



GLENN FOUNDATION
FOR MEDICAL RESEARCH



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

**THE 37TH ANNUAL AFAR GRANTEE CONFERENCE
GLENN WORKSHOP ON THE BIOLOGY OF AGING**

May 29 – 31, 2024

Program Book

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THE PAUL F. GLENN/AFAR CONFERENCE ON THE BIOLOGY OF AGING THE 37TH ANNUAL AFAR GRANTEE CONFERENCE

MAY 29 – 31, 2024
Ritz-Carlton Bacara
Santa Barbara, CA



#AFAR2024 @AFARorg

WEDNESDAY, MAY 29

3:30 – 4:00 p.m.	Registration/reception	Santa Ynez Terrace
4:00 – 4:10 p.m.	Welcome Thomas A. Rando, MD, PhD Director, UCLA Broad Stem Cell Research Center Professor, Neurology University of California, Los Angeles President, American Federation for Aging Research	Santa Ynez
4:10 – 5:10 p.m.	Breakthroughs in Gerontology (BIG) Presentations Cellular Recycling in Aging and Disease - The Importance of Taking Out the Trash Malene Hansen, PhD (2020 BIG awardee) Chief Scientific Officer and Professor Buck Institute Repurposing the Principles of Reproduction to Stall and Reverse Cellular Aging Vittorio Sebastiano, PhD (2020 BIG awardee) Associate Professor of Obstetrics and Gynecology Stanford University	
5:10 – 6:10 p.m.	McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss Presentations Blood-Based Approaches to Rejuvenating the Aging Brain Saul Villeda, PhD (2021 McKnight awardee) Associate Professor, Anatomy University of California, San Francisco	

WEDNESDAY, MAY 29 (CONTINUED)

Detrimental vs. Adaptive Phenotypes of Aging Microglia and their Contribution to Cognitive Decline

[Lindsay De Biase, PhD](#) (2021 McKnight awardee)

Associate Professor, Physiology and Neurobiology
University of California, Los Angeles

6:10 – 8:00 p.m.

Dinner

Rotunda Terrace

Glenn Workshop on the Biology of Aging May 30 - 31

THURSDAY, MAY 30

Joint meeting with the AFAR grantees

7:30 – 9:00 a.m.

Breakfast

Rotunda Terrace

9:00 – 9:10 a.m.

Introduction and Meeting History

Stephanie Lederman, EdM

Executive Director, American Federation for Aging Research

Santa Ynez

9:10 – 9:20 a.m.

Meeting Overview and Goals

Kevin Lee, PhD

Workshop Moderator

Senior Scientific & Programmatic Advisor

Glenn Foundation for Medical Research

9:20 – 9:50 a.m.

Leveraging Multiomic Data to Decode Healthy Aging and Longevity

[Noa Rappaport, PhD](#)

Principal Scientist, Institute for Systems Biology

9:50 – 10:20 a.m.

Translational Geroscience: Human Models of Resilience and Healthy Aging

[Sofiya Milman, MD, MS](#)

Professor, Albert Einstein College of Medicine

10:20 – 10:50 a.m.

Metabolic Signals in Longevity Regulation

[Meng C. Wang, PhD](#)

Janelia Senior Group Leader, Howard Hughes Medical Institute

10:50 – 11:15 a.m.

Break

11:15 – 11:45 a.m.

Reflections on a Quarter Century in Geroscience

[Matt Kaeberlein, PhD](#)

Chief Executive Officer, Optispan, Inc.

11:45 a.m. – 12:15 p.m.

Origins of Life & Death: Multi-Agent Reinforcement Learning in Living Systems

[Morgan Levine, PhD](#)

Founding PI, SDI Institute of Science

Altos Labs

THURSDAY, MAY 30 (CONTINUED)

12:15 – 1:30 p.m.	Lunch	Rotunda Terrace
1:30 – 2:00 p.m.	Life-Long Dietary Restrictions Have Negligible or Damaging Effects on Late-Life Cognitive Performance: A Key Role For Genetics in Outcomes Catherine Kaczorowski, PhD Elinor Levine Professor of Neurology University of Michigan	
2:00 – 4:00 p.m.	Poster Session <i>Kindly remove your poster at the conclusion of the session</i> 2:00 – 2:30 pm: General viewing 2:30 – 3:15 pm: Odd numbers stand by their poster 3:15 – 4:00 pm: Even numbers stand by their poster	Ballroom A/B
4:00 – 6:00 p.m.	Free time	
6:00 – 7:00 p.m.	Reception	Rotunda Terrace
7:00 – 7:30 p.m.	Dinner Speaker Science at the Edge of Uncertainty: An Optimistic View Stuart Firestein, PhD Professor of Biological Sciences, Columbia University Fractal faculty, Santa Fe Institute	
7:30 – 9:00 p.m.	Dinner	Rotunda
FRIDAY, MAY 31		
7:00 – 9:00 a.m.	Breakfast	Santa Ynez Terrace
	ADJOURN	



PAUL F. GLENN

Founded by Paul F. Glenn in 1965, the mission of the Glenn Foundation For Medical Research is to extend the healthy years of life through research on mechanisms of biology that govern normal human aging and its related physiological decline, with the objective of translating research into interventions that will extend healthspan with lifespan.

GLENN FOUNDATION
FOR MEDICAL RESEARCH



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Diane Nixon/Deeds Foundation
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The Irving S. Wright Endowment



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

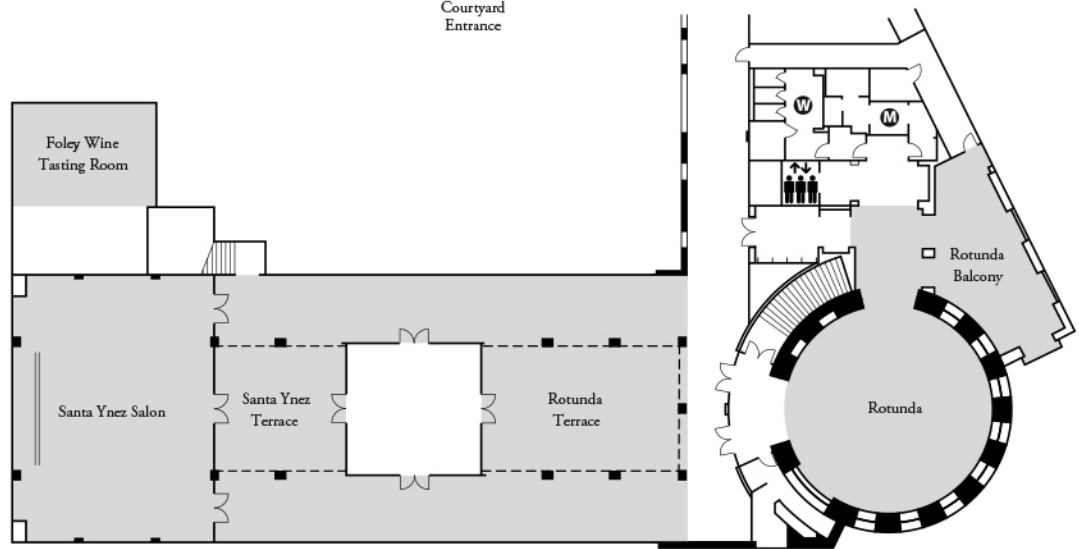
Each of the resort's six restaurants offers its own twist on a successful pairing: ocean views and local flavors. From a modern steakhouse with seasonal sides at Angel Oak, to rustic California cuisine at 'O' Bar and Kitchen, our dining showcases the best of Santa Barbara. For restaurant recommendations,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



Rotunda, Santa Ynez Terrace & Salons



Courtyard
Entrance



THE RITZ-CARLTON

BACARA, SANTA BARBARA

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JF = Glenn and AFAR Grant for Junior Faculty

PD = Glenn Postdoctoral Fellowship Program

NI = Hevolution/AFAR New Investigator Award

NSC = Nathan Shock Center Pilot Award

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NSC = Nathan Shock Center Pilot Award

Platelet Factor 4 (PF4) Rejuvenates Aged Hematopoietic Stem Cells

Sen Zhang¹, Charles E. Ayemoba^{1,3}, Anna M. Di Staulo^{1,3}, Ken Joves², Eva Hw Leung², Chandani M Patel¹, Constantinos Chronis², and Sandra Pinho^{1,3}

¹Department of Pharmacology & Regenerative Medicine, University of Illinois Chicago, Chicago, USA.

²Department of Biochemistry and Molecular Genetics, University of Illinois Chicago, Chicago, USA.

³Cancer Biology Research Program, University of Illinois Cancer Center, Chicago, USA.

Abstract

Hematopoietic stem cells (HSCs) responsible for blood cell production and their bone marrow regulatory niches undergo age-related changes, impacting immune responses and predisposing individuals to hematologic malignancies. Extensive research has elucidated cell-autonomous processes underlying HSC aging, however, the impact of external factors derived from the bone marrow niche on HSC aging remains unclear. HSCs reside within specialized niches in the healthy bone marrow, which encompass megakaryocyte cells that play a crucial role in maintaining HSC quiescence and reconstitution potential via platelet factor 4 (PF4) secretion (Nature Medicine 2014; Developmental Cell 2018).

Here, we explored the impact of megakaryocyte-derived PF4 on HSC aging in both murine and human models. Our studies revealed significant alterations in the aged bone marrow megakaryocytic niche, characterized by compromised megakaryocyte maturation evidenced by smaller size and decreased ploidy levels, alongside an increase in megakaryocyte progenitors ($P < 0.0001$), and reduced PF4 expression (40% reduction; $P = 0.001$). Using PF4-deficient mice, we observed phenotypes reminiscent of accelerated physiological HSC aging including decreased lymphoid output ($P = 0.0023$), increased myeloid output ($P = 0.0014$), and DNA damage ($P < 0.0001$), suggesting that PF4 might be a critical factor in the prevention of hematopoietic aging. Long-term recombinant PF4 administration (1 $\mu\text{g}/\text{day}$) by subcutaneous implantation of Alzet pumps in 18-month-old mice (old) for 42 days restored the serum levels of PF4 in old mice to the levels of 2-month-old mice (young). Notably, this restored phenotypic HSC cell number, and DNA damage to levels comparable to young mice. Remarkably, 4 months after transplantation, HSCs from old PF4-treated mice showed a higher reconstitution ($17\% \pm 6.08$) compared with the old saline administration mice ($2.72\% \pm 0.54$) and a balanced lineage output akin to young HSCs. Consistent with the transplantation data, HSCs from old PF4 treated mice exhibit decreased expression of myeloid genes and increased expression of lymphoid genes, mirroring the profile observed in youthful, balanced HSCs. Mechanistically, we identified CXCR3 and LDLR as the receptors transmitting the PF4 signal in young and old HSCs. Accordingly, *in vitro* antibody blockade of LDLR and CXCR3 abrogates the anti-proliferative effects of PF4 on myeloid-biased HSC, the HSC subset that expands with age. Furthermore, HSCs from LDLR-deficient and CXCR3-deficient mice are insensitive to PF4 signal further validating the role of PF4-CXCR3 and PF4-LDLR in HSC proliferation. Finally, our results further indicate that phenotypic human HSCs are also responsive to the PF4 youthful signal. Altogether, our findings contribute to the development of targeted therapies for stem cell rejuvenation, and potential strategies to prevent or improve the course of age-related hematopoietic diseases.

