The 29th Annual

Henry I Russek

Student Achievement Day

April 21, 2023 // Hiebert Lounge // 9:00am - 5:00pm

"Treating Multiple Sclerosis: An MD-PhD Student Teaches Us"

David Housman, PhD

Virginia and D.K. Ludwig Professor for Cancer Research,
Koch Institute for Integrative Cancer Research,
Massachusetts Institute of Technology

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

"Genius is perseverance in disguise."

BOSTON UNIVERSITY

BU

Boston University
Chobanian & Avedisian
School of Medicine

David Housman, PhD

Virginia and D.K. Ludwig Professor for Cancer Research, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology

Research-at-a-glance

- Genetic approaches to identifying mechanistic bases of human disease pathology
- Development of effective intervention strategies for trinucleotide repeat disorders

Biography

Dr. Housman is a geneticist and a Virginia and D.K. Ludwig Professor for Cancer Research at the Koch Institute for Integrative Cancer Research at Massachusetts Institute of Technology. He earned both his BA and PhD from Brandeis University in 1966 and 1971, respectively. Dr. Housman is credited for his contribution to discovering the HTT gene, which causes Huntington's disease.

In 1992, Dr. Housman received the MIT Science Council Teaching Prize. He has also been honored with the National Biotechnology Award from the National Conference on Biotechnology Ventures. Since 1988, Dr. Housman has been a Fellow of the American Association for the Advancement of Science, as well as a Fellow of the American Academic of Microbiology since 1994. He is a member of the National Academy of Sciences and the Institute of Medicine of the National Academy of Sciences.

Dr. Housman co-founded five biotechnology companies. He is a co-founder of Genzyme Genetics and the former Somatix Therapy Corp. He also co-founded Kenna Technologies in 2000 and serves as its Adviser and founded Integrated Genetics in 1980. Integrated Genetics was acquired by Genzyme in 1989. Since 1993, Dr. Housman has served as the Chairman, Scientific Founder and Principal Scientific Adviser of Variagenics Inc.

Research

The Housman lab uses genetic approaches to identify the molecular basis of human disease pathology. Specifically, Dr. Housman studies the biological underpinnings of diseases like Huntington's, cancer, and cardiovascular disease and develops strategies to fight these three disease areas. In the cancer field, Housman's research has studied Wilms tumor, glioblastoma and melanoma, in which analysis of genetic alterations in germline DNA or in specific tumors identify pathways of particular significance to tumorigenesis.

Research Areas

- Genetics
- Human disease
- Cancer biology

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

HENRY I. RUSSEK STUDENT ACHIEVEMENT DAY 2023

Program of Events

Coffee and pastries available at 8:30 a.m.

9:45-10:00 a.m. Opening Remarks

Welcoming addresses by Dr. C. James McKnight, Associate Provost & Dean of Graduate Medical Sciences; and Dr. Shelley Russek, President of the Russek Foundation, Department of Pharmacology and Experimental Therapeutics

10:00-10:50 a.m. Keynote Lecture

Henry I. Russek Keynote Lecture by Dr. David Housman, PhD,
Virginia and D.K. Ludwig Professor for Cancer Research,
Koch Institute for Integrative Cancer Research,
Massachusetts Institute of Technology:
"*Treating Multiple Sclerosis: An MD-PhD Student Teaches Us*"

11:00 a.m.-1:30 p.m. Poster Session

Presentations by Graduate Medical Sciences students
Winners will have award stickers on their posters.

11:30 a.m.-12:30 p.m. Lunch

*Award winners (First, Second, and Third place, plus Moderators) please get your lunch and take it to **L1008**. You will be having lunch with our Keynote Speaker & Visiting Professor Dr. David Housman!*

1:30-3:30 p.m. Oral Session

Slide presentations by Student Achievement Award first prize recipients.
Each presentation is 8 minutes with an additional 2 minutes for questions.

3:30 p.m. Award Ceremony

Award presentations by Dr. Shelley J. Russek, Russek Foundation President, and photos of our award winners!

Student Presentations (1:30 p.m. - 3:10 p.m.)

1:30-1:40 p.m.
Morgane Butler: UNRAVELLING EARLY MECHANISMS OF HEAD TRAUMA AND CTE PATHOGENESIS: MICROGLIAL SYNAPSE REMODELING, INFLAMMATION, AND T CELL RECRUITMENT
Department of Anatomy & Neurobiology / Advisors: A. McKee & J. Cherry

1:40-1:50 p.m.
Anne Billot: ROBUST DISSOCIATION BETWEEN THE LANGUAGE AND MULTIPLE DEMAND NETWORKS IN AGING AND AFTER A STROKE
Behavioral Neuroscience Program / Advisor: S. Kiran

1:50-2:00 p.m.
Adeline Matschulat: ROLES FOR THE HIPPO PATHWAY KINASES LATS1/2 IN LUNG EPITHELIAL-IMMUNE HOMEOSTASIS
Department of Biochemistry / Advisor: X. Varelas

2:00-2:10 p.m.
Gian Sepulveda: DOT1L ACTIVATES c-MYC MEDIATED TRANSCRIPTION BY PROMOTING ITS DEGRADATION CYCLE ON CHROMATIN
Program in Genetics & Genomics / Advisor: A. Grishok

2:10-2:20 p.m.
Devin Kenney: A SEXUALLY DIMORPHIC MECHANISM OF HETEROLOGOUS SARS-COV-2 IMMUNITY
Department of Microbiology / Advisor: F. Douam

2:20-2:30 p.m.
Martin Ma: REGENERATION OF MOUSE TRACHEAL EPITHELIUM VIA TRANSPLANTATION OF PLURIPOTENT STEM CELL-DERIVED BASAL LIKE CELLS
Graduate Program in Molecular & Translational Medicine / Advisor: D. Kotton

2:30-2:40 p.m.
Beverly Setzer: A TEMPORAL SEQUENCE OF THALAMIC ACTIVITY UNFOLDS AT TRANSITIONS IN BEHAVIORAL AROUSAL STATE
Graduate Program for Neuroscience / Advisor: L. Lewis

2:40-2:50 p.m.
Ioanna Yiannakou: EGGS AS PART OF A HEALTHY EATING PATTERN ARE NOT ADVERSELY ASSOCIATED WITH DYSLIPIDEMIA IN THE FRAMINGHAM OFFSPRING STUDY
Nutrition & Metabolism Program / Advisors: L. L. Moore, M. T. Long

2:50-3:00 p.m.
Guillermo Arroyo Ataz: CHARACTERIZATION OF THE DEVELOPMENTAL ORIGIN OF POPLITEAL LYMPHATIC SMOOTH MUSCLE CELLS
Department of Pathology & Laboratory Medicine / Advisor: D. Jones

3:00-3:10 p.m.
Jenna Libera: RNA DEMETHYLASE, ALKBH5, DECREASES N6-METHYLADENOSINE (M6A) MODIFICATIONS UNDER OXIDATIVE STRESS AND INCREASES TRANSLATION
Department of Pharmacology & Experimental Therapeutics / Advisor: B. Wolozin

3:10-3:20 p.m.
Andrew Chang: NEURONAL IDENTIFICATION IN C. ELEGANS AS AN APPROACH TO INVESTIGATING THE NEURON-CLASS SPECIFICITY OF VOLATILE ANESTHETIC ACTION
Department of Physiology & Biophysics / Advisor: C. Gabel

RECIPIENTS OF THE HENRY I. RUSSEK STUDENT ACHIEVEMENT AWARDS 2023

First Prize

Morgane Butler
Dept. of Anatomy & Neurobiology
Advisors: Ann McKee, M.D.
& Jonathan Cherry, Ph.D.

Anne Billot
Behavioral Neuroscience Program
Advisor: Swathi Kiran, Ph.D.

Adeline Matschulat
Dept. of Biochemistry
Advisor: Xaralabos Varelas, Ph.D.

Gian Sepulveda
Program in Genetics & Genomics
Advisor: Alla Grishok, Ph.D.

Beverly Setzer
Graduate Program for Neuroscience
Advisor: Laura Lewis, Ph.D.

Liang (Martin) Ma
Graduate Program in Molecular & Translational Medicine
Advisor: Darrel Kotton, Ph.D.

Ioanna Yiannakou
Nutrition & Metabolism Program
Advisors: Lynn L. Moore, Ph.D.
& Michelle T. Long, Ph.D.

Guillermo Arroyo Ataz
Dept. of Pathology & Laboratory Medicine
Advisor: Dennis Jones, Ph.D.

Jenna Libera
Dept. of Pharmacology & Experimental Therapeutics
Advisor: Benjamin Wolozin, M.D. Ph.D.

Andrew Chang
Dept. of Physiology & Biophysics
Advisor: Christopher Gabel, Ph.D.

Devin Kenney
Dept. of Microbiology
Advisor: Florian Douam, Ph.D.

RECIPIENTS OF THE HENRY I. RUSSEK STUDENT ACHIEVEMENT AWARDS 2023

Second Prize

JoColl Burgess
Dept. of Anatomy & Neurobiology
Advisors: Ki Goosens, Ph.D.

Jenna Groh
Behavioral Neuroscience Program
Advisor: Michael Alosco, Ph.D.

Anthony Spinella
Dept. of Biochemistry
Advisor: Xaralabos Varelas, Ph.D.

Dylan Steiner
Program in Genetics & Genomics
Advisors: Marc Lenburg, Ph.D. &
Jennifer Beane, Ph.D.

Kaitlyn Dorst
Graduate Program for Neuroscience
Advisor: Steve Ramirez, Ph.D.

Anna Smith
Graduate Program in Molecular & Translational Medicine
Advisor: Valerie Gouan-Evans, Ph.D.

Matthew Reiss
Dept. of Pharmacology & Experimental Therapeutics
Advisor: Rachel L. Flynn, Ph.D.

Jonathan Kilroy
Dept. of Microbiology
Advisor: Andrew Henderson, Ph.D.

RECIPIENTS OF THE HENRY I. RUSSEK STUDENT ACHIEVEMENT AWARDS 2023

Third Prize

Kayla Nist
Department of Anatomy & Neurobiology
Advisor: Richard Wainford, Ph.D.

Robert Fisher
Program in Genetics & Genomics
Advisors: Michael Hasselmo, Ph.D.

Lucius Wilmerding
Graduate Program for Neuroscience
Advisor: Michael Hasselmo, Ph.D.

Jhonatan Henao Vasquez
Graduate Program in Molecular & Translational Medicine
Advisor: Matthew R. Jones, Ph.D. &
Alan Fine, M.D.

Lucy Peterson
Department of Pharmacology & Experimental Therapeutics
Advisor: Heng-Ye, M.D. Ph.D.

Department of Anatomy & Neurobiology

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

Katharine Babcock (26)
JoColl Burgess (12**)
Morgane Butler (*)
Sarah DeVries (33)
Kayla Nist (36***)

26

MULTIPLEX IMMUNOFLUORESCENT CHARACTERIZATION OF REACTIVE ASTROCYTES IN REPETITIVE HEAD TRAUMA AND CHRONIC TRAUMATIC ENCEPHALOPATHY

K. Babcock, M. Butler, B. Abdolmohammadi, J. D. Cherry, A. C. McKee, and B. Huber

Department of Anatomy and Neurobiology

Astrocytes are important mediators of post-traumatic injury processes and following trauma, undergo structural and functional changes in a process known as reactive astrogliosis. We previously found an increase in GFAP+ astrogliosis at the grey-white matter junction in the frontal cortex of contact sport athletes and military veterans exposed to blast injury compared to individuals without head trauma. GFAP is a structural protein considered a canonical marker of astrocyte reactivity, however additional markers are needed to investigate functional changes. Here, we sought to go beyond GFAP and investigate potential changes in astrocytes using a combination of different markers, including GFAP, the pro-inflammatory marker YKL-40, the universal astrocytic marker ALDH1L1, and the astrocytic water channel AQP4. Using multiplex immunofluorescence, we found a significant increase in YKL40+ astrocytes with a decrease in AQP4+ astrocytes at the grey-white matter interface in football players without CTE, suggesting increased inflammation and reduced fluid movement at this tissue junction. Within cohorts, controls had significantly more homeostatic ALDH1L1+/AQP4+ astrocytes in the grey matter compared to the inflammatory GFAP+/YKL40+ phenotype. There was a significant increase in the inflammatory phenotype compared to the homeostatic phenotype at both the subpial surface and the grey-white matter interface in football players with and without CTE. These preliminary results suggest dynamic astrocytic responses following repetitive head injury and in CTE, most notably in biomechanically vulnerable tissue areas such as the subpial surface and the interface of the cortical grey and white matter.

12**

LONGITUDINAL RECORDINGS OF IN VIVO CALCIUM DYNAMICS OF BASOLATERAL AMYGDALA NEURONS DURING UNPREDICTABLE AND PREDICTABLE FEAR CONDITIONING

J. A. Burgess[1,3], YF. Lee[1], SH. Hong[1,3], S. Shah[1,3], A. E. [2], and K. A. [3]

[1]Department of Anatomy and Neurobiology
[2]Department of Epidemiology, School of Public Health
[3]Department of Psychiatry, Icahn School of Medicine at Mount Sinai

Background: Fear conditioning explores the mechanisms that underlie aversive learning and memory, but it typically uses predictable sensory cues to induce learning. Although environmental unpredictability enhances aversive memory, it is unclear how unpredictability leads to stronger aversive memories. The basolateral amygdala (BLA) is one brain region that contributes to aversive memory formation. Here, we compare local network dynamics in the BLA during predictable versus unpredictable fear conditioning.

Methods: We injected AAV in the BLA of CB57BL/6 male mice to express GCAMP6s. A microendoscopic fiber and lens were placed over the BLA to visualize calcium activity during fear conditioning (Day 1) and auditory memory test (Day 4). Mice received either predictable (n=5; 6 paired tones presented with footshock 20-s after tone onset) or unpredictable (n=5; 6 tones with footshock either 6, 12, 18, 24, 30, or 36-s after CS onset) fear conditioning. The memory consisted of 10-tone (40-s) presentations.

Results: A subpopulation of BLA neurons exhibited elevated tone-shock convergence calcium responses in fear conditioning and recall ("**Winners**"), which were also spatially clustered, while another BLA neuronal population displayed convergence during conditioning but failed to display tone responses during recall ("**Losers**"). Winners from mice that received unpredictable training exhibited more correlated activity during the tone, as well as greater 'emergent' convergence across Winner neighbor pairs.
Conclusions: The overall number of neurons recruited to an aversive memory is stable across different memory strengths, but the local network dynamics supporting those memories differ. Our data suggest spatially clustered units operate differently when forming weak versus strong memories.

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UNRAVELLING EARLY MECHANISMS OF HEAD TRAUMA AND CTE PATHOGENESIS: MICROGLIAL SYNAPSE REMODELING, INFLAMMATION, AND T CELL RECRUITMENT

M. Butler, N. Pervaiz, Y. Wang, J. Campbell, A. C. McKee, and J. D. Cherry

Department of Anatomy and Neurobiology

Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy associated with repetitive head impacts (RHI) endured through contact sport play and military service. Microglia, the resident immune cells of the brain, have been implicated in CTE pathogenesis, but their specific early responses to RHI exposure remain poorly understood. We performed single-nucleus RNA sequencing on post-mortem dorsolateral frontal cortical tissue from 27 individuals (1) without RHI or CTE, (2) with RHI but no CTE, and (3) with low-stage CTE, all under the age of 50 to investigate the early microglial responses to RHI and CTE. Our analyses demonstrate that RHI induces a distinct microglial response characterized by synaptic remodeling and reorganization and GRID2 expression. With the onset of tau pathology, microglia display more heterogeneous phenotypes including cells characterized by increased cytokine signaling and antigen presentation genes, and others delineated by upregulation of T cell recruitment and survival-associated genes. These data suggest that synaptic remodeling may comprise an early microglial response to RHI and may underlie the synapse loss and early cognitive changes observed in young CTE and RHI-exposed individuals. Further, elevated pro-inflammatory signaling and T cell recruitment may be associated with the onset of p-tau pathology. These results identify novel microglial responses to RHI, paving the way for targeted investigation of CTE diagnostic biomarkers and therapeutic targets for RHI-exposed individuals during life.

33

IMMUNE PROTEINS C1Q AND CD47 MAY CONTRIBUTE TO ABERRANT MICROGLIA MEDIATED SYNAPSE LOSS AND ASSOCIATED COGNITIVE IMPAIRMENT IN THE AGING MONKEY

S. A. DeVries, B. Conner, M. Medalla, F. Mortazavi, and D. L. Rosene

Department of Anatomy and Neurobiology

Cognitive impairment in learning, memory, and executive function occur during normal aging, even in the absence of Alzheimer's disease (AD). While neurons are not lost in humans or monkeys, there are structural changes such as synapse loss and dendritic atrophy, especially in the dorsolateral prefrontal cortex (dlPFC), that correlates with cognitive impairment. While the mechanism behind age-related synapse loss is unknown, microglia are an obvious suspect since they eliminate cellular material via phagocytosis. These cells have been shown to prune synapses during developmental synaptic remodeling, raising the question if they are also involved in aberrant age-related synapse loss. Microglia-mediated phagocytosis is directed by immune "eat me" and "don't eat me" signaling proteins in an activity-dependent manner, so that less active synapses are eliminated. This study investigated the balance between the "eat me" signal, (…)

Abstract is continued on the following page.

Continued from S. A. DeVries, "Immune Proteins C1Q and CD47 May Contribute to…"

…complement component C1q and the "don't eat me" signal CD47 relative to age-related synapse loss in the dlPFC in a rhesus monkey model of aging. Results show elevated C1q along with diminished CD47 levels colocalized to PSD95+ synapses with age that was associated with cognitive impairment. Reduced neuronal CD47 RNA expression was found, suggesting neurons are less able to produce the protective signal. Interestingly, microglia do not become morphologically hypertrophic with age in the dlPFC, which is indicative of phagocytosis. These findings suggest that in the aging brain, changes in the balance of immunologic proteins give microglia instructions favoring synapse elimination, but microglia may employ a strategy other than phagocytosis to remove them.

36***

AGE-DEPENDENT HYPERTENSION-DRIVEN COGNITIVE IMPAIRMENT CAN BE ATTENUATED USING ANGIOTENSIN II TYPE 1 RECEPTOR ANTAGONISM

K. Nist and R. Wainford

Department of Anatomy and Neurobiology

We hypothesize that age-related hypertension drives cognitive impairment in the aging Sprague-Dawley (SD) rat. We further hypothesize that reducing established blood pressure will improve cognitive performance.

In male and female SD rats aged 3, 8 and 16-months-old (MO) (N=6/gp) and 16 MO male rats treated with losartan (angiotensin II type 1 (AT_1R) antagonist; 21 days; sc 3 mg/kg/day; N=6/gp) or hydrochlorothiazide (thiazide diuretic; 14 days; sc 4 mg/kg/day; N=6/gp), blood pressure (femoral artery cannulation), sympathetic tone to the vasculature (iv hexamethonium) and plasma NE (ELISA) was measured. Cognition was assessed by the novel object recognition and object location memory tasks. BBB disruption was assessed via FITC extravasation and IHC/IF was performed for microglia (CD11b/c), astrocytes (GFAP), IL-6 and TNF-α in the paraventricular nucleus (PVN)- a critical cardio-regulatory region of the brain.

Aged male, but not female, SD rats develop hypertension, sympathoexcitation, and cognitive impairment. Further, PVN neuroinflammation, cytokine production and BBB disruption increased in male, but not female rats with age. While losartan and hydrochlorothiazide both significantly lowered blood pressure, only losartan attenuated BBB disruption, neuroinflammation, and impairments in recognition memory in aged male rats.

Our results support a) recent clinical findings that a reduction in blood pressure improves cognition, b) AT_1R antagonists as an efficacious treatment for cognitive impairment compared to other anti-hypertensive therapeutics, and c) AT_1R antagonists have a potential role in reducing neuroinflammation.

Behavioral Neurosciences Program

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

Anne Billot (*)

Jenna Groh (20**)

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ROBUST DISSOCIATION BETWEEN THE LANGUAGE AND MULTIPLE DEMAND NETWORKS IN AGING AND AFTER A STROKE

A. Billot, N. Jhingan, M. Varkanitsa, I. Falconer, E. Fedorenko, and S. Kiran

Behavioral Neurosciences Program

Previous studies have shown that the large-scale language and Multiple Demand (MD) networks are robustly dissociated in young adults. However, it is unclear whether this dissociation is maintained in healthy older adults and patients with post-stroke aphasia. This study aimed to investigate this hypothesis by comparing the functional profiles of these two networks among three groups: healthy older adults, adults with post-stroke aphasia, and young adults.

Participants completed functional MRI scans, including language comprehension and demanding non-linguistic MD tasks. Language and MD functional regions of interest (fROIs) were defined in each participant using a subject-specific group-constrained approach. The magnitude of response to the conditions of the language (Sentences, Nonwords) and MD (Hard, Easy) tasks were estimated within each fROI. The contrasts between conditions were compared among the three participant groups using linear mixed-effects regression models.

The three groups exhibited differences in the overall magnitude of response, with overall lower responses in patients and older adults. However, similar to previous findings in young adults, older adults and adults with aphasia showed a robust dissociation between the language and MD networks. The language fROIs showed a language-selective profile with strong responses to the language task and little response to the MD task, whereas the MD fROIs showed a strong response to the MD task and responded to the language task in the opposite way from the language network. In conclusion, this study provides evidence that the dissociation between the language and MD networks is maintained in aging and after a stroke.

20**

LONG-TERM HEALTH OUTCOMES OF FORMER COLLEGE ATHLETES FROM KENT STATE UNIVERSITY

J. Groh, E. Yhang, Y. Tripodis, J. Palmisano, B. Martin, E. Burke, U. Bhatia, J. Mez, J. Gunstad, and M. Alosco

Behavioral Neurosciences Program

Objective: Former professional contact sport athletes, particularly football players, are at risk for later-life neurological disorders and chronic conditions. Little is known about health outcomes of amateur athletes. We examined self-reported health conditions among former Kent State University (KSU) contact and non-contact sport athletes.

Participants/Methods: A 36-item survey of neurological, cardiovascular, psychiatric diagnoses and substance use was sent to 8115 former KSU athletes between October 2021 to January 2022. Athletes were grouped into contact sports (American football, soccer, ice hockey, wrestling, lacrosse) and non-contact sports. Binary logistic regression analyses tested the association between playing contact sports at KSU and self-reported survey outcomes, controlling for age and sex.

