mount an immune response against its specifically programmed target antigen on the cell surface of the cancer cells. Although promising, there are a few problems with current CAR T-cell therapies, such as toxcicity, cytokine storm, and tumor lysis syndrome. Using a mass spectrometry-based phosphoproteomics platform, this work aims to unravel the signaling networks that underlie CAR signaling in CAR T cells in collaboration with the Wilson Wong Lab. Understanding the exact signaling pathways of these CARs can help us develop more effective and safer therapies in the future. Current work done employs proteomic and phosphoproteomic analysis of

experiments that attempt to resolve differences in phosphorylation states of proteins and in turn, uncover signal transduction pathways that they represent. Two main experiments are shown; one, a proteomic and phosphoproteomic analysis of a pilot CAR versus T cell receptor (TCR) activation experiment, and two, a proteomic and phosphoproteomic analysis of a TCR activation time course as a proof of concept experiment, showing that we can use our platform to uncover the phosphorylation dynamics involved in TCR signaling.

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MOLECULAR BASIS FOR THE REGULATION OF NEURONAL GPCR SIGNALING BY GINIP

A. Luebbers; P.C Park; V. DiGiacomo; M. Papakonstantinou; S. Broselid; M. Garcia-Marcos

Department of Biochemistry

GINIP is a novel G-protein regulator with an important physiological impact on neuronal GPCR signaling. GINIP is specifically expressed in neurons of the peripheral and central nervous system. We have recently shown loss of GINIP results in increased frequency and severity of seizure episodes upon treatment with $GABA_A$ receptor antagonists suggesting an important role for GINIP in neuronal signaling. The molecular basis for GINIP-mediated regulation of GPCR signaling is unclear, as it has been shown that it exerts paradoxical effects on different signaling branches of G protein signaling, i.e., it dampens Gα- while enhancing Gβγ-dependent signaling. Here, we set out to characterize the molecular mechanism by which GINIP regulates GPCR signaling. We found that GINIP binds specifically to Gαi subunits in their active conformation. Such binding occurs in the α3/SwII binding pocket of Gαi, the canonical binding site where effectors such as adenylyl cyclase bind. Our results suggest that GINIP prevents Gαi-mediated inhibition of cAMP levels by competing with adenylyl cyclase for G protein binding. On the other hand, GINIP also competes with GAPs of the RGS family for binding to Gαi. By binding Gα and competing with GAPs, GINIP prolongs the lifetime of Gβγ in its free state, now competent for engaging downstream effectors (ion channels) to modulate neurotransmission. These findings reveal a new paradigm of GPCR signaling regulation that results in biased signaling to specific effectors. Dysregulation of this mechanism of fine-tuning might underlie the alterations of neuromodulation that give rise to neuropathic pain and epilepsy.

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UNDERSTANDING THE MOLECULAR MECHANISMS OF ABERRANT BASAL CELL EXPANSION IN LUNG CANCERS

A. Matschulat[1], A. Tilston-Lunel [1] and X. Varelas[1]

[1]Department of Biochemistry

Basal cells are resident stem cells in the lungs as they can self-renew and differentiate into the various cell types of the pseudostratified airway epithelium. Aberrant basal cell expansion is associated with a number of common lung diseases, including COPD, cystic fibrosis and cancer and so knowledge into mechanisms controlling basal cell function has important clinical relevance. Current research shows that YAP, a transcriptional regulator which is normally repressed via phosphorylation by Hippo Pathway regulatory kinases LATS1 and LATS2, is a key regulator of basal cell identity. The Hippo Pathway is a developmentally important pathway that controls cell fate, proliferation and survival in various contexts. However, mechanisms controlling YAP and signals regulated by YAP that direct basal cell fate are poorly

understood. My project is to understand YAP function in basal cells by studying the consequences of aberrant nuclear YAP activation via conditional deletion of LATS1/2 in adult murine airway epithelium. Preliminary observations indicate that loss of LATS1/2 drives a phenotype similar to SqCLCs, like uncontrolled basal cell expansion. Analyses of murine lung and trachea samples, along with molecular characterization of global gene expression and proteomic alterations upon LATS1/2 dysregulation has revealed interesting new insights into signals controlling basal cell identity and proliferation.My goal is to understand these signals to gain molecular insight into how basal cell self-renewal and expansion is regulated; this could offer novel directions for preventing basal expansion in disease or guide new methods to expand basal cells ex vivo for regenerative therapy.

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LATS1/2 KINASES ARE ESSENTIAL REGULATORS OF T CELL DEVELOPMENT AND ACT AS SUPPRESSORS OF LYMHPOMAGENESIS

E. Stampouloglou; X. Varelas

Department of Biochemistry

Mechanisms controlling T cell homeostasis are under intense study, but much remains unknown about cell fate decisions during T cell maturation. Here we show essential roles for kinases LATS1 and LATS2 (LATS1/2) in T cell development. We show that CD4-Cre recombinase-driven conditional co-deletion of the *LATS1/2* genes in mice results in defective T cell maturation and tumor development that manifests into animal lethality. In the thymus, lymphoid progenitors progress from a double negative stage (DN, $CD4^-$ $CD8^-$) to a double positive stage (DP, $CD4^+CD8^+$), to a single positive stage (SP, $CD4^+$ or $CD8^+$). Lineage tracing *LATS1/2*-deleted cells within the thymus revealed an increased percentage of cells in the DN stage, suggesting thymocyte de-differentiation from the DP stage upon *LATS1/2* deletion. In *LATS1/2*-deleted mice, the percentage of cells in the SP stage is significantly higher, suggesting that LATS1/2 function is necessary for SP thymocytes to mature and exit the thymus. Further, we found an increased percentage of *LATS1/2*-deleted T cells in the DP and SP stages expressing CD5 and TCRb, markers associated with maturation defects and lymphoma. At 16-weeks of age, *LATS1/2*-deleted mice present with hypotrophic thymuses, hypertrophic spleens and lymphopenia. Interestingly, *LATS1/2*-deleted mice develop solid masses composed of spindle shaped cells expressing CD45, an early pan-leukocyte marker, but are negative for CD3, CD4 or CD8, suggesting that loss of *LATS1/2* drives T cell de-differentiation and oncogenic transformation. Collectively, our study reveals an essential role for LATS1/2 kinases in thymocyte development and maturation and offers a new model of lymphoma development.

Department of Endocrinology

Participants

Lauren Johnson (29)

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AN INVESTIGATION OF GENETIC VARIANTS IN CORTICOTROPH ADENOMAS

L. Johnson; A. Abreu

Department of Endocrinology, Diabetes and Hypertension

Corticotroph adenomas, a subset of pituitary adenomas staining positive for ACTH, are increasing in incidence due to improved detection methods and growing awareness amongst physicians. Corticotrophomas are categorized into functional adenomas (FCA), silent adenomas (SCA) and hyperplasia (CH) which result in Cushing's disease (CD), mass effect and mild hypercortisolism, respectively. Trans sphenoidal surgery (TSS) is the best available treatment for corticotrophomas, but tumor recurrence is common. We sought to identify differential genetic drivers of sporadic FCAs, SCAs, and CHs to better characterize these lesions and determine appropriate treatment options.
Corticotroph lesion samples, collected from Brigham and Woman's Hospital (BWH) surgical patients between 2008 and 2018, were assayed using the Dana-Farber/BWH OncoPanel, versions 2 and 3. We examined 12 FCA, 5 SCA, and 2 corticotroph hyperplasia (CH) tissue samples.
Alterations in epigenetic regulatory genes were identified across all subtypes implicating epigenetic regulation as a therapeutic target. Furthermore, recurrent mutations in *USP8* and *ARID1B* were observed exclusively in FCAs. Copy number gains of 6p, 20q and 21q as well as losses in 11p and 19q were frequently observed in this group. No recurrent mutations were identified in SCAs, but amplification of chromosome 12 and single copy deletions in chromosome 10 were reported. Additionally, we observed diverging genomic disruption between subtypes, associated with functional hormone status.
Corticotroph subclasses presented with novel genetic drivers and distinct genomic profiles associated with hormone secretion and clinical presentation. Further research is required to better elucidate the role of these genetic variants in tumorigenesis and hormone production.

Program in Genetics and Genomics

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

Stefanie Chan (*)
Jiayi Cox (15)
Gian Paolo Sepulveda (62)

*

THE ROLE OF GPS2 IN THE PI3K/AKT PATHWAY IN BREAST CANCER

S. Chan[1,2], E. Smith[2], S. Monti[1,3], D. Cardamone[2], V. Perissi[1,2]

1- Program in Genetics and Genomics
2-Department of Biochemistry, Boston University School of Medicine
3-Department of Computational Biomedicine, Boston University School of Medicine

The PI3K/AKT pathway is well known to be involved in essential cellular processes, including proliferation, metabolism and cell survival. Mutations in this pathway commonly result in AKT hyperactivation, which contributes to breast cancer development and progression. Although activation of AKT through phosphorylation has been well studied, recent studies have also shown that AKT activity can also be regulated through ubiquitination. However, the mechanism and players involved in this process are not well understood. Better understanding of these pathways could inform on better potential therapeutics to target this disease.
Recent work in our lab has identified G-protein suppressor 2 (GPS2), a member of the NCoR/SMRT complex, as a regulator of the insulin-signaling pathway through the inhibition of AKT ubiquitination by the E2 ubiquitin-conjugating enzyme Ubc13. GPS2 has also been identified as a tumor suppressor in liposarcoma and medulloblastoma, and low GPS2 expression is correlated with poorer survival outcomes in breast cancer. Here we explore the role of GPS2 as a tumor suppressor in the context of breast cancer by generating and characterizing a series of GPS2-KO breast cancer cell lines and investigate the role GPS2 plays in regulating the PI3K/AKT pathway through the inhibition of aberrant activation of AKT. Preliminary results indicate that MB231 GPS2-KO cells have increased migration, invasion and proliferation, some of which can be attributed to increased AKT activation. However, further studies are needed to uncover other possible processes impacted by the knockout of GPS2, and to elucidate the mechanism by which AKT ubiquitination regulates its activation.

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FACTORS ASSOCIATED WITH OPIOID CESSATION: A MACHINE LEARNING APPROACH

J. Cox, R. Sherva, K. Lunetta, R. Saitz, M. Kon, H. Kranzler, J. Gelernter, L. Farrer

Program in Genetics and Genomics department

Opioid epidemic has a high mortality rate and is a burden on health care. Treatments for opioid use disorder (OUD) are efficacious, but few research on the environmental and genetic factors that influence successful outcomes. Here, we employed multiple machine learning prediction algorithms in a racially mixed group of individuals who met DSM-5 criteria for OUD and were profiled genetically. Demographic, lifestyle, drug use, and behavioral information, as well as symptom level data that enabled derivation of DSM diagnoses for multiple substance use and other psychiatric disorders, were obtained by a semi-structured interview. Several thousand variables including polygenic risk scores (PRS) for multiple substance use disorders were evaluated using different machine learning methods to identify key factors for opioid cessation. Support vector machine (SVM) models performed marginally better than other machine learning methods with maximum prediction accuracies of 75.4% in African Americans (AAs) and 79.4% in European Americans (EAs). Subsequent stepwise regression analyses identified recency of cocaine use (OR_{AA}=1.91, OR_{EA}=2.30, $p<5 \times 10^{-8}$), duration of opioid use ($OR_{AA,EA}$=0.55, $p<5 \times 10^{-6}$), and current age (OR_{AA}=2, OR_{EA}=2.44, $p<5 \times 10^{-9}$) as the strongest independent predictors of cessation among 83 most highly ranked variables. Variables related to drug use comprised 50% of the significant independent predictors. Other significant predictors were related to non-drug use behaviors, psychiatric problems, overall health, and demographic factors. PRS's for addiction to other substances were comparatively weaker predictors marginally improved prediction accuracy. These proof-of-concept findings suggest strategies for improving disease management and provide a framework for personalized OUD treatment.

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PUTATIVE PROTEOLYTIC CLEAVAGE OF C-MYC

G. Sepulveda; D. Cardamone; R. Esse; K. Gushchanskaia; A. Mora-Martin; A. Grishok

Program in Genetics & Genomics; and Department of Biochemistry

The proto-oncogene c-MYC controls multiple cellular processes ranging from cell cycle regulation, growth and metabolism, to apoptosis. It is implicated in multiple cancer types. c-MYC is thought to be a global transcription regulator. The C-terminus of c-MYC contains its DNA binding domain, called bHLH/LZ, which also participates in heterodimerization with MAX to bind to E-box promoters. The N-terminus contains the trans-activating domain, which is responsible for recruiting transcription factors and chromatin modifiers to the promoter of target genes. While the termini of c-MYC are well characterized, much is still unknown about its middle portion. Specifically, the molecular function of the heavily conserved Myc boxes IIIa, IIb and IV remains enigmatic. Here, we begin to characterize a novel isoform of c-MYC, which is likely produced by a proteolytic cleavage in the middle of the protein, using HEK293 and MDA-MB231 human cell lines. Our data suggest that c-MYC is cleaved to form a 27 kDa C-terminus fragment, which retains the bHLH/LZ domain. Importantly, with siMYC treatment expression of this c-MYC isoform gets reduced. It appears that an elastase-like protein may be the protease that cleaves the C-terminus of c-MYC. Further characterization of the cleaved product is needed, but these findings suggest a new form of post-translation regulation of c-MYC that may be important in its implication in cancer.

Master of Science in Medical Sciences

Participants

Jelena Bogovic (5)
Sarah Caplan (11)
Jessica Cashen (12)
Yashoda Dhole (18)
Gianna Dingillo (19)
John Fernan (21)
Alexandra Greenberg (26)
William Moore (47)

5

ACTIVITY PATTERNS OF CENTRAL AMYGDALA NEURONS IN A MOUSE MODEL OF NARCOLEPSY

J. Begovic; C. Mahoney; T. Scammell

M.S in Medical Science

Narcolepsy is a disorder of unstable wake and sleep states caused by the lack of orexin neurons. The state instability of narcolepsy includes rapid eye movement (REM) sleep intruding into wake in the form of dream-like hallucinations and cataplexy, muscle paralysis (atonia) much like occurs in REM sleep. In mice lacking orexin peptides, cataplexy is also observed with similar presentation as in humans. Prior research showed that the activation of the central amygdala (CeA) is sufficient to promote cataplexy in a mouse model of narcolepsy. We hypothesize that γ-aminobutyric acid-ergic neurons in the CeA expressing the oxytocin receptor (OTR) mediate cataplexy as these neurons project to a known REM sleep atonia-regulating region, the ventrolateral periaqueductal gray/lateral pontine tegmentum, and, as oxytocin sensitive neurons in the amygdala, likely participate in emotional processing and social behavior. In this study, we used fiber photometry to investigate the behavior of these neurons in response to social and rewarding stimuli, and during emotion-triggered cataplexy. Photometry recordings showed increased activity in response to social interaction and reward, prior to REM transitions, and decreased activity during cataplexy. These responses appear to occur before interaction with stimulus mice or reward stimulus. In conclusion, responses of CeA-OTR neurons to social and rewarding stimuli, cataplexy, and at REM transitions are in support of a possible role of these neurons in emotion-triggered cataplexy which can be tested using additional methods, such as optogenetics.