Cardiolipin Remodeling by ALCAT1 Mediates Mitochondrial Dysfunction and Aging

Jun Zhang, Qianqian Ye, Fanyu Zeng, Shou Pan, Yuguang Shi

Sam and Ann Barshop Institute for Longevity and Aging Studies, Department of Pharmacology,
UT Health San Antonio, TX, USA

Cardiolipin (CL), a mitochondrial signature phospholipid, is required for mitochondrial membrane structure and function. Aberrant CL acyl composition from pathological CL remodeling has been implicated in mitochondrial etiology of aging and age-related diseases. However, the mechanisms through which various signals converge in CL remodeling and aging remain largely elusive. In this study, we identified Acyl-CoA:lysocardiolipin Acyltransferase 1 (ALCAT1) as a pivotal mediator of mitochondrial dysfunction contributing to inflammaging. ALCAT1 catalyzes the pathological remodeling of CL commonly linked to metabolic and age-related diseases. We showed that aging increased the accumulation of CL species rich in polyunsaturated fatty acids, leading to excessive reactive oxygen species production, CL oxidation, cytosolic mitochondrial DNA (mtDNA) release, and mitochondrial dysfunction. Importantly, all these defects were significantly attenuated in ALCAT1 knockout mice, resulting in extended lifespan of both the natural aging mice and the mitochondrial DNA polymerase γ mutator (POLG^{m/m}) mice, a premature aging mouse model. In addition, ablation of ALCAT1 significantly mitigated the cytosolic mtDNA-induced cGAS-STING signaling and type-I interferon response in POLG^{m/m} mice and macrophages. Moreover, the inhibition of ALCAT1 using a small molecular inhibitor significantly improved the healthspan of aged mice by reducing cellular senescence and inflammation. Taken together, these results revealed the molecular mechanism of pathological CL remodeling by ALCAT1 in aging and identified ALCAT1 as a potential anti-aging drug target.

Keywords: Cardiolipin, mtDNA, Mitochondrial dysfunction, Inflammaging

The Extracellular Matrix Defines Mitochondrial Homeostasis

Hanlin Zhang¹, C. Kimberly Tsui¹, Gilberto Garcia^{1,2}, Larry K. Joe¹, Haolun Wu¹, Ayane Maruichi¹, Ryo Higuchi-Sanabria^{1,2}, and Andrew Dillin^{1,3}

¹Department of Molecular & Cellular Biology, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA 94720, USA;

²Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089, USA; ³Lead Contact

Abstract

Maintaining proper mitochondrial function is critical to overall organismal health. Mitochondrial stress contributes to aging and the development of numerous diseases. Various intracellular factors, such as oxidative stress, mitochondrial DNA mutations, and disrupted interactions with other organelles are well recognized inducers of mitochondrial dysfunction. However, it remains elusive how changes in the cellular microenvironment, particularly the **extracellular matrix (ECM)**, can alter mitochondrial homeostasis.

In mammals, ECM remodeling occurs during aging and in multiple diseases, including infections, cancers, and neurodegeneration. Remodeled ECM may liberate bioactive fragments that promote tissue damage-related responses, such as wound healing and inflammation. TMEM2 is a plasma membrane-bound hyaluronidase that cleaves **hyaluronan (HA)**, a major glycosaminoglycan constituent of the ECM in vertebrates. Our study shows that TMEM2-induced ECM remodeling alters mitochondrial homeostasis and sensitizes human fibroblasts to mitochondrial stress via activating the TGF- β signaling pathway. Ectopic expression of human TMEM2 in *C. elegans* also leads to mitochondrial functional decline and activates mitochondrial stress responses, suggesting an evolutionarily conserved signaling pathway between the ECM and mitochondria. Interestingly, TMEM2 promotes the longevity and immunity of worms in a tissue-specific manner via inducing mitochondrial and oxidative stress responses. Therefore, this novel ECM-mitochondria pathway can be maneuvered to promote tissue homeostasis in a context-dependent manner.

Identification and characterization of functional non-coding variants associated with human longevity

Jiping Yang¹; Jih-Rong Lin²; Zhengdong Zhang²; Sofiya Milman²; Nir Barzilai²; Yousin Suh¹.

1. Department of Obstetrics and Gynecology, Columbia University Medical Center, New York, USA

2. Department of Genetics, Albert Einstein College of Medicine, Bronx, New York, USA
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Centenarians, despite representing only a tiny proportion of the world's population, hold the key to access longevity. By decoding their genomes, the majority of genetic variations were found in non-coding regions which were once considered "Junk DNA" but are now known to play crucial roles. Recent studies show that non-coding variants are significantly enriched in cis-regulatory elements (CREs), especially within enhancers specific to trait-relevant cell types. However, the functional roles of non-coding variants are difficult to predict due to incomplete knowledge of non-coding regulatory elements, their mechanisms of action, and the cellular states and processes in which they function, let alone the identification of truly causal variants and their target genes. To address this challenge, we employed CRISPR-based screens to discover longevity-associated variant-residing CREs capable of modulating cellular senescence *in vitro*. Leveraging the Cis-element Atlas (CATlas), we prioritized 594 rare non-coding variants identified in the Ashkenazi Jewish (AJ) centenarian cohort, mapping to potential CREs. Pooled activation or inhibition of these longevity-associated variant-residing CREs using CRE-targeting sgRNAs showed ameliorated cellular senescence compared to non-targeting sgRNAs. Further sgRNA enrichment analysis identified CREs with the potential to modulate cellular senescence. These CREs are being tested in single-cell CRISPR screens to identify causal genes and will further be characterized in human stem cell model and mouse model to elucidate their role in human longevity.

Targeting $p21^{Cip1}$ -highly-expressing Senescent Cells to Improve Healthspan in Progeroid Mice

Binsheng Wang^{1,2}, Lichao Wang^{1,2}, Nathan S. Gasek^{1,2}, Taewan Kim^{1,2,3}, Chun Guo¹, Evan R. Jellison⁴,
Laura Haynes^{1,4}, George A. Kuchel¹, Ming Xu^{1,2}

¹UConn Center on Aging, ²Department of Genetics and Genome Sciences, ³Biomedical Science Graduate Program, ⁴Department of Immunology, UConn Health, Farmington, CT

Abstract

Aging is the leading risk factor for a variety of chronic diseases. Emerging evidence suggests that modulating fundamental aging processes such as cellular senescence may delay the onset of most chronic conditions as a group and increase healthy lifespan. Cellular senescence refers to the essentially irreversible growth arrest that occurs when cells experience stress. One common feature of senescent cells is the high expression level of $p16^{Ink4a}$ and/or $p21^{Cip1}$. We and others have demonstrated the causal roles of $p16^{Ink4a}$ -highly-expressing ($p16^{high}$) senescent cells in various age-related conditions. However, the role of $p21^{high}$ senescent cells in aging remains largely unknown. To address these questions, we have generated and validated a novel $p21$ -Cre mouse model containing $p21$ promoter driving inducible Cre. By crossing with floxed mice, we managed to monitor, sort, image, eliminate, or modulate $p21^{high}$ cells *in vivo*. Using our model, we showed that $p21^{high}$ cells accumulate in various tissues in klotho (kl)-/- progeroid mice, and clearance of $p21^{high}$ cells can improve physical function in kl -/- mice. Additionally, inactivation of NF- κ B specifically in $p21^{high}$ cells also can improve tissue function in kl -/- mice. This study will provide invaluable research tools and pinpoint the role of cellular senescence in age-related tissue dysfunction.

Title: Understand the metabolic mechanism of age-associated decline in antiviral T cell immunity.

Tuoqi Wu, PhD
University of Texas Southwestern Medical Center

Abstract: Immunosenescence, the gradual decline of immunity with age, leads to increased infection-related morbidity and mortality. T cells are essential to antiviral immunity; and yet T cells are among the immune cells most negatively impacted by aging. Understanding the molecular mechanism of T cell immunosenescence is required for developing strategies to improve antiviral immunity in the elderly. Using murine hepatitis virus (MHV) infection in mice as a model, we found that aging increased mortality and impaired antiviral T cell responses after MHV infection. Before infection, there was a higher frequency of PD1⁺ exhausted-like T cells in aged mice than in young mice. Upon activation, T cells from aged mice were more prone to TCR induced cell death compared to those from young mice. The defective activation of aged T cells was rescued through shortening duration of TCR stimulation by culturing activated aged CD8 T cells with exogenous IL2 without TCR stimuli. Using single-cell metabolism, we showed that aging lowered the baseline metabolic rate of T cells and impaired the metabolic adaptation to TCR stimulation. In aged mice, exhausted-like PD1⁺ T cells exhibited a greater defect in metabolism than PD1⁻ T cells. Ongoing work in the lab focuses on how aging alters metabolism of virus-specific CD8 T cells before and after infection and whether enhancing IL2 signaling improves metabolic fitness and immune response of virus-specific CD8 T cells in the elderly.

NAD⁺ metabolism and germ-somatic cell interactions in female aging**Lindsay Wu, University of New South Wales**

We previously showed that declining levels of the redox cofactor nicotinamide adenine dinucleotide (NAD⁺) are a reversible cause of declining oocyte quality and female infertility during reproductive ageing, and that NAD⁺ repletion using metabolic precursors could restore functional fertility with age (Bertoldo et al *Cell Reports* 2020). We are currently translating this through an ongoing clinical trial of nicotinamide riboside (NR) treatment in older IVF patients. Here, we show that this strategy can ameliorate accelerated ovarian ageing due to clinically relevant chemotherapy treatment, with impacts on late-life health related to ovarian function including protection against a striking loss of bone integrity (Ho et al, under review at *EMBO Mol. Med*). The clinical development of these NAD⁺ precursors is complicated by PK/PD studies being confounded by background levels of naturally occurring metabolites, where treatment with the amidated precursors NR and NMN leads to sharp spikes in the deamidated precursors NaMN and NaAD. To address this, we have developed stable isotope tracing strategies to identify unexpected aspects of NAD⁺ metabolism, involving contributions from the gut microbiome (Kim LE et al *FEBS* 2023), and base exchange activity from the enzyme CD38 (under preparation). These stable isotope tracing experiments are being extended to human studies, where we present data for measures of NAD⁺ turnover. In addition, we have identified an unexpected role for somatic-germline interactions in NAD⁺ biosynthesis, involving metabolic exchange between the oocyte and somatic nurse cells called cumulus cells.

Finally, we present our new concept of “heterochronic cumulus oocyte complexes” as a new experimental paradigm that acts as a clinically tractable version of the classic heterochronic parabiosis model for studying somatic-germline interactions. In this model, we find that re-aggregating aged oocytes with cumulus cells from young females can rejuvenate oocyte quality. This suggests that biological ageing of mitotically active somatic cells, rather than chronological ageing of the non-renewable and quiescent oocyte reserve, may be the greater determinant of reproductive ageing.

Clearance of *p21*-highly-expressing cells extends lifespan and confers long-term benefits to health and physical function.

Binsheng Wang^{1,2}, Lichao Wang^{1,2}, Nathan S. Gasek^{1,2}, Chia-Ling Kuo^{1,3}, Taewan Kim^{1,2}, Pengyi Yan^{1,2}, Junyu Zhu^{1,2}, Chun Guo¹, Rachel L. Cohn^{1,2}, George A. Kuchel¹, Ming Xu^{1,2}

¹UConn Center on Aging, ²Department of Genetics and Genome Sciences, ³Department of Public Health Sciences, UConn Health; Farmington, CT, 06030, USA

Key words: cellular senescence, morbidity compression, inflammation, frailty, aging

Abstract

A key challenge in aging research is to extend lifespan in tandem with slowing down functional decline so that the life with good health (healthspan) can be extended. Here, we show that clearance of a small number of cells, which highly express *p21*^{Cip1} (*p21*^{high}), starting from 20 months improves cardiac and metabolic function, and extends both median and maximum lifespan in mice. Importantly, by assessing health and physical function of these mice monthly until death, we show that clearance of *p21*^{high} cells improves physical function at all remaining stages of life, suggesting morbidity compression and healthspan extension. Mechanistically, *p21*^{high} cells encompass several cell types, with a conserved pro-inflammatory signature. Clearance of *p21*^{high} cells reduces inflammation, and rejuvenates transcriptomic signatures of various tissues to younger states. These findings demonstrate the feasibility of morbidity compression in mice, and indicate *p21*^{high} cells as a therapeutic target for healthy aging.