Results: 512 (6.3%) completed the survey: 164 played contact sports (median age: 60.5, IQR=48-72, 19.5% reported American football as their primary sport) and 338 played non-contact sports (median age: 56, IQR=41-68, 12.9% reported baseball or basketball as their primary sport). Contact sport athletes reported higher rates of diagnosed sleep apnea ($p<0.01$) and learning disabilities ($p<0.01$) compared to non-contact sport athletes. Additionally, they were more likely to have a neurodegenerative disease diagnosis ($p=0.02$) and recurrent headaches ($p=0.04$). They also reported more concerns of memory problems ($p<0.0001$), attention/concentration ($p<0.001$), problem solving/multi-tasking ($p<0.01$), language ($p<0.01$), impulsivity ($p<0.01$), short-fuse/rage/explosivity ($p<0.0001$), anxiety ($p<0.01$), and violence/aggression ($p<0.01$).

Conclusions: Former KSU collegiate contact sport athletes reported greater cognitive, mood, and behavior complaints and had higher rates of sleep apnea. Millions play college contact sports each year, thus research on chronic health outcomes and risk factors is a high priority.

Department of Biochemistry

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
Nan Cheng (43)
Margaret Downs (25)
Jean Royce Gatdula (21)
Alex Luebbers (6)
Adeline Matschulat (*)
Nadia Mirza (38)
Anthony Spinella (3**)

43

AIRWAY GOBLET CELLS RESULTING FROM CONDITIONAL YAP/TAZ DELETION PROMOTE DISTINCT PULMONARY INFLAMMATION

N. Cheng, J. Hicks-Berthet, X. Varelas

Department of Biochemistry

The aberrant presence of goblet cells, which are epithelial cells of the conducting airways that have specialized roles in secreting mucus, is frequently associated with distinct innate and adaptive immune changes that together are hallmarks of various pulmonary diseases. The transcriptional regulators YAP and TAZ, key effectors of Hippo pathway signaling, play a central role in suppressing the differentiation of airway goblet cells. Here we investigated the consequences of goblet cell production, resulting from the YAP/TAZ conditional depletion in Scgb1a1+ secretory cells, on lung immunity. We observed that loss of YAP/TAZ resulted in the production of goblet cells promoting local CD45+ immune cell expansion. To gain insight into immune changes associated with the production of YAP/TAZ-deleted goblet cells, we performed a single-cell RNA sequencing experiment on control and Yap/Taz-cNull mouse lungs. Data analyses, along with follow-up validation experiments, demonstrated an alveolar immune response, including alveolar macrophage (AM) activation and polarization in Yap/Taz-cNull mice, accompanied with alveolar type 2 cell (AT2) response. We proposed that AMs and AT2s act synergistically to drive pulmonary inflammation in Y/T-cNull mice. Gene expression analysis of YAP/TAZ-deleted airway cells revealed altered expression of notable immune-modulating factors, many of which are implicated in lung diseases. Collectively our study demonstrates that key immune-modulating signals arise from the secretory lung epithelium through reduced YAP/TAZ activity, raising interesting questions about the functions of YAP/TAZ in lung homeostasis and about the contributions of goblet cells to shaping lung immune responses.

25

CONFIDENT GLYCOPEPTIDE IDENTIFICATION USING HOT ELECTRON CAPTURE DISSOCIATION

M. Downs, C. Lin, C. Xia, A. Smyrnakis, D. Papanastasiou, and J. Zaia

Department of Biochemistry

Glycoproteomics presents a challenge for analysis by conventional proteomics methods. Many methods use higher-energy collisional dissociation (HCD) that preferentially fragments the glycan, preventing confident localization on the peptide. Many peptides have clusters of glycosites, further complicating their identification by HCD. In such cases, electron-based dissociation methods preferentially dissociate the glycan provided that the extent of vibrational excitation is kept low. In this work, we used an Omnitrap platform that combines the speed and sensitivity of the Q-Exactive HF with the ability to perform hot electron capture dissociation (hECD).

Proteins were digested using trypsin/LysC and analyzed using a nanoAcquity UPLC coupled to a Q-Exactive HF mass spectrometer with an Omnitrap add-on. Glycoproteomics searches were performed using GlycReSoft. Using a digest of the human neuronal proteoglycan brevican, we confidently identify doubly *O*-glycosylated peptides from the hECD data. The glycopeptide search space was constructed using peptides identified in a search of singly glycosylated peptides, which produced a total of 172 glycopeptide spectrum matches corresponding to 89 glycopeptides. In contrast to HCD analysis of the same digest, very little glycan fragmentation is observed in the hECD data. Additionally, in hECD analysis, doubly glycosylated peptides are identified as the best match for a spectrum more often than they are in HCD analysis, indicating the potential of hECD for mapping densely glycosylated regions of a protein. The Omnitrap allows hECD to be performed on a faster, more sensitive instrument than previously described.

21

USE OF NON-CONVERTIBLE MUTANTS TO TEST THE ROLE OF CELL SURFACE PRP^{SC} IN THE GENERATION OF A NEUROTOXIC SIGNAL

J. R. P. Gatdula, R. C. C. Mercer, J. S. Vultaggio, and D. A. Harris

Department of Biochemistry

The processes accounting for how PrP^{Sc} damages neurons and causes the neuropathological aberrations characteristic of prion diseases remain elusive. Current studies have not yet deciphered which step of the prion-conversion phenomena induces the neurotoxic signal that eventually leads to synaptic dysfunction and neuronal death. My goal is to elucidate the role of the PrP^{C}-PrP^{Sc} conversion process in prion-mediated synaptotoxicity. To address this, I am utilizing PrP^{C} mutants that are blocked from undergoing conversion to PrP^{Sc}. The human G127V PrP mutation, discovered as a kuru-protective variant in Papua New Guinea, is known to be completely refractory to prion conversion in transgenic mice. Another mutation is the experimental V209M variant, which inhibits the conversion to PrP^{Sc} in vitro and in transgenic mice. I first assessed the convertibility of these two mutants in a murine neuroblastoma cell line (N2a) that is capable…

Abstract is continued on the following page.

Continued from J. R. P. Gatdula, "Use of Non-Convertible Mutants to Test the Role of…"

…of prion propagation. I will then go on to test these mutants for their ability to mediate synaptic degeneration in a hippocampal neuronal culture system. Thus far, I have shown that the mouse-equivalent G126V mutant exhibits a complete resistance to conversion in N2a cells when challenged with both RML and 22L mouse-adapted prion strains. In contrast, the mouse-equivalent V208M mutation showed partial resistance to conversion when challenged with these same prion strains. These mutants will therefore be excellent tools for assessing the necessity for prion conversion in mediating acute synaptotoxicity in hippocampal neurons. The meticulous investigation of how PrP^{Sc} elicits a neurotoxic signal on the surface of neurons will aid in the implementation of new therapeutic strategies against prion diseases.

6

DISSECTING THE MOLECULAR BASIS FOR THE MODULATION OF NEUROTRANSMITTER GPCR SIGNALING BY A GAI-BINDING PROTEIN

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Department of Biochemistry

It is well-established that activation of heterotrimeric G-proteins (Gαβγ) by G-protein-coupled receptors (GPCRs) stimulated by neurotransmitters is a key mechanism underlying neuromodulation. Much less is known about how G-protein regulation after receptor-mediated activation contributes to neuromodulation. We have recently identified a regulator of Gi proteins that shapes GPCR inhibitory neuromodulation and underlies neurological processes affecting pain and seizure susceptibility. The molecular basis of this mechanism remains ill-defined because the structural determinants of this regulator responsible for binding Gαi subunits and regulating G-protein signaling are not known. Here, we combined hydrogen-deuterium exchange mass-spectrometry, protein folding predictions, bioluminescence resonance energy transfer assays, and biochemical experiments to identify specific amino acids required for Gαi binding. Surprisingly, our results support a model in which this G-protein regulator undergoes a significant conformational change to accommodate Gαi binding. Using cell-based assays, we validate that the specific amino acids required for G-protein binding are also essential to regulate, differentially, Gαi-GTP and free Gβγ signaling upon neurotransmitter GPCR stimulation. In summary, these findings shed light onto the molecular basis for a post-receptor mechanism of G-protein regulation that fine-tunes inhibitory neuromodulation.

*

ROLES FOR THE HIPPO PATHWAY KINASES LATS1/2 IN LUNG EPITHELIAL-IMMUNE HOMEOSTASIS

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The LATS1 and LATS2 kinases are regulators of the transcriptional effectors YAP and TAZ, and together function as key effectors of Hippo pathway signaling that control essential aspects of organ development and tissue homeostasis. In this study we set out to explore the roles of LATS1/2-YAP/TAZ signaling in lung epithelial homeostasis, focusing on the consequences of hyperactivating YAP/TAZ activity in adult mouse lungs. We interestingly found that conditional deletion of LATS1/2 in CC10+ve secretory airway cells, which results in YAP/TAZ activation, drives dramatic lung inflammation that severely compromises lung function. Airway epithelial cells with LATS1/2 deletion acquire a thin squamous morphology that is associated with the onset of a AT1-like gene expression program. Single cell RNA-sequencing and flow cytometry analysis of LATS1/2 deleted lung have revealed distinct immune cell changes, mirroring aspects of interstitial lung disease. These data have highlighted signals initiated by LATS1/2-deleted cells that recruit innate immune cells that drive inflammation. Our study offers knowledge into lung epithelial homeostasis and identifies epithelial-immune crosstalk that may contribute to severe lung inflammation observed in disease.

38

VISUALZING PRP^{SC} USING N-TERMINAL ANTIBODIES

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Department of Biochemistry

Prion diseases are invariably fatal and untreatable neurodegenerative diseases of humans and other mammals. The central event underlying the disease pathobiology is the structural rearrangement of the primarily alpha-helical prion protein (PrP^{C}) to an infectious conformation composed of primarily beta-sheet (PrP^{Sc}). These conformers can be biochemically differentiated from each other by protease resistance and solubility. Very little of the intracellular signaling induced by PrP^{Sc} on the cell surface is known and the protease resistant core of PrP^{Sc} has made it difficult to visualize in live cells. Visualizing the PrP^{Sc} typically requires denaturation, making it indistinguishable from PrP^{C}. Here, we took advantage of the intrinsically disordered N-terminus to visualize PrP^{Sc} in murine cell line N2a infected with prions using immunofluorescence microscopy, following suit from Rouvinski et al 2014. In infected cells, we find a common strand-like structure of PrP^{Sc} on the surface of the cell which is not present in uninfected cells. Future steps include using this methodology on astrocytes to better understand their infection physiology as well as to test differences in prion stains with N2a cells.

3**

DEFINING ONCOGENIC DRIVERS OF AN AGE-ASSOCIATED, IMMUNE-EVASIVE TUMOR POPULATION IN ORAL SQUAMOUS CELL CARCINOMA

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Head and neck cancer squamous cell carcinomas (HNSCC) are the seventh most common cancers globally with poor survival rates and largely ineffective pharmacological treatments. While older age is associated with worse five-year survival for HNSCC, most preclinical cancer studies focus on outcomes in young animals, potentially contributing to our poor understanding and limited treatment strategies for the disease. To test the influence of age on HNSCC, we utilized syngeneic orthotopic xenograft models of HNSCC, performing hemi-lingual injections of 4MOSC1 and DMBA-MOC1 cells in young (8-14-week-old) and old (75-100-week-old) C57BL/6 mice. We tracked tumor growth, performed scRNA-seq, and histological assays on isolated xenograft tissues. We observed more rapid tumor growth in old compared to young animals with aged xenografts exhibiting reduced CD4+ and CD8+ lymphocytic infiltration. Aged tumors also exhibited a transcriptionally distinct, immune-evasive tumor cell population. This age-associated gene signature correlated with human patient age and worst survival probability in non-HPV HNSCC samples from the TCGA. A Nichenet analysis modeling ligand-expressing tumor cells and receptor-expressing immune cells highlighted a distinct chemokine-expressing signature in young tumor cells predicted to recruit T lymphocytes to tumors, of which was absent in aged xenografts. We observed that re-introduction of these chemokines into aged xenografts reduced tumor growth and promoted CD4+ and CD8+ lymphocyte infiltration. Furthermore, knockdown studies of YAP, a key transcription factor associated with worse HNSCC survival, suggest YAP-mediated suppression of these key lymphocyte-recruiting chemokines thus implicating YAP as a mediator of the pro-tumorigenic, immune-evasive transcriptional alterations observed in aged HNSCC tumors.

13

DOT-1.1 (DOT1L) DEFICIENCY IN C. ELEGANS LEADS TO SMALL RNA-DEPENDENT GENE ACTIVATION

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Graduate Program in Genetics and Genomics

Methylation of histone H3 at lysine 79 (H3K79) is conserved from yeast to humans and is accomplished by Dot1 (disruptor of telomeric silencing-1) methyltransferases. The *C. elegans* enzyme DOT-1.1 and its interacting partners are similar to the mammalian DOT1L (Dot1-like) complex. The *C. elegans* DOT-1.1 complex has been functionally connected to RNA interference. Specifically, we have previously shown that embryonic and larval lethality of *dot-1.1* mutant worms deficient in H3K79 methylation was suppressed by mutations in the RNAi pathway genes responsible for generation (*rde-4*) and function (*rde-1*) of primary small interfering RNAs (siRNAs). This suggests that *dot-1.1* mutant lethality is dependent on the enhanced production of some siRNAs. We have also found that this lethality is suppressed by a loss-of-function of CED-3, a conserved apoptotic protease. Here, we describe a comparison of gene expression and primary siRNA production changes between control and *dot-1.1* deletion mutant embryos. We found that elevated antisense siRNA production occurred more often at upregulated than downregulated genes. Importantly, gene expression changes were dependent on RDE-4 in both instances. Moreover, the upregulated group, which is potentially activated by ectopic siRNAs, was enriched in protease-coding genes. Our findings are consistent with a model where in the absence of H3K79 methylation there is a small RNA-dependent activation of protease genes, which leads to embryonic and larval lethality. DOT1 enzymes' conservation suggests that the interplay between H3K79 methylation and small RNA pathways may exist in higher organisms.

*****

DOT1L ACTIVATES C-MYC MEDIATED TRANSCRIPTION BY PROMOTING ITS DEGRADATION CYCLE ON CHROMATIN

G. Sepulveda, E. Gushchanskaia, A. Mora Martin, R. Esse, A. Ceballos, P. Dholiya, A. Emili, M. D. Cardamone, V. Perissi, and A. Grishok

Graduate Program in Genetics and Genomics

DOT1L is the only known histone 3 lysine 79 methyltransferase in mammals. This methylation mark is found in the gene bodies, promoters, and enhancers of actively transcribed genes and is important for the maintenance of open chromatin structure. DOT1L is recognized as an oncoprotein in numerous cancers. However, our current mechanistic understanding of the oncogenic role of DOT1L is incomplete, with recent studies suggesting that DOT1L can regulate transcription in a methyltransferase-independent manner and can cooperate with c-Myc. Here, we have created a *DOT1L/+* knockout MDA-MB-231 breast cancer cell line in which we observe increased accumulation of c-Myc without a corresponding increase in…

Abstract is continued on the following page.

Continued from G. Sepulveda, "DOT1L Activates c-Myc Mediated Transcription…"

…target gene expression. This c-Myc accumulation under the conditions of global reduction of c-Myc-dependent gene expression suggests that c-Myc promoter binding is not sufficient for inducing transcription when DOT1L levels are reduced. We show that DOT1L works with the ubiquitin-proteasome system to activate c-MYC driven gene transcription. This novel avenue of c-Myc regulation by DOT1L may lead to the development of new approaches for cancer treatment.

30

IFNG-INDUCED IMMUNOSUPPRESSION IN LUNG ADENOCARCINOMA IS MEDIATED BY THE ARYL HYDROCARBON RECEPTOR THROUGH PD-L1 AND INDOLEAMINE 2,3-DIOXYGENASE CONTROL

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Graduate Program in Genetics and Genomics

Immunotherapy has shown dramatic results in treating cancer, but only a minority of patients benefit. Therefore, there is an unmet medical need to understand the regulation of immune suppression in tumors. We have shown that the aryl hydrocarbon receptor (AhR) is a central player in regulating immune checkpoints in a mouse model of lung adenocarcinoma (LUAD). Within the malignant cell, AhR regulates expression of several genes important to suppressive immune signaling, including PD-L1. We find similar immune-related regulatory mechanisms in human malignant cells. Notably, the presence of IFNg boosts the expression of these AhR-regulated suppressive genes. Transplantation of mouse AhR-knockout (KO) LUAD cells leads to partial and up to complete rejection of tumor formation in some mice. AhR-KO-injected mice that do form tumors have increased T cell infiltration, activation, and signaling in the tumor, whereas wild type (WT) LUAD challenge leads to faster and larger tumor formation with less immune infiltration and greater expression AhR agonist, kynurenine. To identify specific T cell subsets involved in AhR-related tumor immunity, we performed single cell RNA sequencing of $CD3^+$ tumor infiltrating lymphocytes. Identifying T cell phenotypes key to this anti-tumor immune response begins to elucidate how AhR in the malignant cell promotes immunosuppression and thus paves the way for future AhR-related therapies and biomarkers relevant to improving patient treatments.

28

EVALUATING A NOVEL MOLECULAR BIOMARKER OF ANGIOINVASIVE LUNG ADENOCARCINOMA WITH SPATIAL TRANSCRIPTOMICS

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Graduate Program in Genetics and Genomics

Microscopic vascular invasion (VI) is predictive of recurrence in stage I lung adenocarcinoma (LUAD) but is difficult to assess in resection specimens and cannot be accurately predicted prior to surgery. To assess molecular and tumor microenvironment (TME) features associated with angioinvasive LUAD we profiled 171 resected tumors with and without VI by RNA-seq, including 16 tumors by high-resolution spatial transcriptomics (ST). We identified a robust molecular signature of VI from the RNA-seq data containing subclusters of genes involved in hallmark programs of tumor suppression, EMT, angiogenesis, growth and metabolism. This VI-associated signature increases across the spectrum of indolent to aggressive stage I LUAD histopathology and is predictive of recurrence-free survival. We found increased expression of the bulk VI signature in invasive tumors profiled by ST, regardless of whether the capture area contained the invasive focus. We then leveraged our bulk RNA-seq dataset to develop a predictor of VI. We applied a nested cross-validation approach within a training cohort to select a machine learning model. Finally, we generated over 200 pseudo-bulked *in silico* biopsies similar in size to standard transthoracic needle biopsies using the spatial data. The scores from these *in silico* biopsies were similar to predictions from matched bulk RNA-seq data. Our combined bulk and ST analysis suggests that VI-associated gene expression extends far from the site of intravasation and can be used to predict the presence of VI. This may enable the prediction of angioinvasive LUAD from small biopsy specimens, allowing for more tailored treatment prior to surgery.

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The accompanying number indicates each abstract's poster board.

Participants

Drew Alessi (87)
Karthik Ashok (77)
Brianna Badalamenti (62)
Ana Isabel Campos (56)
Giulio Cataldo (73)
Sarah Chang (69)
Benjamin Cohen (90)
Patrick Costello (53)
Dylan Dang (75)
Elizabeth Daramola (76)
Katelyn DeMaio (78)
Derrick Ekanayake (54)
Ronell Esquivel (57)
Cassidy Freeman (65)
Michael Gillespie (85)
Mackenzie Hagood (60)
Catherine Hurley (58)
Cara Jones (86)
Savannah Kazemipour (66)
Tafaani Khan (89)
Natalie King (70)
Annabel J. Lee (72)
Christopher Lietz (61)
Priscilla McElhinney (88)
Erika Teresa Minetti (83)
Gloria N. Nabbosa (81)
Janice Nam (82)
Gabriel Luis Ocampo (64)
Sarah Perret (55)
Kimberly Pokstis (59)
Virzhiniya Ruseva (68)
Aarabhi Srinath (67)
Jenny Sun (79)
Kayla Tashjian (84)
Gabriela Taslitsky (63)
Sarah Ultmann Fierstein (74)
Bryan Wang (71)
Gavin Yu (80)

87

QUANTITATIVE INVESTIGATION OF THE EFFECT OF DIET ON THE RISK OF DEVELOPING METABOLIC SYNDROME USING A COMPUTATIONAL WHOLE-BODY MODEL OF METABOLISM

D. Alessi

MS in Medical Sciences Program

Metabolic Syndrome (MetS) is a cluster of metabolic disorders that substantially increase the risk of developing other chronic diseases. Diet is known to play a crucial role in the development of MetS. However, performing extensive dietary intervention studies on humans to investigate the effect of diet on MetS is practically infeasible. To overcome this limitation, we employed an organ-resolved whole-body model (WBM) of metabolism to evaluate computationally, at genome-scale, the effect of ten different diets on the serum levels of five key metabolites implicated in MetS, namely glucose, triacylglycerides (TAG), LDL-C, HDL-C, and palmitoyl-CoA. We performed separate simulations for males and females using sex-specific WBMs. Our analyses elucidated molecular mechanisms that support the current hypothesis that an unhealthy diet can significantly elevate the risk of developing MetS. Our investigation uncovered novel insights into the contribution of specific organs/tissues to the risk of MetS under these diets in males and females. For example, we found that glucose and TAG secretion by adipocytes into the blood are substantially lower and higher, respectively, under the unhealthy diet compared to other diets in males. Striking differences were also observed between the unhealthy diet and other diets for LDL-C, HDL-C, and palmitoyl-CoA. In females, we observed patterns that resembled those in males, although other organs, such as the breast or uterus, also unexpectedly contributed to the serum levels of these metabolites. Overall, our study offers a promising strategy for investigating the effect of various dietary regimens on human metabolism and MetS at organ-level resolution.

77

NEUROPILIN-2 IS A KEY MEDIATOR OF VASCULAR PERMEABILITY

K. Ashok, Y. Gao, J. Omari, S. Coma, A. Bigger-Allen, N. Levonyak, R. Murali, R. M. Adam, and D. R. Bielenberg

MS in Medical Sciences Program

Our goal is to understand the role of Neuropilin-2 (NRP2) in vascular permeability and angiogenic functions *in vivo*. NRP2 is expressed in endothelial cells and mediates signaling from opposing stimulatory and inhibitory ligands. NRP2 has equal binding affinity for VEGFA and SEMA3F. VEGFA binds NRP2 with VEGFR2, while SEMA3F binds NRP2 with plexin A1. We hypothesize that Nrp2 is necessary for VEGFA-mediated vascular permeability and neovascularization and inhibiting endogenous Sema3F signaling will increase vascular leakage and angiogenesis. Our results indicate that knockdown of NRP2 *in vitro* reduced VEGFA-induced activation of VEGFR2 in endothelial cells compared to control siRNA. *In vivo*, *Sema3F*-deficient mice show increased basal vascular leakage compared to wildtype littermates, and *Nrp2*-deficient mice demonstrate reduced vascular leakage in response to VEGFA compared to wildtype controls. (…)

Abstract is continued on the following page.

