THE PAUL F. GLENN/AFAR CONFERENCE ON THE BIOLOGY OF AGING

THE 38TH ANNUAL AFAR GRANTEE CONFERENCE

GLENN WORKSHOP ON THE BIOLOGY OF AGING

May 28 – 30, 2025

Program Book

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GLENN FOUNDATION
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The Paul F. Glenn/AFAR Conference on the Biology of Aging
The 38th Annual AFAR Grantee Conference
Glenn Workshop on the Biology of Aging

May 28 – 30, 2025

Ritz-Carlton Bacara, Santa Barbara, CA

Wednesday, May 28, 2025

3:30 - 4:00 pm *Santa Ynez Terrace*	Registration/reception
4:00 – 4:10 pm *Santa Ynez*	***Welcome*** Catherine Kaczorowski, PhD Elinor Levine Professor of Neurology University of Michigan Board Member, AFAR **Kevin Lee, PhD** Senior Scientific & Programmatic Advisor, Glenn Foundation for Medical Research Board Member, AFAR
4:10 – 5:10 pm	**Glenn Foundation for Medical Research Breakthroughs in Gerontology (BIG) Award Presentations** ***"The Fibrotic Tissue Environment in Aged Mice Limits Muscle Regeneration by Muscle Stem Cells"*** Bradley Olwin, PhD (2021 BIG awardee) Professor, University of Colorado ***"G-quadruplexes in aging"*** Andrey Tsvetkov, PhD (2021 BIG awardee) Associate Professor, The University of Texas McGovern Medical School at Houston

Wednesday, May 28, 2025 (continued)

5:10 – 5:30 pm	Break
5:30 – 6:30 pm	**McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss Presentations** ***"Blood-brain Barrier Breakdown is Associated with Alzheimer's Disease Risk and Brain Microstructure."*** **Emilie Reas, PhD** (2022 McKnight awardee) Assistant Professor, University of California, San Diego ***"KIBRA and Susceptibility to Age-Related Memory Loss"*** **Tara Tracy, PhD** (2022 McKnight awardee) Assistant Professor, The Buck Institute
6:30 – 8:30 pm *Rotunda Terrace*	**Dinner**

Thursday, May 29, 2025

7:30 – 9:00 am *Rotunda Terrace*	Breakfast
9:00 – 9:30 am *Santa Ynez*	***"Harnessing the Neuroprotective Effects of Exercise"*** **Christiane Wrann, DVM, PhD** Associate Professor, Harvard Medical School
9:30 – 10:00 am	***"From Human Data to Therapeutics for Aging-Related Diseases"*** **Kristen Fortney, PhD** CEO, Bioage
10:00 – 10:30 am	Break
10:30 -11:00 am	***"Old Dogs Can Teach Us New Tricks: Results from the Dog Aging Project"*** **Daniel Promislow, DPhil** Senior Scientist Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University
11:00 – 11:30 pm	***"High Resolution Spatial Proteogenomics to Assess Target Engagement and Compare Senolytic Efficacy in the Brain"*** **Miranda Orr, PhD** Associate Professor of Neurology Washington University School of Medicine in St. Louis

Thursday, May 29, 2025 (continued)

11:30 am – 1:00 pm *Rotunda Terrace*	Lunch and poster set-up
1:00 – 3:00 pm *Ballroom A*	**Poster session** *Kindly remove your poster at the conclusion of the session* *1:00 – 1:30 pm: General viewing* *1:30 – 2:15 pm: Odd numbers stand by their poster* *2:15 – 3:00 pm: Even numbers stand by their poster*
3:00 – 5:30 pm	Free time
5:30 – 6:30 pm *Santa Ynez*	***Integrity in Science – Panel Discussion*** **Stuart Firestein, PhD**, *Moderator* **Elisabeth Bik, PhD** Microbiologist and science integrity advocate **Ivan Oransky, MD** Editor in Chief, The Transmitter Co-Founder, Retraction Watch Executive Director, The Center For Scientific Integrity **Charles Piller** Correspondent - *Science Magazine* **Matthew Schrag, MD, PhD** Assistant Professor of Neurology Vanderbilt University Medical Center
6:30 – 7:30 pm *Rotunda Terrace*	Reception
7:30 – 9:00 pm *Rotunda*	Dinner

Friday, May 30, 2025

7:00 – 9:00 am *Santa Ynez Terrace*	Breakfast
	Adjourn

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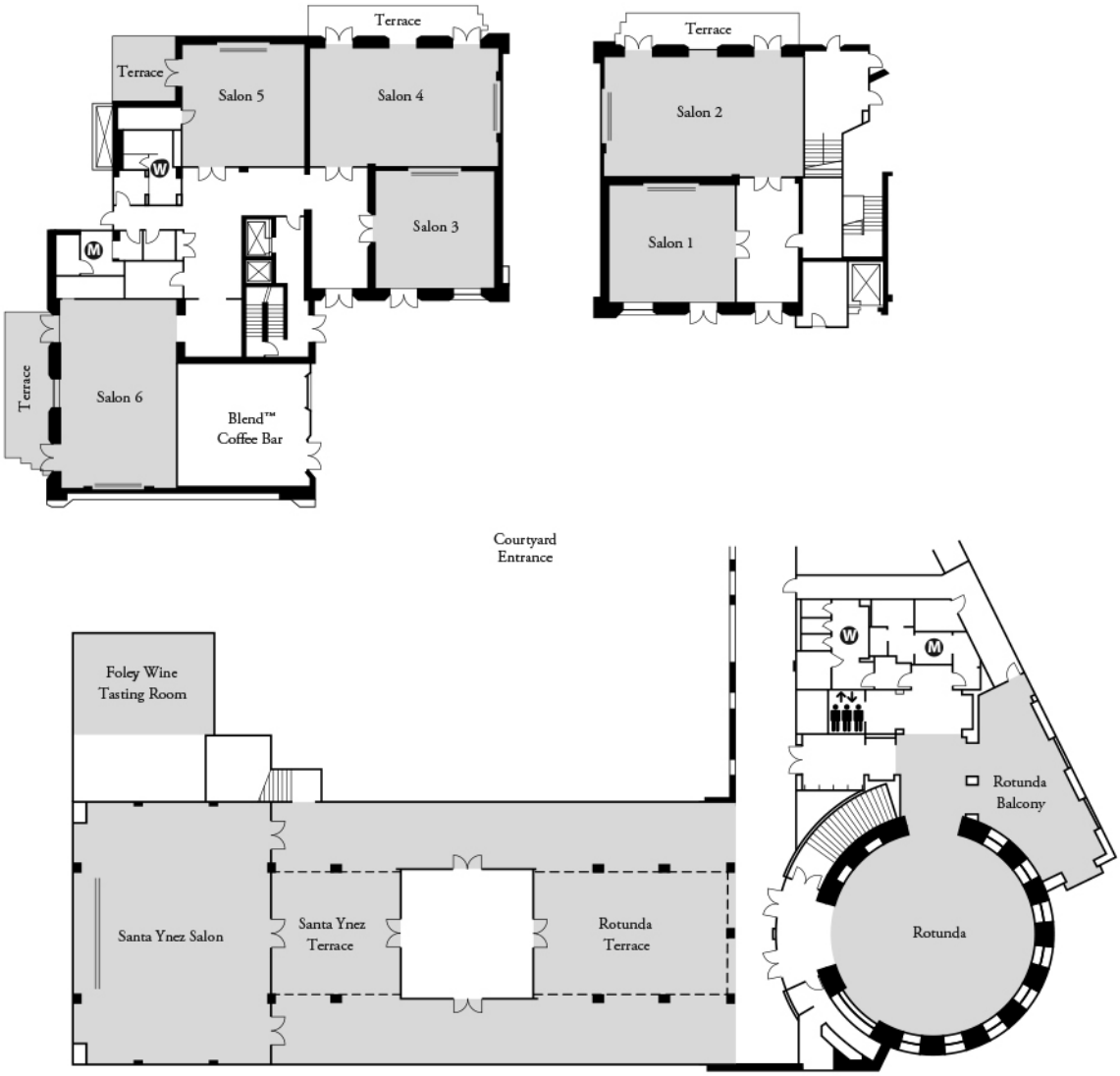
Rotunda, Santa Ynez Terrace & Salons



THE RITZ-CARLTON

BACARA, SANTA BARBARA

BEACHES & POOLS

Set on 78 acres, the resort offers a two-mile natural beach, and three zero-edged saline, heated pools with private cabana rentals. Learn more about cabana rentals by dialing Ext. 43012.

GOLF

Santa Barbara's three 18-hole championship golf courses include Sandpiper Golf Club, located next door to the resort. Tee times at all courses can be arranged by dialing Ext. 0.

OUTDOOR ADVENTURE

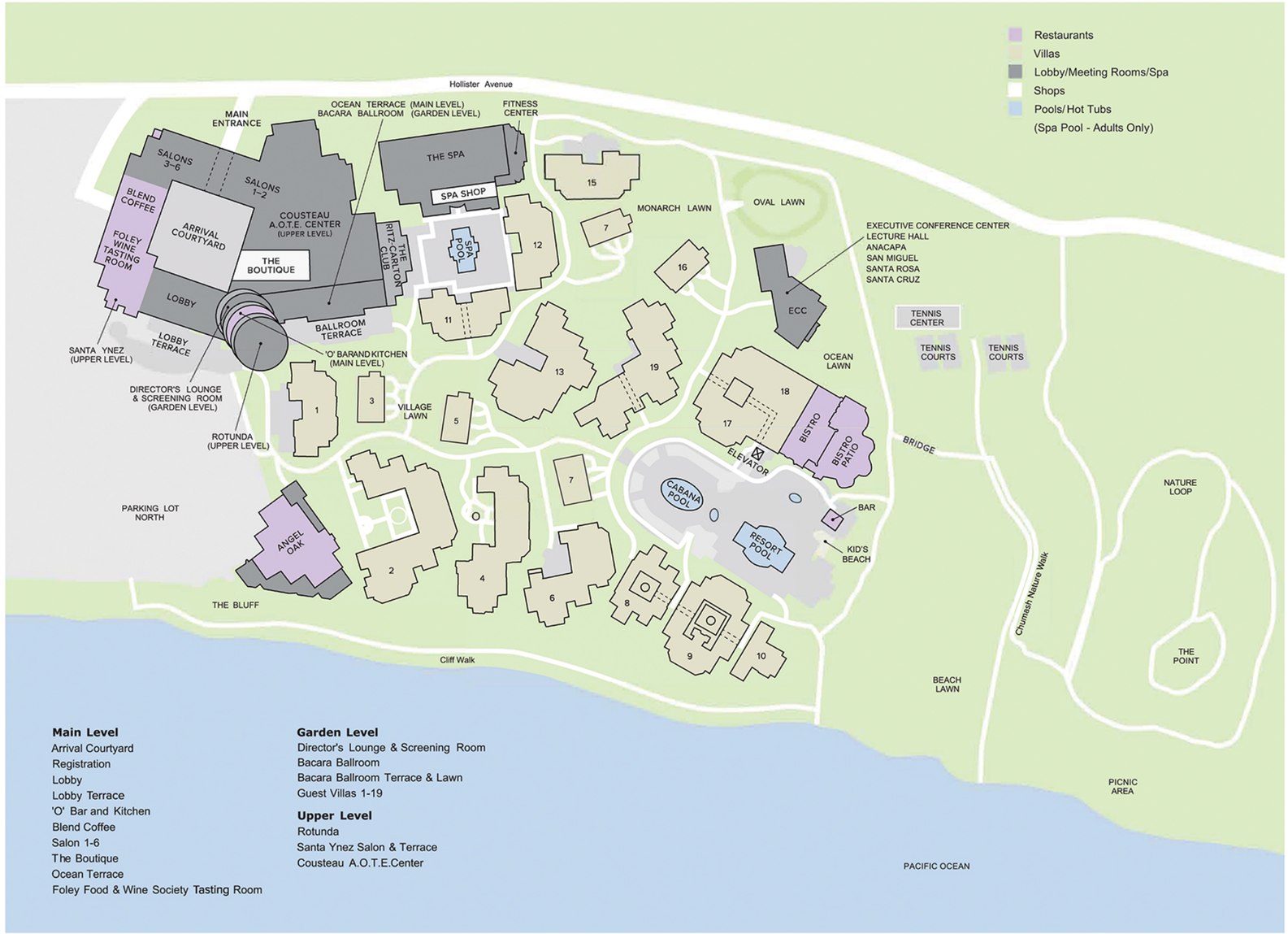
Santa Barbara offers horseback riding, biking, hiking and other activities. Pick up a hiking map. or book activities, including complimentary two-hour bike rentals at the front desk.