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DIFFERENTIAL EXPRESSION OF RIG-I-LIKE RECEPTORS (RLRS) IN THE HUMAN PLACENTA ACROSS GRESTATION

S. Caplan[1], W. Kuohung[1], J. Pudney[1], Z. Masheeb[1], J. Connor[2,3], R. Ingalls[4]

[1]Department of OB/GYN, [2]Department of Microbiology,
[3]National Emerging Infectious Disease Laboratories,

M.S in Medical Sciences

After the discovery that Zika virus (ZIKV) infection in pregnant women may result in severe adverse outcomes such as fetal microcephaly, ZIKV must be added to an ever-expanding list of teratogenic viruses. As only a small minority of newborns will display congenital abnormalities after maternal primary infection, it is clear that innate immune mechanisms exist in the placenta to prevent viral transmission to the fetus. Understanding the innate antiviral defenses of the placenta is critical to improving diagnosis, treatment, and prevention of adverse pregnancy outcomes. We used alkaline phosphatase-based fast red immunohistochemistry to stain placental tissue for the RLRs RIG-I, LGP2, and MDA5, as well as the endosomal Toll-like receptor TLR7, that serve as antiviral innate immune receptors involved in detecting microbial ligands and cytoplasmic viral nucleic acids. We hypothesized that RLRs are expressed in either one or both of the outer cell layers of the chorionic villi, either the syncytiotrophoblast (STB, outermost layer) or in the villous cytotrophoblast (CTB, inner layer), and that expression of these receptors will increase with advancing gestational age. Hofbauer (HB) cells stained positive for all antibodies and served as a positive internal control. TLR7 was not present in either the STB or CTB. MDA5 was localized to the STB. LGP2 was localized to the STB apical membrane and the CTB cytoplasm, especially later in gestation. Lastly, RIG-I was localized to the CTB cytoplasm. The differential expression of these RLRs suggest an innate immune defense system unique to the placenta that is responsible for protecting the conceptus from viral attack. These findings will complement ongoing work in characterizing replication of teratogenic viruses in the placenta.

This work was supported by the National Institute of Allergy and Infectious Diseases 1R21AI134576-01.

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OSTEOGENIC MESENCHYMAL STEM CELL DIFFERENTIATION IN DIFFERENT MEDIA CONDITIONS

J. Cashen; S. Diaz; A. Weissman

Orthopaedic Surgery, Gerstenfeld Laboratory

Osteoporosis is an age dependent disease that leads to fragility fractures. Long-term bone homeostasis is dependent on bone marrow stromal stem cells (BMSCs) that give rise to osteogenic cells. In order to conduct population level studies of human BMSCs, standardized culturing conditions optimized for growth and differentiation of human BMSCs need to be established. This study's objective was to compare the differentiation potential of BMSCs cultured for 21 days in one of three types of growth media: control media supplemented with fetal bovine serum with (CM) and without dexamethasone (CM-D), and an artificial media (AFM) with dexamethasone but devoid of animal product supplementation.

Methods: BMSCs were isolated from acetabular reamings from patients undergoing hip arthroplasty. DNA content was used as a surrogate for cell growth, while osteogenic differentiation was assessed by alkaline phosphatase activity and calcium accumulation. qRT-PCR was used to measure the expression of five genes of interest (COL1A1, RUNX2, SP7, DMP1, SOST) chosen to assess the progression of cellular differentiation from mesenchymal stem cells into osteocytes. **Results:** Cultures showed equal growth in all media. Dexamethasone was a needed additive to promote cellular differentiation. Alkaline phosphatase was higher in CM, and calcium contents were higher in AFM. qRT-PCR analysis showed that AFM cultured cells treated with dexamethasone differentiate into osteocytes more quickly than cells in the other medium conditions. **Conclusions**: From these combined results, we have concluded that a reliable growth media for future osteogenic BMSC studies is AFM with dexamethasone supplementation.

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DIFFERENCES IN ABDOMINAL PAIN AND SENSORY PERCEPTION BETWEEN ADOLESCENT MALES AND FEMALES

Y. Dhole, C. Lunde, C. Sieberg, C. Wong

M. S. in Medical Sciences

The present study aims to collect data on the pain and sensory perception of male and female healthy individuals. Although the overarching project has been testing female controls for longer, males have been added to the protocol with the goal of expanding the understanding of pain norms. This study compares pain and sensory perception between sexes and looks at psychosocial factors that may cause differences among the two. To assess these topics, the protocol includes both quantitative sensory testing (QST) and five questionnaires. QST explores pain and sensory perception resulting from mechanical and thermal stimuli and the questionnaires cover pain sensitivity, demographic information, mental health, and other factors relevant to pain and sensation. Data collected through this protocol was analyzed on SPSS through descriptive statistics and one-way analyses of variance. It showed only three significant differences between the two sexes: thermal sensory threshold of cold on the hand, thermal sensory threshold for heat on the hand, and pressure pain threshold on the hand. The p-values for these were 0.001, 0.013, and 0.044 respectively. The lack of significant variance between genders for the majority of data points shows that both male and female healthy control perceive pain and sensation similarly. Although there may be some differences in anatomy and development, there are no distinct differences in the overall experience of these phenomena.

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META-ANALYSIS: OBSTRUCTIVE SLEEP APNEA AND OCULAR DISEASES

G. Dingillo; JG. Arroyo

M.S in Medical Sciences

PURPOSE: Previous studies have reported an increased prevalence of ocular diseases in patients with obstructive sleep apnea. The purpose of this study was to examine the link between such ocular diseases as diabetic retinopathy, diabetic macular edema, retinal vein occlusion, central serous chorioretinopathy, age-related macular degeneration, non-arteritic anterior ischemic optic neuropathy, and glaucoma.

METHODS: This meta-analysis was conducted through a search using PubMed, Web of Science, Scopus and EMBASE. We identified both retrospective and prospective studies.
RESULTS: The final meta-analysis looked at 30 studies and 7 ocular diseases. The data showed a high prevalence of obstructive sleep apnea for diabetic retinopathy and diabetic macular edema patients. Data for glaucoma and non-arteritic anterior ischemic optic neuropathy patients did not show a statistical increase. There was not enough data for retinal vein occlusion, central serous chorioretinopathy and age-related macular degeneration to calculate statistical significance.
CONCLUSION: These data suggests that patient populations with diabetic retinopathy and macular edema show increased rates of obstructive sleep apnea. Data suggest that hypoxia is an important part of the pathophysiology of diabetic retinopathy and diabetic macular edema. Because obstructive sleep apnea has been shown to affect the progression of the ocular diseases included in this study, ophthalmologists should screen for the presence of obstructive sleep apnea to better help their patients.

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HISTOLOGICAL CHARACTERIZATION OF CT-IDENTIFIED OSTEOARTHRITIC SUBCHONDRAL CYSTS AND CO-REGISTRATION OF CT WITH MRI

J. Fernan; S. Auger, J; E. Morgan; L. Gerstenfeld

Department of Orthopaedic Surgery

Osteoarthritis is a chronic joint disease that commonly affects the hips, and it is one of the most prevalent causes of disability in older populations. The cause of pain in OA is not well understood, but it is known that bone marrow lesions (BMLs) identified in subchondral bone by magnetic resonance imaging (MRI) are an important determinant of pain. Abnormal blood vessel growth may be responsible MRI signature of BMLs, and the commonality between pathways for angiogenesis and neurogenesis suggests this pathologic process may be the source of pain in OA. The objective of this study was to characterize the histologic nature of subchondral cysts identified by micro-computed tomography (micro-CT) and develop a method for co-registering the CT and MRI images. **METHODS:** Femoral heads were collected from 10 patients (6 females and 4 females; age 29-80) who underwent total hip arthroplasty. All patients had MRIs performed within 6 months prior to surgery. The heads were fixed and scanned with micro-CT to identify cysts in the subchondral bone, which were resected and analyzed histologically. The primary compressive group was reliably identified on micro-CT images and served as good indicator for proper orientation between CT and MRI. **RESULTS:** Histopathological findings included vascular fibrosis and peripheral nodules of cartilage in some cysts. **CONCLUSION:** The ability to correlate the histopathology of CT-identified lesions with a signature pattern on MRI will be an important tool for better characterizing the nature of BMLs and understanding the pathogenesis of OA.

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LONGITUDINAL ANALYSIS OF HOST AND VIRAL BIOMARKERS IN LIVER TISSUE SECTIONS OF EBOLA (EBOV) INFECTED RHESUS MACAQUES

Alexandra Greenberg, MSPH, Russ Huber, MD, PhD, Nicholas Crossland, DVM, DACVP

M.S in Medical Sciences

This study characterized the temporal viral pathogenesis of EBOV in the liver of infected rhesus macaques using routine histopathology, multiplex immunohistochemistry (mIHC) and multiplex *In Situ*

Hybridization (mISH), refined by digital pathology (DP) and image analysis (IA). Comparing peracute (≤4 DPE) to acute and terminal (≥ 6 DPE) EBOV infection, there was a statistically significant ($p < 0.05$) increase in hepatic inflammation and fibrin thrombi, correlating with an absolute increase in macrophages (CD68), neutrophils (MPO), and total % of Tissue Factor in the liver. There was also significant increase in severity of necrosis, which correlated with a decrease in Heppar. While there was significant and early colocalization of VP35 and CD68, there was only rare colocalization of VP35 with Heppar, even in terminal animals. Progressive and statistically significant differences were observed in gene expression when comparing peracute to acute and terminal EBOV infection. *IL-6* predominated within periportal fibrovascular compartments and colocalized within cells concurrently expressing EBOV *VP35*. *ISG-15* expression was observed in periportal regions and in proximity to cells expressing EBOV *VP35*, but colocalization within EBOV *VP35* expressing cells was extremely rare. Using digital image analysis, we were able to characterize minute changes that reflect magnitudes of biological variability not feasible to detect with the human eye. Furthermore, spatial context has refined our current understanding of differential gene expression of EBOV, which has the potential to aid in development of host-directed therapies. The establishment of these benchmarks will serve as a guide for validation of cross-institutional EBOV animal models.

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IMPACT OF MUSCLE TRAUMA ON STEM CELL RECRUITMENT DURING POST-NATAL ECTOPIC BONE FORMATION

W. Moore; B. Bragdon; L. Gerstenfeld; Y. Liu

Department of Orthopaedic Surgery

Trauma to the musculoskeletal system can result in heterotopic ossification, a condition where aberrant bone tissue is synthesized and mineralized in the soft tissues of the body. Satellite cells expressing Pax7 are the predominant stem cell population found within adult skeletal muscle tissues and are primarily implicated in skeletal muscle regeneration. Our aim is to identify how muscle trauma effects DBM-induced ectopic bone formation and characterize the contribution of the Pax7 satellite cell population in DBM-induced ectopic bone formation following muscle trauma. Ectopic bone was induced by surgical implantation of DBM (50 mg) with 0.1 μg of bone morphogenic protein 2 (BMP-2) on the femoral periosteum or in the skeletal muscle tissue of the upper hind limb of tamoxifen inducible Pax7/Ai14 reporter Rag1 deficient transgenic mice. Following implantation, mice received varying amounts of blunt force trauma to induce skeletal muscle trauma. Ectopic bone was then evaluated radiologically using plain film and micro-computed tomography, and histologically through fluorescence and brightfield microscopy. Skeletal muscle trauma does not appear to impact the resulting bone volume of BMP-2 supplemented DBM induced ectopic bone formation. However, the decreased dose of BMP-2 that was needed to induce ectopic bone formation within muscle suggests that trauma sensitized the stem cell populations that contribute to ectopic bone to BMP induction. The appearance of Pax7 within the newly formed ectopic bone with muscle trauma suggests that the muscle trauma effects the plasticity of Pax7 satellite enabling them to contribute to ectopic bone formation.

Graduate Program for Neuroscience

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

Jenny Klein (34)
William Mau (*)
Thomas Morin (48)
Shen Ning (50)
Patricia Rein (60)
Michael Rosario (61)
Samantha Shelton (63)
Elizabeth Spencer (64***)
Gregory Wirak (72)
Ellen Witkowski (73**)
Hana Yeh (74)

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ABNORMALITIES IN SPINAL CORD DEVELOPMENT OF THE TS65DN MOUSE MODEL OF DOWN SYNDROME

J. Klein; N. Aziz; M. Brady; T. Haydar.

Graduate Program for Neuroscience

Down syndrome (DS) is caused by the triplication of chromosome 21 and manifests as a constellation of disorders of which intellectual disability and motor dysfunction are the two most prevalent phenotypes. Despite this, there have been few studies that have investigated the etiology of the motor dysfunction. To address this, for the first time, we characterize life-long perturbations in the cellular composition of the spinal cord in Ts65Dn, a mouse model of DS the recapitulates the motor dysfunction observed in people with DS. Ts65Dn animals were sacrificed and collected at various embryonic and postnatal time points in order to be processed for immunohistochemistry. At embryonic day E10.5 the pMN domain, which produces motor neurons and oligodendrocytes, was significantly larger in trisomic animals. Two days later at E12.5, an increase in migrating motor neurons and interneurons produced from domains neighboring pMN was observed. However, by day E14.5 these cellular changes had resolved with no significant differences in cellular populations between trisomic and euploid animals. Postnally, neural degeneration in aged trisomic animals was observed, with fewer motor neurons and interneuron subtypes present in 10-11 month trisomic animals. Additionally, there were differences in oligodendrocyte maturation state between euploid and trisomic animals at all postnatal time points surveyed. Together this data demonstrates that there are marked changes in both white matter and neuronal composition that persist over the life span and may be responsible for the motor deficits seen in humans and mouse models of DS.