Continued from K. Ashok, "Neuropilin-2 is a Key Mediator of Vascular Permeability"

Sema3F-deleted and *Nrp2*-null mice have elevated and prolonged vascular leakage following acute inflammation compared to control mice. The extent of angiogenesis was variable depending on the model used and only some assays were Nrp2-dependent. Novel inhibitors of NRP2 prevent AKT inactivation in the presence of SEMA3F. *In vivo*, vascular permeability is evaluated in *Nrp2*-deficient, and *Sema3F*-deficient mice. Neovascularization is variable in these novel mice strains depending on the type of assay including Matrigel plugs, corneal pellet, wound healing, and tumor xenografts. Taken together, we report that NRP2 is key mediator of vascular permeability and angiogenic sprouting. In the future, blocking the interaction of SEMA3F and NRP2 using novel inhibitors may be useful in diseases such as ischemia or diabetic wound healing.

62

INVESTIGATING THE AGE-DEPENDENT CHARACTERISTICS OF HUMAN MACROPHAGES

B. Badalamenti

MS in Medical Sciences Program

Macrophages are a heterogeneous population of immune cells that play a critical role in our innate and adaptive immune systems. The classical understanding of macrophages was centered around the idea that all macrophages are derived from circulating monocytes produced in the bone marrow. In the last decade, our understanding has changed, and studies have indicated that tissue-resident macrophages (TRMs) are of embryonic origin, not bone marrow-derived. The characteristics of TRMs are well-defined in mice, however not all mouse-related information is applicable to humans. We aim to clearly define the characteristics of human TRMs. This will be accomplished through morphological observations, assessing themmuneophenotypic profile, gene expression, macrophage polarization state, and phagocytic capabilities. Our findings indicate that fetal-derived macrophages display a TRM phenotype, further expanding our understanding of human macrophages.

56

INVESTIGATING THE VALIDITY OF ADAPTIVE THERMAL PAIN CALIBRATION IN SURGICAL PATIENTS AND HEALTHY VOLUNTEERS USING FUNCTIONAL NEAR-INFRARED SPECTROSCOPY (fNIRS)

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Offset analgesia (OA), a phenomenon widely studied in pain research, refers to a disproportionate decrease in pain experience following a small reduction in temperature during noxious thermal stimulation. An attenuation of this response could contribute to chronic pain. Functional Near-Infrared Spectroscopy (fNIRS) is a noninvasive optical imaging technique that measures changes in hemoglobin (Hb) concentrations within the brain using the characteristic absorption spectra of Hb in the near-infrared range. This pilot study investigates whether adaptation exists across four trials of the OA paradigm using fNIRS.

Noxious thermal stimulation was given to 19 participants on the forearm of their non-dominant hand using a 3-temperature OA paradigm including offset, constant and control trials. Each OA paradigm consists of four conditions – A, B, C, and D – with a pseudorandom sequence design of three trials. Real-time fNIRS data was obtained on their bilateral prefrontal and somatosensory cortices, regions known to be involved in pain processing. Hemoglobin responses during the four OA trials were evaluated and compared between experimental trials.

Repeated measures ANOVA was used to analyze the significant differences among trials. Results showed no significant differences among the four OA trials. The findings of this pilot study indicate that brain response from the prefrontal and somatosensory cortices is not affected within the four OA trials.

The consistent brain activation across multiple trials of stimulation suggests an absence of adaptive responses. In line with previous findings, these results suggest the reliability of such thermal pain calibration procedure via fNIRS brain imaging.

73

OXIDATION-REDUCTION POTENTIAL AS AN INDICATOR OF DISEASE ACTIVITY IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

G. Cataldo

MS in Medical Sciences Program

Introduction: Inflammatory bowel disease (IBD) is a complex, chronic, autoimmune disease of the gastrointestinal tract. Reactive oxygen species (ROS), a product of active leukocytes, have been implicated in the pathogenesis of IBD. The ability to reliably measure ROS in blood, urine, and stool samples could represent a new approach to assessing disease activity and response to therapy in pediatric patients with IBD.

Objectives: To assess the relationship between redox measurements and clinical disease activity in pediatric patients with IBD.

Methods: Biological specimens, including stool, urine, blood plasma, and intestinal aspirates, were collected from patients at Boston Children's Hospital. Each sample's oxidation-reduction potential was measured by two oxidation-reduction potential probes (an Arrowdox probe and a Mettler Toledo probe). Probes were directly immersed into the sample, returning a millivolt measurement of oxidation-reduction potential. Linear regression was performed to explore the relationship between patient-reported outcome measures (PROMs) and redox measurements of biological specimens. Patients were also stratified by disease severity, and ANOVA testing was performed to test for differences in oxidation-reduction potential observed in patients with remittent, mild, moderate, and severe disease activity.

Results: Redox values in stool, urine, plasma, and intestinal aspirate did not significantly correlate with PROMs or differ significantly among groups categorized by disease severity.

Conclusions: Measurements of oxidation-reduction potential from stool, urine, plasma, and intestinal aspirate do not appear to be useful for assessing disease severity in pediatric patients with inflammatory bowel disease.

69

EVALUATING THE EFFICACY OF A SMARTPHONE MENTAL HEALTH: APP, MINDLAMP, IN REDUCING ANXIETY AND DEPRESSION SYMPTOMS

S. Chang

MS in Medical Sciences Program

Background: Despite the growing popularity and widespread adoption of mobile mental health apps, there is still insufficient high-quality evidence demonstrating their safety and efficacy.

Aims: This exploratory analysis investigates the potential effect size of mindLAMP, a smartphone mental health app, on reducing symptoms of depression and anxiety by comparing the results of using mindLAMP in a control implementation and in a intervention implementation.

Methods: A total of 238 participants were eligible and finished the study in the control implementation, while 156 participants completed the study in the intervention implementation of the mindLAMP app. All participants (both groups) had access to the same in-app activities, including self-assessments and therapeutic interventions.

Results: After multiple imputation, analysis revealed significant minor effect sizes of Hedge's $g = 0.21$ and Hedge's $g = 0.34$ in the reduction of depression and anxiety symptoms respectively.

Conclusions: MindLAMP demonstrates a promising potential in reducing symptoms of depression and anxiety. Additionally, this study underscores the adaptability, reusability, and scalability of smartphone apps, as they can be implemented in diverse settings. These results serve as a basis for further research to examine the effectiveness of not only mindLAMP but also other mental health apps in addressing symptoms of depression and anxiety.

90

THE EFFECTS OF PERINATAL CHOLINE SUPPLEMENTATION ON NEUROINFLAMMATION IN THE PLAQUE NICHE OF APP-NL-G-F MICE

B. Cohen

MS in Medical Sciences Program

Alzheimer's Disease (AD) is a chronic neurodegenerative disease commonly characterized by the aggregation and deposition of insoluble amyloid-beta (A-beta) plaques throughout the brain, and by an associated neuroinflammatory response to these plaques involving astrocytes and microglia. Choline is an essential nutrient with diverse functional roles that has emerged as a promising candidate for the treatment of AD. Localized plaque regions in the polymorphic layer in the medial dentate gyrus of the hippocampus and in the cortex were examined in 9-month-old $APP^{NL\text{-}G\text{-}F}$ knock-in AD model mice to determine the effects of perinatal choline supplementation on astrocytosis and gliosis associated with A-beta. The size of ionized calcium-binding adaptor molecule 1 (Iba1)-positive cells and clusters were larger in control diet $APP^{NL\text{-}G\text{-}F}$ mice, although the number and total area covered by $Iba1^{+}$ cells/clusters were decreased compared to those of control diet C57BL6/J mice.

Abstract is continued on the following page.

Continued from B. Cohen, "The Effects of Perinatal Choline Supplementation…"

In comparison, choline supplementation significantly reduced the size of $Iba1^{+}$ cells/clusters in $APP^{NL\text{-}G\text{-}F}$ mice. Additionally, choline supplementation reduced hippocampal glial fibrillary acidic protein (GFAP) signal area in C57BL6/J mice. These results suggest that perinatal choline supplementation ameliorates neuroinflammatory processes associated with amyloid plaques in these 9-month-old $APP^{NL\text{-}G\text{-}F}$ mice, and that dietary supplementation of choline might serve as an effective treatment for AD.

53

LONG-TERM TOXICITY PROFILE FOR REAL-WORLD RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH ANTI-BCMA CAR T-CELL THERAPY

P. Costello

MS in Medical Sciences Program

Multiple Myeloma (MM) is a plasma cell malignancy that causes improper production of immunoglobulins and elevated levels of monoclonal protein resulting in organ failure, lytic bone disease, and hematological insufficiencies. This study seeks to elucidate long-term adverse events associated with anti-BCMA CAR T-cell therapy in a real-world patient population.

Fifty-four patients who received a CAR T-cell infusion for their relapsed and refractory multiple myeloma (RRMM) were studied in a retrospective analysis at Dana-Farber Cancer Institute. Data were collected before, during, and after infusion. Analyses of data including complete blood counts, serum protein electrophoresis, fluorescence in-situ hybridization (FISH) data from bone marrow biopsy, and imaging were performed.

This study reports considerably less frequent high-grade cytopenia as compared to clinical trial data. However, instances of low-grade anemia and any-grade thrombocytopenia were greater than in the KarMMa-2 clinical trial study. Compared to KarMMa-2 data, this patient population remained healthier post-infusion in terms of infection with only 23% of patients developing infections. Treatment responses did not significantly differ between the population of patients who developed high-grade cytopenia and those who did not. More data is required to determine the risk-benefit profile of early intervention with CAR T-cell therapy as directly compared to the current standard of care. This study is an encouraging insight into the performance of real-world RRMM patients that should assure patients and clinicians of the safety and uncompromising efficacy of anti-BCMA therapy as a treatment option for multiple myeloma.

75

THE STABILITY AND AGGREGATION OF HUMAN LECT2 PROTEIN

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MS in Medical Sciences Program

Background: The aggregation of the leukocyte cell-derived chemotaxin-2 (LECT2) protein in an amyloid fibril form (ALECT2) causes ALECT2 amyloidosis. This disease is found to disproportionately affect Hispanic populations and these patients often produce a variant of LECT2 that contains a valine residue at position 40 instead of an isoleucine residue. We hypothesized this would increase the propensity of ALECT2 formation by altering protein stability. Another factor to consider is zinc loss as a zinc ion is coordinated by multiple amino acid residues. This implies a potential detrimental effect of zinc loss on LECT2 stability.

Objective: To determine whether there is a difference between the stability of the V40 and I40 variants of LECT2 that could increase the propensity of ALECT2 aggregation.

Methods: The recombinant V40 and I40 variants of the LECT2 were grown in both *E. coli* and human embryonic kidney cells. Purified proteins were then subjected to urea denaturation and amyloid formation assays.

Results: Both LECT2 variants were successfully cloned, expressed, and purified. Urea denaturation revealed similar stability of both proteins and removal of Zn^{2+} destabilized both variants to the same extent at pH 8.

Conclusions: We noted overall similar stabilities for LECT2 I40 and V40. Although LECT2 V40 is associated with ALECT2 amyloidosis, the mild variations in stability and aggregation *in vitro* are not strong evidence that this polymorphism destabilizes LECT2. Additionally, we observed a destabilizing effect in LECT2 variants without zinc, drawing attention to the role of zinc in ALECT2 amyloidosis.

76

A PARADIGM FOR EXPLORING THE IMPACT OF SOCIAL ISOLATION ON OLFACTORY SENSITIVITY IN MICE

E. Daramola, A. Finkelstein, and V. Murthy

MS in Medical Sciences Program

Background: Information from mouse olfactory sensory neurons is sent to the olfactory bulb and then to the olfactory tubercle, which plays a role in goal-directed behaviors. As a result of chronic social isolation, mice have been found to have impaired olfactory bulb neurogenesis, increased Tac2 expression, and decreased prefrontal cortex and hippocampal volumes. Since these neurological deficits alter the processing of olfactory information, using social isolation to induce depression-like phenotypes in mice may provide insight into how changes in mental states are reflected in mouse behavior.

Abstract is continued on the following page.

Continued from E. Daramola, "A Paradigm for Exploring the Impact…"

Objective: To determine the relationship between odor concentration and olfactory sensitivity in mice, and how the relationship is impacted by social isolation.

Materials/Methods: 7 head-fixed mice of either the C57BL/6J or tac1-cre strain were trained on a simple go/no-go odor discrimination task. Mice then went through go/no-go/cheat sessions over decreasing n-butanol concentrations before and after being socially isolated.

Results: Mice were able to achieve an accuracy of at least 85% during the training period. There was an overall downward trend in the performances of mice over decreasing n-butanol concentrations, but there were large and unexpected improvements in performance at lower concentrations before and after isolation. At low odor concentrations, isolated males and females had enhanced and decreased performances, respectively.

Conclusions: Mice can learn to associate odors with a reward. Social isolation does not hinder mice from performing odor discrimination tasks. Future studies with larger sample sizes are needed to further explore the possibility of a sex-dependent impact of social isolation on odor discrimination tasks.

78

CFP1 AND CHONDROCYTE MATURATION: ANALYSIS OF PHENOTYPIC CHANGES IN THE CONTEXT OF GENE DELETION IN EMBRYONIC MICE

K. DeMaio

MS in Medical Sciences Program

Bone dysplasia's affect every 1 in 5,000 babies; most of these dysplasia's are incurable, and some are even lethal (Stembalska et al., 2021). Hundreds of skeletal dysplasia's are heritable, yet the genes involved are not well defined (Krakow, 2015). Most of the skeleton forms through a process called endochondral ossification (EO). There are three parts of EO: chondrogenesis, maturation, and ossification. During chondrogenesis, mesenchymal progenitor cells condense and then differentiate into chondrocytes. After differentiation, chondrocytes will elongate, then proliferate and mature to set up for primary ossification. We know that this process happens through many activated genes, but the sequential steps through which this is achieved has yet to be elucidated. In order to understand the cause of skeletal dysplasia's and find new treatments, the molecular mechanisms controlling EO requires further investigation.
This study focuses on one gene, CXXC1 Finger Protein, *Cfp1*, and its role in chondrocyte maturation during skeletal mouse development. *Cfp1* was specifically deleted in chondrocytes, and the resultant effects on cartilage and bone were analyzed. A mild phenotype was observed in the knockout mouse model. It was found that loss of *Cfp1* in chondrocytes leads to delayed ossification in the vertebrae, tibias, metatarsals, and metacarpals. Therefore, *Cfp1* is necessary for normal chondrocyte maturation.

54

CHONDROCYTE MITOCHONDRIAL DYNAMICS DURING DIFFERENTIATION IN MINERALIZATION

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MS in Medical Sciences Program

Converging evidence in recent years suggests growth chondrocytes, involved in the process of endochondral bone formation, exhibit a dynamic bioenergetic profile. Specifically, chondrocytes show an increased dependence on mitochondrial derived oxidative phosphorylation during differentiating and collagen production, but to a differing extent when mineralizing. Therefore, quantitative analysis of mitochondrial dynamics during these processes serves to corroborate existing metabolic studies and further elucidate the role of oxidative metabolism during the endochondral process.

The murine chondroprogenitor cell line ATDC5 was used, and groups were cultured in differentiating, collagen promoted, and mineralizing conditions. Fluorescence confocal 3D image acquisition and bioimaging analysis was used to quantify changes in mitochondrial volume and branch length per mitochondria along with organization and colocalization changes of the actin cytoskeleton to mitochondria in the various conditions over 21 days.

We showed that chondrocyte differentiation resulted in significantly increased mitochondrial volume and fusion when compared to non-differentiating groups, and in collagen promoted groups, mitochondrial volume was significantly higher. Additionally, we showed that the process of mineralization resulted in a significant decrease in mitochondrial volume and branch length per mitochondria by day 21 of the experiment.

Finally, colocalization analyses of the actin cytoskeleton to mitochondria showed significantly increased overlap in non-differentiating cells when compared to differentiating conditions.

These findings suggest that collagen production is likely an energetically taxing process and mineralization does not heavily rely on oxidative metabolism. Furthermore, mitochondrial fission is associated with actin accumulation to mitochondria and fusion is associated with actin disassociation from the outer mitochondrial membrane.

57

DEPRESSION LEVELS IN LGBTQ+ IDENTIFYING ASIAN AMEICANS AND ITS CORRELATION WITH ASSIMILATION AND ACCULTURATION

R. Esquivel

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The specific aim of this research is to discuss ways that LGBTQ+ Asian Americans' depression is measured and if there is a correlation into assimilating with U.S. Culture. Additionally, a review the current literature will be conducted on factors that contribute to identifying characteristics that are tied to adolescent depression and determine if increased levels of depression are found in Asian American immigrants and their families. This research will delve into the reasons why Asian-Americans who identify as LGBTQ+ have increased depression levels and what factors contribute to an elevated level of depression. Furthermore, we discover what further actions in research can be implemented to guide research in depression intervention specifically in LGBTQ+ youth in America.

65

IS RETINAL PERFUSION A PROXY BIOMARKER OF CEREBRAL PERFUSION IN PSYCHOSIS?

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MS in Medical Sciences Program

Background: The brain and retina have structural and functional similarities. Researchers have separately analyzed brain and retinal perfusion in psychosis, but few studies have investigated the relationship between them. While the retina can serve as a proxy for brain disorders including Alzheimer's or Parkinson's, less is known for psychosis. This study examines the connection between brain and retinal perfusion in psychosis.

Methods: Participants took part in the BSNIP-2 study at Beth Israel Deaconess Medical Center. Participants underwent arterial spin labeling MRI and retinal OCTA imaging to determine brain and retinal perfusion, respectively. Whole retinal and lobe-wise brain perfusion was used for analyses performed in R Studios. Results were summarized using basic descriptive statistics.

Results: In probands, a significant positive correlation between vessel density index (VDI) and frontal lobe perfusion ($p=0.000027$, $r=0.74$) and between vessel diameter (VD) and frontal lobe perfusion ($p=0.00077$, $r=0.64$) were identified. In addition, a significant negative correlation between VDI and temporal lobe perfusion ($p=0.0046$, $r=-0.56$). There were no significant results for healthy controls or probands between retinal perfusion and occipital lobe perfusion.

Abstract is continued on the following page.

Continued from C. Freeman, "Is Retinal Perfusion a Proxy Biomarker of Cerebral Perfusion…"

Conclusion: This study demonstrates retinal perfusion may be a proxy marker for frontal lobe perfusion and could be used for predicting cognitive performance in psychosis populations since the frontal lobe is primarily involved in executive functioning. The absence of a relationship between retinal perfusion and occipital perfusion suggests retinal perfusion does not match visual neuronal pathway connections. These findings demonstrate a step towards connecting the retina and brain in psychosis to better understand psychosis pathology.

85

THE ROLE OF EICOSANOIDS IN INFLAMMATION AND CANCER

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Inflammation is the body's natural response to injury or infection by pathogens. Chronic inflammation is a hallmark of cancer and may act to exacerbate tumor growth and metastasis. The resolution of inflammation is now known to be an active process mediated by small lipid autacoid molecules termed specialized pro-resolving lipid mediators (SPMs). Chronic inflammation can result in a vicious cycle of tissue injury, inflammation, and further tissue injury. We evaluated the SPM, resolvin E1 (RvE1), to stimulate the resolution of inflammation in multiple murine models of pancreatic cancer and metastasis. RvE1 mediated macrophage class switching in the tumor microenvironment, increased immune cell infiltration, and improved immune resistance. On the macroscopic scale, RvE1 treatment inhibited tumor growth and number of metastases. Notably, multiple studies showed that RvE1 improved anti-tumor activity of current frontline cancer treatments such as chemotherapy (e.g. cisplatin and gemcitabine) and immunotherapy (e.g. anti-PD1). There was no observed toxicity associated with RvE1 as both a monotherapy and in combination with other treatments. These results show the efficacy of RvE1 in enhancing the resolution of inflammation within the pancreatic cancer microenvironment and suppressing tumor growth. The current study provides a robust platform for conducting further pre-clinical investigations for SPMs in the treatment of different cancer types.

60

ASSOCIATION BETWEEN BRAIN OSCILLATIONS AND ALERTNESS IN EARLY POST-OPERATIVE RECOVERY

M. C. Hagood

MS in Medical Sciences Program

The aging population and increase of ambulatory surgeries have greatly increased strain on surgical and post-surgical staff that decreases the safety of care. Our overall goal is to find ways to decrease the time…

Abstract is continued on the following page.

Continued from M. C. Hagood, "Association Between Brain Oscillations…"

…of anesthetic recovery to allow for more efficient post-surgical treatment. The specific aims of this study were to assess the correlations between neurocognitive recovery measures of attention and vigilance to brain dynamics. We analyzed reaction time via auditory psychomotor vigilance testing (aPVT) testing and the Richmond agitation-sedation scale (RASS) scores in 145 patients prior to and preceding surgery. Intraoperative electroencephalogram was also recorded for 115 of those patients. Data was analyzed to associate aPVT performance to recovery time and intraoperative brain dynamics. We found an association coefficient between reaction time and RASS recovery of 0.022 (p-value = 0.0001) showing a significant association. Further, we found age to be a significant confounding variable (p=0.04421) and included this in our association model. Lastly, there was no significant association found between intraoperative burst suppression and reaction time values (p=0.497). Overall, aPVT was found to be a robust test to assess recovery timeline in peri-operative anesthesia care unit patients. These results highlighted the potential use of an objective metric to track neurocognitive recovery after anesthesia, especially in elderly patients undergoing surgery.

58

RELATIONSHIP BETWEEN EMOTIONAL INTENSITY AND THE ONSET OF EPISODIC MIGRAINE IN ADULT WOMEN

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MS in Medical Sciences Program

Background: Identifying factors related to migraine onset would allow patients to take prophylactic measures to reduce the likelihood of migraine occurrence. The experience of intense emotions is a potential factor affecting migraine onset. We aimed to explore the relationship between day-to-day experience of emotions (specifically sadness, happiness, anxiety/stress, and interpersonal stress) and migraine onset.

Methods: We recruited 30 adult women with episodic migraine to engage in a 12-week monitoring period answering daily questionnaires via mobile app. The daily questionnaires asked about headache occurrence and triggers, emotional intensity and sleep. We conducted a series of linear regressions to understand overall relationship between emotional intensity and the onset of migraine. Next, we conducted mixed effects models to explore the temporal relationship between emotional intensity one day and migraine occurrence next day.