Title: Investigating age- and species-based molecular differences in synaptic organelles to understand synaptic dysfunction in aging and neurodegenerative disease.

Sarayut Phasuk¹ and Jason D. Vevea^{1*}

¹Neuronal Cell Biology Division, Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA

***Corresponding author: jason.vevea@stjude.org**

The breakdown of synaptic architecture is seen in normal aging and neurodegenerative disease. Investigation into the molecular nature of these changes is largely limited to specific targeted inquiry using immunoblots and immunocytochemistry. Investigation using discovery methods like mass spectrometry-based approaches have limited success due to low sample enrichment and slow preparation times. Additionally, published investigations of age-related changes in biology are plagued by using too few age groups, a lack of species-based comparisons, and low power. This project will investigate the normal age-, sex-, and gene-related molecular changes of synaptic vesicles (SVs) from brains of mice. Synaptic vesicles will be isolated using a newly developed rapid immunoprecipitation method (Bradberry et al., 2022), which is 10x faster and results in 10x higher enrichment of sample relative to classic protocols.

We hypothesize that age-dependent changes in the neurochemical makeup of synapses underlie synaptic dysfunction and cognitive decline in aging, and an understanding of these changes will guide research into age-related neurodegenerative disorders. To begin to test this hypothesis, SVs will be isolated from whole mouse brain in an age-, sex-, and gene-dependent manner using a newly developed rapid immunoprecipitation (SV-RIP) method. These highly enriched samples will be analyzed using mass spectrometry-based approaches to identify their proteome and lipidome, then compared to identify differences. Our goal is to use male, female, and a chemically induced menopause model using VCD and C57BL/6J mice. Importantly, we plan to use rodents of 3, 6, 12, 18, 24, and 30 months of age. Our initial experiments using wild-type mice will inform age and sex selection for a follow-up study using humanized APOE transgenic replacement mice, available from JAX laboratory. These objectives will reveal age-, sex-, and gene-related changes in the synapse during natural aging, leading to a greater understanding of age-related dementias, such as Alzheimer's Disease.

Gap-in-Knowledge

Our research will advance understanding of age-related neurodegenerative diseases like Alzheimer's (AD) and related dementias (ADRD) by addressing gaps in species-based molecular differences of the neuronal synapse. Similar studies don't adequately consider age groups or species differences, leading to knowledge gaps. Examining age-, sex-, and gene-related molecular changes in synaptic vesicles (SVs) from mouse brains will provide insights into factors influencing the onset of age-related cognitive disorders like AD/ADRD.

The new tools and techniques described in this proposal, such as the faster method for isolating SVs and PSDs, will benefit mouse model research and be applicable to human studies, facilitating a broader understanding of sex, gender, disability, race, and ethnicity roles in AD/ADRD pathology, paving the way for inclusive research and targeted interventions.

Title: Intravital characterization of mitochondrial dysfunction in the aged brain endothelium

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Abstract: While the brain only accounts for 2% of the body mass, it consumes 20-25% of the body's total energy requirements. Since brain energy stores are scarce, the brain must rely on the circulation for continuous supply of nutrients as well as oxygen. Energy demand of the brain varies both spatially and temporally with changes in neuronal activity, which require prompt cerebral blood flow (CBF) adjustments in a highly regulated fashion to maintain cellular homeostasis and function. This is accomplished through a process termed neurovascular coupling (NVC), which is orchestrated by an intercellular signaling network comprised of signals from the activated neurons, that induce the release of vasoactive mediators (NO) from the microvascular endothelium. It is now recognized that endothelial cells play a prominent role in signaling cellular responses to environmental cues. An important mode of signaling is the regulated production of mitochondria reactive oxygen species (mtROS). In addition to the age-related impairment of NVC responses and increased mitochondrial dysfunction, disruption of endothelial cells allows entry of unwanted solutes into brain, by damaging the blood-brain barrier (BBB), the highly specialized vascular interface that maintains homeostasis in brain by separating the blood compartment from the central nervous system. Despite this knowledge, how endothelial cell bioenergetics, endothelial mitochondrial dysfunction, and alterations of mitochondrial and glycolytic energy pathways could impact brain homeostasis in aging are not well understood. The long-term goal of this project is therefore to establish a general conceptual framework to ultimately reprogram ECs' bioenergetics and thus improve tissue homeostasis in aging. Our overarching hypothesis is that age-related decrease of glycolysis and mitochondrial dysfunction in brain endothelial cells determine function of the neuro-glial-vascular unit, barrier integrity and angiogenic capacity and contributes to neuronal degeneration. This project will make use of sophisticated novel fluorescent biosensors to allow investigation of glycolysis, oxidative phosphorylation and mitochondrial function in vivo with high spatial and temporal resolution. Metabolic flux analysis & high resolution respirometry will be performed to complete the characterization of bioenergetic changes in aged cerebrovasculature. Mitochondrial and vascular changes will be monitored intra-vitally using two-photon microscopy and ultrafast ultrasound in relevant mice models (mt-Keima, mito-dendra2).

Molecular determinants of memory T cell fitness in older adults

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Generation of a protective and long-lasting immune memory is central to effective vaccination strategies and healthy aging. However, age-associated changes in immune cells, particularly T cells, impair immune memory generation and maintenance rendering older adults vulnerable to infectious diseases.

Here, we investigated signatures of age-related memory T cell dysfunction and uncovered molecular determinants of a protective, persistent T cell memory in older individuals. We leveraged the contrasting T cell memory elicited in younger (<30 years) and older adults (>55 years) by vaccination against varicella zoster virus (VZV). Using *ex vivo* peptide stimulation of peripheral blood mononuclear cells of vaccine recipients several years after vaccination, we collected antigen-specific CD4⁺ and CD8⁺ T cells for high-dimensional spectral flow cytometry and tri-modal single-cell sequencing analyses. We found that older vaccine recipients predominantly suffer from a quantitatively and qualitatively impaired CD8⁺ T cell memory response compared to younger adults receiving the same vaccine type. Conversely, comparisons of two VZV vaccine strategies with contrasting efficacies in older adults demonstrated that a CD4⁺ T cell response rich in self-renewing Th17-like and low in regulatory T cells overcomes the age-associated T cell memory defect and associates with a durable, protective T cell memory.

Collectively, we propose that vaccine strategies targeted towards older adults benefit from a Th17-focused CD4⁺ T cell memory response. Our results not only uncover traits of immune cell dysfunction with aging, but also inform vaccination efforts tailored towards healthy aging.

Single-cell RNA sequencing identifies distinct VAT-resident $\gamma\delta$ T cell subsets with age-dependent shifts

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Adipose tissue dysfunction, such as deleterious changes in cellularity, secretory profile, inflammatory state, and insulin responsiveness, contributes to chronic low-grade systemic inflammation which is linked to the development of numerous age-associated pathologies. Recently, we demonstrated that gamma delta ($\gamma\delta$) T cells expand in visceral adipose tissue (VAT) over the lifespan and contribute to age-related inflammation and metabolic dysfunction. We further determined that VAT $\gamma\delta$ T cells are tissue-resident with minimal recruitment from the periphery and that protection from apoptosis beginning at middle-age appears to be a major driver of age-related accumulation. However, $\gamma\delta$ T cells encompass a group of cells with many functionally distinct subtypes, and age-related changes among these subtypes has not been studied. Here, we employed a single-cell RNAseq approach coupled with feature barcoding and V(D)J profiling to identify age-dependent shifts in $\gamma\delta$ T cell subtypes. Our results indicate eight distinct clusters of $\gamma\delta$ T cells in VAT. Among them, one cluster (C6) was distinctly $\gamma\delta$ T1-type cells characterized by expression of CD27 and IFN γ . The other seven clusters shared features of typical $\gamma\delta$ T17-type cells including expression of Blk, Maf, Sox13, Rorc, Cxcr6, and Zbtb16; however, IL-17 was only expressed in 2 clusters (C4 and C1). After defining clusters using data from all samples, the differences in abundances of these cell subsets across age was analyzed. We found that cells in C4 and C6 showed an age-associated decrease in frequency while cells in C0 and C7 showed an age-associated increase in frequency. C7 is distinguished by expression of Stat1, Ifi47, Igtp, and Tgtp1, yet shared many similarities with C4; thus, we propose that a phenotype shift from C4 to C7 occurs with age. In addition, several genes showed age-associated changes in expression, including Igf1, Fth, Zfp361l1, Ckb, Cxcr6, Il7r, and Bcl2a1b which were upregulated and Rplp0, Rps26, Ctla4, and As4a4b which were downregulated. Pathway analyses indicated upregulation of pathways related to stress response, cell differentiation, proliferation, and $\gamma\delta$ T cell activation. Downregulated pathways related to apoptosis, TNF signaling, and protein metabolic process. Collectively, these data highlight distinct phenotypic differences, not only between different $\gamma\delta$ T cell subsets, but within subsets across age. A better understanding of the VAT $\gamma\delta$ T cell pool is critical to further defining their role in chronic age-associated inflammation and metabolic dysfunction.

Ether Lipid Biosynthesis Promotes Lifespan Extension and Enables Diverse Prolongevity Paradigms in *Caenorhabditis elegans*

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Abstract

Biguanides, including the world's most prescribed drug for type 2 diabetes, metformin, not only lower blood sugar, but also promote longevity in preclinical models. Epidemiologic studies in humans parallel these findings, indicating favorable effects of metformin on longevity and on reducing the incidence and morbidity associated with aging-related diseases. Despite this promise, the full spectrum of molecular effectors responsible for these health benefits remains elusive. Through unbiased screening in *C. elegans*, we uncovered a role for genes necessary for ether lipid biosynthesis in the favorable effects of biguanides. We demonstrate that biguanides prompt lifespan extension by stimulating ether lipid biogenesis. Loss of the ether lipid biosynthetic machinery also mitigates lifespan extension attributable to dietary restriction, target of rapamycin (TOR) inhibition, and mitochondrial electron transport chain inhibition. A possible mechanistic explanation for this finding is that ether lipids are required for activation of longevity-promoting, metabolic stress defenses downstream of the conserved transcription factor *skn-1/Nrf*. In alignment with these findings, overexpression of a single, key, ether lipid biosynthetic enzyme, *fard-1/FAR1*, is sufficient to promote lifespan extension. These findings illuminate the ether lipid biosynthetic machinery as a novel therapeutic target to promote healthy aging.

Determining the role of Dosage Compensation Complex in regulation of sex-specific aging of brain

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Sexual dimorphism has been described in aging and several human diseases, including neurodegeneration, though the fundamental mechanisms remain poorly understood. The aging brain exhibits progressive decline in plasticity, manifesting striking impairments in cognitive abilities. Dosage compensation (DC) regulates sex-specific gene expression, and key genes for neuronal plasticity and cognitive process are located on the X chromosome. Hence, investigating regulatory epigenetic mechanisms that enhance gene-based differences between males and females through single-cell gene expression, chromatin accessibility, and epigenetic features coupled with the remarkable forward genetics tools in the *Drosophila* brain holds the promise of providing profound new understanding of normal aging and pathological aging in neurodegenerative diseases. To define how brain aging is regulated in a sex-specific manner, I am addressing two key questions: 1) What is the mechanism by which DC regulates sex-specific aging in the brain? 2) What sex-specific gene regulatory networks (GRNs) and synaptic genes change during brain aging and how are they related? Our single cell data analysis during aging shows neuronal cells show higher differentially expressed genes on X chromosome than autosomes in males and females. To determine how DC regulates brain aging, my project is focusing on identifying all age- and sex-dependent DC components and defining how synaptic genes are regulated by DC components using genomics, imaging, and behavioral approaches. This study will provide us key insights on defining how genetic and epigenetic networks interact in different sex to drive aging and better understanding of the role of DC in brain aging.