WINE COUNTRY

We encourage you to explore the wineries and tasting rooms located near historic State Street and throughout the diverse Santa Ynez region. Our relationship with select local wineries allows complimentary wine tastings to Resort guests with their room key. For a winery map or more information about the Santa Ynez Wine Country and Urban Wine Trail, see the Concierge.

DINING GUIDE

Each of the resort's six restaurants offers its own twist on a successful pairing: ocean views and local flavors. From a modern steakhouse with seasonal sides at Angel Oak, to rustic California cuisine at 'O' Bar and Kitchen, our dining showcases the best of Santa Barbara. For restaurant recommendations, reservations or a copy of our Dining Guide, please see the Concierge in the main lobby or dial Ext. 44220.



Poster Research Abstracts
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JR = Grant for Junior Faculty
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“Rejuvenating” nuclear speckles to combat proteinopathy

Bokai Zhu
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Aging Institute of UPMC
Pittsburgh Liver Research Center
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Department of Medicine
University of Pittsburgh School of Medicine

Proteinopathies often arise from a decline in proteostasis pathways, including the ubiquitin-proteasome system (UPS), the ER-Golgi protein secretory pathways, and lysosomal quality control. However, therapies targeting singular pathways have limited efficacy, indicating an incomplete understanding of disease mechanisms. We recently discovered that under physiological conditions, the network of proteostasis pathways manifests as cell-autonomous 12-hour (12h) ultradian rhythms, regulated by a dedicated 12h oscillator, independent of the 24h circadian clock. By studying this 12h oscillator, we uncovered an unexpected role of nuclear speckles in global proteostasis control. Nuclear speckles are membrane-less organelles important for mRNA processing and gene regulation, and their 12h liquid-liquid phase separation (LLPS) dynamics dictate the global transcriptional capacity of proteostasis genes. Moderate overexpression of the nuclear speckle scaffolding protein SON is sufficient to decrease nuclear speckle sphericity, increase the recruitment of nuclear speckles to chromatin, amplify proteostasis gene expression, and reduce protein aggregation. Since the decline of *Son* gene expression is associated with aging in mice and humans, we hypothesize that genetically or pharmacologically boosting SON expression and/or function (a term we herein coin as “nuclear speckles rejuvenation”) could augment the entire proteostasis network to delay or even reverse the progression of aging-related proteinopathies. Through a high-throughput drug screen, we discovered pyrvinium pamoate (PP) as a small-molecule rejuvenator of nuclear speckles, which targets the nuclear speckle scaffold protein SON to reduce speckle condensates surface tension. Transcriptome profiling revealed that both PP and SON overexpression robustly amplified the global protein quality control gene expression. In pre-clinical models, PP exhibited potent efficacy in reducing tauopathy and alleviating retina degeneration via enhancing autophagy and the ubiquitin-proteasome system. Our study thus provides proof-of-principle of targeting nuclear speckles to ameliorate proteinopathies.

Abstract

Negative Selection Allows DNA Mismatch Repair-Deficient Mouse Fibroblasts In Vitro to Tolerate High Levels of Somatic Mutations

Lei Zhang[1,2,*], Moonsook Lee[3], Xiaoxiao Hao[3], Xiao Ma[1,4], Chuwei Xia[1,4], Yiwei Zhao[1,4], Joseph Ehlert[5], Zhongxuan Chi[3], Bo Jin[3], Ronald Cutler[3], Alexander Y. Maslov[3], Albert-László Barabási[5,6,7], Jan H. J. Hoeijmakers[8,9,10], Winfried Edelmann[11], Jan Vijg[3,*], Xiao Dong[1,4,*]

[1]Institute on the Biology of Aging and Metabolism, University of Minnesota. [2]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota. [3]Department of Genetics, Albert Einstein College of Medicine. [4]Department of Genetics, Cell Biology and Development, University of Minnesota. [5]Network Science Institute, Northeastern University. [6]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School. [7]Department of Network and Data Science, Central European University. [8]Department of Molecular Genetics, Erasmus University Medical Center. [9]University of Cologne, Faculty of Medicine, Cluster of Excellence for Aging Research, Institute for Genome Stability in Ageing and Disease. [10]Princess Maxima Center for Pediatric Oncology, Oncode Institute. 11Department of Cell Biology, Albert Einstein College of Medicine. *Corresponding Author

Somatic mutations accumulate with age in human tissues. Clonal amplification of some mutations causes cancers and other diseases. However, it is unclear if random mutation accumulation affects cellular function without clonal amplification. We tested this in cell culture, avoiding the limitation that mutation accumulation in vivo leads to cancer. We performed single-cell whole-genome sequencing of fibroblasts from DNA mismatch repair-deficient $Msh2^{-/-}$ mice and controls after long-term passaging. While maintaining the same growth rates, in the $Msh2^{-/-}$ fibroblasts single-nucleotide variants increased up until >50,000 per cell, with small insertions and deletions plateauing to ~16,000 per cell. We provide evidence for genome-wide negative selection and large-scale mutation-driven population changes, including significant clonal expansion of preexisting mutations and widespread cell-strain-specific hotspots, likely caused by positive selection of mutations in specific genes. Since negative selection to prevent mutations from adverse effects in vivo during aging is difficult to envision, these results suggest a causal role of somatic mutations in age-related cell functional decline.

Metformin inhibits nuclear egress of chromatin fragments in senescence and aging

Yanxin Xu[1,2,3], Takuya Kumazawa[1,2,3], Tara C O'Brien[1,2,3], Ji-Won Lee[1,2,3], Yu Wang[1,2,3], Zhixun Dou[1,2,3]

[1]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA
[2]Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA
[3]Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Chronic inflammation is a hallmark of aging and contributes to many age-associated diseases. Metabolic intervention is a strategy to modulate inflammation. However, how metabolism and inflammation are connected during aging is poorly understood. A mechanism that drives chronic inflammation is cytoplasmic chromatin fragments (CCFs) appearing in senescent cells and aged tissues, which activate the cGAS-STING pathway. The size of CCFs greatly exceeds the capacity of the nuclear pore complex, raising the question of how chromatin fragments are shuttled from the nucleus to the cytoplasm. Here we report that CCFs exit the nucleus via nuclear egress, a membrane trafficking process at the nuclear envelope to shuttle large complexes from the nucleus to the cytoplasm. Inactivating ESCRT-III or Torsin proteins required for nuclear egress results in accumulation of CCFs at the nuclear membrane without falling off to the cytoplasm, accompanied by impaired activation of cGAS-STING and senescence-associated inflammation. Furthermore, we discovered that nuclear egress of CCF is inhibited by glucose limitation or metformin treatment. This is due to AMPK phosphorylation of ALIX, a component of ESCRT-III, leading to ALIX autophagic degradation and suppression of CCF nuclear egress. Metformin treatment in naturally aged mice downregulates ALIX protein and blocks cGAS-STING and chronic inflammation in the small intestine. Together, our study directly links nutrient sensing and chronic inflammation, two separate hallmarks of aging, and suggests a new approach to suppress age-associated inflammation by targeting nuclear egress of chromatin fragments.

Role of iPLA2 on store operated Ca^{2+} entry (SOCE) and muscle force generation during aging

Hongyang Xu[1], Shylesh Bhaskaran[1], Jacob Brown[1], Gustavo de Sousa[1], Jessica Thomason[1], Kara Kneuper[1], Holly Van Remmen[1,2]

[1]Aging & Metabolism Research Program, Oklahoma Medical Research Foundation;

[2]Oklahoma City VA Medical Center

Abstract

Age-related muscle atrophy and weakness, termed sarcopenia, substantially compromise healthspan and lifespan in the elderly. Loss of neuromuscular innervation is a key contributor to this degenerative process. We have previously demonstrated that bioactive lipid mediators, particularly oxidized lipids (oxylipins), are elevated during loss of muscle innervation and act as effectors for muscle atrophy and weakness. Phospholipase A2 (PLA2) is the major source for generating oxidized lipids through release of arachidonic acid, and the iPLA2 isoform has been proposed to be involved in intracellular Ca^{2+} homeostasis regulation through mediating store operated Ca^{2+} entry (SOCE) by shortcutting the interaction between Orai1 (Ca^{2+} influx channel on sarcolemma) and STIM1 (activator of Orai1 and sensor of SR Ca^{2+} storage). Our exciting findings reveal that SOCE activity is significantly decreased (~66%) in aged skeletal muscles. Pharmacological inhibition using STIM1 (SKF 96365) and iPLA2 (BEL) inhibitors suggests that STIM1 mediated SOCE activity is the predominant pathway in aging muscles, however, its interaction with Orai1 channel might be interrupted with elevated amount of iPLA2 in aged muscles compared to young. Using a mouse model with muscle specific deletion of iPLA2 (m-iPLA2KO), we found that the association between STIM1 and Orai1 was significantly improved, suggesting an elevation of STIM1 mediated activation of SOCE. Prior to conducting aging studies with these m-iPLA2KO mice, we examined the effect of iPLA2 deletion in a denervation-induced model of sarcopenia. Remarkably, m-iPLA2KO mice exhibited substantial preservation of muscle mass (~20% increase) and force generation (~25% increase) in denervated muscles compared to wildtype controls. Mechanistically, iPLA2 deletion improved cytosolic Ca^{2+} homeostasis, as evidenced by elevated levels of calmodulin (~45%) and calcineurin (~40%), greater RyR stabilization (~2-fold increase in calstabin-to-RyR ratio), and reduced active calpain-3 levels (~55% decrease). Additionally, excitation–contraction coupling was significantly enhanced (~6-fold increase in depolarization-induced force), coinciding with a notable increase in Na^+/K^+-ATPase (NKA) protein content (~1.3-fold) in denervated m-iPLA2KO muscles.

Together, these findings uncover a novel role for lipid metabolism—specifically iPLA2-dependent oxylipin signaling—in regulating Ca^{2+} homeostasis, muscle mass, and contractile function. This work highlights a promising therapeutic target to mitigate sarcopenia and preserve muscle health during aging.

Precision Control of the ISR Uncovers Age-Associated Vulnerabilities in Cellular Stress Adaptation

Max Wilson[1,2*], Debalina Datta[1], Ethan Dickson[3], Gabrielle Daley[1]

[1]Neuroscience Research Institute, University of California, Santa Barbara
[2]Interdisciplinary Program in Quantitative Biosciences, University of California, Santa Barbara
[1]Molecular, Cellular and Developmental Biology, University of California, Santa Barbara
**mzw@ucsb.edu*

The Integrated Stress Response (ISR) is a crucial cell-surveillance system that enables cells to sense diverse physiological and environmental stresses and coordinate appropriate adaptive responses. It is a central regulatory hub that is associated with monitoring, sensing and coordinating responses to cellular stress, and translating them to decisions of adaptation, disease outcome or death. Although the core components of the ISR have been characterized, its broader regulatory mechanism—especially in the context of human aging—remains partially understood. In particular, how the ISR is remodeled with age and how such remodeling contributes to age-related diseases, including neurodegeneration, has yet to be elucidated. In this study, we investigate the relationship between cellular stress signaling and aging by using a combination of state-of-art optogenetic and chemogenetic tools that allow precise spatial and temporal control of ISR activation, in contrast to traditional stress induction methods. Importantly, for our studies, we utilize over 15 well-characterised primary fibroblast cells from donors of 17 years to 90 years old, from the National Institute on Aging's Old Cell Repository. Leveraging the advantage of a consistent genetic background, we are investigating the formation and clearance of stress granules (SGs) in our engineered fibroblasts. Our observations point to potential age-associated differences in SG assembly and dissolution kinetics, as well as in the dynamics of ISR activation. These patterns suggest that aging may impair the coordination and resolution of stress responses at both the molecular and cellular levels. Our ongoing analyses also determine how prior exposure to mild or transient stress influences cellular sensitivity to subsequent stressors, and whether this modulation differs across the aging spectrum. By systematically probing ISR activity in cells from young and aged donors, we aim to uncover whether aging impairs the plasticity of this stress response pathway. Overall, we seek to understand how aging compromises the and responsiveness of ISR and shifts the cellular balance away from adaptive recovery towards maladaptive outcomes. This work also highlights ISR as a potential mechanistic bridge between aging and the pathogenesis of neurodegenerative disorders.