Results: The linear regressions for emotional intensity and migraine occurrence headache were not significant. Mixed effects models showed emotion intensity and migraine onset were significantly associated for happiness (estimate = -0.081; p = 0.027), anxiety/stress (estimate = 0.060; p = 0.040), and interpersonal stress (estimate = 0.120; p = 0.0017), but not sadness (estimate = 0.025; p = 0.46).

Abstract is continued on the following page.

Continued from C. Hurley, "Relationship Between Emotional Intensity…"

Conclusions: Findings suggest high levels of anxiety/stress and interpersonal stress predict onset of migraine next day. Similarly, low happiness levels predict onset of migraine next day. These relationships were not significant when averaged over the monitoring period. These findings support the need for longitudinal research evaluating the temporal relationship between emotion and migraine occurrence, as relationships may be lost with cross-sectional studies.

86

ASSESSING COGNITIVE SURGICAL EXPERTISE USING MENTAL IMAGERY AND FUNCTIONAL NEUROIMAGING

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MS in Medical Sciences Program

Objective: Prior surgical studies have established functional neuroimaging as a validated method to examine brain activation patterns as well as distinctions between novice and expert surgeons during physical skills. The purpose of this study is to examine brain activation during cognitive surgical tasks. Our study utilizes operative dictations (OD) to determine the brain regions activated by cognitive tasks and to distinguish between levels of expertise.

Methods: Junior residents (PGY 1-3), senior residents (PGY 4-5), and attendings were recruited for this study. Participants were tasked to perform a simulated OD of a routine, open inguinal hernia repair utilizing the Lichtenstein technique after baseline measurements were taken. Three trials were completed with a two-minute rest between repetitions. Functional near-infrared spectroscopy (fNIRS) covered prefrontal, occipital, and sensorimotor regions and was used to measure activation during salient events of the OD. Measurement of the fluctuations in deoxyhemoglobin and oxyhemoglobin concentrations was obtained for each participant. Homer3 and AtlasViewer toolboxes were used to process raw data and changes in deoxygenated hemoglobin were evaluated relative to baseline. A general linear model ($p < 0.05$ and $q < 0.05$) was used to evaluate group-level differences.

Results: Ten participants were recruited for each group. Senior residents had significantly more brain activation in premotor areas, including supplementary motor area, parietal area, and right frontal area, compared to junior residents. Attendings demonstrated significantly less activation in medial frontal areas compared to residents.

Conclusion: Functional neuroimaging can examine cognitive functions during simulated OD and discern expertise. This study is the first to connect mental imagery to neuroimaging analysis of cognitive function.

66

IMPROVING ATTENTION FOR PATIENTS WITH FIBROMYALGIA

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MS in Medical Sciences Program

More than three-fourths of fibromyalgia patients complain of cognitive difficulties, memory, and attention problems that often lead to major life impacts including impaired job performance and disability. Cognitive Behavioral Therapy (CBT) is efficacious in treating people with attention deficits, depression, anxiety, and chronic pain (e.g., fibromyalgia). In the current study, we examined the efficacy of CBT in treating attention deficits in patients with fibromyalgia. Participants were randomly assigned into a CBT intervention or an education control, and completed computer attention tasks and surveys at baseline (prior to receiving treatment) and at the follow up (after receiving 8 treatment sessions). We hypothesized that CBT would lead to greater improvement in attention compared to pain education. A repeated measures Analysis of Variance (RM-ANOVA) examined the effects of both treatments in general on outcomes (time effects) and whether the CBT group led to better outcomes (timeXcondition effects) for attention span, attentional switching, and divided attention. Results indicated an effect of time such that patients in both groups improved from baseline to follow up. However, the time by condition interaction was not significant ($ps>0.05$), indicating that the two groups did not differ in change in attention performance from baseline to follow-up. Our results did not support the hypothesis that CBT would result in greater attentional improvement. Our results support practice effects for the attentional tasks which suggest that completing tasks that engage attentional processes could serve as a potential intervention for attentional deficits observed in fibromyalgia.

89

THE IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINE ON PNEUMOCOCCAL NASOPHARYNGEAL ECOLOGY IN CHILDREN 2 MONTHS THROUGH 5 YEARS

T. Khan

MS in Medical Sciences Program

This study evaluates the ecology of *Streptococcus pneumoniae* (SP) nasopharynx (NP) colonization in response to the pneumococcal conjugate vaccines, specifically 7- Valent Pneumococcal Conjugate Vaccine (2000-2009), 13-Valent Pneumococcal Conjugate Vaccine (2010-2023) and 20-Valent Pneumococcal Conjugate Vaccine (2023- future date).

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Continued from T. Khan, "The Impact of Pneumococcal Conjugate Vaccine…"

It is anticipated that the replacement of PCV13 with PCV20, a pneumococcal conjugate vaccine with 7 additional polysaccharide conjugates to CRM197 will enhance the protection against non-vaccine serotypes which are in circulation in communities. The project will evaluate the dynamic changes in pneumococcal colonization over the 5-year time line from 2021-2026. Pneumococcal nasopharynx colonization is detected through nasopharyngeal culture and molecular techniques. The primary source of pneumococcal transmission occurs among the pediatric population and between children and adults. The impact of PCV7 and 13 on pneumococcal colonization over the prior 20 years created a herd effect that resulted in a reduction in pneumococcal disease in unimmunized children and adults. Studies of NP colonization has led to a deeper understanding of pneumococcal conjugate vaccine (PCV) effectiveness and the role of herd immunity in protecting the population, the emergence of replacement serotypes, the variation in invasive capability of each serotype and evolution of antimicrobial resistance resulting from the evolving ecology. In this 5-year- study, researchers at the Pelton Lab in Boston Medical Center set out to understand the prevalence of NP carriage of 13vPnC serotypes, the 7 unique 20vPnC serotypes and NVST (non-vaccine serotypes) within the pediatric population prior to and subsequent to the introduction of PCV 20 (Fall 2023).

70

NEUROPILINS IN BLADDER PHYSIOLOGY

N. King, A. Bigger-Allen, and R. Adam

MS in Medical Sciences Program

Total fat intake should be 20 - 35% of the total calories an individual consumes a day (Dietary guidelines, 2020). On average, it is 35.8% of a person's diet (CDC, 2021). According to Parrish, "dietary fat does not have an immediate effect on blood sugar levels, but consuming a meal high in fat can slow digestion and make it [harder] for insulin to work" (Parrish, 2015). For patients with diabetes, insulin is already not regulating blood sugar adequately. Because of this, patients develop various neurological, urinary, and metabolic symptoms. One symptom in chronic diabetes is a hypocontractile bladder. Contractility is regulated by multiple receptors, ligands, and kinases. One receptor we feel contributes is neuropilin 2 (Nrp2). We have seen it induce cytoskeleton relaxation when expressed in bladder smooth muscle. Potentially, if Nrp2 expression can be reduced, bladder hypocontractility can be attenuated. Through an *in silico* analysis of publicly available data, Nrp2 and diabetes have some differentially expressed genes and pathways in common. To investigate this, we placed mice on a high fat diet. Minimal changes in bladder histology and variable Nrp2 expression was observed. However, when contractile mesenchymal bladder cells were subjected to high glucose, there were significant cytoskeletal changes, increased Nrp2 expression, and decreased contractility. To validate if Nrp2 influenced this, Nrp2 was knocked out using siRNA. This resulted in increased cell contractility on collagen gels. This suggests that Nrp2 signaling is altered under diabetic conditions and potentially could be targeted to attenuate bladder hypocontractility induced by diabetes.

72

DEFINING BIOMARKERS OF MGH-CP1 DRUG SENSITIVITY IN THE TREATMENT OF HUMAN MELANOMA

A. J. Lee

MS in Medical Sciences Program

The Hippo tumor suppressor pathway is a highly conserved signaling pathway that regulates cell proliferation, differentiation, and organ size. Activation of the Hippo pathway leads to the phosphorylation and cytoplasmic sequestration of the pro-growth transcriptional co-activators YAP/TAZ; by contrast, impairment of the Hippo pathway enables YAP/TAZ to enter the nucleus where they bind to the TEAD transcription factors and induce expression of genes involved in proliferation.

Functional impairment of the Hippo pathway, and subsequent hyperactivation of YAP/TAZ, is common in human malignancies, including melanoma. Recently, small molecule inhibitors that disrupt YAP/TAZ-TEAD binding, thus reduce oncogenic transcriptional signaling have been discovered, but their efficacy in preventing cancer cell growth has not yet been well characterized. Moreover, no simple biomarker has been identified that can predict sensitivity to such inhibitors. We hypothesized that cells in which YAP/TAZ are enriched in the nucleus relative to the cytoplasm, indicative of an impaired Hippo pathway, would be more susceptible to TEAD inhibition. This would provide a useful biomarker to identify cancer cell lines most likely to respond to TEAD inhibition. We therefore developed and validated an automated quantification method to score nuclear:cytoplasmic YAP/TAZ localization in melanoma cell lines. This enabled us to identify Hippo activity signatures. We then treated these lines with the TEAD inhibitor MGH-CP1 and performed cell viability assays. Results demonstrated that cell lines that have greater nuclear localization of YAP/TAZ are more susceptible to MGH-CP1 inhibition, suggesting that YAP/TAZ nuclear localization may be a biomarker to identify candidates for TEAD inhibitor treatment.

61

MICRORNA EXPRESSION PATTERNS PREDICT A CHEMOTHERAPY RESISTANCE PHENOTYPE IN OSTEOSARCOMA

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Osteosarcoma is a rare primary bone tumor for which no significant therapeutic advancement has been made since the late 1980's despite ongoing efforts. A subset of patients who share good prognosis respond well to the current chemotherapeutic regimen of methotrexate, doxorubicin, and cisplatin. However, pathologically assessed chemotherapy response has thus far failed as a tool to stratify patients to alternate…

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Continued from C. E. Lietz, "Microrna Expression Patterns Predict a Chemotherapy…"

…therapies and improve patient outcomes. Aside from chemotherapy response, no other validated prognostic factor exists, and genetic studies have not revealed any actionable drug targets. We and others have previously reported that epigenomic biomarkers may be useful prognostic tools in this disease. We thus tested if microRNA transcriptional patterns mark the transition from a chemotherapy sensitive to resistant tumor phenotype. We performed small RNA sequencing on a cohort of paired pre-chemotherapy and post-chemotherapy frozen high-grade osteosarcoma tumor samples and discovered a profile of miRNAs with dynamic transcriptional patterns following chemotherapy exposure in patient samples. An independent dataset of paired pre-chemotherapy and post-chemotherapy formalin fixed paraffin embedded samples we assayed with array-based technology was used to show the miRNA profiles are reproducible. Transcriptional studies of the miRNAs' target genes contextualize the potential biological role of the miRNAs. In a pharmacogenomic screen, both miRNAs and their target genes predict response to drugs which reverse the chemoresistant phenotype and potentially synergize with chemotherapy in otherwise treatment resistant tumors.

88

NOVEL APPROACHES TO ACTIVATE SIRTUIN-1

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Sirtuin-1 (SirT1) is a lysine deacetylase known to promote anti-inflammatory and antioxidant effects. Hence, SirT1 is considered an attractive therapeutic target for vascular, aging-related, and metabolic diseases. However, SirT1 activators fail to reach the clinic because of a lack of efficacy or specificity. Moreover, the development of SirT1 activators is hindered by commercial activity assays that lack specificity and sensitivity. Thus, novel approaches to stimulate and measure SirT1 activity are needed. We sought to optimize a fluorescence-based SirT1 activity assay, with which to test the efficacy of compounds hypothesized to activate SirT1. We also generated wildtype (WT) and redox-resistant (3M) SirT1 adeno-associated viruses (AAV) to analyze the effects of SirT1 overexpression in vascular smooth muscle cells (VSMCs), in normal and oxidative stress conditions. Our results show our activity assay is sensitive and specific in detecting SirT1 activity. Initial trials with small molecule activators showed variable results, in part because of interference of high concentrations of DMSO, in which the compounds were solubilized. SirT1 expression, measured by HA-tag, significantly increased after infection with both WT and 3M SirT1 AAV ($p=0.04$), indicating that the AAVs efficiently infect VSMCs. SirT1 activity, measured by decreased acetylated-histone3, also appeared to increase for both WT and 3M SirT1 AAV, in normal and oxidative stress conditions ($n=3$). In conclusion, we successfully established conditions for a novel SirT1 activity assay and tools for overexpressing SirT1. The overexpression of SirT1 by AAV may become a gene therapy option for in-vivo prevention and treatment of vascular and other diseases.

83

CARDIOMETABOLIC PROTEOMICS AND VASCULAR ENDOTHELIAL HEALTH IN TYPE 2 DIABETES (T2DM)

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Background: T2DM arises from insulin resistance and facilitates progression to cardiovascular disease through endothelial dysfunction. Proteomics is a rapidly improving technique that can help elucidate differences in serum biomarkers, and their relationship to vascular endothelial health.

Objective: To evaluate the proteomic background and implicated pathways in T2DM, and understand how these biomarkers are associated with endothelial cell phenotype and systemic vascular function.

Methods: Individuals with and without T2DM between the ages of 30 and 80 were enrolled. Blood glucose and insulin levels, and two proteomics panels assessing 192 serum biomarkers were obtained. Baseline vascular measures were carried out. Endothelial cells collected from participants were stimulated with insulin and stained with phosphorylated endothelial nitric oxide synthase (p-eNOS). Associations between biomarker levels and insulin-stimulated p-eNOS levels were evaluated.

Results: The study included 37 subjects with T2DM (age 57±8 years) and 32 control subjects (age 53±9 years). Patients with T2DM had lower flow-mediated dilation (6.04±3.41% versus 9.1±4.4%, $p=0.01$). 26 serum biomarkers, involved in metabolism, vascular and fluid homeostasis, immune response, and apoptosis were differentially regulated (adjusted $p<0.05$) in the two groups. Renin and Adrenomedullin were associated with lower insulin stimulated p-eNOS activation ($r=-0.38$, $r=-0.27$, and $p=0.004$, $p=0.049$ respectively). Chymotrypsin C ($r=0.37$, $p=0.006$), Paraoxonase 3 ($r=0.35$, $p=0.009$), Lipoprotein Lipase ($r=0.34$, $p=0.01$), and Superoxide Dismutase 2 ($r=0.31$, $p=0.02$) were associated with higher insulin stimulated p-eNOS activation.

Conclusions: We found associations between serum biomarker levels and insulin-stimulated p-eNOS levels which showed that there is a relationship between altered biomarkers and endothelial cell phenotype.

81

MISSED CARE OPPORTUNITIES WITHIN PEDIATRIC GASTROENTEROLOGY AMBULATORY CLINICS

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Patients in the United States who do not speak English as their first language must contend with many socioeconomic and medical obstacles. These barriers impact how they receive, access, and understand their healthcare needs. These factors also influence the frequency of patients missing their (or their child's) medical appointments. In the medical literature, missed appointments are referred to as Missed Care Opportunities (MCO). Many problems arise when patients miss their scheduled clinical appointments. (…) Previous research has been conducted to better understand why patients miss their appointments. However, most of the available literature focuses on aggregate MCO. Subset analysis stratified by language typically includes only "English", "non-English/Spanish", and "Other". Most studies have not systematically looked at other languages because of the predominance of non-English/Spanish speakers in the United States today. For this reason, this study's focus is to better understand the impact of language on MCO in the ambulatory Gastroenterology, Hepatology, and Nutrition clinics at Boston Children's Hospital. The hypothesis of this study is that the MCO rate is more significant in patients from marginalized communities, including patients who do not speak English as their first language, come from an economically disadvantaged background, or have a minority background. The plan for this study is to translate the statistical findings to develop initiatives to serve this community better and reduce the MCO rate.

82

CHARACTERIZATION OF APOL1 RENAL RISK VARIANT EFFECTS ON PLACENTAL FUNCTION AND PREECLAMPSIA

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African American pregnant individuals have a 1.7-fold increased risk of preeclampsia and 3-fold higher case fatality rate than White people. The highest preeclampsia rates are in sub-Saharan Africa, where common Apolipoprotein L1 (*APOL1*) gene risk variants are linked to a significantly increased risk for non-diabetic chronic kidney disease (CKD). Studies support the association between *APOL1* variants in the placenta and preeclampsia, although the mechanisms are unclear. We hypothesize that APOL1 is expressed in placental villous cytotrophoblast and syncytiotrophoblast in early and later gestation, respectively. APOL1 variants also increase levels of GRP78, an endoplasmic reticulum (ER)-specific protein involved in placental development, and we hypothesize that APOL1 overexpression increases its levels and lead to…

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Continued from J. Nam, "Characterization of Apoli Renal Risk Variant Effects…"

…defective placental invasion linked to preeclampsia. We performed immunofluorescent dual staining for APOL1 and positive controls in early first trimester placenta, and analyzed basal expression of APOL1 and GRP78 in BeWo cells, a trophoblast-derived choriocarcinoma cell line. Immunofluorescent microscopy analysis revealed APOL1 expression in the villous cytotrophoblast early in gestation, indicating a potential role of APOL1 in trophoblast invasion. Additionally, BeWo cells express both *APOL1* and *GRP78* mRNA via RT-PCR, and low expression of APOL1 protein and moderate expression of GRP78 protein via Western blot. Future work will localize APOL1 in placenta of more advanced gestation and employ overexpression of *APOL1* risk variants in BeWo cells to understand how APOL1 expression changes across gestation and its impact on GRP78 expression. These cell models will allow us to interrogate the mechanisms by which *APOL1* variants increase preeclampsia risk.

64

NEUROPILIN-1 MEDIATES RELAXATION IN VASCULAR SMOOTH MUSCLE CELLS

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High blood pressure affects nearly 1 billion people worldwide and increases the risk of heart failure and kidney disease. Dysfunctional vascular smooth muscle cells (VaSMC) cause abnormal and persistent elevation in vascular resistance in patients with hypertension. The transmembrane protein receptor Neuropilin-1 (NRP1) is found in VaSMC in addition to endothelial cells and is widely expressed in tissue biopsies from human and mouse arteries although its function in VaSMC is unclear. We hypothesize that the NRP1 signaling axis is a novel regulator of vascular smooth muscle tone. To study the role of Nrp1 in adult cardiovascular function, we use novel transgenic mice in which the *Nrp1* gene is inducibly deleted specifically in VaSMC. We find increased systolic blood pressure using tail cuff air plethysmography and augmented evoked contraction of aortic smooth muscle using *ex vivo* isometric tension testing, compared to control mice. *In vitro*, silencing of *Nrp1* in primary VaSMC leads to increased contractility in gel contraction assays. Treatment of wildtype primary VaSMC with the soluble NRP1 ligand, Semaphorin-3A (SEMA3A), inhibits the activation of RhoA, a key molecular mediator of muscle contraction. Furthermore, RNA microarray data shows an inverse relationship between *Sema3A* expression and blood pressure with spontaneously hypotensive mice expressing high *Sema3A* and hypertensive mice expressing low *Sema3A.* Taken together, our results demonstrate that the SEMA3A/NRP1 axis is a novel regulator of vascular tone with Sema3A inducing a relaxant signal to SMC while loss of *Nrp1* results in hypertension. Targeting this pathway may provide new therapies for cardiovascular diseases.

55

IMPLENTATION OF DIGITAL OUTREACH FOR OBTAINING SKILLS AND RESOURCES (DOORS) TO IMPROVE THE WELL-BEING AND FUNCTIONAL OUTCOMES IN ADULTS WITH SERIOUS MENTAL ILLNESSES

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Background: The importance of digital inclusion and equity in healthcare is increasing with the rise of new technology. However, many people still lack the necessary digital skills. To address this need, the Digital Outreach for Obtaining Skills and Resources (DOORS), a community-based group, was developed to educate adults with a serious mental illness on digital skills and how to use technology to improve their well-being.

Objective: This thesis aims to summarize the improvements and implementation of DOORS. The program focuses on improving smartphone skills and the overall health of participants.

Methods: There were four phases: (1) development of surveys, (2) accessibility improvement, (3) implementation of the program in two locations, (4) and qualitative feedback.

Results: Improvements to DOORS included an updated curriculum, patient-facing handouts, translation into Spanish, and re-designed surveys. Participants reported improved confidence in 72% of the digital skills taught. Thematic analysis of semi-structured interviews revealed three overall themes: awareness of divide, patient-centered design, and expanded skills and confidence.

Discussion: The updated curriculum focuses on daily life skills and has the potential to improve participants' health. Although it had a positive impact, there is still room for improvement.

Conclusion: Overall, the findings illustrate that programs like DOORS can mitigate the second digital divide and increase technology accessibility. DOORS is a digital literacy program that aims to bridge the second digital divide and promote access to digital health resources. With recent updates and adaptations, DOORS is better equipped to be an accessible program that can positively impact participants through education.

59

ASSESSING THE ACCEPTABILITY AND UTILITY OF CONSUMER SLEEP TRACKING DEVICES IN CHARACTERIZING SLEEP DISTURBANCES IN PEDIATRIC PAIN POPULATIONS

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MS in Medical Sciences Program

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The relationship between sleep, activity, and pain is often cyclical and bi-directional for pediatric patients with chronic pain. Consumer-grade wearable devices such as Fitbits are increasingly being used to assess objective measures of sleep and activity, yet there is still minimal data regarding the acceptability and utility of Fitbits in chronic and post-surgical pediatric pain populations at Boston Children's Hospital. The present study sought to assess the acceptability of Fitbit devices by two post-surgical populations and one intensive rehabilitation population, ages 8-23, as well as characterize various sleep, pain, and activity metrics in these populations as well as select associations between these variables. Participants in all three groups gave an average rating of over 8 out of 10 for Fitbit comfort, and average ratings below 2 out of 10 for Fitbit inconvenience. Decreases in the number of nightly awakenings and pain ratings were seen in both post-surgical populations, along with an increase in step count. The intensive rehabilitation population also demonstrated an increase in steps, along with an increase in amount of deep sleep per night, and a decrease in total sleep time. Significant correlations included those between sleepiness and pain scores, and REM sleep and step counts. The successful implementation of wearable devices that allow researchers and clinicians to assess multifaceted data offers the potential to identify factors contributing to the differences in and transition between acute and chronic pain. Furthering these understandings could impact pain management and clinical decision-making for patients receiving intensive rehabilitation or undergoing surgery.