Title: Single Nucleus RNA Sequencing (snRNAseq) of Mouse Brown Adipose Tissue (BAT) Identifies Anti-Involution Pathways

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Abstract: Aging, chronic western diet feeding, and housing at thermoneutrality have been shown to induce involution of brown adipose tissue (BAT), a process characterized by a reduction in BAT mass and thermogenic function alongside an increase in lipid droplet size. Using single-nuclei RNA sequencing (snRNAseq) of BAT from aged mice, our study identifies a distinct population of brown adipocytes marked by a reduction in *Ucp1* expression and increased pyroptotic signaling, associated with decreased expression of the longevity-associated protein, syntaxin 4 (STX4). These adipocytes exhibit characteristics indicative of an impaired metabolic process and heightened inflammatory response.

The technological core of our research leveraged high-throughput snRNAseq to parse the cellular heterogeneity within BAT including mature adipocytes, enabling the identification of age-related phenotypic shifts in adipocyte subpopulations. Further, through CRISPR-mediated gene editing, we generated *Ucp1*-STX4 knockout mice to model BAT involution mechanistically. These models provided crucial insights, as they replicated the age-induced phenotypic changes and allowed us to explore the therapeutic potential of STX4 restoration.

Functionally, STX4-deficient brown adipocytes showed decreased oxidative phosphorylation, glucose uptake, and glycolysis, leading to reduced ATP levels, a known trigger for pyroptosis activation. We demonstrated that restoration of STX4 expression or pharmacological inhibition of pyroptosis effectively mitigates these declines, preserving BAT mass and function. Mechanistically, this involved the attenuation of Caspase1/11 activation, which are central players in the pyroptosis pathway.

Our findings elucidate a novel mechanism of BAT involution with aging, highlighting a potential therapeutic target in STX4 and the pyroptosis pathway. This could pave the way for developing interventions that not only prevent the loss of BAT mass and function with age but also improve metabolic health in the elderly population.

Title: Metformin inhibits mitochondrial complex I in gut epithelium

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Metformin is the first-line treatment for type II diabetes, but its use is associated with positive health outcomes beyond glycemic control such as reductions in chronic inflammation and cancer incidence. Consistent with these observations, metformin extends healthspan and lifespan in multiple model organisms. Thus, metformin is a promising candidate to delay a broad spectrum of age-related diseases. Despite its therapeutic utility, the mechanism of action of metformin is unclear. In vitro data strongly suggest that mitochondrial complex I is a target of metformin. However, experimental tools to test this in vivo have been lacking. In this study, we leverage the tissue-specific misexpression of yeast NADH dehydrogenase (NDI1) to rescue the suppression of mitochondrial complex I activity. NDI1 recapitulates two of the three main functions of complex I: electron donation and NAD⁺ regeneration (but not proton pumping). Because the biodistribution of metformin is gut-selective, we misexpressed NDI1 in intestinal epithelial cells of mice. Gut NDI1 expression incurred resistance to metformin's glucose-lowering effect, indicating gut mitochondrial complex I inhibition is necessary for the primary therapeutic effect of the drug. Thus, modulating intestinal mitochondrial function can elicit systemic metabolic benefits.

Exploring ribosomal RNA modifications in cellular and organismal aging by Nanopore direct RNA sequencing

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Aging is associated with multiple cellular and physiological changes, including the loss of proteostasis, altered composition of ribosomes, and, consequently, dysregulated translational regulation. Considering the importance of ribosomal RNA (rRNA) modifications for ribosome functionality and the evidence for an adapting translome, rRNA modifications might also change during biological aging. While single rRNA modifications have already been associated with aging phenotypes, global rRNA modification patterns have not yet been studied more comprehensively in this context.

To investigate whether rRNA modifications change throughout organismal aging and cellular senescence, we performed Oxford Nanopore direct RNA sequencing of rRNA in several aging models. Direct RNA sequencing allows the simultaneous detection of different types of RNA modifications and the identification of concerted modification changes at single molecule resolution. First, we induced cellular senescence in normal human epidermal keratinocytes and melanocytes. Direct RNA sequencing revealed that several 2'-O-methylation (2'-O-m) and pseudouridine (Ψ) positions showed altered modification levels in 18S and 28S rRNA. Affected modification sites partly overlapped between the two cell types, and generally, modification levels were higher in senescent than in proliferating cells. Second, we used *Caenorhabditis elegans* to study changes in rRNA modifications throughout an organism's lifespan.

To conclude, we identified several 2'-O-m and Ψ sites of rRNA that show different modification levels between proliferating and senescent primary human skin cells. Additionally, we established *C. elegans* as a model to monitor rRNA modification changes throughout an organism's lifespan.

We aim to correlate those changes in rRNA modifications with translation dynamics and cell physiology. This will contribute to a better understanding of the rRNA epitranscriptome in organismal aging, cellular senescence, and age-associated diseases.

Title: Distinct mechanisms of non-autonomous UPR^{ER} mediated by GABAergic, glutamatergic, and octopaminergic neurons.

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Keywords: Aging, stress, ER, neurons.

Abstract

The capacity to deal with stress declines during the aging process, and preservation of cellular stress responses is critical to healthy aging. The unfolded protein response of the endoplasmic reticulum (UPR^{ER}) is one such conserved mechanism which is critical for the maintenance of several major functions of the ER during stress, including protein folding and lipid metabolism. Hyperactivation of the UPR^{ER} by overexpression of the major transcription factor, *xbp-1s*, solely in neurons drives lifespan extension as neurons send a neurotransmitter-based signal to other tissue to activate UPR^{ER} in a non-autonomous fashion. Previous work identified serotonergic and dopaminergic neurons in this signaling paradigm. To further expand our understanding of the neural circuitry that underlies the non-autonomous signaling of ER stress, we activated UPR^{ER} solely in glutamatergic, octopaminergic, and GABAergic neurons in *C. elegans* and paired whole-body transcriptomic analysis with functional assays. We found that UPR^{ER}-induced signals from glutamatergic neurons increased expression of canonical protein homeostasis pathways and octopaminergic neurons promoted pathogen response pathways, while minor, but statistically significant changes were observed in lipid metabolism-related genes with GABAergic UPR^{ER} activation. These findings provide further evidence for the distinct role neuronal subtypes play in driving the diverse response to ER stress.

Chromatin Structural Landscape of Cardiac Aging

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Aging is a major contributor to the development of cardiac disease and is set to become even more critical as human advancements lead to increased life expectancy. Our recent research has shed new light on the impact of aging on heart health. Through phenotypic studies using embryonic (E18.5), weaning (p0, p3, p7), juvenile (p21), adult (8 weeks old) and aged (1 year and 2 year old) mouse hearts, we confirmed progressive deterioration of heart function, including ventricle dilation, increased fibrosis, and decline in heart performance. Similar effects were observed after induction of cardiac disease by transverse aortic constriction and cardiomyocyte-specific depletion of the chromatin structural factor CTCF. RNA-seq experiments revealed a significant difference between young and aged mice, with the latter resembling the transcriptional landscape observed in animals with induced heart failure. Our study also discovered that aged mice undergo a return to the fetal gene program, akin to what is observed in pathological conditions, suggesting that aging is associated with a reorganization of genome structure, as evidenced by downregulation of key chromatin architectural factors (e.g., CTCF and Cohesins). Our spatiotemporal Hi-C experiments confirmed this hypothesis and additionally showed that topological organization of the genome is similarly organized in embryos, the elderly, and those with heart disease. To deeply explore the intricate relationship between chromatin structure and the repercussions of cardiac senescence, we conducted an integrated analysis of our RNA-seq and Hi-C data. These experiments pinpointed specific 3D structural elements that govern the transcriptional reprogramming linked to aging. To assess the potential of restructuring higher-order chromatin organization in mitigating the effects of cardiac aging, we will employ a mouse with cardiomyocyte-specific Cas9 expression (CMCas9). In this model, we will disrupt chromatin regions strongly associated with gene expression and study the resulting phenotypic changes by echocardiography. Our work revealed how high-order chromatin organization influences heart function as it naturally deteriorates and investigated whether direct genomic structure manipulation can ameliorate the aging-related impacts on cardiac health.

Heme toxicity in the aged spleen impairs T-cell immunity through iron deprivation

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Aging compromises T cell immunity, impacting vaccine responses and infection susceptibility. We demonstrate differential T cell aging trajectories within one host, determined by their metabolic microenvironment; spleen-derived lymphocytes are functionally inferior to lymphocytes derived from lymph nodes. Proteomic analysis reveals overexpression of heme detoxification and iron storage proteins in aged spleen-derived lymphocytes, mirroring accumulation of heme and iron depositions in this tissue. Exposure to the aged spleen microenvironment or to heme itself induces aging phenotypes in young lymphocytes, including reduced proliferation and upregulation of the ectonucleotidases CD39 and CD73. Mechanistically, T cells survive the aged spleen's microenvironment by maintaining low labile iron pools, to resist ferroptosis. Remarkably, timed iron supplementation rescues aged T cells proliferation and vaccination response. Thus, T cells survive the aged spleen's hostile milieu at the expense of critical functions. Understanding these mechanisms may inform strategies to enhance immune responses in the elderly.

Transcriptional regulation of 15-PGDH, a novel gerozyme

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BACKGROUND. As we age, we gradually lose muscle mass and strength, which in its extreme form is clinically categorized as sarcopenia. Progression of sarcopenia is marked by a loss of strength, mobility, independence, and ultimately increases mortality. There are currently no drugs to treat sarcopenia.

Recently, we identified a protein called 15PGDH that directly contributes to muscle atrophy in aging. Functionally, 15PGDH catabolizes a regenerative lipid signaling molecule called Prostaglandin E2 (PGE2) which promotes skeletal muscle regeneration. In aged muscle, we find an accumulation of 15PGDH and reduced abundance of PGE2. Giving aged mice a pharmacologic inhibitor of 15PGDH increases strength, muscle mass, and running endurance, allowing them to perform similarly to healthy, younger mice [2]. Similarly, transgene overexpression of 15PGDH in healthy young mice causes a loss of muscle mass, strength, and endurance, resembling aged muscle. We propose that 15PGDH is an enzyme that directly underlies the biological mechanisms of sarcopenia.

AIMS. While we have shown that 15PGDH activity can be pharmacologically suppressed to restore function of aged skeletal muscle, it would be beneficial to prevent its initial overexpression in aging. The goal of my research is to identify the specific molecular events that drives the overexpression of 15PGDH in aging.

METHODS. I am using a series of single-nuclei multiomics analysis and neural networks to identify transcription factors that drive the age-related upregulation of 15PGDH expression. I am validating upstream regulators and signaling cascades that drive an upregulation of 15-PGDH using a series of *in vitro* assays.

RESULTS. I have identified a cis-regulatory enhancer region that lies upstream of the 15PGDH promoter. This enhancer region is only accessible during muscle atrophy, which suggests it immediately precedes an upregulation of 15PGDH. Further analysis shows this enhancer region harbors critical Runx1 and AP-1 binding motifs, which are the likely activators of the 15PGDH enhancer region. I am currently investigating upstream signaling pathways that activate this enhancer region.