*

VISUALIZATION AND MODULATION OF ENSEMBLES IN THE HIPPOCAMPUS AND AMYGDALA DURING FEAR REINSTATEMENT

W. Mau*; Y. Zaki*; E. Doucette; S.L Grella; A. Hamidi; E. Merfeld; N.J Murawski; S. Ramirez; M. Shpokayte

*Equal contribution

Graduate Program for Neuroscience

Post-traumatic stress disorder is a condition that precipitates from an aversive experience and is manifested by overgeneralized fear in innocuous situations. Interestingly, a striking proportion of patients who undergo exposure therapy - which can lead to the suppression, or "extinction," of the original fear memory - are vulnerable to relapse, especially when the conditioned stimulus is delivered outside a clinical context. Here, we interrogated the neural substrates supporting the acquisition of fear as well as the subsequent extinction of fear to gain a causal understanding of its underlying neural components. We used activity-dependent labeling of neuronal ensembles in brain regions associated with fear-related behaviors (basolateral amygdala, BLA; dorsal dentate gyrus, dDG) and manipulated these ensembles using optogenetics to probe the changes that a fear memory undergoes during extinction and fear reinstatement. We found that inhibition of the fear ensemble in BLA or dDG during a recall test disrupted fear expression in the conditioned context after reinstatement. Additionally, we employed *in vivo* calcium imaging using miniaturized microscopes in BLA to visualize ensemble activity during the reinstatement paradigm. Using population vector correlations, we observed that the overall state of the BLA network evolved over extinction. However, after reinstatement, the BLA reverted to a state resembling that seen during fear conditioning. These convergent data suggest that the original fear ensembles in BLA and dDG contribute to a context-dependent fear response following shock-induced reinstatement, providing key evidence that the neural correlate of fear reinstatement may be a reemergence of the original fear memory trace.

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AN fMRI INVESTIGATION OF SYMBOLIC PROCESSING DURING A ONE-DIMENSIONAL RAVEN'S PROGRESSIVE MATRICES TASK

T. Morin; A. Chang; C. Ster

Graduate Program for Neuroscience

This project presents preliminary data from an ongoing fMRI study of symbolic processing during a simplified version of the Raven's Progressive Matrices (RPM) task. The RPM Task is widely used by neuropsychologists as a test of non-verbal fluid intelligence and abstract reasoning ability. The goal of this study was to investigate activity in regions of prefrontal cortex that underlies abstract reasoning. We developed a simplified, one-dimensional version of the RPM task suitable for testing during an fMRI scan. During the task, subjects were presented with symbolic or texture-like stimuli that varied sequentially across one dimension. Subjects were required to deduce and apply a sequence-rule to select a probe stimulus that correctly fills in the blank location in the stimulus. Participants (n = 20, data collection ongoing) successfully performed this task with virtually no training beforehand. In this preliminary analysis, we observe reasoning-related activation associated with deducing a sequence rule in frontoparietal cortical regions. Notably, this activation is greater when subjects are reasoning about a

symbolic sequence rather than a texture-like sequence. Conversely, increased activity in inferior temporal regions was associated with reasoning about a texture-like sequence. Our preliminary results suggest the existence of separate networks underlying reasoning behavior for symbolic and non-symbolic stimuli.

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RAPID REMOVAL OF AMYLOID-B AGGREGATES USING ANTIBODY CONJUGATED SUPERPARAMAGNETIC NANOPARTICLES

S. Ning[1, 2], A. Rompala[2], N. Shanmugam[2], I. Kim[2], S. Hartman[2], D. Kumar[2], A. Rodriguez[2], S. Patel[2], S.H.Choi[2], R.Tanzi[2] and D.Y Kim[2]

[1] Graduate Program for Neuroscience
[2] Genetics and Aging Research Unit, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School

Multiple Alzheimer's disease (AD) clinical trials target pathogenic amyloid-β (Aβ) species using therapeutic anti-Aβ antibodies. However, recent AD drug clinical trial failures demonstrate an immediate need for innovative therapeutic approaches. Developments in nanotechnology and human stem cell biology have spurred a renewed interest in developing new strategies to overcome this current therapeutic challenge. Here, we developed superparamagnetic iron oxide nanoparticles conjugated with anti-Aβ antibodies that bind to Aβ peptides and aggregated Aβ species *in vitro* and *ex vivo*. Application of this superparamagnetic iron oxide nanoparticles immunotherapy (SPIONi) in our 3D human neural cell culture model of AD, followed by rapid removal of SPIONi-Aβ complex by an external magnet force in real time, efficiently decreased soluble and insoluble Aβ species including Thioflavin-S-positive Aβ. Furthermore, applying an alternating magnetic field, we can remotely interrogate the size of the plaques, allowing the electromagnetic force to induce a shearing effect to dissociate the aggregated plaques. More importantly, acute removal of Aβ species in our 3D culture significantly decreased accumulation of pathogenic phosphorylated tau species. These results show that excess accumulation of Aβ species drives neurofibrillary tangle (NFT) pathology in our 3D cell culture model of AD. Our results clearly demonstrate therapeutic potential of SPIONi technology in reducing both Aβ accumulation and tau pathology.

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PHARMACOLOGICAL INTERVENTION TO PROMOTE MYELINATION IN DOWN SYNDROME

P. Rein[1, 2], N. Aziz[2], C. LaBlang[2], C. Pineda Delgado[2], and T. Haydar[2]

[1]Graduate Program in Neuroscience And
[2]Department of Anatomy and Neurobiology

Insufficient myelination can reduce neuronal communication speed and result in widespread complications implicated in Down syndrome (DS), such as loss of motor skills and cognitive impairment. Our prior work, investigating changes in gene expression due to triplication of human chromosome 21, demonstrated disruption in genes related to oligodendrocyte precursor cells and the maturation of oligodendrocytes. This finding is modeled in Ts65Dn mice, where it was shown that compared to euploid littermates, Ts65Dn animals have a significantly thinner myelin sheath surrounding cortical axons, and a significant increase in the number of immature oligodendrocytes at the expense of mature oligodendrocytes. These cellular changes are correlated with functional changes; measurements of

compound action potentials across the corpus callosum show a reduced velocity in myelinated fibers in Ts65Dn animals, compared to euploid littermates. Together, these findings suggest that in DS there is a deficit in the ability of OPCs to differentiate into mature oligodendrocytes, leading to disruptions in myelin structure and integrity.
Three compounds have been shown to promote OPC differentiation *in vitro* and our preliminary *in vivo* studies illustrate that these compounds increase the number of mature oligodendrocytes in both euploid and Ts65Dn corpus callosum. Treating animals during the postnatal period of active myelination increased the ratio of mature to immature oligodendrocytes. Future studies will examine the functional changes that result from these treatments and develop novel pharmacological therapies that facilitate the healthy development of OPCs to maturation. Finding novel target sites and developing compounds to promote OPC differentiation could serve as a potential therapy for the cognitive and motor deficits found in DS.

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CARDIORESPIRATORY FITNESS IS ASSOCIATED WITH CORTICAL THICKNESS, BUT NOT SURFACE AREA OF THE ENTORHINAL CORTEX IN HEALTHY YOUNG ADULTS.

M. Rosario[1,2], R. Nauer[2,3], M. Dunne[1,3], T. Storer[4], K. Schon[1,2,3]

[1]Graduate Program for Neuroscience,
[2]Brain Plasticity and Neuroimaging Laboratory, Department of Anatomy and Neurobiology
[3]Department of Psychological and Brain Sciences and Center for Memory and Brain, Boston University
[4]Brigham and Women's Hospital, Harvard Medical School

Statement of the Problem/Background: Cardiorespiratory fitness (CRF) is a known modulator of the medial temporal lobe memory system, resulting in structural alterations of the hippocampus and its primary input, the entorhinal cortex (EC). Previous studies in humans have primarily examined the relationship between CRF and cortical volume (CV). Because CV is a composite measure comprised of cortical surface area and thickness that have separate developmental origins, CRF may relate to these two measures differentially. In children and adolescents, CRF appears to preferentially modulate surface area and is associated with decreasing gray matter thickness, whereas in older adults CRF is associated with greater cortical thickness. None of these studies have specifically examined EC morphology. A previous study from our laboratory showed a positive relationship between right EC volume (ECV) and CRF in a sample of young adults, but did not examine cortical thickness or surface area.
Research Question/Hypothesis: Here, we seek to examine whether CRF is equally related to both components of ECV in young adults.
Research Design/Methods: Cross-sectional data were collected from a cohort of 57 young adults (18-35 years, 17 male) enrolled in an exercise study at Boston University School of Medicine. EC thickness and surface area were calculated using T1-weighted structural magnetic resonance images processed with FreeSurfer 6.0. We assessed CRF (estimated-VO_{2max}) using a submaximal-graded treadmill exercise test with a modified-Balke protocol. Multiple regression analyses were used to predict EC thickness and surface area from CRF, controlling for age and sex.
Results/Summary of the Investigation: Multiple regression analysis showed a significant positive relationship between CRF and left EC thickness, while holding age and sex constant (p = .004, β = .314, t (3) = 2.979, *CI* = (.005, .026), *adj.* R^2 = .12)), but not right (p = .912, *CI* = (-3.986, 3.569). CRF was not associated with surface area for left (p = .912, *CI* = (-3.986, 3.569)) or right (p = .475, *CI* = (-1.822, .3.863)) EC. Here we show that, consistent with data from older adults, CRF is associated with cortical thickness but not surface area.

Interpretation/Conclusion: Here we bridge the gap between research conducted in children/adolescents and older adults through examining the association between CRF and brain morphology in young adults. The EC is important for learning and memory and spatial navigation, and EC thickness may be amenable to CRF-induced neuroplasticity in young adulthood. Future research will examine the relationship between structural measures and cognitive outcomes, mediated by CRF, and examine the effect of an exercise intervention on brain morphology.

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NEURAL STEM CELL SUSCEPTABILITY IN CONGENTICAL ZIKA SYNDROME

S. Shelton; J. Connor; T. Haydar; A. Soucy

Graduate Program for Neuroscience

The rapid spread of Zika virus (ZIKV) and its association with abnormal brain development constitute a global health emergency. Congenital ZIKV infection produces a range of mild to severe pathologies, including microcephaly. These pathologies are specific to fetal brain tissue, where neural stem cells and progenitors are targeted. To understand the pathophysiology of ZIKV infection, an *in vivo* mouse model of fetal brain development was used to recapitulate the human cytoarchitecture of early to mid-gestation. ZIKV PRVABC59, isolated from the blood of a patient in Puerto Rico in 2015, was used to characterize ZIKV infection of neural progenitors and resulting changes in mouse brain development. Multiple dose concentrations, inoculation periods, and time points in development have been investigated to characterize the development of congenital Zika syndrome. Gross brain size and cortical thickness have been measured to test what age and inoculation periods result in microcephaly. The targeted neural precursor types were identified at various stages of brain development using in utero electroporation and immunohistochemistry. Changes in neural precursor proliferation and cell death were quantified to determine how these cellular processes may contribute to ZIKV-induced microcephaly. The morphology of infected cells has been perturbed, potentially leading to decreased neural migration. Additionally, differential vulnerability to ZIKV has been found, begging the question of progenitor heterogeneity. Current experiments aim to quantify morphological changes as well as the existence of two distinct neural stem cell populations. These experiments will elucidate the etiology of ZIKV induced microcephaly while adding essential knowledge to the field of cortical development.

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CHARACTERIZING THE RELATIONSHIP BETWEEN FUNCTIONAL CONNECTIVITY AND NEUROCOGNITIVE DEFICITS IN BENIGN EPILEPSY WITH CENTROTEMPORAL SPIKES

E. Spencer; D. Chinappen; L. Ostrowski; D. Song; S. Stoyell; C. Chu; M. Kramer.

Graduate Program for Neuroscience

Benign epilepsy with centro-temporal spikes (BECTS) is a particular type of childhood focal epilepsy where by adolescence all patients spontaneously enter into remission and are no longer at risk for experiencing seizures. BECTS is linked to the development of various sensorimotor deficits that in some cases follow patients into adulthood. There is evidence in other studies of focal epilepsies that the way the brain transiently coordinates the flow of information between cortical regions, i.e. functional connectivity, is disrupted. However, there is limited understanding of the specific differences in functional connectivity between BECTS patients at time of diagnosis and patients in remission, and what is the neurological basis for the neurological deficits. We hypothesize that the impact of BECTS during a

critical period in cognitive development has long-lasting effects on the functional connections and that the differences in functional connectivity provide the neurological basis for why the deficits are present later in life despite the lack of seizure activity. We propose a pipeline to address this hypothesis. We will use electroencephalography recordings to map out the functional connections at different stages of BECTS, compared with age-matched controls to gather information about how signaling between cortical areas has been disrupted. Next, we will determine which differences are predictive of task performance on language and motor tasks. By understanding the differences in brain network organization, we may understand why neurological impairments develop in certain individuals and establish a clinical relationship between functional connectivity and cognitive processes.

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LIFELONG NEUROBEHAVIORAL CHANGES DUE TO ANESTHETIC EXPOSURE IN LARVAL *C. ELEGANS*

G. Wirak[1,2,4,5]; C. Gabel,[1,2,5]; C. Connor, [1,3,6]

[1]Department of Physiology and Biophysics, [2]Department of Pharmacology and Experimental Therapeutics, [3]Department of Biomedical Engineering, [4]Graduate Program for Neuroscience, Boston University School of Medicine; [5]Boston University Photonics Center; [6]Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital

The administration of anesthesia during critical neurodevelopmental periods has been shown to elicit enduring behavioral impairments, in organisms of widely varying neuronal complexity. Indeed, clinical studies suggest that multiply-exposed children experience cognitive deficits and behavioral abnormalities later in life. While it has been presumed that these effects are caused by aberrant neuronal apoptosis, no studies have yet examined the direct effects of developmental anesthetic exposure on neural circuit functionality. The nematode worm *Caenorhabditis elegans* is well suited for such investigations, given its fully-mapped neural connectome and tractability for functional neuronal imaging. We therefore set out to characterize the effects of larval isoflurane exposure on the *C. elegans* circuitry controlling forward and backward movement. Behaviorally, exposed worms responded similarly to controls when confronted with an aversive touch stimulus, indicating that they remain grossly neurologically intact. However, we observed statistically significant reductions in the spontaneous reversal rate of freely moving worms. This deficit persisted into both early and late adulthood. Confocal laser fluorescence microscopy was used to measure spontaneous *in vivo* neuronal activity within a premotor interneuron, known to initiate backward movement in *C. elegans*. Our investigations reveal alterations to the neuronal activity underlying the observed behavioral shift. These findings indicate that larval isoflurane exposure induces persistent changes in neuronal activity dynamics and the relative biasing of this neural network, which are unlikely to be explained by mere apoptotic cell death.