68

CHARACTERIZATION OF A NOVEL TWO-HIT MODEL OF MATERNAL TLR7 ACTIVATION AND EARLY POSTNATAL RESOURCE DEPRIVATION STRESS

V. Ruseva

MS in Medical Sciences Program

Early life adversity during critical periods of early development increases the risk of neuropsychiatric disorders. Adversity such as abuse, neglect and poverty can lead to activation of the immune system and alter the development of the brain. It can also impact the regulation of neuronal networks by microglia, the innate immune cells of the brain. This neuro-immune dysregulation can result in neurodevelopmental disorders such as autism spectrum disorder, schizophrenia, and others. We are also investigating the…

Abstract is continued on the following page.

Continued from V. Ruseva, "Characterization of a Novel Two-hit Model…"

…functions of toll-like receptor 7 (TLR7), a sensor of single-stranded viruses such as influenza and rubella that is highly conserved between humans and rodents. Immune activation induced by *in utero* administration of the TLR7 agonist imiquimod activates the immune system and is characterized by a phenotype of fragmented social behavior and reduced anxiety-like behavior with a sex-bias for males. While it is a strong risk factor, maternal immune activation alone does not always lead to offspring developmental disorders, but it seems to increase the susceptibility to other risk factors. Previous research has shown a mouse model of ELA resulted in sex-specific alterations in behaviors that are relevant to the clinical manifestations of neurodevelopmental disorders. In this study, we employed a two-hit model of early-life resource deprivation, stress in the form of limited bedding and nesting in combination with maternal immune activation via *in utero* TLR7 stimulation. We then investigated the ability of this two-hit paradigm to induce neural and behavioral alterations and investigate offspring communicative, social, and perseverative behaviors, along with brain proteomic alterations.

67

THE ASSOCIATION BETWEEN PAIN-RELATED FUNCTIONING AND PSYCHOLOGICAL DISORDERS IN PEDIATRIC RACIAL/ETHNIC MINORITIES WITH CHRONIC PAIN

A. Srinath

MS in Medical Sciences Program

The association between pain catastrophizing (PC), fear of pain (FOP) and psychological disorders with relation to chronic pain across different race/ethnicity minorities are understudied. Prior research indicates that racial/ethnic minority populations may engage in more PC and FOP than White populations. Less is known about the nuanced differences in PC and FOP across individual racial/ethnic groups. This study explores between-group differences in PC and FOP across diverse racial/ethnic groups of youth with chronic pain while observing the association between anxiety/depression and PC/FOP. Youth with chronic pain presenting for treatment to a tertiary pediatric pain clinic completed the Pain Catastrophizing Scale (PCS) and the Fear of Pain Questionnaire. Racial/ethnic groups were: Black/non-Hispanic (N = 29), Hispanic (N = 58), Asian (N = 17), another race/non-Hispanic (N = 37), and Multiracial (N = 15). One-way ANOVAs tested differences in the PCS and FOP scales among racial/ethnic groups, and Chi-square analyses tested the association between anxiety/depression diagnoses provided. Results revealed non-significant differences in PCS, PCS subscales, and FOP across racial/ethnic minority groups. However, clinically significant differences appeared between mean PCS and FOP scores across certain racial/ethnic groups. Finally, no significant associations emerged between anxiety/depression and race/ethnicities. Findings suggest that youth with chronic pain may experience PC and FOP similarly regardless of their racial/ethnic backgrounds. However, these findings were limited by small sample sizes, and future research with larger sample sizes is warranted. These results can inform continued exploration and sensitivity to diversity, equity, and inclusivity issues in healthcare for pediatric chronic pain patients.

79

ERYTHROMELALGIA IN PEDIATRICS: CASE SERIES OF PATIENTS SEEN AT BOSTON CHILDREN'S HOSPITAL

J. Sun, C. Berde, C. Brownstein, C. Donado, C. Genetti, M. Halpin, A. Kim, K. Lobo, A. Madden, D. D.

MS in Medical Sciences Program

Background: Erythromelalgia is a rare pain disorder characterized by burning pain, erythema, and warmth in distal extremities, exacerbated by heat and improved by cold. There are currently no established diagnosis guidelines, and treatment effectiveness varies. Published pediatric case series have been limited to small samples.

Objective: To determine the clinical characteristics of pediatric patients with erythromelalgia, investigate associated underlying conditions, and identify the current treatments.

Methods: We extracted electronic medical record data for 42 patients. The current sample was already enrolled in the Gene Discovery protocol at the Manton Center for Orphan Disease Research at Boston Children's Hospital.

Results: 3 had confirmed Nav1.7 sodium channelopathies. Investigations are in progress for novel gene candidates in 6 other probands. Our cohort showed a female predominance (2.3:1) with a median onset age of 12 years (IQR=3-14). Patients saw a median of 3 specialists (IQR=2-3) for a diagnosis. The majority (n=37) reported bilateral symptoms and presented with a wide range of other chronic conditions. Cooling methods were most helpful for symptom relief, while heat and exercise exacerbated pain symptoms. Patients often trialed multiple medications with no apparent consistent pattern regarding effectiveness, with the most commonly prescribed being lidocaine (n=24), gabapentin (n=22), and aspirin (n=15).

Conclusions: Based on the currently published literature, we believe this cohort to be the largest pediatric study of erythromelalgia to date. Many findings are consistent with those noted in previous published case series. Work is in progress to establish a prospective cohort and multi-center registry.

84

ANESTHESIA AWARENESS IN TRAUMA PATIENTS

K. T. Tashjian, R. Canelli, and A. Nozari

MS in Medical Sciences Program

Anesthesia awareness is a rare, but severe complication of anesthesia with possible long-term effects that is more commonly reported after surgery. Anesthesia awareness in trauma patients has not been researched on since Bogetz and Katz's study in 1984, which reported a higher risk of anesthesia awareness due to the intolerance of anesthetic agents in these patients who often present with hemodynamic instability and low blood pressure. Given the reported risk of awareness in this population, clinicians continue to administer standard doses of anesthetic agents despite the associated hemodynamic effects and concern for other anesthesia-related complications. It is important to determine if the risk factors and incidence of awareness remains high despite recent advances in anesthetic techniques and monitoring. We hypothesized that awareness under general anesthesia in trauma patients is less common with the use of modern-day anesthetic agents and monitoring devices. The incidence of anesthesia awareness was retrospectively studied in trauma patients requiring emergency surgery at Boston Medical Center (BMC) between January 2020 and February 2022. It was found that the incidence of awareness during general anesthesia in trauma patients at BMC was significantly lower (0%) than the previously reported incidence of 11% by Bogetz and Katz, ($p = 0.028$, CI -0.22-0.00). Further research is warranted to confirm our findings and further explore the incidence and impact of awareness in this vulnerable population. Future prospective studies should examine a greater number of trauma patients, associated risk factors, and the role of processed EEG monitoring in preventing awareness during general anesthesia.

63

PSYCHOSOCIAL RISKS FOR DECREASED MATERNAL BONDING DURING THE COVID-19 PANDEMIC

G. Taslitsky

MS in Medical Sciences Program

Background: The Coronavirus Disease of 2019 (COVID-19) presented the pregnant population with unique challenges and stressors. This study sought to investigate the association of COVID-19, mental health symptoms, and other sociodemographic variables on the change of maternal bonding from the prenatal to postpartum period to better identify pregnant women at risk for decreased bonding.

Methods: This longitudinal analysis used data from the Perinatal Experiences and COVID-19 Effects Study which included data from online survey completed over the course of the pandemic. Over 2,000 women participated in this study, but for the purpose of the longitudinal analysis only women who were pregnant during the first time point and later participated in the second time point once they were postpartum were included, 204 women.

Abstract is continued on the following page.

Continued from G. Taslitsky, "Psychological Risks for Decreased Maternal Bonding…"

Results: Women who reported experiencing their first pregnancy were significantly associated with an increased change in bonding. COVID-19 related worries experienced during pregnancy were associated with a decreased change in bonding. When accounting for the variables in time point two, pandemic related worries showed no association with a decrease in bonding. COVID-19 related grief due to loss of experiences in the postpartum period were associated with a decrease in bonding scores.

Discussion: This study found that mothers being pregnant for the first time is associated with decreased bonding, and COVID-19 related worries during pregnancy and grief during postpartum are risk factors for a decreased maternal bonding during the transition from pregnancy to postpartum.

74

MATURING HEMATOPOIETIC PROGENITORS DERIVED FROM IPSCS TO OPTIMIZE HUMAN MODELS OF MDS.

S. Fierstein

MS in Medical Sciences Program

Myelodysplastic syndromes (MDS) encompass a heterogeneous group of age-related hematopoietic disorders characterized by ineffective and incomplete hematopoiesis leading to an increased risk of acute myeloid leukemia (AML). The development of accurate and easily used in vitro models is crucial for understanding the pathogenesis of MDS and identifying potential therapeutic targets. Induced pluripotent stem cells (iPSCs) can be used to study MDS due to their ability to differentiate into any cell type depending on the environment. The main limitation is that the blood progenitors produced by iPSCs are of a fetal state, which hinders modeling of MDS, a disease of older adulthood. This study aimed to optimize the maturation state of blood progenitors derived from iPSCs by induction of the micro-RNA let-7, which, we hypothesize will increase the maturation and adult phenotypic state of hematopoietic progenitors.

iPSC lines were generated from healthy controls and samples containing the SRSF2 mutation, a common mutation in MDS, containing a doxycycline-inducible, stabilized let-7 transgene. A stepwise differentiation efficiently drove the iPSCs toward hematopoietic progenitors and, subsequently, other mature lineages. The hematopoietic progenitors were characterized by assessing the expression of specific cell surface markers and functional properties using flow cytometry, colony-forming assays, and multilineage differentiation abilities. These findings demonstrate the potential of using iPSC engineering to create a novel model for MDS and other age-biased disorders by inducing let-7 expression in iPSCs and, when differentiating them, exposing them to doxycycline to promote an adult cell phenotype. This approach offers a valuable potential tool for elucidating the molecular mechanisms underlying these disorders and exploring potential therapeutic interventions.

71

INVESTIGATIONS OF ENTEROTOXIGENIC E. COLI (ETEC) INTESTINAL COLONIZATION IN NEONATAL MICE

B. Wang

MS in Medical Sciences Program

Enterotoxigenic *E. Coli* (ETEC) is an enteropathogen that is a global cause of traveler's diarrhea which is responsible for millions of diarrhea cases every year. Children are most vulnerable, as severe infections can lead to stunting and death. ETEC is primarily found in nations with inadequate sanitation systems and little access to clean water. There has been increased interest in outer membrane vesicles as vaccine adjuvants due to their ability to display a range of antigens. Mutation of the Mla pathway (delta-*mlaE*) has been seen to increase OMV production in multiple pathogens. Current animal models of ETEC colonization are lacking to study colonization, bottlenecks and pathogenesis. Barcoded ETEC libraries were constructed to determine founding population sizes and intestinal ETEC burdens. P5 neonatal CD-1 and C57BL/6 mice were infected with 10^4-10^7 CFU of wild type ETEC. Founding population sizes of ETEC strains were compared via sequencing and STAMPR analysis while CFU burdens were determined via serial dilution plating. Outbred CD-1 suckling mice only colonized with a 10^7 dose while C57BL/6 mice had 10^6 CFU per small intestine at inocula sizes of 10^5 or greater. P5 CD57BL/6 were infected with 10^5-10^7 barcoded wild type ETEC and delta-*mlaE* mutant. There was no difference in founding population sizes at the same inoculum between WT ETEC and the mutant, though the founding population size increased with input. We concluded that C57BL/6 P5 mice can serve as a new model to study ETEC intestinal colonization.

80

SHOCK ATTENUATION AT THE KNEE DURING WALKING IN PEOPLE WITH KNEE OSTEOARTHRITIS: ASSOCIATION WITH DISEASE SEVERITY

G. Yu

MS in Medical Sciences Program

Department of Physical Therapy and Athletic Training

Background: Knee osteoarthritis (OA) is a highly prevalent disease in middle- and older-aged adults, impacting their quality of life through joint pain and decreased mobility. While altered loading at the knee during walking is implicated in disease pathogenesis, whether shock attenuation at the knee is altered in people with knee OA is not known. Ineffective shock attenuation due to failure of muscle activity could be a contributor to structural and symptomatic worsening of knee OA.

Purpose: This study aims to examine shock attenuation at the knee during walking and its association with muscle co-contraction and disease severity in people with knee OA.

Methods: 295 individuals with confirmed knee OA were recruited from surrounding communities in this study. Participants underwent 3D motion capture and surface electromyography during walking for estimation of shock attenuation (i.e., coupled vector angle or CVA) and muscle co-contraction at the knee.

Abstract is continued on the following page.

Continued from G. Yu, "Shock Attenuation at the Knee During Walking…"

We examined associations of measures of CVA with muscle co-contraction, pain, and radiographic severity of knee OA.

Results: Worse radiographic knee OA was related to lower shock attenuation at the knee which was positively correlated with increased knee pain. Greater lateral thigh muscle co-contraction was related to a lower shock attenuation at the knee.

Discussion: As the knee OA disease severity progresses, patients may also experience lower shock attenuation and increased pain. In addition, increased muscular co-contraction corresponded with greater shock attenuation. These results suggest that altered shock attenuation at the knee may be a contributing factor to knee OA.

Department of Microbiology

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
David Bean (11)
Elizabeth Chavez (9)
Josiane Fofana (31
Devin Kenney (*)
Jonathan Kilroy (24**)
Stephen Ross (46)

11

PRIOR SARS-COV-2 INFECTION PROTECTS AGAINST SYMPTOMATIC COMMON COLD CORONAVIRUSES

D. Bean, J. Monroe, A. Olson, J. Weinberg, M. Sagar

Department of Microbiology

Majority of the human population now has immunity toward SARS-CoV-2 through infection and/or vaccination, yet little is known how this SARS-CoV-2 specific immunity may protect against future heterologous coronaviruses. We examined the incidence of common cold coronaviruses (ccCoV) infections as a proxy for response against a future emerging CoV among those with SARS-CoV-2 infection, COVID-19 vaccination, or neither exposure. In a retrospective analysis of individuals at Boston Medical Center that underwent a comprehensive respiratory panel polymerase chain reaction (CRP-PCR) test from November 30, 2020 to October 8, 2021, the incidence of symptomatic ccCoV was lower in those individuals with documented prior SARS-CoV-2 infection (1.0%) compared to those with COVID-19 vaccination (2.9%) or no prior SARS-CoV-2 exposure (1.8%, $p = 0.01$). To understand these different infection rates, we compared immune responses to ccCoV between the three groups.

Individuals with prior SARS-CoV-2 infection and those with previous COVID-19 vaccination had similar plasma neutralization against SARS-CoV-2, OC43, and 229E spike bearing pseudoviruses. SARS-CoV-2 ($p<0.0001$) and OC43 nucleocapsid ($p = 0.03$), but not spike specific peptides, yielded higher $CD4^+$ T cell responses in individuals with a prior SARS-CoV-2 infection as compared to those with COVID-19 vaccination or neither exposure. The COVID-19 vaccinated individuals' cells also had lower Th1 (IL-2 and IFN-gamma) responses after exposure to OC43 peptides. Prior SARS-CoV-2 infection, but not COVID-19 vaccination, protects against subsequent ccCoV symptomatic infection. This protection against symptomatic ccCoVs may be mediated by cellular responses to non-spike proteins and provides insight for the design of future pan-coronavirus vaccines.

9

IDENTIFICATION OF FLAVIVIRUS RNA ELEMENTS MEDIATING ESCAPE FROM CELL-INTRINSIC IMMUNITY

E. Chavez[1,2], M. Matsuo[1,2], and F. Douam[1,2]

[1] Department of Microbiology
[2] National Emerging Infectious Diseases Laboratories

Mosquito-borne flaviviruses such as Dengue virus (DENV), Zika virus (ZIKV), or yellow fever virus (YFV) remain a significant public health threat. The ability of these viruses to evade cell-intrinsic immune responses is critical for them to successfully replicate within their host and to sustain their transmission. The ability of flavivirus proteins to orchestrate such escape has been well-characterized and is underlaid by a complex panel of molecular mechanisms involving the inhibition or degradation of several innate immune effectors by flavivirus non-structural proteins. In contrast, how RNA secondary structures within the flavivirus genome contribute to this escape remains elusive. Viral RNA genomes arrange into an assembly of stem-loops of various lengths that regulate several steps of the virus life cycle. We recently discovered that introduction of synonymous mutations within a 400 nucleotide (nt) region of the coding genome of a YFV strain, YFV-17D (17D), significantly attenuates viral infection and impairs viral RNA replication without compromising intrinsic replication competency. A sub-genomic 17D replicon carrying these mutations induces higher expression of interferon-stimulated genes in immune competent cells in comparison to a wild-type replicon, and defective replication can be rescued in cells incompetent for type I interferon (IFN) signaling. Finally, we identified two small genomic segments within this 400nt region, which we named EL1 and EL2, that mediate this phenotype. Therefore, we hypothesize that critical RNA elements within these two segments promote evasion from the type I IFN response. Our ongoing work focuses on defining sequence determinants of these elements and characterizing their ability to drive immune escape.

31

DUAL ANTIRETROVIRAL LOADED POLYMERIC NANOPARTICLES FOR LONG-ACTING SUPPRESSIVE HIV THERAPY

J. Fofana[1], B. Eshaghi[2], E. Schiferle[2], N. R. Chandra[3], Z. Ambrose[3], B. M. Reinhard[2], and S. Gummuluru[1]

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[2] Departments of Chemistry and The Photonics Center
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Viral persistence in secondary lymphoid tissues (SLTs) of patients on cART has been linked to low SLT penetration of antiretrovirals (ARVs). Furthermore, suboptimal adherence to ARVs can lead to drug failure and rapid viral rebound from these SLTs. Therapeutic strategies that sustain ARV concentrations in SLTs are essential. Hence, we designed membrane-wrapped poly-lactic acid nanoparticles expressing the CD169-ligand GM3 ($GM3^+$ NPs) and co-incorporating Rilpivirine (RPV) and Cabotegravir (CAB) to selectively target CD169+ macrophages in SLTs. We hypothesized that $GM3^+$ NP retention within CD169+ CD81+ non-degradative compartments (NPCCs) in macrophages will lead to establishment of myeloid cell-associated ARV-depots for sustained viral suppression in SLTs. GM3-NPs were formulated by one-step nanoprecipitation of lipids, poly-lactic acid, RPV and CAB. Intracellular ARV retention in monocyte-derived macrophages (MDMs) was quantified by high performance liquid chromatography (HPLC). Trafficking of NPs in MDMs was determined by quantifying co-localization of NPs with $CD81^+$ NPCCs via confocal microscopy. Antiviral effect of NPs was assessed in CD169+ MDMs pre-treated with ARV loaded GM3(+/-) NPs or NP-free ARVs and infected weekly with luciferase expressing HIV-1 over 35 days. Dissemination of GM3(+/-) NPs in vivo was determined by IVIS and immunofluorescence microscopy upon single s.c. injection of fluorescently labelled NPs in BALB/c mice. Long-acting antiviral potency was assessed in HIV-1_{CH185}-infected BLT humanized mice by weekly quantification of plasma viral RNA for 21 days, upon single injection of ARV-GM3(+/-) NPs or daily NP-free ARVs.

Temporal HPLC analysis of MDM lysates revealed that $GM3^+$ NPs maintained high intracellular ARV concentration for 28 days, which correlated with persistent localization of $GM3^+$ NPs in $CD81^+$ NPCCs. $GM3^+$ NPs sustained antiviral effect in MDMs, with robust viral suppression for 35 days post NP addition. Furthermore, $GM3^+$ NPs co-localized with $CD169^+$ cells and were retained in SLTs for 21 days unlike $GM3^-$ NPs. Importantly, a single dose of ARV-$GM3^+$ NPs suppressed viremia in HIV-infected BLT mice for 21 days to levels observed with daily NP-free RPV/CAB.

These results suggest that $GM3^+$ NPs are an attractive long-acting delivery platform with the potential to enhance ARV pharmacokinetics and facilitate sustained inhibition of HIV-1 replication by establishing drug depots in $CD169^+$ macrophages in SLTs.

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A SEXUALLY DIMORPHIC MECHANISM OF HETEROLOGOUS SARS-COV-2 IMMUNITY

D. Kenney[1,2], J. Léger[4,5], D. Chen[3,2], CV. Chin[3,2], S. Adams[1,2], N. Chehadeh[1,2], S. Seitz[1,2], J. H. Connor[1,2], C. Harly[4,5], M. Saeed[3,2], F. Douam[1,2 †]

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[2] National Emerging Infectious Diseases Laboratories
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Heterologous immunity upon SARS-CoV-2 reinfection is driven by several circulating immune effectors, from Fc effector functions to memory T-cells. However, many aspects of lung-resident mechanisms of heterologous immunity remain elusive. Here, we used K18-hACE2 mice and the antigenically divergent SARS-CoV-2 Delta and Omicron (BA.1) variants to explore such mechanisms. While Delta infection is highly lethal in naïve mice, mice inoculated with Delta thirty-days following BA.1 primary challenge are protected from severe disease and lethality independently of sex. Protection is associated with a rapid control of viral replication as early as day 2 post-secondary challenge but is not mediated by circulating antibodies. Protection is not recapitulated in mice lacking lymphoid lineages, and depletion of B-cells prior to BA.1 infection results in early and severe disease in a sex-independent manner upon Delta re-challenge. *In vivo* T-cell activation assays suggested that disease upon B-cell depletion is driven by excessive T cell-mediated inflammation. We found that heterologous protection is defined by the induction of robust IFNγ–mediated responses and M1 polarization. Strikingly, this is a sexually dimorphic mechanism, with males displaying less robust M1 polarization than females. Consistently, T-cell depletion prior to BA.1 challenge is highly lethal in male but not in female mice upon Delta challenge. Finally, we uncovered that BA.1 infection primes the establishment of a pool of IFNγ-expressing B cells in the lung (referred to as γB_R), with female mice displaying a more prominent pool of γB_R cells than males. Altogether we uncovered an *adaptive-to-innate* immune barrier in which BA.1-inducible γB_R cells drive rapid M1-mediated antiviral responses while preventing exacerbated host responses upon heterologous challenge. This mechanism operates in a sexually dimorphic manner, with males displaying an increased dependence on the T-cell compartment to drive protection from disease. Our findings can inform the design of novel variant-proof and sex-tailored vaccines against SARS-CoV-2.