Methionine restriction started late in life promotes healthy aging

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Metabolic reprogramming represents one of the major driving forces in aging. Methionine metabolism is affected by aging and restricting methionine consumption extends lifespan across different species. Similarly, the levels of enzymes in the tyrosine degradation pathway (TDP) increase with age and targeting enzymes in the tyrosine degradation pathway extends *Drosophila* lifespan. However, we have limited information on the effects of methionine restriction (MetR) or inhibition of the TDP on age-related deterioration when it is started late in life. To investigate how targeting either methionine metabolism or the TDP affects age-dependent changes in metabolic health, motor function, cumulative frailty, epigenetics clocks, and other healthspan parameters, we applied either dietary MetR or an FDA-approved inhibitor of the TDP, nitisinone, to 18-mo old male and female C57Bl/6J mice for 6 months. We also tested whether dietary MetR may improve clinical manifestations associated with Alzheimer's disease (AD) by applying dietary MetR for six months to 5XFAD mouse model. We have observed multiple healthspan benefits associated with dietary MetR started late in life. Using single-nucleus RNA and ATAC sequencing on the muscle tissues, we have determined potential mechanisms associated with the beneficial effects of dietary MetR. Nitisinone did not elicit any beneficial effects despite significantly upregulated plasma tyrosine levels. Surprisingly, dietary MetR did not significantly affect the epigenetic clocks in either mice or humans. Although it is difficult to translate MetR into a human clinical trial, the development of MetR mimetics holds great promise as an anti-aging intervention in humans.

**The influence of body mass index on biomarkers of cellular senescence
in older adults**

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Obesity accelerates the onset and progression of age-related conditions. In preclinical models, obesity drives cellular senescence, a cell fate that compromises tissue health and function, in part through a robust and diverse secretome. In humans, components of the secretome have been used as senescence biomarkers that are predictive of age-related disease, disability, and mortality. Using biospecimens and clinical data from two large and independent cohorts of older adults, we tested the hypothesis that the circulating concentrations of senescence biomarkers are influenced by body mass index (BMI). After adjusting for age, sex, and race, we observed significant increases in activin A, Fas, MDC, PAI1, PARC, TNFR1, and VEGFA, and a significant decrease in RAGE, from normal weight, to overweight, to obesity BMI categories by linear regression in both cohorts (all $p < 0.05$). These results demonstrate that obesity exacerbates the biology of aging and highlight the influence of BMI on circulating concentrations of senescence biomarkers.

Ceramide accumulation: A novel ~~hallmark~~ driver of Aging?

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Aging is the single greatest risk factor for all major chronic diseases, and thus, understanding the biological mechanisms that modulate aging is critical for the development of health-maximizing interventions. Lipids are small hydrophobic molecules that not only serve as fundamental cellular components, but also act as important cellular signaling molecules, with well-established roles in nutrition, health, and disease. The first longevity gene discovered in yeast, i.e. the longevity-assurance gene (LAG1), encodes an enzyme that synthesizes ceramides, a central class of sphingolipids that has been associated with insulin resistance, apoptosis, mitochondrial dysfunction, senescence, inflammation, sarcopenia, frailty, among other age-related pathways and phenotypes. Using a number of animal models and methodologies, we have amassed a significant amount of data that place ceramides as a novel major driver of aging. Specifically, we found that: (1) ceramides consistently accumulate with age in circulation and in multiple organs in multiple mouse strains and marmosets, as well as in affected organs of age-related diseases including diabetes and Alzheimer's; (2) circulating ceramides are dramatically reduced in long-lived isolated growth hormone deficient mice; (3) hepatic ceramides are substantially reduced in old marmosets treated with rapamycin, a well-established anti-aging drug; (4) low doses of myriocin, a potent inhibitor of ceramide synthesis, improves healthspan (i.e. glucose and insulin tolerance and grip strength) in WT mice (C57BL/6J and Balb/c genetic backgrounds) fed with Western diet. In addition, we are currently testing the effects of myriocin on marmoset healthspan and on mouse lifespan using inbred and heterogenous mice. Taken together, our results place ceramide accumulation as a novel major driver of aging.

The Role of Habitual Feeding Schedule in Mediating the Metabolic Response of a Calorie Restriction Diet**Heidi Pak¹, Joseph Takahashi¹**

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Abstract

Calorie restriction (CR) is recognized as the most effective non-pharmacological intervention that promotes health and extends lifespan across species. The majority of the research done in the aging field has focused its efforts on the effects produced from reducing calories. However, recent studies have highlighted the importance of a prolonged fasting period to gain the full metabolic health benefits of CR – rodents placed on a CR feeding regimen consume their daily food allotment within a period of ~2 hrs and fast for the remainder of the day. This feeding pattern exposes another overlooked feature of a CR feeding regimen: in a laboratory setting, CR groups adhere to a strict, habitual feeding schedule. This consistent routine could potentially entrain the metabolism to develop a nutrient anticipatory memory by circadian synchronization of the peripheral tissues. Therefore, the overall contribution of the feeding schedule of a CR regimen may have an important effect on metabolic health and longevity.

Here, we test the hypothesis that the physiological and metabolic response to CR is produced in part by a habitual eating pattern utilizing three experiment groups: AL (ad libitum), CR (30% restricted, fed once a day at the start of the dark cycle ZT 12) and CR-Sporadic “CR-S” (30% restricted, fed once a day at alternating times). Notably, we find that while energy expenditure is highly circadian regulated, respiratory exchange ratio (RER) followed the pattern of food intake. Furthermore, the irregular feeding pattern of the CR-S group disrupted the classical preference for fuel source, where the CR group has an RER value of ~1 during feeding which drops to a value of 0.7 during the fast. In contrast, the CR-S group consistently falls short of reaching 0.7, indicating inefficient fuel source utilization. These results suggest that the habitual feeding schedule is required for the metabolism to efficiently switch between fuel sources based on the feeding pattern. Therefore, beyond mere caloric intake, the temporal intervals between food intake is as important as the nutritional value in the regulation of health and lifespan.

DL-Buthioninesulfoximine induces lean phenotype in male diet-induced obese mice.

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Our study aims to identify pharmaceutical compounds that induce a lean phenotype based on the mechanisms of well-established antiobesity dietary interventions. Decreasing the dietary concentration of Met in the absence of Cys (SAAR, sulfur amino acid restriction) exerts robust antiadiposity effects in rodents. We previously demonstrated that the SAAR-induced antiadiposity effects were associated with low hepatic Cys levels and its metabolite glutathione (GSH) but not Met levels. Since it is challenging to formulate palatable human diets without Cys, we investigated whether pharmaceutical lowering of hepatic Cys and GSH concentrations would induce antiadiposity effects.

Five groups of C57BL6/NTac mice were treated with a combination of a high-fat (60% Kcal) diet and drugs to modulate the biological availability of Cys and GSH. The first two groups, control diet (CD) and SAAR, were *ad libitum* fed diets with 0.86% w/w and 0.12% w/w Met levels, respectively; both diets were devoid of Cys. To investigate if Cys supplementation reverses the SAAR-induced antiadiposity effects, a third group (NAC) was maintained on the SAAR diet and offered *ad libitum* water with N-acetylcysteine, a precursor of Cys and GSH. To investigate if Cys or GSH depletion results in antiadiposity effects, the last two groups were maintained on the CD diet and offered 2-mercaptoethane sulfonate sodium (Mesna) or DL-buthionine (S, R) sulfoximine (BSO). Mesna decreases the biological availability of Cys by increasing its urinary excretion, while BSO decreases GSH concentration by inhibiting its biosynthesis.

Compared to the CD mice, SAAR mice had lower concentrations of hepatic Cys, hepatic GSH, lower body fat, and lower body weight. This SAAR-induced phenotype was reversed in the NAC group, i.e., all parameters were similar to those in the CD group. Despite being on the control diet, BSO mice had lower levels of hepatic GSH, hepatic Cys, lower body fat, and body weight. Contrary to our expectations, hepatic Cys and GSH concentrations in Mesna mice were similar to those in CD mice. No differences between Mesna and CD were observed in body weight or body fat composition. Overall, preliminary data indicate that lower biological availability of Cys and GSH are associated with the SAAR-induced lean phenotype in mice and that BSO, due to its ability to lower GSH concentrations, might be a potential antiobesity candidate. Investigations on whether BSO induces the lean phenotype through the same molecular mechanisms induced by the SAAR diet are underway.

Lower mitochondrial genome turnover and greater mutation frequency in skeletal muscle of aged compared to adult OKC-HET rats

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Mitochondrial genomic integrity, which is supported by processes such as replication, repair, and turnover, is critical in supporting metabolism and organismal health. Accumulation of mitochondrial DNA (mtDNA) mutations contributes to mitochondrial and whole-tissue dysfunction, which lead to chronic diseases including sarcopenia. The mechanisms that promote generation and age-related accumulation of mtDNA mutations are unclear. mtDNA mutants may have a replicative advantage that propagates the mutation burden. However, there is limited *in vivo* data regarding effects of age on mtDNA replication. We hypothesized mtDNA synthesis rates would increase with age as mutation burden increased in skeletal muscle in both female and male rats.

We measured mtDNA synthesis over 14 days using the stable isotope tracer deuterium oxide and assessed mtDNA copy number and mutation deletion frequency using digital PCR in quadriceps muscle of 9- and 26 month-old (mo) male and female OKC-HET rats, which have heterogenous mtDNA backgrounds.

There were no differences in mtDNA copy number between sexes or ages. However, 26 month-old rats had lower rates of mtDNA synthesis compared to 9 month-old rats (9 mo: 0.509 ± 0.009 %/day, 26 month: 0.371 ± 0.048 %/day; $p=0.0024$) and greater mtDNA half-lives (9 mo: 132 ± 1.79 days, 26 month: 216 ± 25.63 days; $p=0.0009$). Concomitantly, 26 month-old rats had greater ($p=0.003$) mtDNA deletion mutation frequency ($2.023e-004 \pm 7.18e-005$) than 9 month-old rats ($9.310e-005 \pm 2.404e-005$). 26 month-old female rats ($1.309e-004 \pm 2.386e-005$) had a lower ($p=0.008$) mutation burden than male rats ($2.74e-004 \pm 4.484e-005$).

Contrary to our hypothesis, mtDNA synthesis declined with age as mutation burden increased in the quadriceps. Because mtDNA copy number was not different between ages as synthesis decreased, mtDNA turnover declined with age. Altogether, these results suggest lower mtDNA turnover rates contribute to age-related mtDNA mutation burden. Future studies should test how modulating mtDNA turnover affects mutation frequency.

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Effect of aging on dendritic cell heterogeneity and function

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Immune cell populations undergo tissue-specific transcriptional and functional adaptations in aged mice and humans. However, how aging alters antigen-presenting cell 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 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 decreased in old mice. We hypothesize that old tissue niches disturb cDC1 cell functional adaptation, resulting in populations with dysfunctional antigen cross-presentation and CD8 T-cell activation. To identify the mechanisms linking an old tissue microenvironment to the development and function of different subsets of dendritic cells, we sorted progenitors of dendritic cells from bone marrow and differentiated dendritic cell subsets from the spleen of young, middle-aged, and old male and female C57BL/6J mice. Currently, we are performing bulk RNA sequencing of these cell subsets and analyzing differentially expressed gene modules to identify transcriptional programs affected by age and sex. In the future, the results of this analysis will guide us to study how age-sensitive genes and pathways regulate antigen presentation and immunogenic responses in dendritic cells in vitro and in vivo. This project will identify, for the first time, aging-dependent signals and mechanisms altering cDC1 cell function critical for immunosurveillance of infected, damaged, and cancer cells.