Leveraging Kynurenine Pathway Manipulation to Improve Metabolic Dysfunction and Extend Healthspan in Mice

Reyhan Westbrook, Ph.D.
Johns Hopkins University School of Medicine, Division of Geriatric Medicine and Gerontology

Through findings from translational studies on both aged and chronically inflamed mice, as well as on aged and frail older adults, we have identified metabolites of the kynurenine pathway as potential mediators of systemic damage caused by chronic inflammation. Kynurenines are derived from the amino acid tryptophan and are precursors for the important electron carrier and coenzyme molecule NAD+. Some kynurenine pathway intermediate metabolites including kynurenine, 3-hydroxykynurenine, and quinolinic acid are potentially deleterious and accumulate with obesity, inflammation and aging. Genetically reducing levels of kynurenines has been shown to be protective against metabolic decline resulting from high-fat diet (HFD) in mice, however pharmacological approaches to reduce kynurenines in the context of HFD have not been tested. In this study, we will evaluate the changes in energy metabolism caused by genetically and pharmacologically reducing levels of kynurenine pathway metabolites, and determine if pharmacologically reducing the levels of kynurenine pathway metabolites can reduce inflammation, improve glucose metabolism and protect against HFD related functional decline in aged mice.

To this end, we propose to trace $^{13}C_6$ labeled glucose metabolism through mitochondrial bioenergetics pathways, biosynthesis, and redox homeostasis pathways, in mice with genetically and pharmacologically suppressed kynurenine pathway metabolism, on standard diet and HFD (Aim 1). We also propose to put mice with pharmacologically suppressed kynurenine levels, on HFD and to conduct a battery of physiological tests and functional measurements followed by *ex vivo* metabolomic, cytokine, gene expression, morphological and other measurements on tissues to pinpoint specific effects of kynurenine inhibition on energy metabolism and on aging related biological features (Aim 2). We will utilize the indolamine 2,3 dioxygenase (IDO) knockout mouse (IDO -/-) which has significantly reduced levels kynurenines as our genetically reduced kynurenine model for Aim 1. In Aims 1 and 2, we will pharmacologically suppress kynurenine with the IDO enzyme inhibitor, 1-methyl-L-tryptophan (1-MT), which blocks the activity of IDO thereby lowering the levels of kynurenine pathway metabolites. We will determine if kynurenine suppression alone and in tandem with supplementing an NAD+ precursor can prevent/delay functional decline, pathophysiological metabolic changes and aging related changes in cellular structure and mitochondrial function in C57Bl/6 mice (Sub-aim 2a).

The goal of this study is to determine the role kynurenines play in the development of dysregulated energy metabolism, increased inflammation and aging-related functional decline and frailty in mice in the context of high-fat diet. Completing these goals will further the mechanistic understanding of the connections between inflammation, kynurenines, energy metabolism and aging-related functional decline, as well as determine the effect of novel and practical intervention strategies to extend healthspan. If successful, the findings will provide strong rationale for the translation of these therapeutic strategies into interventions to counteract aging-related declines in older adults.

SEQUENCING-FREE WHOLE GENOME SPATIAL TRANSCRIPTOMICS AT MOLECULAR RESOLUTION IN INTACT TISSUE

Yubao Cheng[1], Shengyuan Dang[1,#], Yuan Zhang[1,#], Yanbo Chen[1], Ruihuan Yu[1,2], Miao Liu[1], Shengyan Jin[1], Ailin Han[3], Samuel Katz[4], *Siyuan Wang*[1,5]

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[#]These authors contributed equally to this work.

Recent breakthroughs in spatial transcriptomics technologies have enhanced our understanding of diverse cellular identities, compositions, interactions, spatial organizations, and functions. Yet existing spatial transcriptomics tools are still limited in either transcriptomic coverage or spatial resolution. Leading spatial-capture or spatial-tagging transcriptomics techniques that rely on *in-vitro* sequencing offer whole-transcriptome coverage, in principle, but at the cost of lower spatial resolution compared to image-based techniques. In contrast, high-performance image-based spatial transcriptomics techniques, which rely on *in situ* hybridization or *in situ* sequencing, achieve single-molecule spatial resolution and retain sub-cellular morphologies, but are limited by probe libraries that target only a subset of the transcriptome, typically covering several hundred to a few thousand transcript species. Together, these limitations hinder unbiased, hypothesis-free transcriptomic analyses at high spatial resolution. Here we develop a new image-based spatial transcriptomics technology termed Reverse-padlock Amplicon Encoding FISH (RAEFISH) with whole-genome level coverage while retaining single-molecule spatial resolution in intact tissues. We demonstrate image-based spatial transcriptomics targeting 23,000 human transcript species or 22,000 mouse transcript species, including nearly the entire protein-coding transcriptome and several thousand long-noncoding RNAs, in single cells in cultures and in tissue sections. Our analyses reveal differential subcellular localizations of diverse transcripts, cell-type-specific and cell-type-invariant tissue zonation dependent transcriptome, and gene expression programs underlying preferential cell-cell interactions. Finally, we further develop our technology for direct spatial readout of gRNAs in an image-based high-content CRISPR screen. Overall, these developments provide the research community with a broadly applicable technology that enables high-coverage, high-resolution spatial profiling of both long and short, native and engineered RNA species in many biomedical contexts.

Chronic activation of CaMKII, a key signal transducer for exercise performance and adaptation, drives muscle aging

Michael Bene, Will Fountain, Giovanni Rosales-Soto, Erick Hernandez-Ochoa, Seeun J. Jeong, Corina Antonescu, Liliana Florea, Tae Hwan Chung, Jeremy Walston, Peter Abadir, Qinchuan Wang, Department of Medicine, Johns Hopkins School of Medicine, 21224, qinchuan.wang@jhmi.edu

Aging skeletal muscle exhibits progressive loss of strength and mass, culminating in sarcopenia, a major contributor to physical frailty in older adults. In young muscles, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) integrates contraction-induced calcium and redox signals to enhance contractile performance and promote exercise-induced adaptation. However, in aged muscles, both cytosolic calcium and oxidative stress are chronically elevated, even at rest. Consistent with this altered milieu, we observed significantly higher CaMKII activity in the skeletal muscles of sedentary old mice (20–33 months) than in sedentary young mice (4 months). These findings indicate a shift toward constitutive, dysregulated CaMKII activation in aging muscle.

To test whether elevated CaMKII activity contributes causally to muscle aging, we delivered a CaMKII inhibitor (CN19o) to the tibialis anterior (TA) muscles of 20-month-old mice via AAV9. Inhibition of CaMKII markedly improved muscle contractile force. Conversely, expressing a constitutively active mutant ($CaMKII^{CA}$) in the TA muscles of young (3-month-old) mice induced a pronounced decline in contractility, accompanied by progressive muscle atrophy.

Electromyography revealed a significant yet modest reduction in compound muscle action potential (CMAP) amplitude in $CaMKII^{CA}$-expressing muscles, which was insufficient to explain the loss of contractile force. Short-term (6–7 weeks) $CaMKII^{CA}$ expression resulted in a mild atrophy (~8% reduction in mass), but mediation analyses showed that the initial decline in force was independent of atrophy. This decoupling of strength and mass echoes human muscle aging, where functional decline typically precedes overt muscle wasting. With prolonged $CaMKII^{CA}$ expression, however, marked atrophy (~24% reduction in mass) developed and further impaired muscle function. Histological analysis revealed ragged red fibers and mitochondrial disorganization, hallmarks of aged skeletal muscle, in $CaMKII^{CA}$-expressing muscles.

Transcriptomic profiling showed that $CaMKII^{CA}$ expression in young muscle induced gene expression patterns reminiscent of aged muscle, whereas CaMKII inhibition in old muscle partially restored a youthful transcriptomic signature. Our findings suggest CaMKII hyperactivation is a driver of muscle aging and a potential therapeutic target for sarcopenia. Ongoing proteomic and metabolomic analyses will further delineate the downstream pathways by which CaMKII mediates muscle decline.

Chimeric mitochondrial transcripts: a novel metric of biological aging in RNA-sequencing data

Amy R. Vandiver[1,2], Allen Herbst[3*], Paul Stothard[3], Jonathan Wanagat[2,4]

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[2]Veterans Administration Greater Los Angeles Healthcare System, Los Angeles, California, USA
[3]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada
[4]Department of Medicine, Division of Geriatrics, UCLA, Los Angeles, California, USA
*Current affiliation: US Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA

While many markers of biological age have been proposed, the majority require specialized assays to quantify and cannot be distilled from publicly available genomic or transcriptomic data. One such marker, large deletions within the mitochondrial genome, has clear functional relevance to the aging process and thus the potential to serve as both a metric of biological age and a target for anti-aging interventions. Despite this potential, the challenges of sequencing the mitochondrial genome make mitochondrial genome deletions hard to quantify without specialized assays. Further, the limits of mitochondrial reverse genetics prevent understanding the direct consequences of these mutations.

We hypothesized that mitochondrial deletion mutations are transcribed as chimeric mitochondrial RNAs which may serve as accessible metric of biological aging in RNA sequencing data. To test this hypothesis, we analyzed publicly available and newly generated RNA sequencing data from multiple systems known to have mitochondrial genome deletion mutations. We observed increased chimeric mitochondrial RNA frequency in samples from patients with mitochondrial genetic diseases, multiple aged human tissues and rats with induced skeletal muscle mitochondrial deletion mutations. We observe significant correlations between the frequency of chimeric mitochondrial RNAs and quantification of mitochondrial deletion mutations in multiple settings and between the locations of chimeric RNA fusion sites and known genomic deletion breakpoints. Further, we observe that the frequency of chimeric mitochondrial transcripts predict changes in the nuclear transcriptome associated with cellular metabolism. Together, these data reveal the utility of mitochondrial chimeric transcripts as an accessible method for identifying mitochondrial genome structural mutations and understanding the cellular impact of these events.

Differential changes in autophagy in distinct neurons during aging

Mya N. Rodriguez and Andrea KH Stavoe

Department of Neurobiology and Anatomy, McGovern Medical School, University of Texas Health Science Center at Houston

Autophagy is a cellular degradation and recycling pathway that contributes to homeostasis. It is especially important for neurons, as they are terminally differentiated cells that must maintain their function for a lifetime. While autophagy has been widely explored in non-neuronal cells, it remains largely unknown how autophagy is regulated in neurons. Importantly, neuronal autophagy decreases with age and is misregulated in neurodegenerative diseases such as Alzheimer's and Parkinson's Diseases. Using the model organism *C. elegans*, we have investigated whether distinct neuron types have differential dynamics of autophagy *in vivo* during aging. We quantified autophagosome biogenesis using the canonical autophagosome marker LGG-1 (LC3) in the serotonergic Neurosecretory-Motor (NSM), dopaminergic Cephalic (CEP), and dopaminergic Post-Deirid (PDE) neurons of *C. elegans* during aging. Using *in vivo*, live-animal microscopy, we quantified autophagosomes throughout aging. In addition to differences in neurotransmitter identity, these neurons exhibit distinct morphologies. NSM and PDE have larger axonal than dendritic volumes, while CEP has a larger dendrite and a small axon. Previous studies examining autophagy in the worm nervous system as a whole found broad decreases in autophagy with age. Surprisingly, we observe an increase of autophagosomes during aging in the serotonergic NSM and dopaminergic PDE neurons. In contrast, we observe a decrease in autophagosomes dopaminergic CEP neurons during aging. These results suggest that neuron morphology, and not neurotransmitter identity, is a major driver of age-related changes in neuronal autophagy. Exploring autophagy in additional neurons throughout aging will provide an understanding of how autophagy is differentially regulated in distinct neurons and aid in identifying efficient therapeutic targets for specific age-related neurodegenerative diseases.