24**

EXPRESSION OF DEFECTIVE HIV PROVIRUSES TRIGGER INNATE IMMUNE RESPONSES

J. Kilroy, B. Basukala, and A. Henderson

Department of Microbiology

The persistent HIV reservoir includes a subset of cells harboring transcriptionally repressed latent HIV that contributes to rebound upon treatment interruption presenting a challenge to HIV cure. However, examination of proviral sequences has shown that the reservoir consists of mostly defective viral genomes with point mutations, frame shifts, inversions, and deletions which would limit productive HIV-1 transcription. Furthermore, even in the presence of antiretroviral therapy (ART) and limited HIV transcription, people living with HIV (PLWH) demonstrate chronic comorbidities of the central nervous system, heart, and general inflammaging. It is unknown what drives the dysregulated inflammation in PLWH. In previous work from our lab, we demonstrated the expression of RNAs and proteins from defective proviruses driven by an intragenic promoter. We have employed CRISPR-cas9 to engineer cells harboring defective HIV proviral genomes lacking 5' Long Terminal Repeats (LTR). Induction of IFN-stimulated genes as measured via RTqPCR, including IP10, ISG15, and MX1, was significantly higher in cells harboring defective proviruses than those harboring mostly intact virus. Furthermore, we observed a correlation with the expression of cell-associated cryptic RNAs derived from the intragenic promoter and serum levels of IP-10 and TNFa in samples from PLWH on ART. We propose a model whereby defective proviruses produce RNAs and peptides which activate innate immune activities in T cells and myeloid cells to perpetuate inflammation.

46

LONG-RANGE GENOMIC INTERACTIONS DETERMINE THE POSITION AND STOICHIOMETRY OF TEMPLATE-SWITCHING EVENTS DURING SARS-COV2 DISCONTINUOUS TRANSCRIPTION.

S. Ross[1,2,3], C. Ye[4], L. Sobrino-Martinez[4], E. Mühlberger[1,2], and D. Cifuentes[1,3]

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Coronaviruses are positive-sense RNA viruses which undergo discontinuous transcription to produce negative-sense subgenomic RNAs (sgRNAs). These (-)sgRNAs serve as template for the production of positive-sense viral mRNA. The 3'-end of all (-)sgRNAs is generated during a template-switching event mediated by the viral RNA-dependent RNA polymerase precisely at conserved transcription regulatory sequences (TRSs). The precise genomic location of the template switching event seems to be defined by the interaction of a TRS within the newly transcribed subgenomic RNA (TRS-B) and the leader (TRS-L) in the genome RNA. Yet, the exact determinant of this template switching event has not been fully elucidated. To further define the mechanism of (-)sgRNA production, we established a novel RNA-Seq approach to quantify the (-)sgRNAs of mouse hepatitis virus and SARS-CoV-2 and establish their relative stoichiometry. We observed that the relative stoichiometries of viral (-)sgRNAs remain constant in infection and are refractory to changes in time, MOI, and large deletions or insertions to the viral genome. The stoichiometries of the resulting viral (+)mRNAs mirror the relative abundances of the (-)sgRNAs suggesting that viral (+)mRNA expression is mainly regulated through (-)sgRNA abundance, whereas other factors, such as gene-specific destabilizing elements, do not play a role. Our analysis revealed that SARS-CoV-2 (-)sgRNA expression levels significantly correlate with the published frequencies at which each genomic TRS interacts with the leader sequence. To probe that these TRS-L:TRS-B interactions are dependent on the ΔG of leader-TRS duplex formation, we generated SARS-CoV-2 mutants with altered TRS duplex ΔG values. These mutants show an altered (-)sgRNA expression level in each mutated TRS, while the unchanged sgRNAs remain constant. Altogether, our data suggests that coronavirus (-)sgRNA synthesis and (-)sgRNA abundance during infection are governed, at least in part, by the frequency of leader-genome interactions formed stochastically at a frequency defined by the ΔG of the interacting nucleotides.

Department of Molecular & 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

Claire Burgess (42)
Senegal Carty (2)
Jhonatan Henao Vasquez (14***)
Rachel Ho (15)
Liang Ma (*)
Erik Matson (27)
Emilie Mausser (48)
Carly Merritt (49)
Noah Prince (35)
Yuhan Qiu (45)
Anna Smith (23**)

42

GENERATION OF HUMAN ALVEOLAR EPITHELIAL TYPE I CELLS FROM PLURIPOTENT STEM CELLS

C. Burgess[1,2], P. Bawa[1], X. Varelas[3], E. Morrisey[4], and D. Kotton[1,2]

[1]Center for Regenerative Medicine
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[3]Department of Biochemistry
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In the distal lung, alveolar epithelial type I cells (AT1s) are uniquely flattened to allow efficient diffusion of oxygen into capillaries. This structure has made them challenging to study and isolate, resulting in no established models for the study of human AT1 biology. We sought to engineer a human *in vitro* model of AT1s via directed differentiation of induced pluripotent stem cells (iPSCs). The Hippo signaling pathway has been implicated in mouse AT1 program maintenance and development, thus we tested the effect of nuclear effector protein YAP in iPSC-derived alveolar epithelial type II cells (iAT2s). Cells transduced with lentivirus encoding constitutively active nuclear YAP (YAP5SA) downregulated AT2 genes and upregulated AT1 genes, including AGER, CAV1, and PDPN, by whole-well RT-qPCR. Additionally, we developed a reporter iPSC line containing a knock-in $AGER^{tdTomato}$, detectable post YAP5SA transduction, allowing tracking and isolation of these AT1-marker expressing cells. Latsly, using this reporter line, we developed a serum-free defined iAT1 induction medium containing a LATS inhibitor to activate nuclear YAP signaling, generating $AGER^{tdTomato}$+ cells from iAT2s grown in 3D cultures. These iAT1s clustered separately from iAT2s by scRNA-seq and upregulated expression of AT1 transcripts such as AGER, PDPN, CAV1, and CLIC5. When plated in 2D cultures at air-liquid interface, these iAT1s formed thin, flat monolayers of $AGER^{tdTomato}$+ cells. Our results suggest a role for Hippo signaling in the differentiation of human AT1s and establishes an iPSC reporter cell line and differentiation medium able to serve as a potential human AT1 *in vitro* model system.

2

PULMONARY LYMPHATIC VESSEL REMODELING IN RESPONSE TO INFLUENZA-INDUCED INFLAMMATION

S. Carty, E. Crossey, F. Shao, A. Ysasi, M. Zeng, T. Norman, J. Yuan, J. Henao Vasquez, A. Hinds, A. Fine, and M. R. Jones

Graduate Program in Molecular & Translational Medicine

Influenza A viral infections represent a significant worldwide public health burden. In response to respiratory infection, lung lymphatic endothelial cells (LECs) orchestrate multiple functions, including coordinating immune and parenchymal cell phenotypes, draining interstitial fluid and trafficking immune cells to lymph nodes. Based on our preliminary data, we hypothesize that during influenza, pulmonary lymphatic vessels are remodeled, affecting both pathogen clearance and tissue resilience.

We report that influenza A virus-induced inflammation triggers an increase in the murine pulmonary LEC population. Through immunohistochemical staining for PROX1, the chief transcriptional regulator of LEC fate, and enumeration of LEC nuclei, we show that by the peak of infection at 7 days post infection (dpi), the LEC population has approximately doubled. At 21 dpi, post viral clearance, LEC numbers have approximately tripled. By quantifying LEC EdU incorporation and performing lineage tracing, we determined that proliferation contributes significantly to this lymphangiogenesis, which is distinct from dilation. We also note that levels of soluble VEGFR2 (sVEGFR2), an endogenous inhibitor of lymphangiogenesis, are lower in whole lung homogenates following influenza infection. An antibody blockade against sVEGFR2 increased PROX1 levels in whole lung homogenates, but did not recapitulate the lymphangiogenic response observed to influenza. Preliminary flow cytometry reveals that the LEC marker podoplanin is downregulated during influenza. Since podoplanin binds CLEC-2, inducing platelet aggregation, perhaps this prevents thrombosis. Our upcoming bulk and single nuclei RNA sequencing (snRNAseq) may shed light on the implications of this change. snRNAseq could also reveal LEC subtypes that mount diverse responses to influenza-induced inflammation.

14***

AIRWAY MIWI2+ MULTICILIATED CELLS AND HOST SUSCEPTIBILITY TO INFLUENZA A INFECTION

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Graduate Program in Molecular & Translational Medicine

Influenza is a major public health concern that targets airway multiciliated cells during early infection. MIWI2, a piRNA binding Argonaute family protein that maintains genomic integrity by suppressing retrotransposons, is expressed by 5% of airway multiciliated cells. However, the function of MIWI2 in somatic cells and its impact on exogenous viral RNAs is unclear. We employed a model of murine A/Puerto Rico/8/1934 H1N1 (PR8) infection in Miwi2$^{+/+}$ (wild-type), Miwi2$^{+/tom}$ (haplosufficient), and a Miwi2$^{tom/tom}$ (deficient) knock-in reporter mice. We found MIWI2 deficient mice exhibited significantly decreased…

Abstract is continued on the following page.

Continued from J. Henao Vasquez, "Airway MIWI2+ Multiciliated Cells…"

…viral burden compared to wild-type and haplosufficient mice. To understand this further, we performed small and bulk RNA sequencing of uninfected sorted MIWI2 haplosufficient and deficient multiciliated cells. A limited number of mRNAs and no miRNAs were differentially expressed in a MIWI2-dependent manner. In addition, we found no MIWI2-dependent changes in expression of LINE-1 nor did we find changes in the distribution of infected epithelial cell populations. In contrast, reductions in levels of tRNA fragments and piRNAs in MIWI2 expressing cells were observed. These data suggest a potential role for MIWI2 in the biogenesis and/or turnover of specific classes of small RNAs during homeostasis. Future studies will determine whether PR8 infection modifies host and viral genomic expression, including small RNAs, within MIWI2+ and MIWI2- multiciliated cells. We anticipate these studies will provide novel information regarding the lung host response to viral infections, and the role of Argonaute family proteins and small RNAs in immune regulation.

15

SERINE/THREONINE KINASE STK25 NEGATIVELY REGULATES MTORC1 ACTIVITY TO SUPPRESS TISSUE OVERGROWTH AND TUMORIGENESIS

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The maintenance of cellular and tissue homeostasis relies on tight regulation of cell growth and proliferation. These processes are predominantly regulated by two evolutionarily conserved pathways: the mammalian Target of Rapamycin (mTOR) pathway and Hippo pathway. Dysregulation of both mTOR and Hippo pathways promotes aberrant cell proliferation, tissue overgrowth and tumorigenesis. Elucidating how the mTOR and Hippo signaling pathways coordinate their activities is therefore a vitally important, yet poorly understood, area of cell cancer biology. Our lab previously demonstrated that STK25, a serine/threonine kinase, functions as an upstream activator of Hippo signaling to limit cell growth. We now demonstrate that STK25 also functions to regulate mTORC1 signaling. We observe that genetic deletion of *STK25* hyperactivates the mTOR pathway, both *in vitro* and *in vivo*. To elucidate the molecular mechanisms of STK25, we utilized a BioID2 proximity labeling system and comparative LC MS/MS analysis to identify a list of proteins that interact significantly with STK25. Based on those findings, we propose that STK25 disrupts the 55tabilityy of the mTORC1 protein complex by interfering with the CCT/TRiC chaperone protein complex, ultimately attenuating mTOR signaling. Furthermore, using a conditional *STK25* knockout mouse model, we found that deletion of *STK25* alone is sufficient induce tumorigenesis. Taken together, our data suggests that STK25 is critical in modulating cell growth and tumor suppression by negatively regulating the mTORC1 pathway. This would establish STK25 as an upstream regulator of both the Hippo and mTOR pathways, providing further insights to the coordinated regulation of these two crucial pathways.

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REGENERATION OF MOUSE TRACHEAL EPITHELIUM VIA TRANSPLANTATION OF PLURIPOTENT STEM CELL-DERIVED BASAL LIKE CELLS

L. Ma, P. Bawa, ML. Beermann, A. Berical, B. Thapa, F. Hawkins, M. Herriges, J. Le Suer, H. Kiyokawa, A. Kohn, D. Kotton, T. Matte, A. Tilston-Lünel, X. Varelas, F. Wang, and A. Ysasi

Graduate Program in Molecular & Translational Medicine

Lung diseases resulting from airway epithelial dysfunction such as cystic fibrosis are responsible for significant morbidity and mortality. In vivo engraftment of mutation-corrected airway stem cells generated from pluripotent stem cells (PSC) could provide a novel autologous cell-based therapy. To simulate future transplantation approaches for treating recipients without immunosuppression, we sought to develop a "pre-clinical" model by transplanting syngeneic engineered mouse cells into immunocompetent mice.

Mouse PSCs were differentiated into airway basal-like cells (iBCs) via in vitro directed differentiation. When transplanted into syngeneic immunocompetent mice following tracheal epithelial injury by polidocanol, iBC-derived cells can be observed more than 1-year post-transplantation. Flow cytometry, immunofluorescence, and scRNA-Seq revealed transplanted cells can contribute to more than 50% of recipients' tracheal epithelium. They maintained their airway epithelial cell identity and expressed both the morphologic and molecular phenotypes of differentiated airway basal, secretory, and ciliated lineages while assuming quiescence. iBC-derived basal cell can participate in repair after injury, and iBC-derived ciliated cells can participate in the concerted mucociliary clearance with normal cilia length and beating frequency. Human iBCs, when transplanted with the same approach into NSG mice trachea, can be detected for at least 6 weeks post-transplantation and contributed to basal, secretory, and ciliated populations in recipient airway epithelium.

Overall, our findings suggest that airway basal-like cells generated from PSC can engraft in airway epithelium in vivo. These results set the stage for disease model rescue experiments, transplantation model establishment in large animals, and may serve as a tentative first-step towards potential future regenerative therapy.

27

INVESTIGATING THE ROLE OF TACI AS A KEY RHEOSTAT FOR BAFF SIGNALING IN NON-INFECTIOUS COMPLICATIONS OF COMMON VARIABLE IMMUNODEFICIENCY

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Common variable immunodeficiency (CVID) is the most frequently diagnosed primary antibody deficiency, defined by a lack of serum immunoglobulin and poor responses to vaccination. About half of CVID patients develop chronic non-infectious complications associated with autoimmunity and lymphoproliferative disease through mechanisms that remain unclear but are the significant cause of morbidity and mortality. Genetic variants of *Tnfrsf13b*, encoding Transmembrane activator and CAML interactor (TACI), are associated with increased risk of non-infectious complications in CVID. We hypothesize that TACI provides key regulation of B cell activating factor (BAFF) that prevents autoimmunity and lymphoid hyperplasia in the context of CVID-associated BAFF elevations. Plasma assays indicate that CVID subjects with BAFF elevations and low soluble TACI have greater incidence of autoimmune thrombocytopenia and interstitial lung disease compared to CVID subjects with low plasma BAFF or plasma elevations of both BAFF and TACI. Spectral flow demonstrates expanded activated naïve B cell populations in CVID subjects with autoimmune and lymphoproliferative complications. From these studies, we have identified a subset of CVID patients with increased incidence of autoimmune and lymphoproliferative complications in association with their plasma ratio of BAFF to soluble TACI, supporting the importance of TACI as a soluble neutralizing receptor for BAFF in CVID. We begin to illustrate differences in transcriptional identities of naïve B cell subpopulations in CVID that may be contributing to pathogenesis of autoimmune and lymphoproliferative complications. Further studies will investigate the role of BAFF signaling and *Tnfrsf13b* variants in contributing to abnormalities in the naïve B cell compartment in CVID.

48

CYCLICAL VARIATIONS AND CYTOLYTIC FUNCTION OF COMPLEMENT PROTEINS IN HUMAN CERVICAL MUCUS

E. Mausser, D. Anderson, J. Marathe, J. Politch, and M. Tjilos

Graduate Program in Molecular & Translational Medicine

The complement system mediates complement dependent cytotoxicity (CDC) to eliminate pathogens and foreign cells. However, complement concentrations and function in cervical mucus are not well characterized. Ten healthy women of reproductive age provided three paired samples of serum and cervical mucus during each phase of their menstrual cycle: follicular, ovulatory, and luteal. Thirteen complement components were quantified using bead-based multiplex assays. In serum, concentrations of complement proteins remained consistent over the course of the menstrual cycle. However, in cervical mucus, average…

Abstract is continued on the following page.

Continued from E. Mausser, "Cyclical Variations and Cytolytic Function…"

…concentrations of components were often decreased in the ovulatory sample compared to the follicular sample, with MBL, C2, C4b, and C5a all being significantly lower at ovulation. Cervical mucus complement concentrations were on average 308-fold (median= 84) lower than in serum and the distribution of complement proteins also varied between the two sample types. The most abundant classical complement proteins in serum, in descending order, were C4, C1q, and C3 while in cervical mucus, C3b/iC3b, C4, C3, and C2 were the most abundant. Despite these differences, ovulatory cervical mucus effectively induced CDC against two antibody-coated target cells: sperm and red blood cells. Cervical mucus significantly reduced sperm motility by 53% and lysed opsonized red blood cells with 10.6% the hemolytic potency of serum. This study showed that human cervical mucus contains detectable and functional levels of complement proteins. These findings may inform future development of antibody-based drugs by demonstrating the possibility of mucus to induce complement-mediated lysis of pathogens at and sperm in the female reproductive tract.

49

EXPRESSION OF ALPHA-1 ANTITRYPSIN AND RESULTING CELLULAR STRESS IN ALPHA-1 ANTITRYPSIN DEFICIENT TYPE 2 ALVEOLAR EPITHELIAL CELLS

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Graduate Program in Molecular & Translational Medicine

Alpha-1 antitrypsin deficiency (AATD) is a monogenic disease that leads to both liver disease and emphysema. While AATD liver disease is the result of misfolded mutant "ZAAT" accumulation and resulting proteotoxicity in hepatocytes, AATD emphysema has classically been attributed to reduced circulating AAT levels and associated protease/antiprotease imbalance in the lung. There is evidence for ZAAT-driven proteotoxicity in extrahepatic cells, such as monocytes. Although it has recently been shown that type 2 alveolar epithelial cells (AT2s) express the gene encoding AAT, the consequences of ZAAT protein expression in AATD patient cells has not been examined. We hypothesize that ZAAT accumulates intracellularly in AATD AT2s, inducing proteotoxicity that contributes to emphysema. Using immunofluorescent microscopy, we identified co-localization of AAT and AT2s in AATD but not in healthy lung tissue, consistent with the possibility that intracellular AAT protein levels are elevated in AATD AT2s. To determine the impact of ZAAT expression in AT2s, AATD patient-derived induced pluripotent stem cells, along with CRISPR/Cas9-corrected syngeneic controls, were differentiated into AT2-like cells (iAT2s). iAT2s were plated at an air-liquid interface and exposed to cigarette smoke injury before undergoing single cell RNA sequencing. Analysis of DEGs identified enrichment of hallmark gene sets indicating cellular stress in AATD iAT2s compared to their gene-corrected counterparts. Overall, our findings identify heterogeneous AT2 intracellular AAT protein in AATD explant lung tissue, and transcriptional evidence of cellular stress in AATD iAT2s. Future studies will investigate the functional impacts of ZAAT expression on AT2s, and how this expression contributes to emphysema.

35

THE TRANSCRIPTION FACTOR PAX3 AS A REGULATOR OF NONSENSE-MEDIATED DECAY IN MELANOMA

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The transcription factor PAX3 regulates melanoma development via binding and modifying the activity of other transcription factors. While PAX3 functions by regulating genes through regulatory enhancers, the molecular mechanisms by which PAX3 regulates if and how much a gene is expressed are not well understood. Our group carried out a PAX3 immunoprecipitation coupled with mass spectrometry analysis in two melanoma lines to reveal melanoma-specific binding partners and found several proteins involved in RNA degradation and the exon junction complex (EJC), suggesting that the function of PAX3 in melanoma is not limited to transcription regulation. Several PAX3 candidate binding partners are involved in the nonsense-mediated decay (NMD) pathway, an EJC-regulated RNA degradation program that promotes tumor progression by suppressing the expression of tumor suppressor genes. In three melanoma lines PAX3 overexpression (OE) resulted in a 48-89% increase in NMD activity across all cell lines. PAX3 OE resulted in increased phosphorylation of UPF1, a core NMD factor that is phosphorylated during NMD activation. PAX3 OE also resulted in downregulation of known NMD target transcripts. PAX3 was also shown to interact with EJC factors in the cytoplasm. This supports that PAX3 transcriptional function is not necessary for NMD, but overexpression leads to a significant rise in NMD activity, thereby promoting tumor survival. Identifying the role PAX3 plays in regulating NMD is important as it represents a new PAX3 function outside of transcriptional regulation, and it could serve as an explanation for PAX3's near-universal overexpression in melanomas.

45

EXOSOMES PRODUCED BY ADIPOCYTES INDUCE EMT, AND TUMOR METASTASIS, IN BOTH *IN VIVO* AND *IN VITRO* MODELS OF TNBC

Y. Qiu, R. Yu, A. Chen, P. Llevenes, M. Kolla, N. Jafari, I. Pompa, C. Ennis, C. S. Mazzeo, K. Mahdavian, N. Y. Kg, and G. V. Denis

Graduate Program in Molecular & Translational Medicine

Type 2 Diabetes (T2D) is a chronic disease characterized by inflamed adipose tissue. Patients with triple negative breast cancer (TNBC) and comorbid T2D have higher risk of metastasis and shorter survival. However, mechanisms that couple T2D to TNBC outcomes are unknown. Here we hypothesize that exosomes, small vesicles secreted by tumor microenvironment (TME) breast adipocytes, drive epithelial-to-mesenchymal transition (EMT), metastasis in TNBC.

Exosomes were purified from conditioned media of 3T3-L1 mature adipocytes, either insulin-sensitive (IS) or insulin-resistant (IR), then characterized and quantified by NanoSight. Murine 4T1 cells, a TNBC model, were treated with exosomes *in vitro* (3 days). For *in vivo* models, mammary fat pads of BALB/c mice were injected with 4T1 cells. Histology and immunohistochemistry detected TME differences (angiogenesis; EMT and proliferation marker). Metastases in distant organs were quantified by clonogenic assay, and mRNA were extracted for RNA-seq analysis. Exosomal RNAs were profiled by miRNA array to identify potential candidates responsible for driving metastasis.

Tumor-bearing mice exhibited more metastasis in exosome-treated groups. In primary tumors, vimentin(EMT marker), ki67(proliferation marker) and angiogenesis biomarker CD31 were elevated in IR exosome group vs. control and IS exosomes groups. Clonogenic assay of brain metastases showed more mesenchymal morphology characteristics and RNA-seq revealed enriched EMT pathway genes in IR exosome treated group compared to other groups. miR-let-7b is highly differentially expressed between IS and IR, and potentially regulates metastasis.