The pathophysiological role of gut microbiota-derived acetate under aging

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Gut microbiota and its metabolic products directly affect host pathophysiology. Dysbiosis of the gut microbiota under aging is strongly associated with many clinical manifestations and aging-related illnesses (e.g., cardiovascular diseases, diabetes/obesity, and neurodegenerative diseases). Acetate is the primary fermentation product in the gut. Interestingly, gut-derived acetate has been found to drive insulin secretion in the brain and modulate host lipid metabolism. However, how the gut senses acetate and alters the gut-brain axis for hormonal and metabolic control is unknown. Interestingly, my previous finding suggest that acetate level is decreased in aged flies. As aging is an inevitable risk factor for insulin resistance, whether dysfunction of acetate-mediated gut-brain communication contributes to abnormal insulin signaling during aging is not clear. Fruit fly *Drosophila* is a well-established model for studying aging, metabolism, and inter-organ communications. We find that acetate significantly reduces lipid storage of adult flies, and this effect is through the production of the neuropeptide Allatostatin A (AstA) from the enteroendocrine cells (EEs) in the midgut epithelium. Notably, silencing the AstA receptor *DAR-2* in the insulin-producing cells in the brain eradicates the reduction of fat storage by acetate. These results suggest that EEs sense acetate and produce AstA, which triggers insulin secretion in the brain. In addition, aging is highly associated with increased hepatic fat accumulation or liver steatosis. Consistently, we observe aging-induced steatosis in fly oenocytes, the equivalence of human liver, which is largely eliminated by acetate feeding. Based on these observations, I hypothesize that the level of intestinal acetate is reduced during aging, which abolishes the acetate-mediated gut-brain communication and results in dysregulated endocrine control and visceral adiposities such as liver steatosis.

Title: The role of the propionate metabolism pathway in aging

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The aging-metabolism link has solidified itself as one of the new challenges in understanding the complexity of aging as a systemic disease. Aging-associated metabolic dysfunction negatively alters the body's processing and distribution of macronutrients, inducing abnormal accumulation of metabolites. These dysregulated metabolites can regulate signaling pathways and be used as substrates for protein modification and have been found to contribute to aging-related syndromes. The propionate metabolism pathway is a highly conserved pathway critical for the metabolism of nutrients. However, the relevance of propionate metabolism in pathologies was largely unknown. Recently, our lab identified methylmalonic acid (MMA), a byproduct of propionate metabolism, as a driver of breast cancer metastasis. Additionally, ongoing projects have revealed that propionyl-carnitine, the reversible form of propionyl-CoA, can modulate lipid metabolism and influence the progression of prostate cancer. While an essential role of the propionate metabolism pathway in cancer is established, the function of this pathway and its metabolites in cell senescence and aging remains unknown.

The comprehensive population cohort study reveals that serum MMA levels increase with age and correlate with all-cause mortality. In our findings, both MMA and propionyl-carnitine levels are elevated in 18-month-old mice compared to 3-month-old counterparts. At the tissue level, the increase of these two metabolites with age varies depending on the tissue. To elucidate the mechanism behind this phenotype, we examined the expression of enzymes involved in the propionate metabolism pathway. While there's a general downtrend in enzyme expression with age, the extent of change varies across tissues. To investigate MMA's role in aging, we supplemented 6-month-old mice with MMA until they reached 12 months. This intervention significantly boosted both serum and tissue MMA levels, concomitant with elevated IL-6 levels in the serum. Tissue staining further demonstrated that MMA supplementation fosters fibrosis in multiple organs and tissues, a common feature of aging associated with the accumulation of senescent cells. At the cellular level, increasing intracellular MMA levels induced senescence, evidenced by altered proliferation, beta-galactosidase staining, mitochondrial dysfunction and expression of senescence markers. These findings collectively underscore the pivotal involvement of MMA and the propionate metabolism pathway in cell senescence and aging. Manipulating this pathway and inhibiting MMA production emerge as promising avenues for interventions targeting age-related diseases.

Nutrient Abundance Signals the Changing of the Seasons by Phosphorylating PER2

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The circadian clock synchronizes metabolic and behavioral cycles with the rotation of the Earth by integrating environmental cues, such as light. Nutrient content also regulates the clock, though how and why this environmental signal affects the clock remains incompletely understood. As genetic abrogation of the clock causes premature aging and calorie restriction extends lifespan, understanding the mechanisms whereby nutrient affects clock function is critically important for combatting aging. The axial tilt of the Earth relative to the solar plane drives seasonal differences in nutrient abundance and the duration of the daily light and dark phases. Animals must advance or delay circadian rhythms throughout the year to maintain alignment between organismal rhythms and the light cycle. As PER2-S662 phosphorylation regulates behavioral phase relative to the prevailing light cycle and is modulated by nutrient signaling pathways, we hypothesized that nutrient shifts circadian rhythms to anticipate changing seasonal photoperiods by phosphorylating PER2-S662. Here, we elucidate a role for nutrient in regulating circadian alignment to seasonal photoperiods by phosphorylating PER2. High fat diet (HFD) promoted entrainment to a summer light cycle and inhibited entrainment to a winter light cycle by phosphorylating PER2 on serine 662. Mice on a calorie restricted diet had the opposite phenotype, suggesting that caloric content or nutrient composition drives entrainment to the season in which that nutrient state predominates. PER2-S662 phospho-mimetic mutant mice were incapable of entraining to a winter photoperiod, while PER2-S662 phospho-null mutant mice were incapable of entraining to a summer photoperiod, even in the presence of HFD. Multi-omic experimentation in conjunction with isocaloric hydrogenated-fat feeding, revealed a role for hypothalamic polyunsaturated fatty acids in nutrient-dependent seasonal entrainment. Altogether, we identify PER2-S662 phosphorylation and transcriptional control of polyunsaturated fatty acid metabolism as a key mechanism whereby mice maintain synchrony between the nutritive state, the external light/dark cycle, and circadian rhythms across the year.

Heterogeneity of autophagy during aging

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Autophagy, a cellular recycling process that degrades damaged cellular components, is upregulated upon stress, including heat shock (HS), and plays a pivotal role in maintaining cellular homeostasis and healthspan. Although autophagy is known to decline with age and is linked to age-related diseases such as neurodegeneration, the individual variability in autophagy during aging remains poorly understood. To address this, our study assessed autophagy flux across the lifespan cohort (San Diego Nathan Shock Center) using peripheral blood mononuclear cells (PBMCs) from 19 participants aged 24 to 70 years. We monitored the lipidation status of the autophagy-related protein LC3B, which revealed a negative correlation between autophagy flux and age, indicating a decline in autophagy capacity with advancing age.

Furthermore, we explored how aging affects the inducibility of autophagy by heat shock, using human dermal fibroblasts isolated from individuals of different ages. RNA sequencing of these fibroblasts under heat shock conditions revealed a distinct transcriptional response that varies by age. This included changes not directly in autophagy-related genes, but rather in genes associated with metabolism and stress response, suggesting a broader regulatory mechanism at play that could impact autophagy indirectly through cellular stress pathways. Currently, we are comparing autophagy flux in human patient-matched PBMCs, primary fibroblasts, and induced neurons from young and senior donors to identify when autophagy changes with age in humans and to establish human aging cell models for testing autophagy-boosting interventions.

Identification of pathways connecting age-associated accumulation of mtDNA mutations with aging phenotypes.

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Cells and organisms depend on flawless performance of mitochondria, which, in turn, relies on the integrity of a small DNA molecule housed inside them - the mitochondrial genome (mtDNA). A rich literature shows progressive increases in the number and abundance of mtDNA mutations with age and implicates this accumulation in age-related conditions such as sarcopenia, diabetes mellitus and Parkinson's disease (Kauppila et al., 2017; Khrapko and Vijg, 2009). However, it is unclear how such intra-organellar damage might translate into systemic dysfunction observed in aging. For a mtDNA mutation to have an impact on OXPHOS function, levels of mutant mtDNA usually must climb above 60% of the total mtDNA population in a cell (Rossignol et al., 2003). Cells with such high levels of mutations are rare (in mice, fewer than 1 in 10³). Systemic aging phenotypes are unlikely to be the direct result of such rare defects. Instead, it is thought that mitochondrial dysfunction exerts adverse effects on the organism through stress signaling such that even minor populations of defective mitochondria can trigger cellular responses which can be further amplified by cellular stress signals to provoke systemic inflammatory events (Bar-Ziv et al., 2020). A number of signaling pathways relaying mitochondrial stress induced by various stressors have been described. Yet, it remains unknown which pathways are affected by age-associated accumulation of mutant mtDNAs.

To address this question, I developed a 10X based method to profile mtDNA mutations and gene expression in single cells. This method uses ATAC-seq to profile mtDNA mutations (Buenrostro et al., 2013; Ludwig et al., 2019; Lareau et al., 2021), RNA-seq to profile gene expression and barcoding for sample multiplexing (modified version of McGinnis et al., 2019). Recently, we identified a useful biological system for this study. Aged beta-2 microglobulin knock-out mice accumulate a minor arc deletion that deletes multiple mitochondrial genes (spans p1105 – p4925). I profiled single hepatocytes from these mice to identify genes whose expression was altered in the cells carrying the deletion at levels above 60%. In these cells, all the mtDNA encoded protein-coding genes that were not deleted, were upregulated by 2-fold. This is in line with other findings (Herbst et al., 2007; Durham et al., 2007) suggesting that deletions in mtDNA lead to upregulation of mtDNA copy number to compensate for the deficient functions which results in an excess of the intact functions. I also detected changes in nuclear gene expression, particularly genes involved in OXPHOS, adipogenesis and fatty acid metabolism. One of the top 5 upregulated genes was *Plin4* encoding protein that coats storage lipid droplets. These apparent changes in fat metabolism are in line with previous work showing that various mitochondrial stressors lead to lipid droplets accumulation (Seon-Jin Lee et al., 2013). While these preliminary data do not identify hallmarks of a known retrograde signaling pathway, they do identify downstream modulation of metabolism with features characterizing senescent cells (Wiley and Campisi, 2021) and demonstrate that the developed method works to identify cellular responses to specific mtDNA mutations. Because of their longer lifespan and much higher level of accumulated mutations, we expect that aged human cells will allow us to profile cellular responses to multiple different types of mtDNA lesions and uncover the signaling pathways they induce.

Examining Age- and Cell-Type Specific Changes in Translation in Hematopoietic Stem Cells

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Hematopoietic stem cells (HSCs) regenerate blood and immune cells throughout life. Age-related changes in HSCs diminish their fitness and regenerative potential which contributes to blood disorders and hematological malignancies in older adults. However, the underlying molecular changes which affect HSC fitness during aging are not completely understood. Previously, we discovered that young adult HSCs preferentially depend on low protein synthesis rates to maintain protein homeostasis and self-renewal activity. In preliminary work, we determined that a substantial number of genes involved in translational control are differentially expressed in young and old adult HSCs. This raised the possibility that aging results in significant proteomic alterations in HSCs. To test this, we optimized mass spectrometry conditions for low input freshly isolated HSCs and progenitors. Using this approach, we identified over 4500 unique proteins in 2×10^4 HSCs. Comparing protein diversity between young and old HSCs, 613 and 133 proteins were exclusively identified in young and old adult HSCs, respectively. Proteins identified exclusively in young adult HSCs were associated with autophagy, mRNA processing, nucleotide biosynthesis and translation regulation, whereas proteins exclusive to old HSCs were associated with immunological responses. Using paired RNA-sequencing, we identified multiple proteins whose expression is post-transcriptionally regulated in both a cell-type- and age-specific manner. HSCs post-transcriptionally suppress expression of ribosome biogenesis associated genes as compared to restricted progenitors, suggesting a mechanism underlying their unusually low protein synthesis rates. We also found that proteins involved in amino acid metabolism were suppressed in HSCs during aging. Furthermore, multiple proteins related to protein transport, transcriptional repression, DNA damage repair, and lipid metabolism were post-transcriptionally downregulated in old as compared to young HSCs. These studies are beginning to unravel how translational control, beyond protein quality, influences proteome content to regulate HSC identity, function and aging.