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IN VIVO NEUROVASCULAR CHANGES IN THE VISUAL CORTEX FOLLOWING CLOSED-SKULL MILD TRAUMATIC BRAIN INJURY

E. Witkowski; E. Erdener; K. Kiliç; S. Kura; J. Tang; D. Boas; I. Davison

From war zones to athletics to everyday falls, traumatic brain injury (TBI) is increasingly recognized as a major health concern and financial burden. However, many questions remain as to what changes are occurring in the brain, especially within the first minutes to hours after injury. To better understand what

changes are happening in the brain and when, we measured neural activity and cerebral blood flow *in vivo* in the visual cortex of mice after a closed-skull weight drop model of mild TBI. *In vivo* calcium imaging revealed a dichotomy in neural activity where the majority of the layer 2/3 excitatory cells had significantly less spontaneous activity after injury, but a minority of cells became hyperactivated with extremely long duration repeated calcium transients. Cerebral blood flow in the visual cortex was measured with laser speckle and optical coherence tomography (OCT) which allow for *in vivo* measurements at high temporal and spatial resolution. Laser speckle and OCT showed a sudden drop in cerebral blood flow down to ~50% of baseline levels after mild TBI before gradually recovering by 2 hours. Previous work indicates that the prolonged decrease in blood flow was extensive enough to induce oxidative stress and disrupt normal neural function, and thus may be responsible for the lower neural activity we detected with calcium imaging. Use of minimally invasive, high resolution imaging revealed that even mild TBI induces robust changes in neural activity and blood flow and that the first 2 hours is a critical time window for treating TBI.

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RENEWAL OF MICROGLIA CORRECTS MATERNAL INFLAMMATION-INDUCED MORPHOLOGICAL AND TRANSCRIPTOMIC MICROGLIAL ABNORMALITIES IN MOUSE OFFSPRING

H. Yeh; T. Ikezu

Central immune dysfunction has been implicated in the pathogenesis of neurodevelopmental disorders. Maternal immune activation (MIA) due to viral infection during pregnancy is associated with increased risk of autism or schizophrenia. As brain immune cells, microglia survey the immune landscape and maintain homeostasis. Early prenatal exposure to elevated levels of cytokines from maternal infection leads to abnormal microglial function that contributes to aberrant brain development of progeny, although the biological mechanism of microglial involvement has not been clearly defined. We hypothesize that MIA alters microglial morphology and function, and that renewal of microglia can restore their homeostatic function in offspring. Here, we show that MIA alters microglial gene expression and morphological phenotype in the cortex of MIA offspring. MIA microglia have increased branching complexity and interactions with dendritic spines of intrinsically bursting pyramidal neurons in layer V of the medial prefrontal cortex. RNA-sequencing analysis of acutely isolated microglia revealed enriched neuritogenic signaling pathways and down-regulated interferon-gamma signaling. Renewal of microglia by treatment of MIA offspring with colony stimulating factor 1 inhibitor reversed the MIA microglial phenotype by normalizing branching complexity and reverting transcriptional alterations. This was validated by intrathecal injection of interferon-gamma, which reduced hyper-ramification of MIA microglia and demonstrates its role as an upstream regulator in mediating the effect of MIA in microglia. Using dual pulse labeling of newly repopulated cells with 5-bromo-2'-deoxyuridine (BrdU) and 5-Ethynyl-2´-deoxyuridine (EdU), we showed that MIA microglia repopulate from a non-proliferating pool that is distinct from the pool of saline microglia repopulation. These data demonstrate that maternal immune challenge results in aberrant microglial phenotype lasting into adulthood, and reveals the potent effect of microglial depletion and repopulation on correcting the MIA phenotype of microglia.

Department of Microbiology

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

Bukola Adeoye (1)
Molly Braun (*)
Michael Breen (9)
Neelou Etesami (20)
Alexander Gold (25)
Jeffrey Kuniholm (35)
Whitney Manhart (44**)
Sarah Nodder (51***)
Christine Odom (53)
Kyle Pedro (54)
Allison Thomas (66)
Rachel Yuen (75)

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MYCOBACTERIUM TUBERCULOSIS (MTB) INFECTION IMPACTS HIV-SPECIFIC ANTIBODY RESPONSE

A. Bukola [1], A. Olson[2]: M. Sagar[1],[2]

1 Department of Microbiology and
2 Division of Infectious Diseases, Department of Medicine

Mtb infected macrophages and dendritic cells secrete proinflammatory cytokine, IL-6, which promotes the polarization of T follicular helper (T_{fh}) cells. T_{fh} cells are essential for B cell development and humoral responses. We hypothesized that HIV-1 infected individuals with as compared to without Mtb have different HIV-specific B cell responses. We compared humoral responses among HIV-1 infected Ugandan individuals either with (n= 16) or without Mtb (n= 15) at baseline and six-month follow-up. At follow-up, individuals with pulmonary Mtb had finished their Mtb treatment. All patients were also placed on antiretroviral treatment at presentation. Patient plasma was assessed for its neutralization capacity against a panel of HIV-1 strains (n= 10). We compared a breadth and potency (BP) score which normalizes the average percent neutralization over the entire panel of HIV-1 variants, among the two groups. HIV-1 infected individuals without Mtb (n = 2) pre-treatment BP neutralization score (mean= 0.49 $\pm$ 0.15) was not significantly different (p= 0.62) as compared to HIV-1 infected individuals with Mtb (n= 5) pre-treatment BP neutralization score (mean= 0.44 $\pm$ 0.11). Baseline HIV-1 specific BP neutralization score demonstrated no significant correlation with baseline plasma IL-6 (r= 0.54, p= 0.34) or another myeloid cytokine, sCD163, (r= 0.46, p= 0.43). HIV-1 infected individuals with Mtb co-infection have heightened myeloid cell activation, and analysis of a larger number of samples may reveal that Mtb co-infection impacts HIV-1 humoral responses. These studies may yield novel insights into the generation of HIV-1 neutralizing antibodies.

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DIFFERENT POPULATIONS OF THE RESPIRATORY SYNCYTIAL VIRUS TRANSCRIPTION ELONGATION FACTOR, M2-1, DURING INFECTION

M. Braun[1], E. Blanchard[2], P. Santangelo[2], R. Fearns[1]

1 Boston University School of Medicine and
2 Georgia Institute of Technology

Department of Microbiology

Respiratory syncytial virus (RSV) is the leading cause of pediatric acute lower respiratory tract infection and is a major cause of hospitalization in children. The RSV genome is transcribed and replicated by the viral polymerase in inclusions within the cell cytoplasm. M2-1, a viral mRNA-binding protein, is a transcription elongation factor that aids the polymerase in synthesizing full length mRNA. We have demonstrated that there are two populations of M2-1, which are preferentially recognized by different antibodies. At early times in infection, both populations localize in the viral inclusions, but at later times, one population is found within inclusions whereas the other has a diffuse cytoplasmic distribution. It is unknown if M2-1's role as a transcription elongation factor changes at early versus late times in infection, in correlation with its sub-cellular localization. We sought to understand if these two populations play different roles by examining if M2-1 binds exclusively to viral mRNAs or additionally binds to cellular mRNA. To do this, we used cross-linking immunoprecipitation followed by RNA sequencing (CLIP-seq) to characterized M2-1 bound RNA at early and late times in infection. Our analysis identified RSV and cellular RNA at both times post infection. However, M2-1 bound proportionally more cellular RNA at later times in infection than at earlier times, correlating with the second population of M2-1 residing in the cell cytoplasm. We conclude that in addition to its previously described role in transcription elongation, a subpopulation of M2-1 has an additional role that involves cellular RNA interactions.

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LUNG MEMORY B CELLS POPULATIONS AFTER INFLUENZA INFECTION IN MICE

M. Breen[1,2], F. Feng[2], K. Barker[1,2], A. Hua[2], Y.M.Wang[2], T. Kepler[2], J. Mizgerd [1,2], R. Fearns[2]

[1]Pulmonary Center, Boston University School of Medicine
[2]Microbiology, Boston University School of Medicine

Influenza remains a global health burden despite modern interventions. B cells (BCs) are pivotal protection effectors against reinfection of influenza. They are found in the lung after recovery from infection, but their regulation and function are only beginning to be defined. We hypothesize repeat influenza infection generates lung BC populations that reflect infection history and involve distinct patterns of immunoglobulin (Ig) gene rearrangement and mutations.
Balb/c mice received two intratracheal, sub-lethal infections of influenza A virus (IAV) four weeks apart. Lung and spleen BCs were sorted on memory markers PD-L2 and CD80 while using an intravenous CD45 antibody to reject intravascular leukocytes. Finally, immunoglobulin heavy chain transcript sequencing (IgSeq) from these BCs was used to analyze the clonal repertoire.
IAV-experienced mice ("IAVexp-mice") showed significant accumulation of lung extravascular CD19+ BCs. Of these, IAVexp-mice had higher proportions that were memory-marked with CD80+ and/or PD-L2+ expression versus naive controls. There were no significant differences across treatment groups

between the total numbers of splenic BCs or their memory-marked proportions. Early IgSeq analysis points to increased clonal dominance in the IAVexp-mice's lung PDL2+ and CD80+ populations while only the former population was dominated in the spleen. Interestingly, the data suggests that the dominating BC clones of each organ are not the same. Altogether, these data suggest repeat IAV infections result in altered extravascular BC populations in the lung that differ from the spleen in proportion and clonal dominance. Further analysis of the IgSeq data is required to determine mutation rate and clonal ancestry.

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EVALUATING OUTER MEMBRANE VESICLES ISOLATED FROM RMP-DEFICIENT NEISSERIA GONORRHOEAE AS A GONOCOCCAL VACCINE CANDIDATE

N. Etesami; I. Francis; L. Wetzler

Department of Microbiology

Neisseria gonorrhoeae (GC) is a Gram-negative human pathogen that causes the sexually transmitted disease (STD), gonorrhea. Despite the recent emergence of multidrug resistant strains worldwide, there have been no successful vaccines developed for the prevention of gonorrhea. We have isolated and purified naturally-released outer membrane vesicles (nOMV) derived from the outer membrane of a genetically-modified strain of GC to be used as a potential vaccine. Currently, we are in the process of *in vitro* and *in vivo* characterization of immune responses, as well as testing their protective ability in murine models of upper and lower genital tract infections. We show that nOMV vaccinations via intranasal and subcutaneous routes induce large titers of GC-specific IgG and IgG subtypes indicative of both Th1 and Th2 responses. Preliminary data suggest the presence of IgA titers in mice vaccinated intranasally, but not subcutaneously, in both sera and vaginal secretions. Additionally, mice vaccinated with nOMV generate GC-specific antibodies against an amalgam of different proteins across a variety of strains—including a meningococcal lab strain. Thus far the prospects of using nOMV in vaccines for gonorrhea seem promising as we provide evidence of potentially protective immune mobilization in response to these candidate formulae.

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FURTHER EXPLORATION OF THE RESISTANCE OF AEDES MOSQUITOES TO FILOVIRUS INFECTION

A. Gold; S. Asad; K. Chandran; T. Colpitts; F. Feitosa-Suntheimer; R. Jangra

Department of Microbiology

Aedes aegypti is one of the most prolific vectors of viral disease in the world, and arboviruses transmitted by *Aedes aegypti* are emerging and reemerging at a rate greater than ever. Yet, *Aedes aegypti* remains unable to vector a multitude of clinically relevant viruses, including Ebolavirus (EboV). EboV is a highly virulent filovirus responsible for multiple outbreaks of zoonotic infection over the last century. Previous research identified the Niemann-Pick C1 protein (NPC1), as the necessary intracellular entry receptor for EboV and the closely related Marburgvirus. However, key residues present only in the NPC1 of certain mammalian species have made these species the preferential hosts of filovirus infection, rendering multiple non-mammalian species, such as *Aedes*, resistant to infection. Nonetheless, significant sequence homology between *Aedes* NPC1 and human NPC1 (hNPC1) indicated at the possibility of a gain-of-function mutation that would allow the *Aedes* NPC1 to function as the filovirus entry receptor. With this in mind, we aimed to determine whether this potential gain-of-function would be sufficient for filovirus

entry into *Aedes* cells. By creating a model system of *Aedes* cells that expressed hNPC1 and subjecting these cells to infection by filovirus-pseudotyped vesicular stomatitis virus, it was determined that the expression of hNPC1 in non-permissive cells was not alone sufficient for filovirus entry. This conclusion indicated at the presence of an additional block or multiple blocks of filovirus entry within these cells.

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THE EFFECT OF VACCINE ADJUVANT PorB ON ANTIGEN SPECIFIC MEMORY T CELLS

J. Kuniholm; R. Yuen; C. Lisk; L. Wetzler
Department of Microbiology

Memory T cells persist after retraction of a primary immune response and help hasten the immune response to future exposures. CD4+ Memory T cells ($CD4^+T_M$) recognize extracellular antigen loaded on MHC class II molecules of antigen presenting cells (APCs). Upon antigen recognition, $CD4^+T_M$'s promote antibody secretion by antigen-specific B cells. Accordingly, we expect that an expansion of the antigen-specific $CD4^+T_M$ pool will contribute to an increase in antigen specific antibody production and affinity. Production of these high affinity antibodies is a useful metric when characterizing the efficacy of vaccines.
Our laboratory has previously demonstrated the properties of a bacterial porin protein from *Neisseria Meningitidis*, Porin B (PorB), as a vaccine adjuvant for the weakly immunogenic model antigen, ovalbumin (OVA). These properties include enhancing antigen trafficking to secondary lymphoid organs (SLOs), promoting germinal center (GC) formation, and increasing the amount of co-stimulatory cytokines secreted by splenocytes *in vitro* after OVA stimulation. In this study, we vaccinated OT-II transgenic mice which express T cell receptor specific for OVA (323-339) with PBS, OVA alone, OVA+PorB, and OVA+Alum. We used PE-conjugated MHC Class II tetramers to stain OVA-specific $CD4^+T_M$'s isolated from mouse lymph nodes and spleen. We observed that PorB increases the number of antigen specific $CD4^+T_M$'s in the lymph nodes of OT-II mice 3 weeks after vaccination when used as an adjuvant. This expansion of OVA-specific $CD4^+T_M$'s likely contributes to the observed increase of OVA-specific IgG and supports that PorB improves T cell memory as a vaccine adjuvant.

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DEVELOPMENT OF AN EBOLA VIRUS INFECTION MODEL USING IPSC-DERIVED HEPATOCYTES

W. Manhart; J. Bernbaum; S. Bhatia; N. Crossland; R. Johnson; L. Manci; S. Mazur; G. Mostoslavsky; E. Mühlberger

Department of Microbiology and; National Emerging Infectious Disease Laboratory

Background
Ebola virus (EBOV) infection causes a severe disease in humans. The liver is an early target organ of EBOV infection, at which time viremia spikes and symptoms worsen. Therefore, it is thought that hepatocytes are a key site of viral replication *in vivo* and potentially a promising target for therapeutic interventions. Primary human liver samples are difficult to acquire, and animal models of EBOV infection either incompletely recapitulate disease or are costly. Our goal is to use induced pluripotent stem cell (iPSC)-derived hepatocytes (iHeps) to develop a disease-relevant infection platform for modeling EBOV pathogenesis.
Methods

We differentiated hepatocytes from human iPSCs using a published directed differentiation protocol. iHeps were characterized using flow cytometry, intracellular staining, qRT-PCR, and functional assays. iHeps, primary hepatocytes (PHH), and immortalized Huh7 cells were infected with EBOV at an MOI of 3 and harvested for analysis after 24 hours. Infected cells were analyzed by immunofluorescence, electron microscopy, and 3' RNA-seq (DGE, Broad Institute).