IR adipocyte exosomes modify TME, increase EMT and promote metastasis to distant organs, likely through miRNA pathways. We suggest metabolic diseases (e.g T2D) reshape TME, promoting metastasis and decreasing survival. Therefore, TNBC patients with T2D should be closely monitored for metastasis, with metabolic medications considered.

23**

HOST PRECONDITIONING AND TRANSIENT MITOGEN EXPRESSION VIA MRNA-LNP LEAD TO ROBUST PRIMARY HUMAN HEPATOCYTE ENGRAFTMENT AND TRANSIENT IPSC-DERIVED HEPATOCYTE-LIKE CELL SURVIVAL IN MICE

A. R. Smith, F. Rizvi, E. Everton, A. Adeagbo, H. Liu, Y. Tam, N. Pardi, D. Weissman, and V. Gouon-Evans

Graduate Program in Molecular & Translational Medicine and Center for Regenerative Medicine

Liver transplantation is the primary treatment for end-stage liver disease, but limited availability of donor organs highlights the need for alternatives such as transplantation of healthy liver cells. However, challenges in low efficiency and lack of sustained benefit limit the success of primary human hepatocyte (PHH) transplantation, while hepatocyte-like cells (HLC) derived from induced pluripotent stem cells (iPSC) remain in the preclinical stage with poor survival, proliferation, and maturation in liver disease mouse models. To overcome these limitations, we hypothesize that stimulating regenerative pathways in transplanted hepatocytes with hepatocyte growth factor (HGF) and epidermal growth factor (EGF) and preconditioning the host liver with P21 expression to prevent host hepatocyte proliferation will improve engraftment of PHHs and HLCs in an injured mouse liver. We established safe way to express HGF and EGF specifically in the liver using nonintegrative nucleoside-modified mRNA encapsulated in lipid nanoparticles(mRNA-LNP), and used AAV8-Tbg-P21 to precondition the host liver with long-lasting P21 expression specifically in hepatocytes. NSG-PiZ mice were used as an injury model recapitulating alpha-1 antitrypsin deficiency associated liver disease. We find that both AAV8-Tbg-P21 and HGF and EGF mRNA-LNP treatments significantly improved transplanted PHH survival and proliferation in NSG-PiZ mice, leading to robust and functional human cell repopulation (~30%) and improved liver disease. Furthermore, HGF and EGF mRNA-LNP transiently improves transplanted HLC survival in NSG-PiZ mice. Overall, stimulating survival and proliferation in transplanted hepatocytes and inhibiting host hepatocyte proliferation are highly effective strategies to improve PHH engraftment, and thus may be promising for promoting HLC engraftment.

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

Lucas Carstensen (8)
Kaitlyn Dorst (17**)
William Lynch (32)
Ryan McCann (4)
Beverly Setzer (*)
Nicole Tomassi (37)
Lucius Kelton Wilmerding (41***)

8

ADVANCING TRACKING IN RODENT STUDIES: 3-DIMENSIONAL RECONSTRUCTION OF ANIMAL POSE DURING FREELY MOVING BEHAVIOR

L. Carstensen, M. Patel, Y. Guo, M. Betke, and M. Hasselmo

Graduate Program for Neuroscience

Accurate and precise measurement of animal behavior, such as the position, orientation and movement of their head, body, and limbs, is essential for studies in neuroscience, ecology, and psychology. Computer vision has long been used to facilitate these studies with two-dimensional analysis of laboratory animals, three-dimensional analysis of wild animals, and deep learning to estimate the 3D pose of animals. Progress has been hindered by the lack of publicly available video datasets and the difficulty of obtaining accurate 3D data. In our study, we created a multi-camera setup to capture rodents in a laboratory using three types of cameras: RGB, depth, and thermal infrared. We also provide a novel dataset named Rodent3D containing videos of rodents foraging in an open environment. This unique dataset includes high-speed multi-view thermal and RGB-Depth videos, calibration data obtained using a thermoelectric calibration cube that we constructed, synchronization data, hand-labeled 2D keypoints, and 3D reconstructions. We also propose a deep-learned model called OptiPose, which utilizes representations of 3D pose and a self-attention mechanism to interpret spatial and temporal patterns for 3D pose sequence optimization. Additionally, we provide tools for analyzing animal behavior that can be used alongside popular tracking methods. The dataset and code are also publicly accessible. This new multi-modal dataset enables researchers in the field of animal pose estimation, tracking, and/or multi-modal computer vision to have access to an additional dataset and our method of 3D pose tracking will greatly benefit other researchers in neuroscience, computer vision, and other disciplines.

17**

HIPPOCAMPAL ENGRAMS GENERATE FLEXIBLE BEHAVIORAL RESPONSES AND BRAIN-WIDE NETWORK STATES

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Memory engrams are both necessary and sufficient to mediate behavioral outputs. Defensive behaviors such as freezing and avoidance are commonly examined during hippocampal-mediated fear engram reactivation, yet how reactivation of these cellular populations across different contexts engages the brain to produce a variety of defensive behaviors is relatively unclear. To address this, we first optogenetically reactivated a tagged fear engram in the dentate gyrus (DG) subregion of the hippocampus across three spatially distinct contexts. We found that there were differential amounts of light-induced freezing depending on the size of the context in which reactivation occurred; mice demonstrated robust light-induced freezing in the most spatially restricted of the three contexts but not in the largest. In lieu of freezing, these mice exhibited ambulatory responses during hippocampal-mediated fear engram reactivation. We then utilized graph theoretical analyses to identify brain-wide alterations in cFos co-activation during engram reactivation across the smallest and largest contexts. Our manipulations conferred greater positive cFos correlations and recruited regions spanning putative fear and defense systems as hubs in the respective networks. Moreover, reactivating DG-mediated engrams generated network topologies across experimental conditions, emphasizing both shared and distinct features. By identifying and manipulating the circuits supporting memory function, as well as their corresponding brain-wide activity patterns, it is thereby possible to resolve systems-level biological mechanisms mediating memory's capacity to modulate behavioral states.

32

VALIDATING ZHX2 IN OXYCODONE METABOLITE (OXYMORPHONE) BRAIN CONCENTRATION AND BEHAVIOR VIA RECIPROCAL GENE EDITING AND VIRAL MANIPULATION OF GENE EXPRESSION IN BALB/C SUBSTRAINS

W. Lynch, J. Beierle, R. Bhandari, A. Farnan, I. Kazerani, S. Miracle, BM. Nguyen, G. Saavedra, and C. Bryant

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Department of Pharmacology and Experimental Therapeutics

Opioid Use Disorder **(OUD)** maintains epidemic proportions in the U.S., with current pharmacological treatments limited to opioid substitution therapy. Sensitivity to the subjective and physiological responses to opioids has a genetic component that could influence addiction liability. We identified *Zhx2* as a candidate gene underlying increased oxycodone **(OXY)** metabolite brain concentration in BALB/cJ (**J**) vs. BALB/cByJ (**By**) females. The metabolite, oxymorphone (**OMOR**), is more potent and efficacious and…

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…could enhance state-dependent learning and recall of OXY-induced conditioned place preference (CPP) in J vs. By females. A structural intronic variant causes a significant reduction in Zhx2 expression in J vs. By mice. Thus, here, we tested the role of this variant in OMOR levels and OXY behaviors through gene editing of the variant, through modeling Zhx2 loss-of-function via exon 3 deletion, and through virally manipulating *Zhx2*. We are still validating the Zhx2 variant on OMOR and behavior. Following AAV-mediated liver overexpression of Zhx2, J females showed an increase in state-dependent OXY reward learning and a decrease in OXY-induced locomotor sensitivity. We also observed an increase in Cyp2d22 RNA, thus providing a potential intermediary mechanism linking Zhx2 with differential brain OMOR concentration. Complementary to these results, there was an increase in OXY-induced locomotor sensitivity when *Zhx2* was knocked out and an increase in state-dependent reward learning. Our work supports validation of *Zhx2* as a quantitative trait gene underlying brain OMOR concentration and behavior, which could increase our understanding of OXY addiction liability in humans.

4

MESENCHYMAL STEM CELL EXTRACELLULAR VESICLES IMPROVE MOTOR FUNCTION, REDUCE INFLAMMATION AND INCREASE REPAIR FOLLOWING CORTICAL INJURY IN THE RHESUS MONKEY

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Cortical injury is a leading cause of functional disability and there is a critical need for interventions. Previous work in our rhesus monkey model of cortical injury demonstrated that systemic treatment with mesenchymal stromal cell extracellular vesicles (MSC-EVs) facilitated full functional motor recovery in 3-5 weeks post-injury. However, the temporal changes that occur with MSC-EV treatment are largely unknown. Therefore, it is important to determine whether MSC-Evs facilitated recovery through preventing damage, or enhancing repair. We hypothesized that MSC-Evs play an immunomodulatory and regenerative role early in the post-injury period. To assess this hypothesis, 8 middle-aged female monkeys were trained to complete a fine motor function task, and then a lesion was made in the primary motor cortex. Monkeys then received MSC-Evs (n=4) or vehicle (n=4), completed fine motor function testing for 4 weeks and then were euthanized and the brains harvested. Blood and CSF samples were also collected prior to and during recovery. Treated monkeys demonstrated a similar rate and pattern of recovery as the monkeys in the previous study. ELISA quantification of neurofilament light in CSF suggested an increase in damage clearance in the MSC-EV treated animals. Using the Olink proximity extension assay, we found an overall suppression of the immune response in plasma from MSC-EV treated animals. Real time quantitative polymerase chain reaction was performed on brain tissue and treated monkeys had increases in myelin transcripts, suggesting a pro-myelination environment. These findings demonstrate that MSC-Evs lower inflammation and increase markers of myelination, underscoring their potential as a therapeutic for cortical injury.

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A TEMPORAL SEQUENCE OF THALAMIC ACTIVITY UNFOLDS AT TRANSITIONS IN BEHAVIORAL AROUSAL STATE

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Awakening from sleep reflects a profound transformation in neural activity and behavior. The thalamus is a key controller of arousal state, but whether its diverse nuclei exhibit coordinated or distinct activity at transitions in behavioral arousal state is unknown. Using fast fMRI at ultra-high field (7 Tesla), we measured sub-second activity across thalamocortical networks and within nine thalamic nuclei to delineate these dynamics during spontaneous transitions in behavioral arousal state. We discovered a stereotyped sequence of activity across thalamic nuclei and cingulate cortex that preceded behavioral arousal after a period of inactivity, followed by widespread deactivation. These thalamic dynamics were linked to whether participants subsequently fell back into unresponsiveness, with unified thalamic activation reflecting maintenance of behavior. These results provide an outline of the complex interactions across thalamocortical circuits that orchestrate behavioral arousal state transitions, and additionally, demonstrate that fast fMRI can resolve sub-second subcortical dynamics in the human brain.

37

EFFECTS OF AUTONOMIC AROUSAL AND COGNITIVE LOAD ON SENORIMOTOR ADAPTATION OF SPEECH

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[1]Graduate Program for Neuroscience
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Shared neural substrates suggest possible relationships among cognition, autonomic arousal, and speech motor control, but these systems have yet to be studied cohesively. Thus, the purpose of this study was to investigate the effects of cognitive load and autonomic arousal on sensorimotor adaptation of speech. Adults with typical speech (n = 30) were exposed to persistent errors to voice fundamental frequency (f_o) and the first formant frequency (*F1*) in two cognitive load conditions. Physiological measures of the autonomic nervous system (ANS) were simultaneously recorded to determine if changes in autonomic arousal and…

Abstract is continued on the following page.

Continued from N. Tomassi, "Effects of Autonomic Arousal and Cognitive Load…"

…cognitive load were associated with the ability to adapt to these errors. Results indicated cognitive load condition as a statistically significant predictor for f_0 responses and ANS arousal changed significantly between conditions. However, an order effect was found in the *F1* adaptive responses and changes were found to be driven by which condition was presented first. Further investigation revealed changing of articulatory targets was driving this effect and the amount of target change was influenced by the condition. This study was the first to describe the relationships among cognition, autonomic arousal, and sensorimotor adaptation of speech and poses important clinical implications for error-based learning.

41***

EXPLORING THE CONTRIBUTION OF DENTATE GYRUS TO MEMORY GUIDED SPATIAL NAVIGATION IN MICE

L. K. Wilmerding[1,2,3], I. Kondratyev[1,3,4], W. B. [1,3,4], S. Ramirez[1,2,3,4], M. E. Hasselmo[1,2,3,4]

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The hippocampus has been studied extensively for its role in navigation and episodic memory, in particular the CA1 region. To understand the influence of upstream regions on memory-guided navigation in CA1, we manipulated ensembles of learning-associated cells in mouse dentate gyrus (DG) using optogenetics. The DG is necessary for memory-guided spatial navigation tasks such as the Delayed-Non-Match-to-Place (DNMP) T-maze task. Each DNMP trial requires a mouse to traverse one route of the T-maze in the sample phase, and after a short delay choose the opposite route during the subsequent test phase, allowing dissection of the encoding and retrieval stages of memory respectively. Using the fos-tet-tag activity-dependent labeling system in male and female mice, multiple ensembles of DG cells were tagged across learning and experimental days with channelrhodopsin2-eYFP (experimental) or eYFP (control). Across five experimental days, 20Hz pulsatile optogenetic stimulation was delivered bilaterally to DG during pseudo-randomly chosen sample phases of the DNMP task to stimulate the tagged 'promiscuous' ensembles. Within-subjects analyses of trial accuracy, speed, and choice point occupancy were used to assess the behavioral outcomes of this 'nonsense' stimulation during ongoing navigation, revealing decreased accuracy during stimulation trials in the experimental group only. Additionally, we tested for correlation between the degree of behavioral impairment and the size of the labeled population. These results extend previous findings on the role of the DG in spatial navigation tasks, in particular its contribution to the encoding of spatial memories for subsequent retrieval in memory-guided decision making.

Nutrition & Metabolism Program

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

*1st Prize

The accompanying number indicates each abstract's poster board.

Participants

Ting-Yu Fan (7)
Ioanna Yiannakou (*)
Xinyi Zhou (19)

7

ADIPOCYTE DERIVED FACTORS INHIBIT GLUCOSE-STIMULATED INSULIN SECRETION FROM CLONA-PANCREATIC BETA-CELLS (INS-1)

T. Fan, J. English, J. T. Deeney, and V. Perissi

Nutrition and Metabolism Program

Background: Obesity has similar causative factors and is linked to increased risk for type 2 diabetes (T2D). Pancreatic beta-cells exposed to chronic excess nutrients (glucose and fatty acid) exhibit characteristics of glucolipotoxicity (GLT), including elevated basal, increased glucose sensitivity and decreased glucose-stimulated insulin secretion (GSIS) that may precede the full development of T2D. Adipose tissue, as an endocrine organ, plays an impactful role in intercellular communications and interorgan crosstalk. Here we examine whether crosstalk between adipocytes and pancreatic beta-cells contribute to beta-cell dysfunction leading to insulin resistance and T2D.

Methods: Clonal pancreatic beta-cells (INS-1) were cultured in low (4 mM) and high (11 mM) glucose and further incubated for 18 hrs with conditioned media or exosomes collected from mouse epididymal white adipose tissue (eWAT). Conditioned media was collected from both explant (minced eWAT) and primary isolated adipocytes from both chow and high-fat diet (HFD) mice. Exosomes were extracted from adipocyte-conditioned media from HFD mice. GSIS was measured at 1, 4 and 12 mM glucose for 2 hrs using an insulin HTRF immunoassay kit (Cisbio).

Results: GSIS was inhibited from INS-1 cells cultured in 4 mM glucose and exposed to both explant and adipocyte-conditioned media from chow-fed mice. There was a greater inhibition of GSIS when conditioned media was collected from adipose tissue from HFD mice. Exosomes extracted from adipocytes from HFD mice showed a trend to decrease GSIS.

Conclusion: Crosstalk between adipocytes and beta-cells may play an important role in beta-cell function and the regulation of GSIS.

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EGGS AS PART OF A HEALTHY EATING PATTERN ARE NOT ADVERSELY ASSOCIATED WITH DYSLIPIDEMIA IN THE FRAMINGHAM OFFSPRING STUDY

I. Yiannakou[1,2], M. Mott[3], X. Zhou[1], M. R. Singer[1], and L. L. Moore

[1] Department of Medicine, Section of Preventive Medicine and Epidemiology
[2] Nutrition and Metabolism Program
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Eggs have been considered unhealthy out of concern about the high content of dietary cholesterol; however, recent evidence questions this. We examined the association of egg consumption with plasma lipid levels and risk of dyslipidemia in the Framingham Offspring Study and determined whether other dietary factors modify these effects. We included 1,852 adults aged 30-64 with available 3-day food records without prevalent CVD or use of lipid- or glucose-lowering medications. Multivariable Cox proportional hazard models were used for elevated fasting LDL-C and triglyceride risks. Analysis of covariance models were used to estimate mean plasma levels of total cholesterol, LDL, LDL:HDL ratio, and triglycerides adjusted for age, BMI, and other dietary factors. Over approximately 6 years of follow-up, 372 and 306 adults developed new-onset elevated LDL and triglyceride levels, respectively. Those with higher egg consumption (vs. lower) had very similar intakes of other healthy foods at baseline (e.g., whole grains, fruits, vegetables, dairy, and fish). Men and women consuming ≥5 eggs per week (vs. <0.5) had no increased risk of elevated LDL (HR=0.85, 95% CI: 0.59-1.22) or triglycerides (HR=1.25, 95% CI: 0.83-1.86). In fact, men with higher egg intakes (≥5 vs. <0.5 per week) had a total cholesterol level that was 8.6 mg/dL lower, an LDL level that was 5.9 mg/dL lower, and a log-transformed triglyceride level that was 0.13 units lower ($p<0.05$ for all differences) after 4 years of follow-up. Further, participants with higher egg consumption in combination with higher fiber intake had lower levels of all lipids ($p<0.05$). This study suggests that higher egg intakes had no adverse effects on serum lipids in healthy adults and may be beneficial as part of a healthy eating pattern.

19

BUTTER, MARGARINE, AND OILS: EFFECTS ON CARDIOMETABOLIC RISK

X. Zhou, L. L. Moore, M. R. Singer, and M. Yuan

Nutrition and Metabolism Program

Cardiovascular diseases (CVD) and type 2 diabetes (T2DM) pose significant public health challenges but the effects of different dietary fats, particularly saturated fats, on these outcomes are controversial. Butter is an important source of saturated fat. Margarines contain both polyunsaturated fats and trans fats, while oils include polyunsaturated, monounsaturated, and saturated fats, depending on the type of oil. In this study, we compare the effects of butter with those of margarine and oils on biomarkers of cardiometabolic risk as well as risks of CVD and T2DM. After exclusions, 2459 subjects (≥30 years) from the prospective Framingham Offspring Study were examined. Analysis of covariance and Cox proportional hazards models estimated the effects of butter, margarine, and oils on cardiometabolic risk factors and CVD/T2DM risks. We found that higher intakes of butter (>5 vs. 0 g/day) were associated with less insulin resistance (p=0.0011), higher HDL levels (p=0.0021), lower triglyceride (TG) levels (p=0.0032), and a lower TG:HDL ratio (p=0.0052), as well as a 31% lower risk of developing T2DM (95% CI: 0.49-0.97). Higher margarine intakes (>7 vs. <2g/day) were not associated with biomarker levels but showed a 29% increased risk of CVD (95% CI:1.02-1.63) and a 41% increased risk of T2DM (95% CI:1.02-1.95). Finally, higher oil consumption (>7 vs. ≤2g/day) was associated with a 0.6 kg/m^2 higher BMI and 8 mg/dL higher LDL-C levels. Neither CVD nor T2DM risk was associated with oil intake. These results suggest that butter may be a better fat choice for lowering cardiometabolic risk.

Department of Pathology & Laboratory Medicine

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

*1st Prize

The accompanying number indicates each abstract's poster board.

Participants
Guillermo Arroyo Ataz (*)

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CHARACTERIZATION OF THE DEVELOPMENTAL ORIGIN OF POPLITEAL LYMPHATIC SMOOTH MUSCLE CELLS

G. Arroyo-Ataz[1]; D. Jones[1]; T. Padera[2]; J. Rajotte[2] and K. Ruscic[2]

[1] Department of Pathology and Laboratory Medicine
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Dysfunctional lymphatic smooth muscle cells (LMCs) have been linked to lymphedema (1-3), a debilitating disease of lymphatic vessel insufficiency. LMCs cover large collecting lymphatic vessels and are the principal drivers of lymphatic vessel contractility. Restoring the contractility of lymphatic vessels is an attractive treatment for lymphedema patients with LMC damage, but little is known about the unique developmental program and molecular profile of LMCs.

We generated Nkx2.5 reporter mice and Wilms' tumor 1 (WT1) reporter mice to investigate whether LMCs originate from cardiac muscle or smooth muscle progenitors, respectively (1,4,5). Whole-mount immunofluorescence analysis of collecting lymphatic vessels from neonatal and adult mice revealed that LMCs originated from $WT1^+$, but not $Nkx2.5^+$ progenitors. These data suggest that WT1-derived LMCs arise during neonatal development and are maintained into adulthood. Next, we assessed the contribution of WT1-derived cells to the LMC population by using diphtheria toxin to conditionally deplete cells derived from progenitors that expressed WT1. We found that after ablation, the collecting lymphatic vessels of these mice showed significantly lower LMC coverage (63% vs 43%, p=0.006). We then assessed the role of WT1-derived cells on lymphatic function by measuring collecting lymphatic vessel contractility with intravital fluorescence microscopy. Ablation of WT1-derived cells significantly decreased lymphatic vessel contraction, as measured by ejection fraction (0.47% vs 0.15%, p=0.002), compared to controls.

Finally, we optimized a method to extract collecting lymphatic vessels and obtain WT1-derived cells from the vessels for further characterization (6). Future studies will use single-cell RNA sequencing and flow cytometry to molecularly profile WT1-derived cells from collecting lymphatic vessels.