A nanoscale approach to understanding the biology of senescence

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A hallmark of aging is the accumulation of senescent cells. Senescence is a cell-fate decision characterized by permanent proliferation arrest, resistance to apoptosis, and secretion of inflammatory cytokines, chemokines, and proteases collectively termed senescence-associated secretory phenotype (SASP). Although elimination of senescent cells improved health and delayed onset-of several diseases in animal models, translation of this intervention is challenging. There is a critical lack of knowledge about senescent load, as well as the efficacy, dosage, and side-effects of these senotherapeutics. To date, a unified molecular fingerprint (i.e. specific gene/protein expression pattern) for senescence is unknown. Senescent cells display distinct morphological features, including enlarged cell size, increased mitochondrial and lysosomal (organelle) mass, and accumulation of intracellular iron. However, these features are not easily captured in living organisms with existing technology. To overcome these challenges, we will develop and validate a nanodetection tool that can identify senescent cells based on subtle changes in size, shape, volume, through mapping of the magnetic field. Magnetic nanoparticles (MNP) have been widely used in nanomedicine including for diagnostics, drug delivery, magnetic imaging, magnetic separation, because of their low toxicity and good biodegradability. Our proposed nanoscale tool will be able to detect hyperfine changes in the magnetic properties of MNPs allowing us to correlate these changes to the alterations that a cell undergoes during senescence. Three types of MNP - nanocrystalline maghemite (Mh) with different amounts of Co substitutions were synthesized. The nano-crystalline nature of the MNPs was confirmed by a variety of characterization methods such as FT-IR, X-ray Diffraction (XRD). Atomic Force Microscopy (AFM) and Scanning Electron Microscopy. The MNPs did not affect cell viability or senescence associated galactosidase, suggesting that the nanoparticles are compatible with cells and do not induce cellular changes. To further examine spatial location of MNP, we have synthesized Rhodamine isothiocyanate conjugated MNPs. Future studies in defining magnetic signatures of senescent versus quiescent and proliferating cells are underway. This unique and innovative approach may serve as a 'one-stop-shop platform' for detecting senescence.

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A Urinary Extracellular Vesicle Proteomic Signature of Inflammation and Senescence in People with HIV

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Background:

People with HIV (PWH) are living longer but have a higher burden of age-related comorbidities, including chronic kidney disease (CKD) in the modern era. Moreover they have higher levels of systemic inflammation than people without HIV. Inflammation promotes senescence and development of age-related comorbidities. Extracellular vesicles (EV) are released from activated cells and contain cargo from their parent cell representative of its molecular state of stress. The majority of urine EVs originate from kidney tubular epithelial cells, which are infected by HIV and vulnerable to senescence. We therefore investigated whether PWH without clinical CKD would have a urine EV proteomic signature of inflammation and senescence.

Methods:

Clean catch urine samples from a morning void were collected from 10 HIV+ and 9 HIV- persons matched on age, sex and race and stored at -80 °C. Enrichment for urine EVs was performed using low speed centrifugation and precipitation and was confirmed by verifying CD9, Alix and TSG101 protein expression by western blot. Urine EVs were then characterized using transmission electron microscopy (TEM) and tandem liquid chromatography mass spectrometry (LC/MS). Gene ontology (GO) analysis was performed to assess for cellular pathways enriched in biological function in HIV+ samples.

Results:

Median age was 36 (IQR 32.5, 42.5), 85% were men, 50% were Black, 50% were Hispanic and median creatinine was 1.0 mg/dL (IQR 0.8, 1.2). There were no significant differences in demographics or kidney function between the groups ($p > 0.05$). Among PWH, median CD4 count was 569 cells/mm³ (IQR 238, 880) and all had plasma HIV RNA <200 copies/mL. Five HIV- persons were receiving pre-exposure prophylaxis antiretroviral therapy. Urine EVs ranged from 60-100 nm in size. A total of 3238 proteins were found in HIV+ samples compared to 1787 proteins in the HIV- samples. No HIV viral proteins were detected. Among 562 unique proteins identified, 59 proteins had >2-fold higher abundance in HIV+ samples. Biological pathways that were enriched in HIV+ samples included nitric oxide mediated signal transduction and SMAD protein signal transduction. Several of the most abundant proteins in the HIV+ samples have a role in inflammation, senescence and kidney fibrosis.

Conclusion:

In a pilot study of PWH and people without HIV without age-related diseases, we found significant differences in urinary EV proteomic profiles. PWH had significantly more EV proteins than people without HIV. Two of the top biological function pathways enriched in PWH play important roles in senescence. Future studies are needed to determine if urine EV inflammation and senescence proteins are associated with development of CKD in PWH.

Title: The role of vitamin B12 in supporting skeletal muscle mitochondrial genome integrity and mitochondrial function

Authors: Martha S. Field, Anna E. Thalacker-Mercer, Katarina E. Heyden, Luisa F. Castillo, and Joanna L. Fiddler

Deterioration of skeletal muscle (SkM) mass, strength, and function (i.e., sarcopenia) is a common and debilitating manifestation of advancing age. Aging is also accompanied by disrupted mitochondrial function, one mechanism underlying SkM deterioration. Intriguingly, age-related SkM deterioration and mitochondrial decline are associated with low vitamin B₁₂ (B12) status, which is common in older adults and as a result of use of pharmaceuticals including metformin.

Folate and vitamin B₁₂ (B12) are essential cofactors required for folate-mediated one-carbon metabolism (FOCM), which provides one-carbon groups for biosynthesis of nucleotides and amino acids. Perturbed *de novo* thymidylate (dTMP) synthesis results in an accumulation of dUMP and subsequently dUTP, and then misincorporation of uracil during DNA replication and repair. Repair of nuclear DNA (nDNA) uracil misincorporation leads to DNA strand breaks and chromosome instability. The consequences of uracil misincorporation into mitochondrial DNA (mtDNA) are largely uncharacterized. Moreover, a direct link between low B12 status and SkM deterioration has not been defined.

We have recently shown that either genetic perturbation to B12 metabolism or dietary B12 deficiency in young mice (3 mos) increased liver and skeletal muscle uracil misincorporation into mouse mitochondrial DNA (mtDNA) by up to 40-fold. In all tissues, uracil levels in mtDNA increased without concomitant changes in uracil in nuclear DNA (nDNA), indicating that mtDNA is more sensitive to impaired B12 status than is nDNA. Importantly, mtDNA uracil misincorporation in mouse models is associated with decreased skeletal muscle oxidative phosphorylation complex activity, a key determinant of mitochondrial ATP production capacity. More specifically, dietary B12 deficiency decreased tibialis anterior complex I (CI) and complex IV (CIV) maximal oxygen consumption rate by 50%. In addition, B12 supplementation in older mice (age 22 mos) increased gastrocnemius CIV maximal activity by 2-fold, suggesting that B12 supplementation may improve skeletal muscle function in aged mice. Taken together, these data indicate that B12 deficiency contributes to age-related skeletal muscle deterioration by limiting dTMP synthesis which ultimately impairs mtDNA integrity and impairs mitochondrial function.

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Human skeletal muscle methylome and transcriptome changes after exercise during ageing

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Introduction: Ageing represents an important health and economic burden on society. Sedentary behaviour and lack of physical activity accelerate the widespread cellular and molecular changes induced by ageing, resulting in the increased prevalence of many chronic diseases. Epigenetics (particularly DNA methylation) is one of the hallmarks of ageing. Targeting epigenetic ageing is a promising actionable mechanism and late-life exercise mitigates epigenetic aging in rodent muscle. Whether exercise training can decelerate, or reverse epigenetic aging in humans is unknown.

Methods and Results: We performed a powerful meta-analysis of the methylome and transcriptome of an unprecedented number of human skeletal muscle samples (n = 3176). We show that: (1) individuals with higher baseline aerobic fitness have younger epigenetic and transcriptomic profiles, (2) exercise training leads to significant shifts of epigenetic and transcriptomic patterns toward a younger profile, and (3) muscle disuse "ages" the transcriptome. Higher fitness levels were associated with attenuated differential methylation and transcription during aging. Furthermore, both epigenetic and transcriptomic profiles shifted toward a younger state after exercise training interventions, while the transcriptome shifted toward an older state after forced muscle disuse. We demonstrate that exercise training targets many of the age-related transcripts and DNA methylation loci to maintain younger methylome and transcriptome profiles, specifically in genes related to muscle structure, metabolism, and mitochondrial function. Next, we are building a multi-tissue (18 tissues) ageing methylome atlas (more than 75K samples). Preliminary results suggest there is sex-specific DNA methylation responses to exercise in human muscle.

Discussion: Our comprehensive analysis will inform future studies aiming to identify the best combination of therapeutics and exercise regimes to optimize longevity.

Disruption of GHR in “middle aged” mice enhances insulin sensitivity and extends lifespan

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Congenital reduction of growth hormone (GH) action extends healthspan and lifespan in several organisms. In fact, mice and humans with a congenital disruption in the GH receptor (R) gene (GHRKO mice and patients with Laron Syndrome, respectively) have improved insulin sensitivity and are resistant to diabetes, cancer and age-associated cognitive decline. Importantly, GHRKO mice hold the record for the longest-lived laboratory mouse, with a lifespan one week short of five years of age. GHRKO mice also show reduced markers of aging such as adipose tissue senescence and mTORC1 signaling in liver, kidney, heart and muscle. However, as post-natal GH action is necessary for normal longitudinal growth, we and others have hypothesized that targeted inhibition of GH action at an adult age would allow normal stature and yet extend healthy lifespan. To test this hypothesis our laboratory has been developing a systematic series of mouse models of inducible GHR ablation at either 1.5-months or 6-months or 12-months of age. We found and reported that ablation of the GHR at 6-months of age remarkably recapitulated critical physiologic advantages of congenital GH resistance including extended lifespan in females and improved insulin sensitivity and suppressed neoplastic occurrences in males. We most recently ablated the GHR at 12-months of age in middle-aged (12mGHRKO) mice. Preliminary data show that 12mGHRKO mice have extended longevity in both male and female mice, with improved insulin sensitivity and reduced glucose levels in males as well as decreased adipose tissue fibrosis in females compared to males. Body composition measurements showed that 12mGHRKO mice have increased adipose tissue and reduced lean mass compared to controls. However, despite the obese phenotype of 12mGHRKO mice, health-span parameters of frailty index, and motor coordination and grip strength show no deficit in 12mGHRKO mice compared to the controls. Furthermore, in liver markers of senescence such as p21 and p16 are also reduced in 12mGHRKO. Single-nuclei RNAseq and pathology studies are currently being performed to elucidate the cancer incidence and the molecular mechanisms responsible for the healthy aging of 12mGHRKO mice. Collectively, our results indicate that reduced GH action in middle aged mice is an effective intervention extending health-span and lifespan, without affecting growth and development.