Results

iHeps expressed mature hepatic markers and key hepatic enzymes were active. iHeps and PHHs were less susceptible to EBOV infection compared to Huh7s. By transcriptomics, iHeps and PHHs respond more similarly to infection compared to Huh7 cells, and iHeps express an interferon signature upon infection that is not observed in Huh7 cells but is observed *in vivo*.

Conclusions

iHeps are a suitable *in vitro* model for EBOV infection of hepatocytes. We can now use this model to better understand the molecular mechanisms leading to liver damage during human EBOV infection.

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HIV-2/SIV_{mac} VPR SUPPRESSES A TYPE III IFN RESPONSE IN MONOCYTE-DERIVED DENDRITIC CELLS IN A DCAF-DEPENDENT MANNER

S. Nodder; H. kiyama; J. Berrigan; M. Lei; C. Miller; R. Gummuluru

Department of Microbiology

Vpr is a 14 kDa accessory protein conserved amongst all primate lentiviruses. Packaging of Vpr into virions allows for its immediate delivery into the target cell cytoplasm upon viral fusion. As such, Vpr is poised to facilitate early steps in the lentiviral life cycle. Virion-associated Vpr is known to induce a DNA damage response (DDR) that results in a G_2 to M cell cycle arrest in cycling cells. Vpr activates the DDR by interacting with the E3-ubiquitin ligase CUL4A-DDB1 DCAF, allowing for degradation or modification of select host proteins. However, the consequences of Vpr-mediated DCAF-dependent ubiquitination of host proteins and activation of a DDR on lentiviral replication are not fully understood. In this study, we sought to determine the effect of Vpr-deficiency on Vpx-expressing lentivirus (HIV-2/SIV_{mac}) infection of MDDCs. Whilst infection of MDDCs with Vpx-deficient HIV-2 or SIV_{mac} was severely attenuated, Vpr-deficiency had negligible impact on HIV-2 or SIV_{mac} virus infection. Rather, infection of MDDCs with HIV-2/Vpr^{neg} or SIV_{mac}/Vpr^{neg} resulted in robust induction of type III IFN at 24 h post infection. Induction of type III IFN was abrogated upon pre-treatment with tenofovir (RT inhibitor) but not raltegravir (integrase inhibitor), suggesting that sensing of HIV-2 or SIV_{mac} reverse transcripts in the absence of Vpr induces type III IFN responses. We posit that the HIV-2/SIV_{mac} Vpr has evolved to mitigate host detection of viral infection in the context of robust reverse transcription in MDDCs, thereby allowing for efficient systemic dissemination of virus from mucosal sites of virus transmission.

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LIVER ACTIVITY REMODELS THE LUNG TRANSCRIPTOME AND LIMITS PNEUMONIA SUSCEPTIBILITY DURING SEPSIS

C. Odom; Y. Kim; F. Korkmaz; E. Na; L. Baird; M. Jones; J. Mizgerd; K. Traber; L. Quinton

Department of Microbiology

Septic patients are highly susceptible to nosocomial pneumonia, demanding a better understanding of host pathways influencing this connection. The liver acute phase response (APR) is robustly initiated during sepsis, and we have shown that pneumonia susceptibility is considerably elevated in genetically modified mice lacking an intact liver response. We hypothesized that liver activation remodels the lung transcriptome during sepsis to fortify pulmonary host defense.
Wildtype (WT) mice and APR-deficient littermates lacking hepatocyte STAT3 (hepSTAT3$^{-/-}$), a transcription factor necessary for APR initiation, were systemically challenged with LPS for 18 hours to model sepsis, after which pneumonia was induced by intratracheal *E. coli* instillation. Lungs were collected at multiple timepoints after the *E. coli* challenge to assess the influence of STAT3-dependent liver activity on the pulmonary response to endotoxemia and pneumonia.
RNAseq revealed nearly 2000 significant gene changes between endotoxemic WT and hepSTAT3-/- mice. Subsequent pneumonia in mice with endotoxemia a led to transcriptional remodeling by 24 hours of infection but without observable differences between genotypes. Following endotoxemia alone, bioinformatic analyses of differentially expressed genes in the lungs revealed strong enrichment of pathways related to immune signaling and tissue homeostasis. Despite large transcriptomic differences in lungs collected from hepSTAT3-/- mice during endotoxemia, lung architecture was unaffected, with no histological evidence of pneumonia prior to *E. coli* inoculation.
These results indicate that liver activation reshapes the lung transcriptome during sepsis. Future studies will focus on whether and how such gene changes directly influence pneumonia susceptibility.

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IDENTIFICATION OF NOVEL TRANSCRIPTION FACTORS THAT REGULATE HIV-2 EXPRESSION

K. Pedro[1], J. Sewell[3], J. F. Bass[3], A. Henderson[1,2]

[1]Department of Microbiology
[2]Department of Medicine and,
[3]Department of Biology, Boston University

For the nearly 2 million people infected with HIV-2 there is no cure. Though related to HIV-1, the clinical presentation of HIV-2 infection is distinct. Relative to HIV-1, HIV-2 infection exhibits a delayed onset of immunodeficiency, lower rates of transmission, lower levels of viremia and generates a less inflammatory immune response, despite similar amounts of viral DNA. We hypothesized that these differences in HIV-2 pathogenesis are, at least in part, explained by differences in transcriptional regulation and that HIV-2 expression is influenced by a unique set of transcriptional networks. However, the HIV-2 promoter, the long terminal repeat (LTR), is poorly described. We utilized a high throughput, functional yeast screen to identify potential transcription factors influencing HIV-2 expression. This screen suggested that there are several distinct factors that regulate HIV-2 transcription compared to HIV-1 transcription. We have

focused on validating roles for two transcription factor hits from the screen: Krüppel-like factor 3 (KLF3) and PLAGL1. We first confirmed that these transcription factors are expressed in relevant cell types, including $CD4^+$ T cells, monocyte-derived macrophages (MDMs) and dendritic cells (MDDCs). Next, through transcription factor overexpression and silencing experiments, we determined their effect on HIV-2 expression. Finally, using ChIP-PCR we have characterized binding properties of these factors to HIV-2. These experiments indicate that KLF3 is a transcriptional repressor, whereas PLAGL1 is a transcriptional activator in MDMs. Mechanisms by which KLF3 and PLAGL1 interact for cell type-specific HIV-2 expression and their relationship with other identified candidates will be discussed.

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HIGH-THROUGHPUT ASSAY TO ASSESS ADCC ACTIVITY AGAINST CLINICAL ISOLATES OF HIV-1

A. Thomas, M. Ghulam-Smith, M. Sagar

Department of Microbiology

High throughput antibody dependent cellular cytotoxicity (ADCC) assays are needed to gain insights into the role of ADCC in preventing transmission. Current infection-based assays often use CEM-NKr-CCR5 cells as target cells, but transmitted viruses, such as those circulating in infected individuals, often cannot replicate in these cells. Two different CD4 T cell lines, PM1 and MT4, were transduced with a CCR5 and a tat-inducible luciferase expression plasmid. Target cells were exposed to primary and lab-adapted HIV-1 strains and cultured with the NK cell line, KHYG-1. Percent ADCC was calculated as the loss of luciferase expression in the presence as compared to the absence of antibodies. Incubation with NK cells, without HIV-1 antibodies, decreased luciferase only in infected PM1-CCR5-Luc (43.87%, range=31.5-56.7), suggesting PM1 but not MT4 cells were highly susceptible to background NK cell killing. Thus, PM1-CCR5-Luc cells were not examined further and MT4-CCR5-Luc cells were deemed NK cell resistant. NL4-3 infected CEM-NKr-CCR5-Luc (68.2%, range=55.4-83.8) and MT4-CCR5-Luc (70.6%, range=60.8-78.0) yielded similar ADCC estimates in the presence of 500ug/ml HIV-1 IgG ($p=0.79$). While NL4-3 replicated in both CEM-NKr-CCR5-Luc and MT4-CCR5-Luc cells, fold luciferase expression over background was only elevated in the MT4-CCR5-Luc cells after infection with primary CCR5-using variants. In MT4-CCR5-Luc cells, similar ADCC estimates were obtained in the presence of heat inactivated plasma compared to isolated IgG ($p=0.31$). Our MT4-CCR5-Luc cell line can be used to estimate ADCC activity present in plasma, breast milk and among immunoglobulins against both primary and T/F strains.

GERMINAL CENTER B CELL AND T FOLLICULAR HELPER CELL RESPONSES INDUCED BY TLR-BASED ADJUVANTS

R. Yuen[1]; C. Lisk[2]; L. Wetzler[1,2]

[1]Department of Microbiology and,
[2]Department of Medicine, Section of Infectious Diseases, Boston Medical Center

Germinal centers (GC) are essential for the development of high-affinity antibodies and long-lived plasma cells (PC) and memory B cells (MBC), the main contributors to an efficacious vaccine. Adjuvants, the main stimulatory component of a vaccine, have been shown by many groups to promote the GC response. However, the mechanism and alteration of GC cellular dynamics in which adjuvants promote this response is not well understood. Toll-like receptor (TLR) agonists have long been appreciated as adjuvants due to their well characterized signaling pathways. Our group has recently shown that the TLR2 ligand, PorB is able to increase GC formation and GC B cells, 7 days after a second immunization. In this study, we set out to investigate the kinetics of this enhanced GC formation and the overall GC response. Animals were immunized in a prime-boost regimen with the model antigen, ovalbumin (OVA) alone or in combination with PorB or the endosomal TLR9 ligand, CpG. We observed that OVA+PorB treatment caused the greatest enhancement of GC formation and recruitment of T follicular helper cells (Tfh) into GCs. OVA+CpG treatment also showed enhanced GC formation compared to OVA alone treatment groups, however there were less Tfhs cells within these GCs overall. In our flow cytometric evaluation of non-antigen specific MBC and PC populations, OVA+PorB treatment showed the greatest increase compared to vehicle control. A similar trend is also seen with the OVA-specific IgG levels. Overall, these results show that the addition of adjuvants, particularly TLR-based adjuvants, promotes the development of humoral immunity.

Graduate Program in Molecular and Translational Medicine

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

Gabriela Baldeon Vaca (4)
Batbold Boldbaatar (6)
Jess Floro (22)
Richard Giadone (23**)
Christina Lisk (41***)
Elim Na (49)
Rekha Raghunath (*)

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TOPICAL USE OF MONOCLONAL ANTIBODIES AS A MULTIPURPOSE PREVENTION TECHNOLOGY OFFERING CONTRACEPTION AND DECREASED TRANSMISSION OF HIV-1 AND *TRICHOMONAS VAGINALIS, IN VITRO*

G. Baldeon Vaca; J. Marathe; J. Samuelson; E. Mausser; J. Politch; J. Doud; K. Whaley; D. Anderson

Program in Molecular Translational Medicine and Department of Medicine

New transgenic antibody production platforms enable cost-effective, rapid manufacturing of antibodies for clinical applications. Our lab is investigating a human monoclonal antibody, the Human Contraceptive Antibody (HCA), produced in *Nicotiana benthamiana*, as a candidate for a topical Multipurpose Prevention Technology offering both contraception and protection against sexually transmitted infections (STIs). HCA was developed from a sperm-agglutinating antibody known to immobilize sperm cells preventing conception. The antibody targets a GPI-anchored glycoprotein, CD52g, produced specifically by epithelial cells in the male reproductive tract (MRT). Due to its anchor, the hypermobile protein is known to coat sperm as they migrate along the MRT. We hypothesized that CD52g incorporates into other cells in the MRT, including certain enveloped STI pathogens, and that the agglutinating property of HCA could entrap the CD52g-coated pathogens affording STI protection alongside contraception. We tested HCA's contraceptive properties under physiologically relevant conditions, *in vitro*. We demonstrated that HCA agglutinates sperm in under a minute at $\geq$25 μg/mL regardless of sperm concentration or exposure to low pH as is found in the vaginal tract. We further demonstrated that GPI-anchored CD52g is present on the surface of *Trichomonas vaginalis* and HIV-1 virions that have been exposed to seminal plasma (source of CD52g). While HCA alone did not agglutinate the pathogens, the antibody trapped both STI pathogens in sperm agglutinates, decreasing the likelihood of infection. These data indicate that HCA is a promising candidate for use in topical microbicides to achieve contraception and prevent the male-to-female transmission of certain STIs

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EXPRESSION OF TRANSTHYRETIN FROM PATIENT-DERIVED CELL MODEL OF WILD-TYPE TRANSTHYRETIN AMYLOIDOSIS

B. Boldbaatar; L. Connors

Program in Molecular and Translational Medicine

Wild-type transthyretin amyloidosis (ATTRwt) is a protein misfolding disease that commonly features cardiocentric symptoms due to deposition of transthyretin (TTR) fibrils in the myocardium. Recently, the single nucleotide polymorphism (SNP), rs3764479, located in the *TTR* proximal promoter was shown to be significantly associated with clinical features of ATTRwt disease. Preliminary results from functional testing experiments indicated that rs3764479 may disrupt the binding of liver-specific transcription factors, such as HNF1 and HNF3b, at this regulatory site in the *TTR* gene. To further investigate the functional consequences of rs3764479 in ATTRwt, we reprogrammed patient peripheral blood mononuclear cells into an induced pluripotent stem cell (iPSC) line that is homozygous for the SNP; patient-derived cell-based studies should provide more clinically relevant results. We are measuring TTR expression at gene (mRNA) and protein (TTR) levels in hepatocyte-like cells derived from the ATTRwt patient cell line (ATTR7 or 2013080) along with controls from healthy individuals that are either homozygous wt (BU6) or heterozygous for rs3764479 (BU5). At Day 16, both ATTRwt and control cell lines consistently showed elevated levels of transcriptional markers characteristically expressed in mature hepatocytes (e.g., AAT, albumin) and coincidently low concentrations of stem cell markers (OCT4). These results indicate that differentiation was achieved and appropriate cell type was obtained in all study groups (patient and controls). We anticipate additional data using this iPSC-based model will provide insight into the molecular mechanisms driving the pathogenesis of ATTRwt disease and enable us to specifically define the functional effect of rs3764479 SNP on regulation of the *TTR* gene.