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

Jonique George (40)
Sabrina Kistler (44)
Huang Lee (29)
Jenna Libera (*)
Lucy Peterson (1***)
Matthew Reiss (22**)
Christine Sanganoo (47)
Nicholas Sterge (29)
Chaithanya Vedula (18)
Anna Vitantonio (34)
Rose Zhao (5)

40

TGF344-AD RATS DISPLAY DIFFERING LEVELS OF GABRA5 TRANSCRIPT AND PROTEIN IN THE TRISYNAPTIC HIPPOCAMPAL PATHWAY DURING DISEASE PROGRESSION

J. George, A. Tipton, V. Kumaresan, M. Ratner, D. Farb, and S. Russek

Department of Pharmacology & Experimental Therapeutics

There is no cure for the debilitating and lethal disorder, Alzheimer's Disease (AD), which robs patients of their memories and brain functions. Studies have shown that in the transgenic AD rat model (TgF344-AD), there may be a time dependent loss in the ability of the cognitive enhancer α5IA to increase the amplitude of sharp wave ripples, a property of the hippocampal trisynaptic circuit (HTC) that is associated with memory consolidation. α5IA is a negative modulator of γ-aminobutyric acid type A receptors ($GABA_ARs$) that contain the α5-subunit ($\alpha5GABA_ARs$). Given that this drug enhances memory consolidation in wild-type F344 rats (WT), but is not effective in TgF344-AD rats at 9 months and 16 months, we hypothesized that the loss in α5IA responsivity may be due to a change in the amount and/or localization of $\alpha5GABA_ARs$ in the HTC. Therefore, our overarching hypothesis is that there may be a relationship between $\alpha5GABA_AR$ function and the cognitive decline seen in AD. Towards this goal, we examined the spatial distribution of $\alpha5GABA_AR$ transcripts, *Gabra5*, at the single cell level during disease progression, and used immunofluorescence to measure corresponding protein levels. In addition, we examined the spatial…

Abstract is continued on the following page.

J. George, "TgF344-AD Rats Display Differing Levels of Gabra5 Transcript…"

…overlap of α5GABA$_A$Rs with pre-synaptic and post-synaptic markers to assess synaptic localization of receptor populations, including extrasynaptic. In WT, there may be an age dependent increase in α5GABA$_A$Rs at the post-synaptic site of the synapse at 16 months ~2-fold (CA1, p=0.01; DG, p=0.05) and a trend towards an age dependent decrease in extrasynaptic receptors ~0.7-fold (27% decrease) (CA1, p=0.06). In contrast, in TgF344-AD rats in both the CA1 and DG, there is no age dependent increase in post-synaptic α5GABA$_A$Rs as seen in WT because the levels are the same between 9 months and 16 months. These results suggest that the alteration in the hippocampal trisynaptic circuit of TgF344-AD rats may involve an age dependent reorganization of α5GABA$_A$Rs at post-synaptic sites in pyramidal neurons. Future studies will test this hypothesis and look at the role of α5GABA$_A$Rs directly in the local circuit activity that underlies memory consolidation and the cognitive impairment that accompanies AD.

44

HETEROGENEITY OF *APOER2* ISOFORMS IN THE HUMAN ALZHEIMER'S DISEASE BRAIN AND THEIR CELLULAR AND FUNCTIONAL IMPLICATIONS

S. A. Kistler, U. Beffert, C. M. Gallo, A. Ho, A. T. Labadorf, and A. Marinescu

Department of Pharmacology & Experimental Therapeutics

The two largest risk factors for Alzheimer's disease (AD) are age and the e4 allele of apolipoprotein E (*APOE*). APOE is involved in neuronal lipid homeostasis and is known to bind to transmembrane lipoprotein receptors such as APOER2. Recent studies have implicated alternative splicing defects in AD and other neurodegenerative diseases. One such splicing defect lies within exon 18 in *APOER2*, where AD individuals demonstrate less exon 18 inclusion. Due to *APOER2*'s high degree of cassette exon splicing events and enrichment in the brain, we hypothesized that alternative splicing of *APOER2* may be altered in AD brains, and these unique isoforms might impact cellular changes and affect synaptic function. To profile the entire *APOER2* transcript, we used single molecule long-read RNA sequencing from the hippocampus and parietal cortex of three human female control and three female Braak stage IV AD brain tissue. Additionally, we examined novel *APOER2* isoforms at the cellular level to determine changes in APOE induced receptor cleavage and effects on synaptic function. Our data indicates *APOER2* is dysregulated in both individual exon alternative splicing and full-length transcripts in the hippocampus and parietal cortex of AD brains compared to control. We also found different combinations of *APOER2* splicing events give rise to changes in APOE induced receptor cleavage and synapse prevalence potentially influencing overall synaptic function. We conclude that atypical alternative splicing of *APOER2* in neurodegeneration could provide key insight into the association between APOE genotype and AD.

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RNA DEMETHYLASE, ALKBH5, DECREASES N6-METHYLADENOSINE (M6A) MODIFICATIONS UNDER OXIDATIVE STRESS AND INCREASES TRANSLATION

J. Libera, E. Buoso, L. Dorrian, L. Jiang, G. Socorro, C. Webber, and B. Wolozin

Department of Pharmacology & Experimental Therapeutics

The processes of RNA metabolism (e.g., splicing, export from nucleus to cytoplasm, translation, degradation) are precisely controlled and regulate cellular homeostasis. **N^6-methyladenosine (m^6A)** modifications of RNA have been demonstrated to help regulate RNA metabolism.[1][2][3][4] However, in cellular stress and disease, RNA metabolism and m^6A levels become dysregulated; our lab discovered that m^6A modifications are increased in Alzheimer's Disease.[5] We have sought to investigate the consequential **translational stress responses (TSRs)** that occur with dysregulation of RNA metabolism (e.g., mislocalization of RNA-binding proteins, formation of stress granules, changes in post-translational modifications, reduced protein synthesis).[6] While these TSRs have been well characterized in several cell lines using a variety of stressors, the role of m^6A in TSRs remains less clear. **We hypothesize that reducing m^6A modifications in oxidative stress can alleviate TSRs**. Using immortalized cells treated with sodium arsenite to induce oxidative stress, we reduced m^6A level by overexpressing **ALKBH5**, an m^6A demethylase. Here, we show that m^6A reduction does not abrogate TSRs as defined by stress granule formation, phosphorylation of eIF2α, and decreased translation. However, we discovered that ALKBH5 overexpression increases protein synthesis under basal conditions. Since decreased protein synthesis occurs in many neurodegenerative diseases, we aim to identify m^6A-dependent differentially expressed genes with ALKBH5 overexpression and investigate their function using *in vitro* models of Alzheimer's Disease. Investigating the role of m^6A and ALKBH5 overexpression in TSRs could provide insights toward restoring aberrant RNA metabolism and could be extrapolated to broader pathological applications in neurodegenerative diseases.

1***

DOWNREGULATION OF GLUA2 MRNA EDITING IS REQUIRED FOR THE EXPRESSION OF HOMEOSTATIC SYNAPTIC PLASTICITY

L. Peterson, and H. Man

Department of Pharmacology & Experimental Therapeutics

Homeostatic synaptic plasticity (HSP) is a negative feedback mechanism to control neuronal firing after exposure to destabilizing stimuli, and is necessary to maintain neuronal stability. HSP is carried out by changes to postsynaptic expression of AMPA receptors (AMPARs). Our lab has shown that calcium permeable AMPARs (CP-AMPARs) are necessary for the initiation of HSP after activity deprivation. However, the mechanisms underlying the formation of CP-AMPARs remain unknown. The calcium permeability of AMPARs is determined by the presence and subtype of the GluA2 subunit. GluA2 is subject to A-to-I mRNA editing by the enzyme ADAR2. This editing event converts a glutamine to an arginine in the ion pore, rendering the edited GluA2 (GluA2R)-containing AMPAR calcium impermeable. Here, we identify a novel role for unedited GluA2 (GluA2Q) in HSP. We show that GluA2Q expression increases *in vitro* in cortical neurons after chemical activity deprivation and *in vivo* in the visual cortex (V1) after binocular deprivation. This change is mediated by mislocalization and activity inhibition of ADAR2. In addition, decreased ADAR2 is necessary for deprivation-induced calcium influx, as well as HSP expression. both *in vitro* and *in vivo*. In addition, we find that this increased AMPAR-mediated calcium is necessary to increase GluA1 acetylation, which stabilizes synaptic GluA1 by blocking ubiquitination. GluA1 acetylation is necessary for HSP expression and is mediated by calcium-dependent translocation of the acetyltransferase p300.

22**

THE EXORIBONUCLEASE XRN2 MEDIATES DEGRADATION OF THE LONG NON-CODING TELOMERIC RNA, TERRA

M. Reiss and R. L. Flynn

Department of Pharmacology & Experimental Therapeutics

Telomere dysfunction is a significant source of genomic instability and contributes to the development of cancer. The multi-protein complex shelterin binds telomeric DNA to mitigate telomere dysfunction and ensure telomere stability. In addition to shelterin, the telomeric cap includes the telomeric repeat-containing RNA, TERRA, which associates with telomeric proteins and the DNA itself, often forming RNA:DNA hybrids (R-loops). TERRA is most abundant in cancer cells that utilize the alternative lengthening of telomeres (ALT) pathway, where it has been suggested that TERRA R-loops act as a source of replication stress at telomeric DNA that ultimately contributes to the activation of the ALT mechanism. In an effort to evaluate the effect TERRA may have on the emergence of the ALT phenotype, we sought to identify the enzyme(s) that regulate TERRA degradation in mammalian cells. Here, we leveraged an auxin-inducible degron (AID) system to identify the 5'-3' exoribonuclease XRN2 as a direct modulator of TERRA stability in mammalian cells. Following XRN2 depletion, we demonstrate a significant increase in TERRA…

Abstract is continued on the following page.

Continued from M. Reiss, "The Exoribonuclease XRN2 Mediates Degradation…"

…on chromatin in both non-ALT and ALT-positive cell lines. While the stabilization of TERRA on chromatin alone was insufficient to drive replication stress and activation of ALT in telomerase cells, depletion of XRN2 in the ALT-positive context led to a significant increase in R-loops and DNA damage signaling at telomeric DNA. Thus, increased TERRA stability alone is unlikely to activate ALT but may instead exacerbate ALT activity. Taken together, we demonstrate that XRN2 regulates TERRA stability, that defects in TERRA metabolism can alter telomere stability, and dysfunction in both factors drives telomere dysfunction in cells that rely on the ALT pathway.

47

MODULATING FERROPTOSIS WITH AN IRON RESPONSIVE MRNA TO PROMOTE CELL DEATH IN PANCREATIC CANCER

C. Sanganoo, Z. Mattes, and M. Grinstaff

Department of Pharmacology & Experimental Therapeutics

Pancreatic cancer has one of the lowest five-year survival rates across all cancers and current treatments have minimal effect. As the disease progresses, pancreatic cancer cells acquire mutations allowing for evasion of cell death. Ferroptosis is an iron-dependent mechanism of cell death that is triggered by dysregulated intracellular reactive oxygen species (ROS). Despite pancreatic cancer cells harboring high levels of intracellular ROS and labile iron, attaining aspartate from the tumor microenvironment allows for escape from ferroptosis by blocking redox signaling. In this study, we explore methods to increase intracellular ROS and to design an iron responsive mRNA therapeutic to promote targeted ferroptosis in pancreatic cancer. We found that treatment with an aspartate metabolism inhibitor and labile iron contributes to the dysregulation of ROS in PANC1 and BxPC3 cell lines. The dysregulation of ROS led to increased cell death that was rescued with the availability of aspartate. We also designed a luciferase mRNA library containing iron responsive elements at varying locations in the mRNA 5' untranslated region. Each of these mRNAs show preferential expression in cells with high levels of labile iron. Moving forward, we will optimize the selectivity of the iron responsive mRNAs and replicate our results in a 3D cell culture model and *in vivo*. We will also explore mRNA sequences that encode a pro-ferroptotic protein. Altogether, this project will provide more insight to the interface of aspartate metabolism and redox homeostasis and explore a novel means of targeted mRNA expression to selectively promote ferroptosis in pancreatic cancer cells.

29

YAP/TAZ ACTIVITY DICTATES CELL SURVIVAL FOLLOWING PROLONGED MITOSIS

N. Sterge*, L. Huang*, A. Bolgioni, S. Lim, M. Vittoria, and N. Ganem

Department of Pharmacology & Experimental Therapeutics
**These authors contributed equally.*

Anti-mitotic drugs have long been used as therapeutics to treat a variety of cancers; however, tumor response to anti-mitotics is unpredictable and drug resistance commonly occurs. Most anti-mitotics prevent inactivation of the spindle assembly checkpoint and induce prolonged mitotic arrest. Cells arrested in mitosis undergo one of two fates: they either die during mitosis, or exit mitosis without undergoing division, a phenomenon termed mitotic slippage. Mitotic slippage is one mechanism by which cells acquire resistance to anti-mitotic therapeutics. Therefore, elucidating the underlying mechanisms that determine whether a cell slips or dies from an arrested mitosis is critical to developing more effective anti-mitotic therapies. Here we demonstrate that functional inactivation of the Hippo pathway, and consequent hyperactivation of YAP and TAZ oncoproteins, is sufficient to alter cell fate in non-transformed cells following treatment with anti-mitotics. Tracking single-cells using live-cell microscopy, we find that while non-transformed cells commonly undergo mitotic cell death following prolonged mitosis, inactivation of the Hippo pathway alone makes cells more prone to mitotic slippage. Furthermore, we demonstrate that hyperactivation of YAP and TAZ leads to increased anti-apoptotic proteins Mcl-1 and Bcl-xL. These anti-apoptotic proteins stave off apoptosis during prolonged mitosis to promote slippage. Our data suggest that inactivation of the Hippo pathway, which is common in many solid tumors, may promote resistance to anti-mitotic therapies by promoting mitotic slippage. Interestingly, we find that suppressing YAP/TAZ activity in cancer cells that normally undergo mitotic slippage is sufficient to sensitize them to mitotic cell death following treatment with anti-mitotics.

18

KIF18A INHIBITION RESULTS IN MITOTIC DEFECTS AND IMPAIRED VIABILITY IN SELECT WHOLE-GENOME DOUBLED ESOPHAGEAL CANCER CELLS

C. Vedula, R. Quinton, and N. Ganem

Department of Pharmacology & Experimental Therapeutics

Whole-genome doubling (WGD) events, often arising from catastrophic failures in cell division, generate unstable tetraploid cells that are known to fuel tumorigenesis. A classic example of this progression is the well-documented transition from the pre-malignant condition Barrett's esophagus to esophageal adenocarcinoma, which is known to involve a tumor-initiating WGD event in ~60% of all cases. Our lab recently demonstrated that loss of the mitotic kinesin KIF18A specifically impairs the viability of WGD+ cancer cells while having no effect on normal diploid cells. Here, we investigate the impact of pharmacological inhibition of KIF18A (KIF18Ai) in WGD+ esophageal cancer (EC) cells. We found that KIF18Ai induced chromosome misalignment, micronuclei formation, and prolonged mitoses. (…)

Abstract is continued on the following page.

Continued from C. Vedula, "KIF18A Inhibition Results in Mitotic Defects…"

Interestingly, only a select few WGD+ EC cell lines experienced a dramatic viability loss, indicating that WGD status is not the sole factor that confers sensitivity to KIF18Ai. To explore possible KIF18Ai resistance mechanisms that may also explain the disparity seen in the EC cell lines, two KIF18Ai-sensitive cell lines were grown to be KIF18Ai-resistant. The KIF18Ai-resistant cell lines retained wild-type ploidy and KIF18A expression. Upon KIF18Ai treatment, the resistant cell lines experienced an increased mitotic index without a significant decrease in viability. This demonstrated that despite KIF18Ai, the cells successfully progressed through mitosis and proliferated. Taken together, our data suggest that although KIF18Ai generates extensive mitotic defects in WGD+ cells, WGD alone is not enough to confer KIF18Ai-mediated lethality in EC. Other unknown mechanisms allow certain EC cells to proliferate even in the presence of an impaired mitosis.

34

LIFELONG CALORIE RESTRICTION REDUCES OXIDATIVE DNA DAMAGE IN THE AGING MONKEY BRAIN

A. Vitantonio[1,2], C. Dimovasili[2], K. Vaughn[3], J. Mattison[3], D. Rosene[2]

[1]Department of Pharmacology & Experimental Therapeutics
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[3]Nonhuman Primate Core, Translational Gerontology Branch, National Institute on Aging

Aging in monkeys and humans, free of neurodegenerative disease, is accompanied by progressive, cognitive decline despite the preservation of neurons. Instead there is a significant accumulation of myelin pathology beginning in middle age and progressing through senescence. Normally myelin is supported and maintained by oligodendrocytes, microglia, and astrocytes. During aging, these all undergo functional alterations that contribute to myelin pathology. An unanswered question is what interventions can retard the accumulation of glial dysfunction and associated myelin pathology during aging. Calorie restriction (CR) is recognized as the most robust, non-genetic intervention to reduce the rate of aging by improving mitochondrial function and reducing oxidative stress. Here we report the effects of lifelong CR on the brain in 20 male and female rhesus monkeys ranging in age from 22 to 37. All monkeys had been on a 30% reduced calorie or control diet from adolescence. Frozen sections were processed using an antibody against 8-hydroxyguanosine (8-OHG), a marker of oxidative damage to DNA. We quantified 8-OHG positive cells in the corpus callosum (CC) and the cingulum bundle (CB) using unbiased stereology. We found that monkeys subject to lifelong CR displayed a significantly lower number of 8-OHG positive cells in both white matter regions. Furthermore, immunofluorescent studies combining markers of glia subtypes with 8-OHG suggests that oligodendrocytes and microglia are more vulnerable to oxidative DNA damage, while astrocytes are less affected. This suggests that CR may act be an effective intervention to reduce myelin pathology and associated cognitive decline in the aging primate brain.

5

CHARACTERIZATION OF YAP-TEAD DEPENDENCY AND PHARMACOLOGICAL INHIBITION IN CUTANEOUS MELANOMA

R. Zhao, M. Vittoria, A. Spinella, X. Varelas, and N. Ganem

Pharmacology and Experimental Therapeutics

The Hippo tumor suppressor pathway is a highly conserved signaling cascade that regulates organ size and cell proliferation. Inactivation of the Hippo pathway results in nuclear translocation of the transcriptional co-activators YAP and TAZ, which interact with TEAD transcription factors to promote proliferative gene expression programs. Inhibition of this tumor suppressor pathway has been implicated in many cancers, including cutaneous melanoma as shown by recent work in our lab, yet small molecule inhibitors of this pathway have not been well characterized, especially in melanoma. We test two recently developed small molecule TEAD auto-palmitoylation inhibitors in both *in vitro* and *in vivo* melanoma models and characterize the YAP dependency of different melanoma cell lines through immunofluorescence, siRNA knockdown, and gene expression analysis. We also show that YAP-dependent melanoma cells and animal models show decreased cell proliferation and tumor growth after TEAD inhibitor administration. Finally, we also demonstrate that melanoma cells that acquire increased YAP-TEAD activity can circumvent commonly used BRAF and MEK targeted therapies, and we are currently testing the efficacy of YAP-TEAD pharmacological inhibition in treating acquired MAPK inhibitor resistance. Together, this work will help characterize the efficacy of using TEAD inhibitors for treatment of YAP-TEAD dependent or MAPK inhibitor-resistant melanomas.

Department of Physiology & Biophysics

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

*1st Prize

The accompanying number indicates each abstract's poster board.

Participants

Andrew Chang (*)

Emily Lewkowicz (10)

*

NEURONAL IDENTIFICATION IN C. ELEGANS AS AN APPROACH TO INVESTIGATING THE NEURON-CLASS SPECIFICITY OF VOLATILE ANESTHETIC ACTION

A. Chang, C. Connor, and C. Gabel

Department of Physiology & Biophysics

The molecular mechanism and the effects of the volatile anesthetics at the local neuronal network level have yet to be thoroughly explicated. Through the implementation of high-speed volumetric whole nervous-system calcium imaging in *C. elegans*, our previous work has characterized how exposure to isoflurane, a commonly used volatile anesthetic, results in breakdown of neuron-to-neuron communication, as captured by traditional metrics of signal coherence, such as Pearson correlation, as well as by novel Shannon entropy-based metrics. We have also shown that emergence from anesthesia is characterized by distinct early and late states, with early states exhibiting quickly resolving high-frequency activity. In this current study, we extend this work by implementing a fluorescent landmark system for neuron identification (NeuroPAL). This approach takes advantage of the highly comprehensive developmental fate mapping of neurons within the *C. elegans* nervous system. Neuron identification allows for the categorization of neuron activity traces by developmental fate and expression of markers of neuron class. This will allow for the investigation of how isoflurane alters the neuronal activity of isolated classes of cells, including those expressing specific receptors and neurons of particular neurotransmitter types (e.g. GABAergic, glutamatergic).

10

IN SILICO STUDIES SUGGEST STRUCTURAL BASIS FOR AMYLOID-APOLIPOPROTEIN CO-DEPOSITION

E. Lewkowicz, O. Gursky, M. Nakamura, M. Rynkiewicz

Department of Physiology & Biophysics

In Alzheimer's disease (AD), insoluble amyloid-beta (Aβ) fibrils deposit in the brain and co-deposit with apolipoproteins, yet the structural basis for apolipoprotein-amyloid interactions is unclear. We created potential models of these interactions using protein-protein docking of human apoE4 or apoC-III with patient-derived $A\beta_{1-40}$ and $A\beta_{1-42}$ fibril structures. Docking of intact apolipoproteins was non-specific. Next we omitted flexible linkers and docked α-helical apoE fragments. This showed that hydrophobic faces of apoE4 α-helices bind to hydrophobic surfaces on the sides or ends of Aβ fibrils, while basic residues flanking these apoE hydrophobic helical faces interacted with acidic or aromatic arrays in some Aβ fibrils. Similar results for apoC-III fragment docking suggests this binding mode applies to other apolipoprotein-amyloid interactions. Combining fragment docking poses into contiguous models of open conformation apoE4 or apoC-III consistently showed specific binding to hydrophobic surfaces. Apolipoproteins can adopt this open conformation via domain movement around flexible linkers between amphipathic α-helices. This movement closely resembles apolipoprotein-lipid interactions. Molecular dynamics simulations showed that open apoE4 models docked to $A\beta_{1-42}$ fibrils remained stably bound to the fibril and increased fibril rigidity. We propose that apolipoprotein binding to hydrophobic arrays along the fibril axis stabilizes fibrils and interferes with secondary nucleation and fragmentation, while apolipoprotein binding at fibril ends halts elongation and dissolution. This mechanism helps reconcile conflicting reports regarding apoE's effects on A□ aggregation and is supported by extensive prior experimental evidence. ApoE domain opening and direct involvement of Arg/Cys158 in amyloid binding may contribute to apoE isoform-specific effects in AD.

Notes