A non-canonical STING pathway drives cellular and organismal aging.

Lilian Silva, Rafael Cancado de Faria, Barbara Teodoro-Castro, Elena V. Shashkova and Susana Gonzalo.

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Aging is characterized by gradual cellular and tissue decline, driven in part by chronic sterile inflammation linked to DNA damage and cytosolic self-DNA accumulation. A central player in this response is the cGAS-STING pathway, which senses cytosolic DNA and triggers inflammation. In the classical pathway, the first step is the activation of the DNA sensor cGAS. Upon binding to cytosolic DNA, cGAS produces cGAMP, which binds to STING (STimulator of INterferon Genes) leading to STING trafficking from endoplasmic reticulum (ER) to a perinuclear compartment (PNC) where it activates downstream factors that in turn trigger the IFN response. However, our recent discoveries in Hutchinson-Gilford Progeria Syndrome (HGPS) patients' cells and in aged/senescent cells reveal that a non-canonical cGAS-STING pathway drives aging phenotypes. This non-canonical cGAS-STING pathway in aged/progeria/senescent fibroblasts lacks classical hallmarks such as increased cGAMP production, STING trafficking to PNC, and phosphorylation of STING and partners TBK1 and IRF3. Despite reduced cGAMP synthesis and altered STING behavior, cGAS and STING remain essential for inflammation in progeria cells. STING depletion or inhibition reduces hallmarks of aging in these cells, and ameliorates tissue and organismal decline in progeria mice, increasing their lifespan. Moreover, forced activation of cGAS with synthetic DNA resulted in lower cGAMP production in progeria/aged cells compared to young cells, suggesting that aged cells have a reduction in cGAS enzymatic activity. In addition, STING is found at the ER and accumulated at the nuclear envelope (NE), interacting with transcriptional regulators potentially driving pro-inflammatory gene expression and genomic instability. Using progeria cells as a model of aging, we found that STING contributes to replication stress (RS). These findings suggest that nuclear and non-canonical roles of cGAS and STING are key contributors to aging and age-related diseases, offering promising therapeutic targets to mitigate sterile inflammation and genomic instability in aging.

The role of histone succinylation in regulating transcription in longevity

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Our overarching hypothesis is that histone succinylation accumulates on chromatin on cells that have delayed biological aging, and it could potentially be a key player in preventing spurious transcription, loss of chromatin homeostasis and cell loss in functionalities during aging.

Building on the hypothesis that succinylation is not merely a byproduct of metabolic aging, but a functional modifier of chromatin state, we initiated a multi-layered investigation integrating in vivo mouse models, single cell proteomics, transcriptomic profiling, and biochemical validation. We developed a robust single cell histone proteomics workflow, now under revision at Nature Communications (**PMID: 39211145**). This method enables detection of over 50 histone post-translational modifications in individual cells and, importantly, captures the covariation between succinylation and other histone modifications, a metric that indicates coordinated regulation or loss of chromatin integrity during aging. In parallel, we studied the impact on transcription of succinylation by using a model with high levels of histone succinylation (HepG2/C3A cells treated with 10 mM sodium succinate). RNA-seq revealed an overall reduction of transcripts from genes associated with metabolism and histone modification pathways (**data not shown**).

We then revealed that histone succinylation inhibits the activity of lysine demethylases KDM4D and KDM6B (**Fig. 1**). Structural modeling suggests that succinylated peptides bind tightly within the KDM active site, effectively blocking enzymatic function. These enzymes are responsible for removing H3K9me3 and H3K27me3, key repressive histone marks, and our data show that increased succinylation stabilizes these silencing modifications in chromatin. This mechanism presents a potential model by which succinylation preserves transcriptional repression during aging identifying actionable targets for gerotherapeutic intervention.

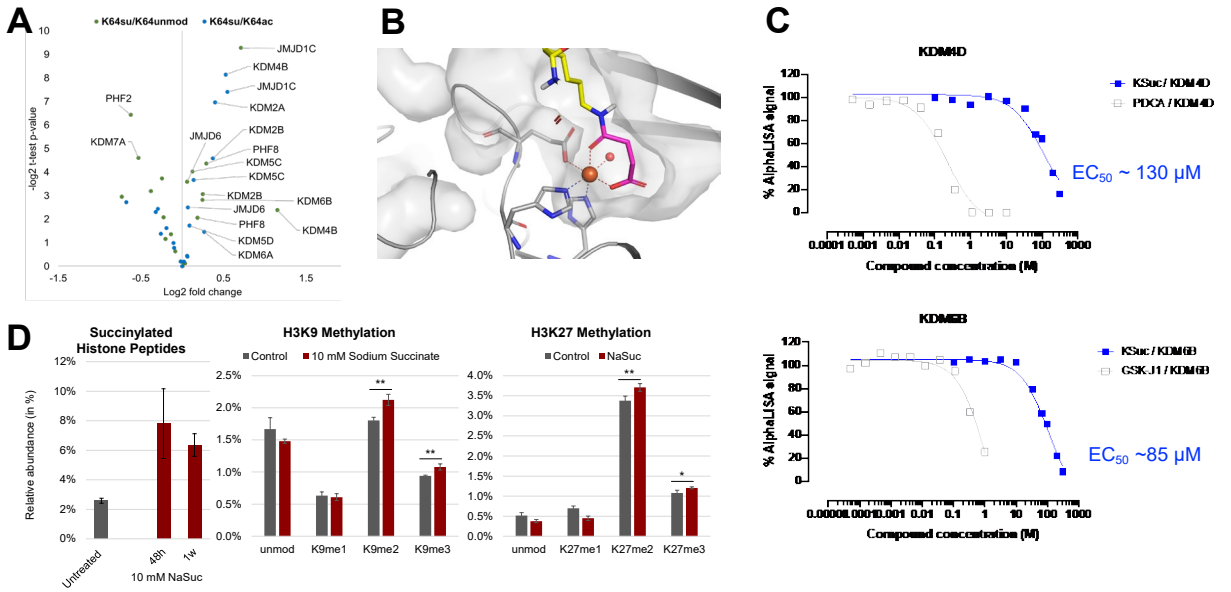

Figure 1: **Histone succinylation inhibits KDMs.** (A) Peptide pull-down with H3K64suc, H3K64ac and unmodified peptide. The nuclear proteome was used as input and lysine demethylases (KDMs) were found to be enriched by the H3K64suc peptide. KDM4D and KDM6B were below detection limit. (B) 3D model of succinylation (pink) binding to the iron atom in the active site of KDM4D. (C) Enzymatic assay of KDM4D and 6B, showing that a succinylated peptide inhibits the demethylation. PDCA and GSK-J1 are inhibitor controls. (D) Relative abundance of histone marks in cells treated with 10 mM sodium succinate.

Human tau pathology is associated with lonely, non-traveling slow waves linked to memory impairment

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Age-related memory decline is a hallmark of human aging, yet the neurophysiological mechanisms linking pathology to cognitive impairment remain unresolved. Here, we identify a novel neurophysiological signature of memory impairment in aging: disruption of the coordinated propagation of NREM sleep slow waves, linked to tau pathology.

Using, overnight EEG, we first show that older adults (N=77) show reduced slow wave travel compared with younger adults (N=61), revealing a normative age effect. However, within older individuals, we observed marked variability in slow-wave propagation. Tau [^{18}F]Flortaucipir (FTP) PET imaging in these unimpaired older adults (N=48) reveals that higher frontal tau burden is associated with a shift in slow-wave dynamics — from widespread, traveling waves to spatially isolated, “lonely” slow waves, , even after adjusting for age, sex, amyloid-β (PET-PiB), and sleep apnea severity. These disruptions in slow-wave dynamics statistically mediated the relationship between tau burden and overnight episodic memory retention.

We replicate and extend these findings in an independent clinical cohort (N=62), from the BASE study, using CSF-derived AD biomarkers. Here, greater AD pathology (tau/Aβ42 ratio) levels predicted a similar shift toward slow-wave isolation, correlating with worse cognitive scores. Furthermore, in a subset of the unimpaired sample with longitudinal PET and EEG (N=19), increases in frontal tau burden over ~4 years predicted progressive reductions in slow-wave propagation and declines in overnight memory retention, suggesting a progressive loss of slow-wave spread as tau pathology accumulates.

Together, these results identify a novel, sleep-based physiological marker of brain aging: the breakdown of large-scale traveling slow waves in NREM sleep linked to tau pathology. These disruptions precede clinical impairment and may represent an early, modifiable mechanism of cognitive decline. Building on this, we are now collecting AFAR-supported data in healthy older adults to examine whether disruption of large-scale, traveling slow waves during NREM sleep impairs CSF flow dynamics, thereby contributing to reduced brain clearance and greater accumulation of tau and amyloid pathology. This study combines simultaneous EEG with fast fMRI to quantify individual differences in sleep-related CSF flow and their relationship to elevated AD pathology.

Disrupted Gut-Brain Communication Underlies Memory Impairment in Aging

Andrea Francesca Makimkim Salvador, PhD, Stanford University

Aging is a complex process characterized by a gradual decline in physiological integrity and cognitive function, leading to significant emotional, social, and economic burdens. Despite increasing prevalence, effective treatments for age-associated cognitive decline remain elusive. Emerging evidence suggests that brain-extrinsic factors—including the gut microbiome and peripheral sensory pathways—play a pivotal role in the aging process and represent promising targets for intervention. Interoception, the nervous system's ability to sense and respond to internal physiological states via bidirectional communication pathways such as the vagus nerve, may be impaired with age. We propose that dysregulation of interoceptive signaling contributes to the brain's reduced capacity to maintain homeostasis and adapt to age-related stressors.

Using an integrative approach combining metagenomics, metatranscriptomics, untargeted metabolomics, and mouse models of microbiome transfer, we show that age-related alterations in the gut microbiome impair memory encoding. Specific bacterial species enriched in the aged microbiome are sufficient to drive cognitive decline, potentially through the production of metabolites that induce peripheral inflammation. We hypothesize that these inflammatory signals are sensed by interoceptive pathways and ultimately disrupt brain function. Together, our research may help uncover novel microbiome-derived and neural mechanisms of aging, offering actionable insights for the development of peripheral interventions that restore cognitive resilience in the aging population.

RNA processing, metabolic flexibility, and caloric restriction

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Caloric Restriction (CR) without malnutrition delays aging and the incidence of age-related diseases. A substantial body of work suggests that metabolic reprogramming is a critical aspect of the mechanisms of CR; however, the details of the regulation of metabolism during CR and how this might lead to enhanced lifespan are still unclear. Emerging evidence points to RNA processing also playing a role as a mechanism of CR, and given the role of alternative splicing in proteoform diversity and the adaptability of the cell, we hypothesize that RNA processing may serve as a link between metabolic and longevity regulation. An AFAR-funded study in my lab, currently ongoing, seeks to address this question by examining hepatic RNA processing, systemic metabolism, and biological age in a cohort of mice undergoing CR. This cross-sectional study involves collection of samples from mice at 3 different ages, along with longitudinal body weight, blood sampling, and metabolic chamber-based measurements of respiratory exchange rate.

In addition to reporting details of the hypotheses and design of the above study, I will report on results from a similar multi-omics analysis in the brains of male mice that was also designed to investigate RNA processing in response to CR and partly served as the inspiration for the AFAR funded project. Brain tissue from mice on CR were collected at 10 mo, 20 mo, and 30 mo of age. Samples were then processed for deep-read RNA sequencing and shotgun lipidomics. We identified widespread transcriptional changes in response to diet, as well as significant changes to lipid composition. Hundreds of diet-responsive alternative splicing events were statistically significant, although the most prominent alternative splice event type was distinct at 30 mo of age compared to younger animals. In addition, expression of both core and ancillary spliceosome components did not display a consistent pattern across the age groups. These data suggest that changes to RNA processing may be an adaptive response that is engaged in a life-stage specific manner. Further analyses and future plans will also be discussed.