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SILENCING DEFECTIVE 2 (SDE2) IN RNA SPLICING AND TRANSLATIONAL EFFICIENCY

J. Floro; A. Dai; R. Flynn; A. Labadorf; A. Metzger

Program in Molecular and Translational Medicine

RNA splicing is an essential component of cellular viability. Deficiencies in splicing can lead to defects in development, promote the formation and progression of cancer and other diseases, and/or compromise cellular and organismal viability. The regulation governing accurate and efficient RNA splicing is extraordinarily complex, and requires a vast repertoire of protein factors. Here, we identify Silencing Defective 2 (SDE2) as an essential gene and major player in the regulation of transcriptional integrity and RNA splicing. Specifically, we demonstrate that loss of SDE2 results in widespread defects in mRNA splicing and alternative polyadenylation in mammalian cells. Furthermore, these defects in RNA splicing have significant effects on protein translation, leading to globally decreased protein production and ultimately, complete loss of cell viability. While the exact molecular function of SDE2 is unknown, future studies aim to elucidate its role in RNA metabolism and translational efficiency.

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HEPATIC PROTEOSTASIS IN THE PATHOGENESIS OF ATTR AMYLOIDOSIS

R. Giadone; D. Liberti; T. Matte; J. Rosarda; N. Skvir; J.C. Jean; A.Wilson; D. Kotton; L. Wiseman; G. Murphy

Program in Molecular and Translational Medicine

Hereditary transthyretin amyloidosis (ATTR) is a multi-system protein folding disorder in which misfolded transthyretin (TTR) is secreted from the liver and deposited as toxic protein aggregates at downstream target organs. Our laboratory has developed the first, comprehensive induced pluripotent stem cell (iPSC)-based model of ATTR, capable of recapitulating key aspects of human amyloid disease pathogenesis. Here, we utilized this model, in combination with gene editing, to develop a universal correction strategy that eliminates the production of all destabilized TTRs and consequently reduces downstream target cell toxicity. We further applied this approach in parallel with single cell RNA sequencing to compare transcriptomic profiles between control iPSC-derived hepatocyte-like cells (HLCs) and those expressing destabilized TTR species, with the only difference being the presence or absence of the disease-causing mutation. In doing so, we found distinct transcriptional changes in cells expressing the disease-associated TTR mutant, including activation of unfolded protein response (UPR) signaling pathways (ATF6 and IRE1) shown to protect the extracellular space from proteotoxic TTR aggregation. To assess the potential protective effects of hepatic activation of adaptive UPR signaling pathways, we then generated an inducible ATF6 patient-specific iPSC line. Upon differentiating this line into HLCs and activating ATF6 signaling, we observed a reduction in the secretion of destabilized TTR moieties, accompanied by a decrease in the formation of extracellular TTR aggregates. Together, these results present potential novel therapeutic strategies for ATTR and suggest a role for the liver in modulating pathogenesis of this disease and other liver-based systemic amyloid diseases.

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DETERMINING THE ROLE OF SUBCAPSULAR MACROPHAGES IN ADJUVANT-DEPENDENT ANTIBODY PRODUCTION

C. Lisk[1], D. Antos[1], J. Kuniholm[2], R. Yuen[2], L. Wetzler[1, 2]

[1] Department of Medicine, Section of Infectious Diseases, Boston Medical Center
[2] Department of Microbiology, Boston University School of Medicine

Program in Molecular and Translational Medicine

While vaccines are arguably the most significant advancement in modern medicine, deadly pathogens exist. Vaccine development has paralleled with greater understanding of the immune system – specifically how innate immune cells interact with adaptive immune cells providing protection. The discovery of Toll-like receptors (TLRs) and use of TLR agonists has been a cornerstone of vaccine research via adjuvants. We investigated subcapsular sinus macrophages ($CD169^+$), and how their presence or absence influences antibody production induced by TLR-adjuvanted vaccines. Two mouse models were used – CD169 knockout mice and low dose clodronate treatment of B6 mice. Low dose clodronate causes apoptosis of $CD169^+$ macrophages. Vaccines consisted of ovalbumin (OVA) or OVA plus an adjuvant. The adjuvants used were a TLR2 agonist (PorB), a TLR9 agonist (CpG), and aluminum salts (alum). PorB and alum vaccines induced significant increases in OVA-specific IgGs levels which were abated in CD169 knockout

and clodronate treated mice. One explanation for these results could be a lack of antigen deposition on the follicular dendritic cells (FDC). Mice were injected with fluorescently labelled OVA +/- adjuvants. Antigen deposition on FDCs was quantified via immunohistochemistry and flow cytometry. PorB increased antigen deposition in wild type mice. CD169 knockout mice and low dose clodronate treated mice showed decreased antigen present in lymph nodes compared to wild-type controls. These studies stress the continuous need for investigating the roles of adjuvants and cellular interactions to allow for better vaccine development.

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DETERMINING EPITHELIAL-SPECIFIC ROLES FOR THE LUNG-PROTECTIVE CYTOKINE LEUKEMIA INHIBITORY FACTOR (LIF) DURING PNEUMONIA

E. Na; L. Baird; M. Jones; Y. Kim; F. Korkmaz; J. Mizgerd; C. Odom; L. Quinton; K. Traber;

Program in Molecular and Translational Medicine

Pneumonia is responsible for an incredibly large burden of disease across the globe, particularly for children and seniors. It has been established that leukemia inhibitory factor (LIF), an IL-6 family cytokine, is essential for minimizing acute lung injury during pneumonia. Furthermore, lung epithelium is the primary source of LIF in pneumonic mice. However, it is unknown which target cells and signaling networks are responsible for LIF-mediated protection. We hypothesize that LIF signaling through its unique receptor, LIFR, in lung epithelium induces cytoprotective pathways to limit acute lung injury during pneumonia. To test this, a novel mouse model lacking LIFR in lung epithelium (EpiLIFR$^{\Delta/\Delta}$) was generated and challenged. Pneumonic outcomes were measured after 15- and 24-hr intratracheal *E. coli* inoculation. Compared to Cre-negative littermates or non-pulmonary tissues of either genotype, EpiLIFR$^{\Delta/\Delta}$ mice exhibited *Lifr* gene rearrangement and concomitant expression of mutant mRNA. LIFR whole-lung protein levels trended lower in EpiLIFR$^{\Delta/\Delta}$ mice ($p = 0.23$) but did not reach statistical significance, requiring a more epithelial-specific approach for this outcome. Moreover, initial pilot studies did not reveal differences in pneumonia susceptibility, as measured by pulmonary edema, airspace cellularity, and inflammatory cytokines. To date, pneumonia susceptibility in EpiLIFR$^{\Delta/\Delta}$ mice has only been addressed in response to a single pathogen. Thus, additional time points and pathogens are required to fully determine the functional significance of epithelial LIFR deletion. These findings support EpiLIFR$^{\Delta/\Delta}$ mice as an effective tool for examining LIF biology during pneumonia.

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INTEGRATED GLYCOMIC, PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS IN AGING AND PARKINSON'S DISEASE.

R. Raghunathan[1]; J. Hogan[3], A. Labadorf[3]; R. Myers[4]; J. Zaia[1,2,3.]

[1] Program in Molecular and Translational Medicine

[2] Department of Biochemistry

[3] Bioinformatics Program

[4] Department of Neurology, Boston Medical Center

Department: Molecular and Translational Medicine.

Age is the primary risk factor in Parkinson's disease (PD). Many molecular changes that occur with age are similar to those with neuropathology. To understand changes in age linked to risk or early markers of neuropathology, we studied glycomic, proteomic, and transcriptomic patterns in a cohort of 37 human prefrontal cortex grey matter brain specimens ranging in age from 36-97 years. We compared these findings with changes in 25 control versus 28 PD brains. The results showed no significant changes in heparan sulfate (HS) and chondroitin sulfate (CS) abundances and sulfation with age and PD. We observed age-associated changes in proteins linked with pathways of metabolism, oxidative phosphorylation that have been associated previously with Parkinson's to be upregulated. By contrast, pathways associated with PI-3 kinase-Akt signaling, endocytosis and dopamine synapses, were downregulated with age. We correlated the transcriptomic and proteomic information for changes with age and PD. The combined transcriptomics, glycomics, proteomics data highlight normal versus pathological neurological aging and PD.

Nutrition and Metabolism Program

Participants

Grace Datu Tasik (16)
Yuhan Qiu (56)

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EFFECT OF DIFFERENT TYPES OF STATINS: SIMVASTATIN, LOVASTATIN AND PITAVASTATIN ON GLUCOSE-STIMULATED INSULIN SECRETION AND INSULIN CONTENT FROM CLONAL PANCREATIC BETA-CELLS (INS-1)

G. Tasik, J. Bodde, J. Deeney, J. Hamilton, E. Kolar, N. Burritt, K. Erion, M. Sitaram, B. Corkey, D. Hajjar, A.M. Gotto, C. Sponseller

Nutrition and Metabolism Program

Cardiovascular disease (CVD) remains the leading cause of death globally. Statins are known as first-choice agents to reduce high blood cholesterol, which is a dominant risk factor for CVD. However, clinical trials report that some statins increased the risk for type 2 diabetes (T2D). Our objective was to investigate the effect of different statins on insulin secretion and content from pancreatic β-cells and the underlying mechanism behind it.

The effects of simvastatin, lovastatin and pitavastatin on GSIS and content were studied in clonal pancreatic β-cells (INS-1 832/13) cultured in high glucose (11 mM). Insulin content and secretion were measured after chronic and acute incubation of statins using homogenous time-resolved fluorescence (HTRF) insulin assay kit. Intracellular Ca^{2+} was measured using fura-2 AM (Invitrogen).

Simvastatin (25-200 nM) and lovastatin (50-200 nM) significantly inhibited GSIS and depleted insulin content in a dose-dependent manner after 72-hour exposure. When the secretion level was normalized for content, the inhibitory effect was not observed. Simvastatin (200 nM) also increased the amplitude of intracellular Ca^{2+} oscillations at low glucose, but this was not reflected in the amplitude of oscillatory insulin release. In contrast, pitavastatin (25-200 nM) did not affect GSIS and only decreased insulin content at the highest dose tested.

Inhibition of GSIS by simvastatin and lovastatin could be potentially due to depletion in content and decreased Ca^{2+} sensitivity. Pitavastatin had less detrimental impact on GSIS and content. The results from our studies revealed cellular mechanisms of distinct statins that could explain the differential risk of developing T2D.

THE EFFECT OF A PUTATIVE ACYL-COA SYNTHETASE 5 INHIBITOR ON LIPID ACCUMULATION AND INSULIN RELEASE FROM CLONAL PANCREATIC BETA-CELL

Y. Qiu; V. Waiyaki

Nutrition and Metabolism Program

A single nucleotide polymorphism of the transcription factor 7 like 2 (TCF7L2) is seen as the causal variant that is associated with increased risk for type 2 diabetes (T2D) and has recently been shown to increase acyl CoA synthetase 5 (ACSL5) mRNA level. This regulation of ACSL5 gene expression highlights the importance of investigating the role of ACSL5 in T2D. ACSL5 is one of a family of enzymes that activates fatty acid (FA) to its CoA ester for FA metabolism within cells. ACSL5 knock-out mice have reduced fat mass and are more insulin sensitive.

Chronic exposure of clonal pancreatic β-cells to excess nutrients has been shown to result in increased intracellular lipid droplets, reduced insulin content and a left-shift in the glucose dose-dependent insulin secretion curve characterized by basal insulin hypersecretion (IH) and blunted glucose stimulated insulin secretion (GSIS). We hypothesisized that the use of a putative ACSL5 inhibitor (AdipoC) would reduce lipid droplets, rescue insulin content and reverse the left-shift in GSIS.

AdipoC (10-25 μM) reduced acute fatty acid incorporation and lipid accumulation accompanied by increased insulin content and a right-shift in GSIS in □-cells. Intracellular Ca^{2+} activity was dampened at low glucose and increased at high glucose by AdipoC, which resembled □-cells cultured in 4 mM glucose having reduced lipid stores. These results all indicate possible protective effects on □-cells exposed to excess nutrients. Islets of T2D patients are exposed to a similar excess nutrient environment. Therefore, these results warrant further exploration of AdipoC and its therapeutic potential.

Department of Pathology & Laboratory Medicine

Participants

Elysia Heilig (27)
Jessica Kenison-White (30)

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THE ROLE OF SERPINA3 IN THE PATHOGENESIS OF KIDNEY DISEASE

E. Heilig; M. Belghasem; V. Chitalia

Department of Pathology and Laboratory Medicine

Chronic kidney disease (CKD) is a global issue. The treatment of CKD imparts a costly burden on the American healthcare system, therefore the need for therapeutics that prevent the progression of CKD is urgent. Microarray studies have established that SERPINA3 is transcriptionally upregulated in kidney injury. We hypothesize that SERPINA3 might not only be a transcriptional biomarker for kidney injury, but might act as an upstream regulator in the advancement of CKD. Our research characterizes the expression patterns of SERPINA3 in four models of kidney injury through immunoblotting and immunohistochemistry. The unilateral ureteral obstruction (UUO) model of renal injury displays glomerular localization of SERPINA3. The adenine diet model and the renal ischemic reperfusion injury (RIRI) model of kidney injury portray tubular upregulation of SERPINA3. The DOCA-salt hypertension model was performed on two strains of mice, C57BL/6 and 129/sv, both of which display tubular and glomerular upregulation of SERPINA3. The C57BL/6 strain, known for its resistance to glomerular sclerosis, displays higher renal localization of SERPINA3 when exposed to DOCA-salt hypertension, than does the 129/sv strain. Our data suggests that SERPINA3 protein is upregulated in kidney injury. The role of SERPINA3 in these models remains unknown, however, we theorize that SERPINA3 protein may be renoprotective in instances of kidney injury. Functional assays must be performed to elucidate the role of SERPINA3 in these models. Characterizing the function of SERPINA3 in kidney injury might aid in the development of novel therapeutics to prevent the advancement of CKD.