Novel Biosensors Enable Super-Resolution Imaging of Innate Immunity in Aging

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Brain aging is driven in part by neuroinflammation. The cGAS-STING pathway plays a pivotal role in neuroinflammatory innate immune signalling. This neuroinflammatory pathway functions across multiple organelles, including mitochondria, the endoplasmic reticulum, the Golgi, endosomes, autophagosomes, lysosomes, and possibly also inside the nucleus. To dissect the cell biological mechanisms underlying neuroinflammation in brain aging, we designed and developed novel biosensors enabling super-resolution timelapse fluorescence microscopy of cGAS-STING innate immune signalling in live brain cells. Upon stimulation of the cGAS-STING pathway in human and mouse microglia by extracellular or intracellular sources of double-stranded DNA, we show that cGAS phase separates with dsDNA into liquid-like condensates that interact with the endoplasmic reticulum and mitochondria and produce 2'3'-cGAMP. By multiplexing our cGAS and STING biosensors within single cells, we also reveal the dynamics of STING activation, oligomerization, and trafficking from the endoplasmic reticulum into the Golgi and post-Golgi vesicles. We show that fluorescence turn-on of our STING biosensor reports on STING activity and requires STING oligomerization. Our biosensors keep the cell biology of the cGAS-STING pathway intact, while enabling quantitative imaging of cGAS-STING innate immune signalling activity with unprecedented temporal and spatial resolution in live cells. We are currently applying our biosensors in aged brain cells *in vitro* and *in vivo*, in models of age-associated illness such as neurodegeneration, and in neurotropic viral infection, which will reveal molecular and cell biological mechanisms of innate immunity driving neuroinflammation in brain aging.

Title: Profound Rewiring of the DNA Methylome and Transcriptome and Decelerated Methylation Age After Regeneration in Aged Skeletal Muscle

Authors: Chambers, Toby; Wells, Jaden; Koopmans, Pieter Jan; Morena, Francielly; Malik, Zain; Greene, Nicholas; Brooke, Robert; Milčiūtė, Milda; Gordevičius, Juozas; Horvath, Steve; Wen, Yuan; Dungan, Cory; and Murach, Kevin.

Abstract (294/300 words)

Background Resident muscle stem cells (satellite cells) are necessary for skeletal muscle regeneration after injury. Whether skeletal muscle tissue that is regenerated by satellite cells maintains a pre-injury molecular phenotype is uncertain. **Methods** We address this fundamental question by assessing the CpG methylome, transcriptome, and DNA methylation age (DNAmAGE) changes in aged tibialis anterior muscle of male mice (OM; 24-25 months) 35d after unilateral injury ($BaCl_2$-induced regeneration). Young, injured mice (YM; 5-6 months) served as comparators. Methylome-transcriptome integration (BETA) was used to infer epigenetic regulation of gene expression. **Results** A quarter of all CpGs measures were persistently altered 35 days after injury in OM, and <10% in YM. In OM, injury then regeneration decelerated DNAmAGE by ~50-60% compared to uninjured muscle. Regeneration had a contrasting effect in young mice and tended to not influence or accelerate DNAmAGE. In OM, BETA was significant for upregulated genes after muscle regeneration (p=0.007, 453 genes) and was trending toward significance for downregulated genes (p=0.089). In YM, BETA was significant for upregulated genes after muscle regeneration (p=0.006, 475 genes) but not for downregulated genes (p=0.578). Of the upregulated genes in YM and OM, there was overlapping expression of 139 genes between groups after regeneration (~30% shared upregulation). Molecular function categories for shared upregulated genes were largely related to platelet-derived growth factor binding and extracellular matrix (ECM) structural constituents. Differential regulation of genes implicated in muscle stem cell performance between YM and OM were primarily related to stem cell performance: *Axin2*, *Egr1*, *Fzd4*, and *Spry1*. **Conclusions** Muscle injury with aging differentially affects DNAmAGE and rewires the transcriptomic-methylomic landscape after regeneration, but to the greatest extent in aged muscle. Our data have implications for understanding muscle plasticity with aging and developing therapies aimed at ECM organization and muscle stem cell performance.

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Effect of aging on dendritic cell heterogeneity and function

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Aging reshapes immune cell populations and their functions through tissue-specific transcriptional alterations in mice and humans. However, how aging regulates antigen-presenting cell heterogeneity and functions remains understudied. We recently showed in mice that aging altered antigen-presenting cell subsets, including conventional dendritic cells (cDC), paralleled by the accumulation of exhausted-like pro-inflammatory $CD8^+$ T cells in multiple organs. Here we used experimental techniques and systems immunology approaches to decipher subset- and tissue-specific changes in aging antigen presenting cells in mice. We show that proportions of cDC type 1 (cDC1) cells – a subset superior in cross-presenting viral and tumor antigens to $CD8^+$ T cells – but not cDC2 are decreased in tissues of old mice. Single-cell transcriptomics analysis revealed two phenotypically distinct subsets of cDC1 with different developmental programs. Interestingly, old mice had specifically reduced the cDC1 subset that depends on transcription factor BATF3 in their development (a population known to be critical for antigen cross-presentation), whereas the BATF3-independent cDC1 subset was not reduced in old mice. We hypothesize that old tissue niches disturb cDC1 functional adaptation, resulting in populations with dysfunctional antigen cross-presentation and CD8 T-cell activation. This project is focused on transcriptional changes in cDC1 and cDC2 subset across mouse lifespan to reveal differentially expressed gene modules affected by age and sex. Identification and understanding of aging-dependent signals and pathways altering cDC1 and cDC2 functions is important for understanding mechanisms immunosurveillance of infected, damaged, and cancer cells in older individuals.

Orthogonal senescence- and aging-associated gene regulatory programs operate in monocytes of older humans to promote inflammatory gene expression

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Aging is the major cause of disease and morbidity and is defined by the gradual decline of tissue function. Dysfunctional senescent cells accumulate in tissues of aging humans, a process that is linked to weakening of the immune system and disease susceptibility. Monocytes are a bone-marrow derived population of the innate immune system which play critical roles in the initial response to pathogenic challenges and removal of damaged cells from tissues. The ability of monocytes to perform their function is, however, markedly perturbed with age. However, whether monocytes undergo senescence as a result of aging or whether these processes occur independently of each other is not known. Using senescent cell isolation followed by multiomic profiling and functional assays, we characterized the senescence and aging features of CD14+ monocytes isolated from the peripheral blood of a cohort of younger (20s) and older (55+) donors. We identified independently-operating inflammatory gene expression programs in SA-ßGal-high monocytes as well as monocytes from older individuals, indicating that both senescence and age synergize to promote constitutive monocyte-dependent inflammation. The regulation of senescence- and age-associated transcriptional programs required upregulation of dedicated sets of transcription factor: AP1, KLF and STAT for senescence; NF-kappaB and PU.1 for age, which acted on a preestablished chromatin. Functionally, SA-ßGal-low and SA-ßGal-high monocytes exhibited similar kinetic responses to endotoxin tolerance challenge but with differential gene expression output, suggesting that senescent monocytes react abnormally to environmental stimuli. Collectively, our findings demonstrate the potential of multiomic profiling in identifying key regulators of senescence and aging phenotypes across cell types and open inroads into assessing the contribution of dysfunctional monocyte subsets to age-related disease.

Combining Dietary Isoleucine Restriction and Resistance Exercise to Enhance Skeletal Muscle Metabolic Resilience to Aging

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Dietary isoleucine restriction (IleR) extends the healthspan and lifespan of diverse species and is associated with improved metabolic health in both young and aged rodents. Furthermore, exercise in the form of resistance training (RT) is an established countermeasure to offset age-related sarcopenia and frailty. However, our laboratory to date, has not explored the influence of IleR and/or RT on skeletal muscle health. Here, we examined the combination of two geroprotective strategies using weight pulling, a validated progressive resistance exercise training regimen, in young and aged mice fed either a control (Ctrl) or IleR diet for 12 weeks. We find that despite food consumption being higher in IleR, both young and aged mice lost considerable amounts of body mass. This resulted in lower adiposity and leaner body composition compared to control chow-fed mice, which is partially explained by the increased energy expenditure associated with IleR. The IleR diet enhanced glycemic control in young and aged mice, with IleR + RT facilitating improvements in insulin sensitivity in the old group only. Skeletal muscle function, assessed by maximal grip strength and inverted cling test, observed notable improvements in muscle function with IleR + RT in both young and aged mice, overall showing an offsetting of age-related decline in muscle function. Future analysis of isotopically labeled D_2O-enriched skeletal muscle samples will allow us to explore muscle protein synthesis, breakdown, and overall proteostatic health with IleR and RT. Coupled with histological assessment of skeletal muscle fiber-type morphology and mTORC1-related nutrient sensing, may give us further insight into the synergistic effect of IleR and RT to improve muscle metabolic resilience to aging.

Effect of Ketogenic Diet on Hippocampal Oxidative Metabolism

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Abstract

The senescence and the resultant increase in inflammation is associated with cognitive decline in aging and disease. Obesity and high-fat diet induced adipocyte senescence have been associated with poor cognitive outcomes in humans and mice. Importantly, ketogenic diets have been shown to improve lipid metabolism and cognitive outcomes in humans and mice. Yet the role of ketogenic diets on the brain-adipose axis senescence and SASP phenotype in cognitive impairment has not been thoroughly investigated. To address effects ketogenic diets on brain energy metabolism and senescence, male mice (16 mo) were fed a ketogenic diet (KD; 10% protein, <1% carbohydrate, and 89% fat), high-fat diet (HFD; 60% fat, 40% carbohydrate) or chow diet *ad libitum* for 6 months. Body weight was measured every week for 24 weeks, body composition once a month, and basal ketones after 4 months of diet. After 6 months of diet (22 mo), mice were tested for cognitive function and hippocampi were assessed for mitochondrial function by high-resolution respirometry. Mice fed with KD gained significant body mass compared to chow but at a lower rate than HFD mice, which tapered off by 24 weeks. Fat mass was significantly higher in both HFD and KD animals compared to chow. Blood ketones significantly higher in the KD group compared to HFD and chow diet controls. High fat diet and chow fed aged animals showed insulin resistance, while ketogenic diet animals showed increased insulin sensitivity. Glucose tolerance was not different between groups. Importantly, HFD reduced hippocampal mitochondrial function which was restored to young levels with KD with a concomitant improvement in cognitive outcomes. These data highlight the importance of dietary interventions to mitigate cognitive impairment in aging by improving brain energy metabolism.

Title: Improving memory flexibility in old age

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Abstract: Aging is accompanied by cognitive decline, including drastic impairments in long-term memory. One aspect of memory that is especially vulnerable to aging is the ability to update existing memories with new information. Very little is known about the mechanisms that underlie age-related impairments in memory updating, however. To address this critical gap, my group has developed a novel memory updating task called Objects in Updated Locations (OUL) that is specifically designed to assess memory updating in young and old mice. Using OUL, we discovered that neuronal co-allocation is a critical mechanism for memory updating, that is the original memory and the memory update are stored in overlapping neuronal ensembles within the dorsal hippocampus. Here, we hypothesized that this process of co-allocation is disrupted in old age, leading to age-related impairments in memory updating. First, we used *Arc* catfish to track the neuronal ensembles engaged by an original memory and a memory update and show that old mice show reduced co-allocation compared to young mice. Next, we artificially bolstered co-allocation in old mice using two strategies. First, we used DREADDs to force the same population of hippocampal cells to encode both the original memory and the update. Next, we reduced the delay between training and updating, as past work (Cai et al., 2016) has demonstrated that learning events occurring close in time are co-allocated. Both strategies improved memory updating in old mice, consistent with our hypothesis that reduced co-allocation underlies age-related impairments in memory updating. We are currently testing whether this process is regulated by the repressive histone deacetylase HDAC3 and using a viral TetTag system to capture training engram cells and manipulate their activity during the update session. Together, our work suggests that successful memory updating requires faithful reactivation and modulation of original-memory neurons, a process that is impaired in the old brain.

POSTER 23

PUTZ: a novel mitochondrial microprotein linking skeletal muscle to healthspan and lifespan

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Although the Human Genome Project identified over 20,000 genes in the human genome, multiple elements were overlooked, such as microRNA, long non-coding RNAs, and microproteins. Recent technological advances have identified overlooked microproteins encoded in the small open reading frames (smORFs). We have identified and characterized mitochondrial-derived microproteins, such as MOTS-c for obesity/diabetes and SHMOOSE for Alzheimer's disease. Here we demonstrate that a novel mitochondrial-derived microprotein called PUTZ is a negative regulator of skeletal muscle and lifespan. By utilizing mito-smORF-focused genomic and transcriptomic approaches, we identified PUTZ as a myo-regulating myokine and detected it in human muscle cells by mass spectrometry and western blotting. Aging, obesity, and physical inactivity increase muscle PUTZ smORF expression levels, while exercise training decreases PUTZ levels in human skeletal muscle. PUTZ decreased metabolic activity, impaired mitochondrial respiratory function, and prevented muscle growth in human skeletal muscle cells. In young mice, PUTZ caused muscle loss and weakness by directly interacting with the PDK1/AKT/FOXOs pathway. PUTZ treatment also increased fat mass and systemic inflammation in young mice. There is a gain-of-function genetic variant in PUTZ, and this variant caused muscle weakness and increased all-cause mortality risk. PUTZ inhibition by its neutralizing antibody and antisense oligonucleotides exhibits opposite biological functions to synthetic PUTZ treatment, demonstrating a potential for becoming a therapeutic for sarcopenia, frailty, and lifespan. In summary, PUTZ is a novel mitochondrial microprotein that negatively regulates skeletal muscle function and lifespan, and PUTZ inhibition could be a novel therapeutic strategy for sarcopenia and healthy aging.

Title: Identification of inter-cellular signaling axes that suppress senescent cell clearance by macrophages

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Senescent cells secrete molecules that attract and activate macrophages, triggering senescent cell clearance. Why macrophages eliminate senescent cells in young organisms but fail to do so in aged organisms remains a key unresolved question. One possible explanation is provided by recent findings that certain senescent cell types suppress macrophage phagocytosis by upregulating the anti-phagocytic factor (APF) CD47. However, other senescent cell types resist phagocytosis in a CD47-independent manner, suggesting that additional unidentified APFs may be more important in these contexts. To identify senescent cell APFs, we developed a CRISPR screening platform involving co-incubation of senescent hepatocytes with macrophages in vitro. By conducting a comparative screen in proliferating hepatocytes, we were able to identify APFs that protect both proliferating and senescent cells against phagocytosis – such as CD47 and APMAP – as well as APFs that are only required for senescent cell evasion of phagocytosis. Interestingly, we found that several novel senescent-cell-specific APFs are induced to a greater degree than CD47 during the induction of senescence. Ongoing efforts focus on evaluating the contributions of these novel APFs to senescent cell immune evasion in vivo and to understanding their mechanistic functions in phagocytosis suppression.

Elucidating mechanistic signatures of resilience to 'normal' age-related cognitive decline

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Resilience to brain aging is a phenomenon whereby cognitive functioning is better than predicted based on chronological age, likely because of the presence of as yet unidentified protective molecular factors. These factors, once identified, may provide key targets novel therapeutic strategies that promote resiliency in the aging population. The hippocampus and prefrontal cortex are brain regions known to be critically involved in learning and memory functions, but a **critical outstanding question** in the cognitive aging and resilience fields is which neurons or neuronal subtypes exhibit the earliest vulnerability to the aging process – as the mechanisms that maintain or retore the function of these neurons would be powerful targets for therapeutic interventions.

To address this question, we have recently developed a sophisticated patch-proteomics pipeline to identify and profile cell type-specific electrophysiological and proteomic signatures associated with cognitive resilience. In this, pipeline, we first characterize cognitive function using learning and memory assays like contextual fear conditioning, and then, in acute brains slices from those cognitively characterized animals, we directly target and profile individual neurons. We assess intrinsic neuronal excitability via visualized whole-cell patch-clamp recordings and collect cellular contents (nucleus and cytoplasm) to analyze the cell-specific proteome using ultra-high sensitivity liquid chromatography mass spectrometry (LC-MS).

We have recently completed a series of benchmark experiments recording from 39 individual CA1 neurons to identify novel proteomic candidates that mediate alterations in neuronal excitability. We successfully detected and identified thousands of proteins across our population of recorded cells, and analysis of functional annotations revealed a broad and diverse set of biologically relevant processes as well as a selective and robust enrichment of neuron-specific proteins. To identify novel candidate proteins that mediate alterations in neuronal excitability related to learning and memory, we prioritized proteins strongly correlated with sAHP size because prior literature shows the size of the sAHP is inversely related to memory performance in aging mice. Results yielded a list of nearly 30 proteins significantly correlated with the amplitude of the sAHP; of these, our top nomination is ANXA7, as it is associated with increased neuronal excitability based on sAHP amplitude and measures of firing rate.

To interrogate changes in neuronal excitability and proteomic signatures of cognitive resilience, we, for the first time, characterized cognition across the lifespan of a large group of genetically diverse UM-HET3 mice and demonstrated individual differences in susceptibility and resilience to learning and memory deficits, validating this panel as a model of heterogeneity of cognitive aging observed in humans. Further, we found that the earliest detectable age-related impairments (at 24 mo of age) involve the movement (or transfer) of hippocampal-dependent contextual fear memory (CFM) to the neocortex for long-term storage (at 7 days after training), rather than the initial encoding or more immediate recall (24 hr after training). Notably, if we age UM-HET3 mice further (~28-30 mo), deficits in recent CFM appear (24 hr after training). Taken together, these results importantly suggest that 'normal' age-related cognitive decline originates from dysfunction of neurons that reside outside of the hippocampus proper and hypothesize that resilience signatures in subicular neurons (which functions as the primary output from hippocampal CA1 neurons) underlie ***cognitive resilience*** in the subset of aging UM-HET3 mice that perform similarly to young mice.

POSTER 26

Complement Aggregates in Endothelial Cells and Inflammaging

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Loss of vascular proteostasis contributes to age-related inflammation and tissue dysfunction. With increasing age, protein aggregates accumulate in endothelial cells (ECs), cells forming the inner lining of blood vessels. While the root cause(s) of aging are unknown, protein aggregates accumulating in ECs are believed to contribute to multi-system organ dysfunction, leading to a broad spectrum of age-associated disease conditions like myocardial infarction and stroke. FDA-approved drugs modulating proteostasis have excitingly advanced the paradigm that protein aggregates such as those occurring in ECs are amenable to therapeutic manipulation. Defining new proteins undergoing age-related aggregation in ECs will uncover targets for enhancing vascular health with aging.

This proposal explores the disruptive concept that immune proteins called membrane attack complexes (MACs) form aggregates that contribute to age-related inflammation and tissue dysfunction. Complement (C') are immune proteins involved in host defense. Upon activation, C' proteins assemble into MACs that insert into EC surfaces as large, transmembrane pores. Diverse stimuli encountered during aging cause MACs to form on ECs. Due to their well-known cytolytic properties, MACs are widely understood to promote inflammation by mediating EC death. However, recent analyses of elderly patients and aged mice have demonstrated increased MAC formation on ECs occurring in the absence of lytic cell death. These findings indicated an undefined, non-cytolytic immune function for MACs.

MACs are comprised of ~20-25 C' proteins, and we found that this large *de novo* burden of MAC proteins on ECs disrupted proteostasis, causing MAC proteins to form intracellular aggregates within the endolysosomal system. Intracellular MAC aggregates were non-cytolytic and activated NF-κB, a pro-inflammatory pathway inducing VCAM-1, an adhesion molecule, to promote age-related inflammation. C9, a MAC protein, formed aggregates in human tissues from aged patients. To address the role(s) of C9 aggregates, we propose to use novel tools to study the immune mechanisms and clinical relevance of MAC aggregates in ECs patient tissues, *in vitro,* and *in vivo*. Using these novel tools, our exciting findings will define a new process contributing to age-related loss of vascular proteostasis.

The Role of Diminished Dopamine Levels on Age-Related Sleep Disturbances

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As individuals age, they often experience sleep disturbances such as difficulty falling or staying asleep. However, the underlying mechanisms driving these sleep deficits remain unclear. Dopamine (DA), a key neuromodulator in the brain, declines with age and has been implicated in sleep regulation. My previous research in mouse models revealed that reduced DA levels are associated with shorter sleep duration and fragmented sleep patterns, resembling those observed in older adults. In this project, I investigated how diminished dopamine signaling contributes to these sleep deficits. I found that reduced DA levels impair the dynamic range of neural activity in the medial substantia nigra pars reticulata (mSNr), a region involved in both motor control and sleep regulation. Additionally, I found that DA neuron activation promotes adenosine accumulation, a process that facilitates sleep. Consequently, reduced dopaminergic signaling may impair adenosine buildup, contributing to sleep disturbances. To further elucidate these mechanisms, I am currently employing fiber photometry and in vivo optogenetics to investigate the interplay between dopaminergic signaling, adenosine dynamics, mSNr neural activity, and sleep regulation.

Single-molecule DNA modification patterns in the aging brain

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Epigenomic alterations are believed to be fundamental to human aging. Epigenetic clocks have demonstrated relationships between DNA modifications and chronological aging across organ systems and species. The loss of epigenetic information content has been proposed as an epigenetic mechanism of aging, but this hypothesis lacks a quantitative definition of epigenetic information and is therefore untested. Mechanistic studies of DNA modifications have been limited, in part, by the lack of cell type-specific studies, and most studies do not discriminate between methylcytosine (mC) and hydroxymethylcytosine (hmC). Because cell populations in the brain are not readily turned over, faithful epigenomic maintenance is particularly important to healthy brain aging. Hydroxymethylation is highest in the brain and comprises nearly ¼ of the modifications typically "called" as methylation with conventional techniques, despite having a positive correlation to gene expression, rather than the generally suppressive role of methylation. Short-read next-generation sequencing methods cannot analyze paired mC/hmC patterns from the same molecule. The Geroscience field is therefore missing rich epigenetic information contained within long-range, single-molecule mC/hmC epigenetic modification patterns. To examine DNA modification profiles in astrocytes, neurons, and microglia, inducible NuTRAP models (Aldh1l1-, CamkIIa-, and Cx3cr1-cre, respectively) were used to collect cell-type-specific DNA and RNA from mice aged 6-25 months. A combination of oxidative bisulfite sequencing (OxBS) and native long-read nanopore sequencing was used to measure DNA modifications. OxBS data were paired with TRAP-Seq gene expression measurements. Promoter methylation was negatively correlated with gene expression in all cell types, and we identified no evidence of genome-wide hypomethylation during aging. Cell-type-specific differences in genome-wide mC and hmC were reproducible in both OxBS and nanopore sequencing datasets. To test whether epigenetic information entropy increases during brain aging, deep nanopore sequencing was performed on DNA isolated from bulk hippocampus tissue of WT mice aged 7 or 25 months and neuronal DNA isolated from the whole brain of CAMKIIA/NuTRAP mice aged 6, 12, and 24 months. Epigenetic information entropy was computed for mCG/hmCG modification patterns identified by nanopore sequencing. In bulk hippocampus tissue, epigenetic information entropy increased during aging. In neurons, epigenetic information entropy was highest in old age, and lowest in adults. Our results support the hypothesis that epigenetic information entropy increases during brain aging—including within post-mitotic cell-types.