THE ARYL HYDROCARBON RECEPTOR (AHR) AS A DRIVER OF CANCER IMMUNITY

F. Kenison-White; Z. Wang; D. Sherr

Department of Pathology, Program in Immunology

The Aryl Hydrocarbon Receptor (AHR) has been identified as a driver of cancer progression and cancer immunity in the tryptophan-IDO/TDO-kynurenine-AHR pathway. Over the last 10 years, studies have shown that AHR mediates immune suppression, at least in part, through regulation of inflammatory and inhibitory T cell subsets, macrophages and immune suppressive MDSCs. These results suggest that the AHR may be a driver of tumor-mediated immunosuppression and cancer immunity. To test this hypothesis, we used molecular and pharmacological approaches to regulate AHR activity in multiple murine cancer models and evaluate the immune phenotypes. The resulting data indicate that a novel, non-toxic AHR inhibitor, HP163 (Hercules Pharmaceuticals), reduces tumor growth in orthotopic models of oral (MOC1), breast (4T1), and skin (B16) cancers in immunocompetent hosts. In the MOC1 oral cancer model, HP163 significantly reduces the number of $CD11b^{+}PD\text{-}L1^{+}$ or $CD11b^{+}CCR2^{+}$ cells in the tumor draining lymph node. AHR knockdown with CRISPR/Cas9 in MOC1 cells decreases the percentage of $CD11b^{+}PD\text{-}L1^{+}$ cells, as well as $CD4^{+}PD\text{-}1^{+}$ and $CD4^{+}CTLA4^{+}$ cells. This decrease in immunosuppressive cells is accompanied by a complete lack of tumor growth. Furthermore, mice having received AHR^{KO} MOC1 cells are completely resistant to a second challenge with wildtype AHR^{+} MOC1 cells several months after the primary inoculation. These data suggest that the presence of AHR in the tumor itself is sufficient to induce immunosuppression. Preliminary data suggest that AHR^{+} myeloid cells may mediate suppression of tumor immunity. These results collectively suggest that targeting the AHR may be a novel approach to cancer immunotherapy.

Department of Pharmacology & Experimental Therapeutics

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

Kristyn Borelli (7)
Veronica Go (24)
Yoonjoo Lee (39)
Sanghee Lim (40***)
Ying Jie Lock (42)
Emily Mason-Osann (45**)
Brandon Maziuk (*)
Kathryn Odamah (52)
Franco Puleo (55)
Sema Quadir (57)
Ryan Quinton (58)
Margarita Tararina (65)
Marc Vittoria (70)

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REWARD SENSITIVITY IN *HNRNPH1*$^{+/-}$ MICE FOLLOWING ACUTE METHAMPHETAMINE ADMINISTRATION AS MEASURED VIA INTRACRANIAL SELF-STIMULATION

K. Borrelli[1], C. Bryant[1]

[1]Department of Pharmacology and Experimental Therapeutics

Psychostimulant addiction is a heritable substance use disorder whose genetic basis is largely unknown. Several quantitative trait loci (QTL) in mice have been linked to addiction-related behaviors. The genes underlying these loci could provide clinical insight to the contribution of genetic factors in addictive disorders. We previously mapped and validated *Hnrnph1* (heterogeneous nuclear ribonucleoprotein H1) as a quantitative trait gene underlying variance in methamphetamine (MA)-induced locomotor activity. Mice heterozygous for a frameshift deletion in coding exon IV of *Hnrnph1* (*Hnrnph1*$^{+/-}$) display decreased locomotor activity in response to MA compared to their wild-type littermates (*Hnrnph1*$^{+/+}$). Microdialysis studies additionally show reduced MA-induced dopamine release in *Hnrnph1*$^{+/-}$ mice. We employed Intracranial self-stimulation (ICSS), an operant behavioral paradigm commonly used to assess shifts in sensitivity to stimulation of the dopaminergic mesolimbic reward circuit, to assess changes in ICSS responding following acute MA administration in *Hnrnph1*$^{+/-}$ mice. Using stereotaxic surgery, we implanted a unilateral, stimulating electrode into the medial forebrain bundle (MFB). Activation of these fibers produces robust brain stimulation reward (BSR). Mice were trained on a fixed ratio 1 (FR1) schedule to receive MFB stimulation for each 1/4 turn of a response wheel. Increasing doses of MA were then administered every other day to detect MA-induced changes in BSR-associated reinforcement. We identified dose-dependent changes in the rate of ICSS responding in *Hnrnph1*$^{+/-}$ mice compared to wild-type littermates following acute MA administration. These findings support our previous work suggesting that *Hnrnph1* dysfunction disrupts the rewarding properties of MA, further implicating this RNA binding protein in reward circuitry modulation.

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MESENCHYMAL STEM CELL DERIVED EXOSOMES FOR RECOVERY AFTER CORTICAL INJURY

V. Go[1], B. Bowley[2], M. Pessina[2], D. Rosene[2], M. Medalla[2], B. Buller[3], T. Moore[2]

1 Department of Pharmacology & Experimental Therapeutics
2 Department of Anatomy & Neurobiology
3 Henry Ford Health Systems, Detroit, MI

Exosomes are extracellular vesicles that mediate cell-to-cell communication, and have promising therapeutic effects for cortical injury. Specifically, in our rhesus monkey model of cortical injury exosome-treated monkeys showed a significantly greater degree of recovery of fine motor recovery than monkeys receiving a vehicle control. To assess potential mechanisms mediating this recovery, we analyzed blood and CSF samples collected across recovery and terminal brain tissue to quantify Myelin Basic Protein (MBP), inflammatory cytokines and microglia. Exosome treated monkeys had reduced MBP in CSF and RANTES and Eotaxin in serum. Optical density quantification of microglial markers Iba1, P2RY12, and the MHCII antigen presenting marker, LN3, showed that exosome-treated monkeys had lower expression of LN3 ($p=0.02$). Evaluation of microglial morphologies confirmed that there were less hypertrophic "inflammatory" LN3+ microglia in exosome monkeys ($p<0.05$), and more ramified "homeostatic" microglia (LN3- and LN3+) ($p<0.05$) in vehicle control monkeys. Further, 3D Sholl analyses demonstrated increased microglial branching complexity, supporting a shift to from an inflammatory to a homeostatic phenotype, in exosome-treated monkeys. These results suggest that exosomes reduce myelin damage and inflammation following cortical injury and provide compelling data for the translational value of exosomes as a treatment for human stroke patients.

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PURINORECEPTOR ACTIVATION INDUCES SUSTAINED CALCIUM OSCILLATIONS WHICH DRIVES COLLECTIVE CELL MIGRATION DURING EPITHELIAL WOUND REPAIR

Y. Lee; K. Sack; C. Gabel; V. Trinkaus-Randall

Department of Pharmacology and Experimental Therapeutics and Department of Biochemistry

Improper wound repair of the corneal epithelium is the 4th highest cause of preventable blindness. Epithelial wound healing requires the coordination of cells to migrate as a unit over the basement membrane after injury. To understand the process of this coordinated movement, it is critical to study the dynamics of cell-cell communication. We developed a method to characterize the injury-induced sustained Ca2+ mobilizations that travel between cells for periods of time up to several hours. These events of communication are concentrated along the wound edge and are reduced in cells further away from the wound. Our goal was to delineate the role and contribution of these sustained mobilizations and using MATLAB analyses, we determined the probability of cell-cell communication events in in vitro models and ex vivo organ culture models. We demonstrated that the injury response was complex and represented the activation of a number of receptors. In addition, we found that pannexin channels mediated the cell-cell communication and motility. Furthermore, the sustained Ca2+ mobilizations are associated with changes in cell morphology and motility during wound healing. The results demonstrate that both purinoreceptors and pannexins regulate the sustained Ca2+ mobilization necessary for cell-cell communication in wound healing.

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IDENTIFICATION OF THE KINASE STK25 AS AN UPSTREAM ACTIVATOR OF LATS SIGNALING

S. Lim; and N. Ganem

Department of Pharmacology and Experimental Therapeutics

The Hippo pathway maintains tissue homeostasis by negatively regulating the oncogenic transcriptional co-activators YAP and TAZ. Though functional inactivation of the Hippo pathway is common in tumors, mutations in core pathway components are rare. Thus, understanding how tumor cells inactivate Hippo signaling remains a key unresolved question. Here, we identify the kinase STK25 as an activator of Hippo signaling. We demonstrate that loss of STK25 promotes YAP/TAZ activation and enhanced cellular proliferation, even under normally growth-suppressive conditions both in vitro and in vivo. Notably, STK25 activates LATS by promoting LATS activation loop phosphorylation independent of a preceding phosphorylation event at the hydrophobic motif, which represents a form of Hippo activation distinct from other kinase activators of LATS. *STK25* is significantly focally deleted across a wide spectrum of human cancers, suggesting *STK25* loss may represent a common mechanism by which tumor cells functionally impair the Hippo tumor suppressor pathway.

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DEFINING THE ROLES OF ETAA1 AND TOPBP1 AT ALT TELOMERES

Y.J Lock; R. Flynn

Department of Pharmacology and Experimental Therapeutics

About 15% of human cancers rely on a telomere maintenance mechanism called the alternative lengthening of telomeres (ALT). Telomeres in these cancer cells are prone to replication stress and are hypersensitive to ATR inhibitors. These findings suggest dependence on ATR, a DNA damage response protein and checkpoint kinase, and points to a potential therapeutic target for ALT cancers. ATR kinase activity is known to be directly stimulated by TopBP1 and ETAA1 in response to DNA damage and replication stress. However, little is known about what regulates ATR activity at telomeres in ALT cancers.

Preliminary findings show that both TopBP1 and ETAA1 localize to telomeres in ALT cancer cell lines, but not in non-ALT cancer cell lines. This suggests that TopBP1 and ETAA1 are responding to the increased replication stress occurring at ALT telomeres and are responsible for driving ATR-mediated damage signaling to initiate ALT-mediated telomere elongation. Thus, our hypothesis is that TopBP1 and ETAA1 both activate ATR at ALT telomeres to drive telomere elongation. In future studies, we hope to further understand how TopBP1 and ETAA1 are recruited to ALT telomeres and how whether they work together or independently to affect ALT phenotypes. Understanding the roles of TopBP1 and ETAA1 at the telomere will better inform our efforts to target ALT-positive cancers.

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RAD54 PROMOTES TELOMERE ELONGATION BY THE ALTERNATIVE LENGTHENING OF TELOMERES PATHWAY

E. Mason-Osann; R. Flynn

Department of Pharmacology and Experimental Therapeutics

Cancer cells overcome progressive telomere shortening, inducing replicative immortality, by promoting telomere elongation either through enzymatic activity of telomerase or through the Alternative Lengthening of Telomeres (ALT) pathway. ALT is a recombination-based mechanism whereby one telomere uses telomeric DNA from a homologous or non-homologous chromosome as a template for elongation. Recombination at ALT telomeres resembles break-induced replication (BIR), a pathway initiated to repair a single-ended DNA double-strand break (DSB). During BIR, a single-ended DSB is resected, leaving a 3' single-stranded DNA overhang, which can invade a region of homology in a duplexed DNA template. The single-stranded end is extended by DNA polymerases eta and delta and then resolved by dissolution or nucleolytic cleavage. In vitro, the formation and resolution of recombination intermediates are regulated, in part, by the ATP-dependent DNA translocase, RAD54. Therefore, we hypothesized that RAD54 contributes to ALT activity by regulating BIR at telomeric DNA. Here, we demonstrate that RAD54 is enriched at ALT telomeres, and while RAD54 is dispensable for the formation of early recombination intermediates, it is critical for the maintenance and resolution of later recombination intermediates. Specifically, we found that depletion of RAD54, or loss of its ATP hydrolysis activity, inhibit telomeric DNA synthesis during BIR. Notably, the combined depletion of RAD54 and SLX4, a nuclease that cleaves recombination intermediates, leads to the accumulation of ultrafine anaphase bridges, indicative of persistent and unresolved recombination intermediates. Together, we conclude that RAD54 contributes to the ALT pathway by promoting extension and resolution of telomeric recombination intermediates.

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DEREGULATION OF RNA BINDING PROTEIN FUNCTION AND mRNA PROCESSING IS A MAJOR PATHOLOGICAL FEATURE OF TAUOPATHY

B. Maziuk,; J. Abisambra,; D. Apicco,; P. Ash; S. Boudeau; AL Cruz,; EL. da Rocha; L. Jiang; J. Leszyk; H. Li; C. Ung,; B. Wolozin; WH. Yu; C. Zhang

Department of Pharmacology and Experimental Therapeutics

Recently, our lab has identified ribonucleoprotein granules, particularly stress granules (SGs), as major components of AD pathology which coincide with the development of tau pathology. We have recently reported that *in vivo* reduction of the stress granule nucleating protein TIA1 rescues the neurodegenerative phenotype of mice which develop NFT pathology. Following this, we sought to determine if other stress granule and/or RNA binding proteins (RBPs) may be involved in the progression of tauopathy as well as characterize the impact of these interactions on mRNA function. We used a proteomic approach followed by biochemical solubility fractionation and immunohistochemical validation to identify disease relevant tau-RBP complexes in the PS19 mouse model of tauopathy. We then used RNA sequencing and bioinformatics analysis to identify mRNA deregulation in tauopathy mice.
From these studies we report that significant changes in the association between tau and RPBs occur as a part of tau pathophysiology. Notably, there are significant alterations in how tau associates with ribosomal subunit proteins, initiation factor proteins, and stress granule proteins including DDX6, EWSR1, HNRNPA0, and PABP. Striking deregulation of mRNA expression and splicing are also

observed in PS19 mice, but are rescued *in vivo* with reduction of TIA1. We suggest a model in which tau initially interacts with RBPs in small complexes, but evolves into isolated inclusions as tau pathology matures. These interactions may both facilitate the formation of fibrillar tau structures and lead to mRNA dysregulation via the sequestration of RPBs into persistent aggregates.

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THE ENCODING OF NOVEL MEMORIES: EVIDENCE FOR ENHANCEMENT OF ENCODING VIA MODULATION OF TONIC EXTRASYNAPTIC INHIBITION

Guo, Ouyang; **Odamah, Kathryn**; Downing, Scott; Kumaresan, Vidhya; Ratner, Marcia; Farb, David

Department of Pharmacology & Experimental Therapeutics

Increased hyperactivity of the hippocampus has been associated with memory impairment during aging and in Alzheimer's disease. We have shown previously that aged memory-impaired rat "CA3 place cells" exhibit hyperactivity, decreased spatial information content (a measure of memory), and rigid place fields as compared with young adult animals. Strikingly, acute administration of levetiracetam + valproic acid to aged animals decreases CA3 place cell hyperactivity, increases spatial information content, and refines place fields when transitioned from a "Familiar" to a "Novel" environment to now resemble young adults (Robitsek 2015). If it is hyperactivity alone that interferes with memory, how can reducing tonic inhibition (either by knockout of tonic inhibitory extrasynaptic a5-containing GABA-A receptors or via pharmacological blockade of such receptors with the selective inhibitor a5IA) cause memory enhancement rather than impairment in young adults? To address this contradiction, we asked whether a5IA might exert its memory enhancing effects in young adults by modulating the dynamics of the hippocampal trisynaptic circuit (HTC) function. We demonstrate that administration of a5IA to young adult rats induces hyperactivity in CA1 pyramidal cells, while enhancing spatial memory (as measured by the novel location recognition task) and spatial correlations between two novel environments. We find that single CA1 neurons respond differentially to a5IA with either hyperactivity or hypoactivity, indicating a modulatory role of the local circuitry in response to drug. These results suggest that enhanced encoding of novel information can be achieved via modulation of tonic extrasynaptic inhibition as a control point in HTC memory circuitry.