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Systemic Regulation of Organismal Aging and Health by Mitochondrial Stress in a Specific Subset of Neurons

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Aging is a systemic process influenced by the functional status of specific tissues and their communication with other parts of the body. Mitochondrial dysfunction in individual tissues is known to exert non-cell-autonomous effects that influence organismal health and longevity. The nervous system, in particular, plays a critical role in regulating aging. Experimental induction of mitochondrial stress across the entire nervous system in *Caenorhabditis elegans* (*C. elegans*) leads to significant alterations in lifespan, yet the specific neuronal subtypes mediating these systemic effects have remained poorly defined. In this study, we demonstrate that targeted mitochondrial stress in γ-aminobutyric acid-producing (GABAergic) neurons is sufficient to reduce reproductive output, enhance stress sensitivity, and shorten lifespan in *C. elegans*. This neuronal mitochondrial stress also causes widespread physiological alterations, including increased mitochondrial mass, disrupted energy metabolism, and elevated reactive oxygen species (ROS) levels through conserved transcription factor DAF-16/FoxO. To further dissect neuron-specific contributions to aging, we are generating transgenic *C. elegans* strains expressing polyQ40 or harboring electron transport chain (ETC) defects in defined subsets of the 37 known sensory neurons. We are currently evaluating how these single neuron-specific perturbations affect lifespan and activation of the mitochondrial unfolded protein response (mitoUPR) and DAF-16 activity. Additionally, we will examine how individual sensory neurons regulate mitochondrial ROS homeostasis across aging.

Title: Decipher the chemical language of gut bacteria in aging and longevity

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The human gut microbiota encodes diverse metabolic pathways, where gut microbes make numerous compounds that are relevant to human health and hold untapped therapeutic potential. Fecal microbiota transplantation has been shown to delay age-associated decline in mouse and fish models. However, how specific gut bacteria and their metabolites impact host physiology represents a new frontier that remains to be fully explored. Leveraging our expertise in the gut microbiome and aging biology, we tackle this underexplored area from both the microbial and host's perspectives. Specifically, our lab seeks to 1) mechanistically characterize gut bacteria and small bioactive molecules in host aging and physiology, and 2) identify gut microbial-dependent, host cellular mechanisms underlying organismal health-span and lifespan. We aim to understand how gut bacteria mechanistically contribute to host aging and longevity and to identify new molecular targets to delay age-associated decline.

POSTER 31

Time-dependent cognitive decline following autophagy deactivation

Hilary Grosso Jasutkar, Elizabeth M. Wasserlein, Azeez Ishola, Nicole Litt, Agnieszka Staniszewski, Ottavio Arancio & Ai Yamamoto

Normal aging is associated with a decline in cognitive function, but the neurobiological underpinnings of this phenomenon are unclear. Cognitive function is dependent upon proper synaptic function and plasticity, and there is evidence that the essential homeostatic process, macroautophagy, herein referred to as autophagy, is particularly important for the synapse [1]. There is a vast literature about the essential role of synaptic autophagy in neurodevelopment [2], but less is known about autophagy in synaptic maintenance and function in adulthood or with aging. As we age, there is a shift from a reliance on the ubiquitin-proteosome system to the autophagy-lysosomal pathway for protein turnover in neurons [3], suggesting that even though autophagic function declines naturally with age, its importance to the synapse may rise. Due to the role of autophagy in maintaining synapse homeostasis, we hypothesized that age-related decline in autophagic function contributes to normal cognitive aging. To investigate this, we deactivated autophagy in adult mice and evaluated them using biochemical, histochemical, and behavioral outcome measures. We found that there was a time-dependent accumulation of synaptic proteins and impairment in cognition-based behaviors, which correlated with changes in ubiquitin-proteasome activity. This was in the absence of neuroinflammatory changes or cell or synapse loss. Further, when autophagy deactivation was limited to neurons, we found a less severe cognitive behavioral phenotype than when autophagy was deactivated in the whole body. Taken together, we conclude that the brain can compensate for autophagy loss initially, but with time, this compensation fails, leading to loss of synapse homeostasis and a resultant decline in cognitive abilities. This decline is not a degenerative phenomenon, given the absence of neuroinflammation and the absence of cell or synapse loss. However, synapse homeostasis is not dependent upon neuronal autophagy alone, as the phenotype is mitigated in animals with neuron-specific autophagy deactivation. Ongoing work is investigating how synaptic autophagy changes with aging and whether improving synaptic efficiency can rescue age-related changes in synaptic health.

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Understanding cellular aging through perturbation screening and virtual cell models

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The pursuit of extending healthspan and mitigating age-related decline presents significant challenges, particularly in drug development and understanding complex biological processes. Combining transcriptomic profiling, pooled screening approaches, and *in silico* modeling, we create a framework for the nomination of new potential mechanisms and interventions for aging. We develop this framework in three stages. First, by using transcriptomic profiling on patient samples across a range of ages, we develop "transcriptomic clocks" to quantify biological age at the single-cell level. Second, we apply these clocks to pooled screening approaches, allowing interrogation across all transcription factors or secreted factors. These screening approaches enable the testing of novel, therapeutically relevant interventions in aging models. Third, we leverage this data to generate "virtual cell" models of aging biology to nominate new rejuvenating interventions in silico. We apply this framework to multiple tissues, including aging hematopoietic blood cells, muscle, hair, and liver tissue. By combining quantitative profiling tools, broad screening modalities, and machine learning-enabled prediction, we hope to discover new and generalizable targets for aging therapeutics.

Title: Gut-Brain Axis in Aging

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Aging is determined by complex interactions among genetic and environmental factors. Increasing evidence suggests that the gut microbiome mediates many age-associated changes, and can drive immune system dysregulation and susceptibility to diseases with advance age. The gut microbiota undergoes extensive changes across the lifespan, and age-related processes may influence the gut microbiota and its metabolites produced. Prior research found that older adults who had a more unique pattern of changes to their gut microbiome, especially increased lactobacillus, bifidobacteria and symbionts, also tend to be healthier and live longer than peers with less microbiome divergence. Healthier participants had higher levels of beneficial microbially-derived metabolites like short chain fatty acids, tryptophan derivatives, glutamate derivatives. We in our lab found that the imbalance in gut microbiota at an advanced age causes increase in peripheral antigen presenting cells and increased inflammatory responses in both periphery and the brain. Therefore, we set to understand if the supplementation of probiotic bacteria (bacteria from young population or isolated from breast milk) may enhance immune response by suppressing inflammation via increased beneficial metabolite production that in turn improves quality of aging. To do so we treated middle aged WT animals with probiotic cocktail until they are aged to 22-months old. We used both males and females. We found increased phagocytosis activity after probiotic treatment in microglia which shows improved clearance within the brain. In addition, we found improved gut barrier integrity measured by enhanced mucus thickness. Since microbial-derived tryptophan metabolites have direct impact on reducing inflammatory response we found probiotic treatment increased indole-derivatives in the circulation which corelated with reduced inflammatory cytokines. The probiotic intervention also showed significant reduction in senescence markers in the gut. We found mild impact on gut microbiota composition after probiotic administration. As a next step we are working towards colonizing the animals with healthy microbiota transplants and see if we can improve quality aging.

Title: Genome-wide and genome-wide interaction study of age-related frailty.

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Frailty summarizes an individual's susceptibility to stressors and is typically measured as a frailty index (FI). Frailty is an age-related, developmental phenotype – low prior to the midlife transition, increasing during or after midlife, with accelerations in FI eventually plateauing typically in late life. However, while frailty genome-wide association studies (GWAS) have identified genetic influences on FIs, no existing GWAS of frailty has evaluated this developmental progression, leaving uncertain whether genetics influences why some individuals become frail earlier than others, or why some are ultimately less frail than others. Characterizing the etiology of these differences in *why* some individuals age earlier or later, and *why* some ultimately become more or less frail has critical implications for care and improving the healthspan.

We generated FIs of frailty for the Health and Retirement Study (HRS) and the UK Biobank (UKB) in both European and diverse genetic ancestry cohorts. In the HRS (up to 16 waves & 30 years of data), we fit a Gompertz developmental growth model to distinguish the age of frailty increase from the late life frailty plateau. In the UKB (up to 4 waves including Online Follow Up, ~10-15 years of data), we estimated the late life frailty plateau. We then performed GWAS on these two developmental phenotypes.

The two developmental phenotypes were at least partially genetically distinguishable using genetic correlation analyses. We performed gene prioritization and gene set enrichment using DEPICT, and identified the SWI/SNF complex (involved in DNA repair and chromatin remodeling) and specifically *PHF2*, as well as ADP signaling through P2Y purinoceptor 12 (which affects vascular endothelial function and promotes inflammatory cytokines). Neither has been identified in previous GWAS.

We are extending these analyses to additional datasets and to genome-wide gene-gene interaction associations, where we aim to identify genes whose interactions integrate genetic influences from multiple molecular pathways within gene-gene networks to affect the developmental progression of age-related frailty.

Mechanisms of Degradation across the Lifespan: The Role of Descending Tract Function on Age-Related Sensory-Motor Deficits

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Abstract: There are multiple descending pathways from the brain that are responsible for motor control and modulating sensory inputs within the spinal cord. The roles each of these pathways play varies substantially across various animals. One pathway in particular, the Corticospinal Tract (CST), is significantly more evolved and developed in humans, even compared to other mammals. This makes humans a unique model to study this pathway, its role in motor control, and how degradation of that pathway with age affects sensory-motor integration throughout the nervous system. Furthermore, there may be compensatory adaptations from other pathways (e.g. brainstem pathways) that alter these complex circuits. Using paired stimulations of the brain, spinal cord, and peripheral nerves, we first aim to determine the role various descending pathways have on human spinal cord sensory-motor circuitry. We believe that young, neurologically intact adults will show modulation of spinal reflexes from the CST, and that the brainstem pathways will have a minor role overall in fine motor control. We also believe that the brainstem pathways may play an important role in force modulation. Second, given that CST has shown to get slower and weaker with age, we aim to quantify how the role of these other neural pathways change in an aged model to compensate. We believe that older adults will exhibit an age-related reduction in the modulation of sensory inputs in the spinal cord (which will lead to sensory deficits), and that a compensatory adaptation from brainstem pathways may occur in some to preserve function in the presence of CST degradation. All of our measures will be performed in 2 groups: young neurologically intact adults, and older adults. They will also be performed across multiple muscle groups that are thought to rely on different neural pathways (CST vs. brainstem). Improving our understanding of the role each descending pathway has in motor control, and how those roles may change with aging, may provide researchers with specific targets for future interventions.

Association of Metabolism and Age-Related Brain Iron Accumulation: Insights from Individual Differences in Mitochondrial Genetic Haplogroup and Imaging Biomarkers

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Abstract:

Several competing mechanisms of neuroanatomical aging have been advanced in animal models and postmortem study, including oxidative damage and mitochondrial metabolic dysfunction. Yet few studies have forward translated to test these mechanisms in relation to human cognitive decline—this remains a substantial barrier in unifying a theory of brain and cognitive aging. When considered as a combined mechanism, oxidative damage and mitochondrial dysfunction converge on the action and homeostasis of non-heme iron. This work directly builds from the Free-Radical-Induced Energetic and Neural Decline in Senescence (FRIENDS) model of aging, that posits brain iron accumulation as an MRI biomarker of risk for neurodegeneration and cognitive decline is, in part, related to disrupted mitochondrial energetics and metabolism. This study leveraged data from the Detroit Aging Brain Study, a community-based, longitudinal study of typical aging across the adult lifespan. Cohorts enrolled in the study had multi-modal MRI data, including multi-echo susceptibility weighted imaging to estimate regional iron concentration via quantitative susceptibility mapping as a biomarker related to oxidative damage, and 31-P magnetic resonance spectroscopy for indicators of regional brain metabolism and energetics. Additional insights on the putative neurodegenerative mechanism were gleaned from individual differences by mitochondrial DNA haplogroup, which reflect maternal lineage and carry phenotypic differences in adenosine triphosphate production and oxidative stress. Key findings from the study will be summarized on age-related differences in regional brain iron accumulation in association with the genetic variants and MRI biomarkers related to mitochondrial energetics and brain metabolism.