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SYMPATHETIC NERVOUS SYSTEM REGULATION OF THE NCC IN SALT SENSITIVE HYPERTENSION

F. Puleo; E. Comsti; E. Faudoa; A. Frame; R. Wainford

Department of Pharmacology & Experimental Therapeutics

High blood pressure (BP) or hypertension (HTN) is present in 1 in 2 Americans and represents a critical public health issue. Dietary sodium intake plays a significant role in influencing BP, and is a well-established precursor for HTN risk. For some individuals that demonstrate salt sensitivity of BP, increased sodium intake drives the development of salt sensitive hypertension (SSH). Excess sympathetic nervous system (SNS) release of norepinephrine (NE) has been shown to drive increases in renal sodium retention via the actions of the sodium chloride cotransporter (NCC) that promotes the development of SSH. The signal transduction pathway by which NE exerts its effects on the NCC may involve alpha or beta adrenoceptors. We hypothesize that SNS release of NE stimulates an alpha1 adrenoceptor pathway to

mediate increases in NCC activity that drives the development and maintenance of SSH. Using a pharmacological approach, Dahl salt sensitive rats were treated with alpha or beta adrenoceptor antagonists prior to a high salt challenge or following the establishment of SSH in order to assess the respective role of these adrenoceptors. Our data reveals that alpha1 adreoceptor antagonism alone can attenuate the development of SSH by evoking downregulation of NCC activity and expression. In a model of established HTN, alpha1 adreoceptor antagonism reduces NCC activity and BP. Taken together, these findings suggest that SNS release of NE activates an alpha1 adreoceptor pathway that stimulates NCC activity to drive the development and maintenance of SSH.

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OPPOSING ROLES OF THE SIGMA-1 AND SIGMA-2 RECEPTOR IN HEAVY ALCOHOL DRINKING AND ASSOCIATED ALLODYNIA

S. Quadir; V. Sabino; P. Cottone; M. Iadarola; S. Martin; C. Moore; C. Rohl; J. Sahn; S. Tanino; E. Yao

Department of Pharmacology & Experimental Therapeutics

Alcohol Use Disorder (AUD) is a complex psychiatric disease characterized by increased alcohol intake, inability to control consumption, and a negative emotional state during withdrawal. Sigma receptors (SigRs) mediate several properties of psychostimulant, although these effects have classically been attributed to the direct binding of these drugs to the receptors. However, more recently, a role for SigRs in the rewarding and reinforcing effects of alcohol has also started to emerge, as SigR hyperactivity has been proposed to result in excessive alcohol drinking. In this study, we investigated the role of the sigma-1 receptor (Sig-1R) and sigma-2 receptor (Sig-2R) in alcohol drinking and associated allodynia in mice, using an intermittent access 2-bottle choice model. The effects of the Sig-1R *antagonist* BD-1063 (0-30 mg/kg, i.p.) and the Sig-2R *agonist* JVW-1034 (0-30 mg/kg, i.p.) were evaluated. Both BD-1063 and JVW-1034 dose-dependently reduced alcohol intake and preference, without affecting water intake. Neither drug affected sucrose intake or locomotor activity, suggesting that the effects are specific for alcohol. In addition, both drugs were able to rescue alcohol withdrawal-induced allodynia (assessed using the automatic Von Frey test) without affecting mechanical sensitivity in alcohol-naïve animals. These data suggest that these two receptor subtypes may exert an opposite role in alcohol addiction and lay the foundation for more extensive studies examining their potential therapeutic role in heavy alcohol drinking and associated allodynia.

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COMPREHENSIVE ANALYSIS OF WHOLE GENOME DOUBLING IN TUMOR EVOLUTION UNCOVERS UNIQUE GENETIC VULNERABILITIES

R. Quinton; S. Patel; Y. Koga; N. Parulekar; J. Campbell; N. Ganem

Department of Pharmacology & Experimental Therapeutics

Human cells are inherently diploid, containing 2 sets of 23 chromosomes. The maintenance of proper chromosome number through cellular division is vital to cellular fitness. In human cells, a failure to divide into 2 daughter cells after a duplication of the genome results in tetraploidy, wherein 4 sets of 23 chromosomes are retained in a single cell. Cells that have experienced a whole genome doubling (WGD) and become tetraploid bear unique characteristics that can favor tumorigenesis. Indeed, several experiments have confirmed that proliferating tetraploid cells give rise to tumors *in vivo*. We performed a

comprehensive computational analysis to characterize tetraploidy in human cancers using the ABSOLUTE algorithm to stratify WGD tumors in ~10,000 tumor samples in The Cancer Genome Atlas. Our analysis reveals that 36% of all solid tumors experience a WGD during their evolution. We further identified a WGD transcriptional signature and integrated that with gene essentiality data mined from the Broad Institute's Project Achilles where we again utilized ABSOLUTE to identify genes with enriched essentiality in WGD tumors. Our analyses indicate that WGD tumors overexpress, amplify, and become more reliant on genes essential for mitotic spindle assembly and spindle assembly checkpoint activation. Specifically, we identified that WGD confers enriched dependence on the genes *MAD2L1*, *BUB1B*, and *KIF18A*. We depleted these genes via siRNA and observed significant impairment of chromosome segregation during mitosis and resultant proliferative decline in WGD cells. These findings represent novel therapeutic targets with the potential to specifically target WGD tumor cells while sparing normal diploid tissue.

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STRUCTURE AND MECHANISM OF A NICOTINE-DEGRADING ENZYME, NICA2: TOWARDS DESIGN OF TOOLS AND THERAPEUTICS

M. Tararina[†,] S. Xue[‡,] K. Janda,[‡,] B. Palfey[⊥] ; K. Allen[†,§]

[†]Program in Biomolecular Pharmacology
[‡]Departments of Chemistry and Immunology, The Scripps Research Institute, La Jolla, CA
[⊥]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI
[§]Department of Chemistry, Boston University

Several tobacco soil bacteria have adapted to using nicotine as a primary growth substrate and source of carbon and nitrogen. Among these bacteria is *Pseudomonas putida*, which uses the enzyme nicotine oxidoreductase (NicA2) to catalyze the first step of nicotine degradation via the pyrrolidine pathway. NicA2 is a flavoenzyme part of the monoamine oxidase (MAO) family, oxidizing *S*-nicotine to *N*-methyl-myosmine, followed by non-enzymatic hydrolysis to pseudooxynicotine. We aim to take advantage of its unique evolutionary adaptation to develop a biotherapeutic for nicotine addiction, nicotine poisoning, and tools for biosensor development. Preliminary mechanistic studies report that NicA2 is specific for *S*-nicotine with a K_m of 44 nM, but with a very slow catalytic rate (k_{cat} of 6.64×10^{-3} s^{-1}), yielding an "apparently efficient" enzyme ($k_{cat}/K_m = 1.5 \times 10^5$ $M^{-1}s^{-1}$). Our goal is to identify factors contributing to the mechanistic and substrate binding properties of NicA2 in efforts to improve its biotherapeutic potential. To address the low k_{cat}, the apparent tight binding and slow rate of NicA2 was further explored using stopped-flow and single-turnover kinetics. Additionally, site-directed mutagenesis and mechanistic studies probe the functional role of the aromatic cage in catalysis. Site-directed mutagenesis of the residues W427 and N462 to resemble those of MAO family members is used to investigate their role in substrate binding and specificity. Overall, these studies reveal the rate-limiting reaction step to be in the half-reaction with oxygen, yielding insight into the specificity of NicA2 and highlighting the possibilities for protein engineering to enhance catalytic activity.

HIPPO INACTIVATION PROMOTES MELANOMAGENESIS

M. Vittoria*, N. Kingston*, S. McDonald, X. Varelas, N. Ganem

*These authors contributed equally.

Department of Pharmacology and Experimental Therapeutics

Melanoma, a malignant growth of melanocytes, is the most lethal form of skin cancer, accounting for >75% of all skin cancer-related deaths. Once fully developed, melanomas exhibit multiple somatic mutations, the most frequent of which are activating mutations in kinases that govern proliferation (*BRAF, NRAS)*. Decades of research have largely focused on how these oncogenic kinases promote unrestrained cellular proliferation through hyper-activation of MAPK signaling. Despite the frequency of activating mutations in *BRAF* or *NRAS*, which are found in almost 80% of all melanomas, addition of oncogenic *BRAF* to mouse melanocytes is insufficient to promote melanomagenesis. Instead, oncogenic BRAF expression results in the formation of growth-arrested nevi (moles). All the factors that restrain the growth of oncogenic BRAF-containing melanocytes remains unknown. Our data suggests that *in vitro* expression of oncogenic *BRAF* results in activation of the Hippo Tumor Suppressor pathway. Once activated, the Hippo pathway serves to restrain the function of two pro-growth, oncogenic proteins YAP and TAZ. Inactivation of YAP/TAZ signaling is known to inhibit proliferation and activation of YAP/TAZ is found in multiple cancers. Analysis of melanoma tumor data has shown frequent copy-number loss of the main Hippo pathway kinases, LATS1 and LATS2. To study this further, we established a melanocyte-specific LATS1/2$^{fl/fl}$ mouse. We observed that melanocytic loss of LATS1/2 is incredibly tumorigenic, resulting the formation of dermal, S100B-positive tumors. Collectively, our data shows the Hippo pathway may play a role in melanomagenesis and that inactivation of the Hippo pathway potently promotes tumorigenesis in a melanoma mouse model.

Department of Physiology and Biophysics

NOTE: Prize winners are noted in the following list of Abstracts by:

*1st Prize, **2nd Prize

The accompanying number indicates each abstract's poster board.

Participants

Mehraj Awal (*)
Angela Urdaneta (69**)
Reeder Wells (71)

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THE BREAKDOWN OF NEURAL FUNCTION UNDER VOLATILE ANESTHESIA: IN VIVO, MULTI-NEURONAL IMAGING IN *C. ELEGANS*

M. Awal; G. Wirak; J. Florman; M. Alkema; C. Connor; C. Gabel

Department of Physiology and Biophysics

Volatile anesthetics produce all stages of general anesthesia, including unconsciousness, amnesia, analgesia, and muscle relaxation. In this state, the experience, memory, and physical response to pain are all lost, yet patients can be returned to consciousness, making it an essential tool in modern medicine. However the mechanism by which neuronal systems are disrupted to cause such effects remains a mystery. Current methods of interrogation are limited by resolution, as fMRI and EEG measurements report the average activity of millions of neurons. We hypothesize that the mechanism of action of volatile anesthetics exists at the level of neuronal circuits, whose baseline function and dysfunction under anesthesia can be investigated through imaging techniques that operate at the resolution of individual neurons. We use *C. elegans*, a small nematode, for our investigations, given its previous establishment as an effective model of anesthesia and its well-known capabilities in functional multi-neuronal imaging. We have demonstrated that under moderate anesthesia (defined as the point at which the nematode no longer responds to external stimuli and is still), the activity in a neural circuit that controls forward and backward locomotion is not abolished. Rather, it is randomized, as measured through a loss in coordination between neurons. Furthermore, we have expanded our measurements to pan-neuronal imaging of *C. elegans*, which has allowed us to functionally image 150 neurons of the nematode brain in parallel during the progressive induction into and emergence from anesthesia. In this way, we seek a comprehensive understanding of the system-wide effects of volatile anesthetics.

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OPTIMIZING ATP BOUND ABCA1 FOR STRUCTURAL STUDIES

A. Urdaneta; C. Akey; D. Atkinson

Department of Physiology & Biophysics

Heart disease causes 1 in every 4 deaths in the United States. High-density-lipoprotein (HDL) levels, through its role in Reverse Cholesterol Transport (RCT), have emerged as a negative risk factor for heart disease. In the first and vital step in RCT, interactions between the ATPase cassette transporter ABCA1 and apolipoprotein A-1 (apoA-I), the major protein of HDL, mediate the formation of HDL particles. ABCA1 functions as a lipid transporter to remodel the plasma membrane for HDL particle formation by apoA-I. However, the underlying molecular mechanisms behind these processes are unknown. To understand these mechanisms, we will elucidate the structure of ABCA1 bound to ATP in its active state, and ultimately with apoA-I bound. Ideally, we require a structure for ABCA1 in a membrane-like lipid environment and we are using several strategies to create the appropriate system for structural studies by electron cryo-microscopy. ABCA1 is expressed and purified from sf9 cells using a specific C-terminus Rho tag. We have developed three different systems: ABCA1 solubilized in detergent, reconstituted into a lipid nanoparticle with Saposin A, and in nanoparticles with Peptidisc. The ABCA1 system is frozen in the presence of ATP and $MgCl_2$ on thin carbon grids for electron cryo-microscopy. Grid optimization shows that ABCA1 in detergent is viable for high-resolution structural studies and there is potential for using the Peptidisc or similar reconstitution systems in the future. Determining the structure of ABCA1 in its active state will allow us to understand the mechanisms behind lipid transport and HDL formation.

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HIGH DOSE INTERVAL VITAMIN D SUPPLEMENTATION IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE RECEIVING REMICADE

R. Wells; P. Rufo; S. Levy

Department of Physiology and Biophysics

Background: Patients with inflammatory bowel disease (IBD) are at increased risk of vitamin D deficiency. Circulating vitamin D is crucial to bone health in children and adolescents, but more recent data has demonstrated that vitamin D also plays a significant role in the regulation of the immune system. **Objectives**: The primary aim of this study is to investigate the safety and efficacy of administering high dose oral vitamin D therapy in pediatric patients with IBD. We chose to study patients receiving Remicade, an immunosuppressive therapy administered intravenously, as the need for scheduled hospital-based infusions provides a unique opportunity to ensure compliance in our study population. **Methods**: We identified pediatric patients with IBD with a recent 25-hydroxyvitamin D (25OHD) level < 30ng/mL, maintained on Remicade, and with no history of kidney or liver disease from November 2017 to November 2018. Patients received either 50,000 international units (IU) or 100,000 IU (assigned by infusion interval) vitamin D_3 orally at their infusions for one-year. Spot urine samples, quality of life metrics, and surveys pertaining to dietary vitamin D intake and ultraviolet B radiation exposure were also collected.

Results: Subjects reached steady-state 25OHD after three doses, administered over a span of 4 to 8 months, in which we saw a 9 ng/mL increase in 25OHD. The improvement in vitamin D status did not correlate with changes in quality of life or disease activity. The response to therapy was independent of diet, sun exposure, race, gender, diagnosis, or season of enrollment. There were no adverse events. **Conclusion**: High dose, interval vitamin D supplementation increased steady-state 25OHD, with no signs of toxicity, in this pilot study. These data suggest that high-dose interval therapy may be a treatment option that bypasses limitations related to patient compliance. Further studies are necessary to assess endpoints related to immune function and gastrointestinal health.

Notes