Microbial Pathogenesis in Age-dependent Protein Conformational Diseases

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ABSTRACT:

The accumulation of oxidative damage and dysregulation of the heat shock response lead to protein damage, collectively contributing to aging and a decline in protein folding homeostasis (proteostasis). We found that colonization of the *Caenorhabditis elegans* gut with gram-negative enteric bacteria disrupts proteostasis, leading to toxic protein aggregation not only in the intestine but also in the muscles, neurons, and subsequent generations. In humans, one example of such a non-autonomous effect of bacteria on host tissues takes place at the gut-brain axis, where microbial residents of the human gut influence protein folding in distant tissues. We discovered that bacteria can secrete protein aggregates that disrupt host proteostasis, possibly by titrating host chaperones away from the endogenous proteome. To further elucidate the role of individual residents of the human microbiome on host proteostasis, we characterized the effects of 229 unique bacterial isolates on their ability to disrupt protein folding in *C. elegans*. We identified bacterial species affecting host proteostasis, leading to either enhancement or suppression of proteins associated with neurodegenerative diseases, namely polyglutamine, $A\beta_{42}$, tau, and α-synuclein. Further analysis of the most robust proteoprotective and proteotoxic bacterial strains for their ability to activate oxidative stress response, endoplasmic reticulum unfolded protein response, and cytoplasmic heat shock response (HSR) revealed several species within the *Prevotella* genus that activate protective HSR. Among them, *P. corporis proved to be* the most robust. While *P. corporis* did not affect lifespan, it enhanced thermotolerance and disassembled existing aggregates, suggesting an overall HSR-mediated enhancement of proteostasis. The proteoprotective role of *P. corporis* was confirmed in the *Drosophila melanogaster* models expressing polyglutamine and $A\beta_{42}$. Collectively, we reveal how bacteria affect host proteostasis and influence disease pathology, potentially offering microbiome-targeted approaches that can influence health span.

Risk factors and consequences of age-related mosaic chromosomal alterations in >1 million individuals

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With aging, hematopoietic stem cells accumulate somatic mutations. Single-nucleotide variants and small insertions or deletions in certain preleukemic genes are termed clonal hematopoiesis of indeterminate potential (CHIP). Larger >1 megabase gains (+), losses (-), or copy-neutral losses of heterozygosity (=) are termed mosaic chromosomal alterations (mCAs). Compared to CHIP and mosaic loss of the sex chromosomes, the prevalence, risk factors, and health consequences of autosomal mCAs across diverse populations remain incompletely understood.

Here, we analyzed autosomal mCAs in 850,810 individuals aged 40-90 years in four large cohorts NIH All of Us, UK Biobank, Vanderbilt BioVU, and NHLBI TOPMed, identifying 23,766 (2.70%) individuals with at least one mCA. We found that specific mCA types show distinct age, sex, and ancestry-dependent patterns - for example, chr15+ and chr20q- occurred more frequently in older males and were associated with genetically predicted longer leukocyte telomere length.

In the largest genome-wide association study of mCAs, we identified novel risk loci in *CHI3L2* and *HLA* class II genes and confirmed known loci in *TERT* and *SP140L*. We also identified oncogenic cis germline variants– that is, on the chromosome affected by mCA – which were under positive selection and increased risk of specific mCAs, such as *JAK2* for chr9p, *MPL* for chr1p, *ATM* for chr11q, and *TCL1A* and chr14q.

In phenome-wide association studies of autosomal mCAs, we found that they increased risk of chronic lymphocytic leukemia (CLL) by 22-fold. Using mediation analysis, we showed that autosomal mCAs mediate ~24% of genetic predisposition to CLL. Individuals with an autosomal mCA and with normal blood counts at the time of DNA extraction had twice the risk of developing incident persistent complete blood count abnormalities compared to matched individuals without an autosomal mCA. Different autosomal mCA types demonstrated distinct cytopenia and cytosis patterns, with lymphoid-cancer-associated autosomal mCAs increasing risk of leukocytosis and myeloid-cancer-associated autosomal mCAs increasing risk of anemia. These findings provide new insights into the germline risk, underlying biology, and clinical significance of age related autosomal mCAs.

Do aging-related neuropathologies spread through neuronal synapses?

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Normal aging is often accompanied by cognitive impairment, perhaps because of abnormal protein aggregation in the brain. Though the specific proteins that aggregate differ between diseases, in each of these diseases, pathology appears to spread along connected brain regions. However, the basic question of whether disease spreads between connected neurons in the brain is unknown. If disease does spread specifically between connected neurons, this means that we may be able to develop drugs to inhibit this process and prevent or slow disease, providing a completely new mechanistic target to combat a wide variety of aging-related diseases. To do this, we are testing whether pathology linked with age-associated neuronal senescence spreads specifically between connected neurons. We are also assessing whether cells connected to cells with a senescent phenotype preferentially exhibit changes in cellular physiological properties. Last, we are exploring whether neurodegenerative disease mechanisms also spread specifically between connected cells. Our study will provide critical insight into basic mechanisms of how pathology of both normal aging and neurodegenerative states spreads in the brain and provide potential druggable targets to combat age-associated brain dysfunction.

Exploring the Molecular Mechanisms that Drive Adaptations to Exercise

Cory W. Baumann, Michael L. York, Thomas A. Krauss, Muni Swamy Ganjayi

Institutions: Ohio University, Heritage College of Osteopathic Medicine

Background: Resistance training is crucial for treating and preventing age-related weakness and enhancing physiological capacity. It increases muscle mass, strength, and resting metabolic rate, thereby improving overall health. However, its effects vary with age, as older adults often show a limited response compared to younger individuals. The reasons for these differences remain unclear. To better understand these adaptations, we conducted a cross-sectional study on young and old C57BL/6 mice. The muscles were subjected to repeated bouts of maximal, in vivo, eccentric contractions. Analysis of muscular strength (torque) after the initial and final bouts revealed a robust adaptive capacity in young muscle, with strength increasing by 20%–36%. In contrast, older muscle exhibited up to an 18% decrease in strength, indicating reduced adaptive potential. Unbiased transcriptomic analyses after repeated eccentric contractions showed that HuR activity, an RNA-binding protein, varied in parallel with positive muscle adaptations in an age-dependent manner. Therefore, we aimed to determine the functional significance of HuR activity in skeletal muscle and the molecular mechanisms underlying this effect.

Methods: Skeletal muscle HuR activity in young mice was blocked or impaired using two distinct methods: a genetic approach and a pharmacological intervention. The genetic approach involved creating a skeletal muscle-specific HuR knockout mouse, while the pharmacological intervention used KH-3, a second-generation HuR-RNA binding inhibitor. All mice were subjected to either one or two bouts of 150 maximal eccentric contractions in vivo, with peak isometric torque assessed before and after the injury. This assay measured susceptibility to injury and adaptation following repeated injuries (i.e., training). Tandem mass tag (TMT) proteomics was performed using tibialis anterior muscles to assess the effects of training and HuR knockout. More direct assays (e.g., ELISA) were completed to confirm the TMT results. Correlational analyses were conducted on proteins regulated by HuR to determine their contribution to skeletal muscle susceptibility and adaptability to eccentric contraction-induced strength loss.

Results: Strength deficits normally found immediately after eccentric contraction-induced injury were significantly attenuated in young skeletal muscle-specific HuR knockout mice, providing protection equivalent to that observed in healthy, HuR-intact, exercise-trained muscle. Importantly, this protection did not compromise other functional parameters, as peak isometric and eccentric strength, recovery from injury, and metabolic indices did not differ between young HuR knockout and control mice. The TMT proteomics revealed that five proteins regulated by training were similarly regulated by HuR knockout (four upregulated and one downregulated), with the most significantly upregulated being myosin binding protein-H (MyBP-H). A subsequent MyBP-H ELISA confirmed the TMT proteomic results for HuR knockout and training. It was also noted that approximately 90% of the variance in eccentric contraction-induced strength loss could be explained by the variance in MyBP-H; higher MyBP-H content resulted in skeletal muscle experiencing less strength loss. As with genetic HuR knockout, KH-3 protected the muscle of young mice from eccentric contraction-induced strength loss.

Conclusions: HuR-dependent expression of MyBP-H appears to be a primary molecular mechanism contributing to exercise-induced adaptations in skeletal muscle. Ultimately, we hope to use this data to develop new pharmacological strategies that enhance resiliency in aging individuals and restore older muscle to a younger phenotype with a robust adaptive capacity.

Targeting Iron Overload in Aged Macrophages to Alleviate Bone Marrow Senescence and Inflammaging

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Iron is essential for oxygen transport, mitochondrial function, and DNA synthesis, yet its dysregulation is a hallmark of aging. Excessive iron has been implicated in age-related cognitive decline and reduced lifespan, likely due to its role in triggering cellular senescence and ferroptosis. We report striking iron accumulation in the bone marrow of adult (6 months) and aged (>2 years) male and female C57BL/6 mice, predominantly within macrophages. These bone marrow macrophages are crucial for immune cell production, inflammatory regulation, and clearance of senescent cells - all functions known to deteriorate with age. Our preliminary data suggest that iron loading alters macrophage phenotype and function including impaired phagocytosis and lysosomal degradation, theoretically contributing to senescent cell accumulation and inflammaging. Interestingly, C3H/HeJ mice, which carry a mutation in TLR4 - a receptor implicated in both iron regulation and senescence - do not accumulate iron in the bone marrow at 6 months of age. Immunophenotyping of 12-week-old female C3H/HeJ bone marrow reveals a marked reduction in macrophage/monocyte, B cell, and T cell populations, accompanied by an increase in common myeloid and lymphoid progenitors, granulocytes, and dendritic cells compared to C57BL/6 mice - indicative of a fundamentally different hematopoietic landscape. Further analyses are underway to determine whether these immunological differences in C3H/HeJ mice extend to males and persist into adulthood, particularly at 6 months of age when iron accumulation becomes evident in C57BL/6 mice. To investigate therapeutic avenues, we are currently analyzing in vivo studies in 6-month-old C57Bl/6 mice treated with senolytics (Dasatinib and Quercetin), alone or in combination with iron chelation (deferasirox) for 2 months. These studies aim to determine whether such interventions can reduce iron overload and senescent cell burden in the bone marrow. Collectively, our findings highlight a central role for iron-laden macrophages in bone marrow aging and support a dual-intervention strategy targeting both iron overload and cellular senescence. The proposed interventions include compounds that are either FDA-approved or have favorable safety profiles, enhancing their translational potential in the context of aging-related disorders.

Identification of functional rare coding variants in insulin/insulin-like growth factor-1 (IGF-1) pathway in longevity cohorts

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Abstract

Genetic variants in the insulin/IGF-1 signaling pathway delay aging in invertebrates and rodents. However, the role of the insulin/IGF-1 variants in human aging has not been confirmed. We studied a cohort of Ashkenazi Jewish centenarians, their offspring, and controls (n=2,487) to identify functional rare coding variants in the insulin/IGF-1 pathway. Rare genetic variants (MAF ≤0.01) were identified using WES, and their function was predicted using Combined Annotation Dependent Depletion (CADD) score ≥ 20 and protein modeling approaches. Subsequently, the identified functional rare genetic variants were evaluated in relationship to longevity and resilience to age-related diseases. We applied this pipeline to 32 genes in the insulin/IGF-1 pathway and found that individuals with at least one functional variant in this pathway compared to individuals without these variants had a significantly lower risk of mortality (p=0.019) and a 44% lower hazard of all-cause mortality in a Cox proportional hazard model adjusted for sex and age, with hazard ratio (HR), (95% confidence interval (95% CI)) of 0.56 (0.37 - 0.91). Additionally, individuals with at least one of these functional variants had a significantly lower risk of CVD (p=0.02) and a 40% lower hazard of CVD in a Cox proportional hazard model adjusted for sex and age, HR (95% CI) 0.60 (0.34 - 0.86), than those without these variants. We identified a novel longevity variant IR:p.V1012M using protein modeling, that was located in tyrosine kinase domain (TKD) of insulin receptor (IR) and was found to be enriched among centenarians (p=0.04). We experimentally confirmed the effect of IR:p.Val1012Met on downstream signaling of the insulin/IGF-1 pathway. We generated IR/IGF1R double knockout mouse preadipocytes (DKO) expressing hIR-A-WT-miniTurbo-V5 and hIR-A-Val1012Met-miniTurbo-V5. IR:p.Val1012Met did not alter the autophosphorylation of IR, but significantly decreased pIRS1, pAKT and pERK, supporting an inhibitory effect of longevity variant Val1012Met on insulin/ IGF-1 signaling. This study highlights the significance of identifying rare functional variants in longevity pathways in humans with exceptional longevity, reinforcing their role in human aging and provide new opportunities for the development of therapeutic interventions to target aging.

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