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Untangling the interorgan communication network

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The secreted proteome represents a poorly-characterized interorgan communication network, which is central to physiology and in the etiology of various disorders. Intriguingly, aging significantly impacts these interorgan communications networks, and vice versa. Despite this, the composition and activity of interorgan communication networks and their impact on aging remain poorly understood. One of the major contributors to interorgan communication is the white adipose tissue (WAT). WAT stores and senses energy, regulates metabolism of other organs, and influences aging. However, it has historically been difficult to identify the secreted proteins involved due to their low abundance and lack of information on their organ(s) of origin. To address this, we established in mice a conditional BirA*G3 engineered biotin ligase system that generally labels secreted proteins within the endoplasmic reticulum of one organ, allowing identification of proteins that traffic to distal organs using affinity enrichment and quantitative mass spectrometry proteomics. This transgenic system can be expressed in specific tissues to identify key secreted factors that communicate to distal organs. Using this approach in mice, the Droujinine Lab has identified hundreds of proteins trafficking between organs in response to food intake, including known low-abundance hormones. We identified a novel secreted protein that specifically targets the WAT, and acts through a receptor to impact WAT metabolism. Altogether, our approach can discover mechanisms by which organs are modulated in physiology. Importantly, these methods are widely applicable to proteins secreted from multiple organs, establishing a strategy to broadly define aging-dependent changes in whole-body interorgan networks.

Intestinal control of neuronal redox in age determination

Juhee Kim*, Lexus Tatge*, Rene Solano Fonseca, Patrick Metang, Kielen Zuurbier, **Peter Douglas**

Inter-organ communication along the gut-brain axis is increasingly recognized as a potent regulator of the aging process. Nevertheless, elucidating the molecular mechanisms governing this signaling network has remained elusive. Our preliminary investigations involving microbiome isolates have identified a signaling cascade conducive to longevity, originating within the intestinal milieu, which holds promise for modulating neuronal health and activity. Through targeted manipulation of a single intestinal peptide transporter during development, we can modulate redox potential and the NAD⁺/NADH dinucleotide ratio within six dopaminergic head neurons of the *C. elegans*, resulting in a doubling of lifespan. Importantly, our initial findings in murine models demonstrate that genetic ablation of this evolutionarily conserved peptide transporter specifically within the villus and crypt epithelial cells of the small and large intestine elicits similar alterations in NAD⁺/NADH ratios within the murine brain. Collectively, our interdisciplinary investigations delineate intricate signaling mechanisms along the gut-brain axis with the potential to modulate brain function and foster healthy aging trajectories.

Common chronic inflammatory diseases converge on nuclear autophagy to amplify inflammation**Yu Wang^{1,2}, Vinay V. Eapen², Yaosi Liang^{1,2,3}, Xiao-Fan Wang^{1,3}, Terje Johansen⁴, Zhixun Dou^{1,2}**

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Abstract

Acute inflammation is an essential response our bodies use to combat infections. However, in the absence of infections, chronic inflammation can play a pivotal role in the onset and progression of chronic diseases, such as arthritis, cancer, autoimmune disorders, nonalcoholic steatohepatitis (NASH), and most ageing-associated pathologies. The underlying mechanisms that distinguish chronic inflammation from its acute counterpart remain unclear, posing challenges to the development of targeted therapies for these major diseases. Here we identify a new mechanism that separates the two responses: during chronic but not acute inflammation, chromatin remodeling is influenced by nuclear autophagy, in which the WSTF protein of the ISWI chromatin remodeling complex interacts with the ATG8 autophagy protein family. This interaction triggers a chain of events, amplifying inflammatory responses. We show that cell-penetrating peptides that block this interaction do not affect acute inflammation but suppress chronic inflammation in senescence as well as NASH and osteoarthritis in mouse models and patient samples. The ability to specifically target chronic inflammation without blunting acute inflammation offers a new approach to treating common chronic inflammatory diseases.

Casting light on energy metabolism throughout the anatomy of aging neuronsCarlos Manlio Díaz-García^{1,2,3}, Jenna Gray^{1,2}, Hadyn Rose^{1,2}

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Cognitive decline in aging is associated with impaired glucose utilization in the brain parenchyma. Neurons are particularly vulnerable to these energy deficits, considering their large energy demands due to the firing of action potentials and the events related to neurotransmission. However, the ability of adult neurons to directly consume glucose remains controversial due to a decades-old hypothesis that posits a preferential use of astrocytic lactate in active states. In addition, energy demands are heterogeneous throughout the anatomy of a neuron, considering its marked cellular polarization that is manifest in well-defined anatomic regions including dendrites/spines, neuronal somata, and axons/synaptic terminals. The thinnest and smallest of these structures (both pre- and post-synaptic compartments) appear to be the most vulnerable parts of the neuron, given the significant loss of neuronal arborization but marginal loss of neuronal somata during aging. Furthermore, changes in the fuel preference of these anatomic compartments during aging remain an enigma. Using fluorescent indicators for Ca^{2+} and the NADH/NAD⁺ ratio, as well as NAD(P)H autofluorescence in hippocampal tissue, we have begun to explore the fuel flexibility of dentate granule cells from young and aging mice. We have performed proof-of-principle experiments selectively expressing the NADH/NAD⁺ biosensor Peredox in hippocampal dentate granule cells at different ages, and we have optimized the simultaneous recordings of local field potentials and mitochondrial NAD(P)H autofluorescence in acute hippocampal slices. Our preliminary results (mostly performed in dendrites and neuronal somata) suggest direct glucose utilization throughout the neuronal anatomy, which can become impaired in aging neurons. Upon completion of our ongoing experiments, we will determine the time course of this metabolic deregulation for each anatomic compartment. Information from these studies can be leveraged for metabolic interventions aimed at preserving the integrity of dendrites and axons during aging.

Title: Macrophages represent a key source of p21⁺senescent cells in aging visceral tissues and metabolic dysfunction associated with steatohepatitis (MASH)

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Research: Senescent cells play key roles in multiple biological processes, including development, tissue homeostasis, and acting as an anti-cancer mechanism. Moreover, senescent cells have been causally linked to sterile inflammation and disease due to their Senescence-Associated Secretory Phenotype (SASP). An open question in the aging field is the identity of the cell types that undergo senescence during the aging process. This is particularly important in metabolic tissues such as the liver, which has been shown to be affected by senescent cell burden. Interestingly, macrophages are emerging as a primary source of senescent cells in multiple settings, including tissue regeneration, wound healing, cancer, atherosclerosis, Alzheimer's, and the aging process. However, much remains unknown about the basic biology of macrophage senescence, including the genes and signaling pathways that regulate the senescent state, accurate biomarkers for defining them, and the underlying biology regulating their functions, including the SASP. To address this question, our lab has generated and validated a novel *in vitro* senescent macrophage system and used an unbiased omic approach to carefully define senescent macrophage phenotypes and specific biomarkers that define macrophage senescence in both mice and humans. Leveraging our defined biomarkers of macrophage senescence, we revealed that a large fraction of Kupffer cells (macrophages), and not other cell types found in the aging livers of old mice have increased expression of senescent macrophage markers. We also demonstrate that the senescent macrophage signature is increased in mice fed a pro-MASH diet. Thus, our data suggest that macrophages represent a key source of senescent cells in aged metabolic tissues and contribute to inflammaging and metabolic disease. Our project provides greater insight into the role of the immune system and cellular senescence in aging and metabolic disease, a better understanding of the novel biology associated with senescent macrophages, and potential therapeutic targets for treating inflammaging and metabolic diseases.

AGING AND NUTRITION IN INTESTINAL PATHOGENESIS: ROLE OF NICHE CELLS

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Continual regeneration of the intestinal epithelium relies on actively cycling Lgr5^{hi} intestinal stem cells (ISCs). The balance between self-renewal and differentiation of these stem cells is controlled by a combination of cell-intrinsic and extrinsic cues derived from the stem cell niche. Aging contributes to impaired precision of regulatory mechanisms, and environmental mediators such as nutritional risk factors can accelerate the impairment that further drives pathogenesis in aging. Therefore, decline of regenerative capacity of ISCs is one of the hallmarks of aging and is a critical contributor to induce age-associated functional impairment of the tissue, which includes elevated risk for tumor development. We recently established that in the aging mouse, there is a major reprogramming of intestinal Lgr5^{hi} ISCs causing their repressed function and retarded developmental maturation of the daughter cells that they generate, remodeling the intestinal mucosa (Aging Cell, 2023).

Our research demonstrated that the nutritional environment is also a critical contributor that alters the function of Lgr5^{hi} ISCs (Molecular Cancer Res, 2023; Nature Genetics, 2024). Specifically, feeding NWD1, a purified rodent diet establishing mouse exposure to key nutrients recapitulating their levels that increase human risk for intestinal cancer. This extensively, rapidly, and reversibly reprogrammed Lgr5^{hi} ISCs via epigenetically down-regulating *Ppargc1a* expression, a gene that controls mitochondria biogenesis and function. Dysregulation of mitochondrial function suppressed regenerative capacity of Lgr5^{hi} ISCs and subsequent lineage maturation of their progeny. Dietary perturbation is recapitulated by *Ppargc1a* genetic inactivation in Lgr5^{hi} ISCs *in vivo*, consequently mobilizing Bmi1+, Ascl2^{hi} cells which adapted lineages to the nutritional environment and elevated antigen processing and presentation pathways, especially in mature enterocytes, causing chronic, pro-tumorigenic low-level inflammation. There were multiple parallels between NWD1-driven remodeling of stem cells and lineages with pathogenic mechanisms in human inflammatory bowel disease, also pro-tumorigenic. Therefore, understanding how nutritional risk factors accelerate development of age-associated pathogenesis in compromising mucosal function and elevating risk for disease is fundamental.

Based on new data, I hypothesize that an unhealthy diet that mimics human nutritional exposures accelerates age-associated pathogenesis by reprogramming mesenchymal cells and their functional interaction with intestinal stem and progenitor cells and lineages. Hence, the goal is to determine the role of mesenchymal cells as an extrinsic driver that remodels intestinal adaptation responding to the nutritional environment during aging and evaluate the efficacy of metformin in restoring normal homeostasis under different nutritional exposures. I will focus on the role of mesenchymal cells as a cell non-autonomous factor regulating adaptive responses and unravel effective strategies to restore mucosal homeostasis and the efficacy of targeting mesenchymal cells to enhance normal stem cell function and mucosal homeostasis.

Cardiac SLC25A51 deficiency causes severe cardiac hypertrophy and shortens lifespan

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Cardiac mitochondrial function declines with age, impacting the heart's ability to efficiently generate energy. This decline leads to mitochondrial dysfunction, a key feature of the aging heart and a significant contributor to various cardiovascular diseases. The mitochondrial NAD⁺ (mtNAD⁺) pool plays a crucial role in maintaining mitochondrial function by supporting processes such as mitochondrial respiration, protein deacetylation and the TCA cycle. The identification of SLC25A51 as the mitochondrial NAD⁺ transporter highlighted its importance in maintaining these essential NAD-dependent metabolic processes. However, the specific role of SLC25A51 in cardiac aging remains unknown. Our findings revealed that 26-month-old wild-type (WT) mice exhibit diminished SLC25A51 expression compared to young (3-month-old) counterparts along with significantly reduced mtNAD⁺ levels and heightened mitochondrial protein acetylation. To further examine the role of SLC25A51 in cardiac aging, we generated a cardiac-specific SLC25A51 knockout (SLC25A51 cKO) mouse model by crossing newly generated *Slc25a51* floxed mice with mice expressing Cre recombinase under cardiac-specific α -myosin heavy chain promoter. SLC25A51 cKO mice died prematurely at approximately 2 months of age with severe cardiac hypertrophy and failure. Additionally, we observed ~85% lower mtNAD⁺ levels and mitochondrial protein hyperacetylation in SLC25A51 cKO mice compared to controls. Isolated mitochondria from SLC25A51 cKO hearts showed impaired NADH-dependent Complex I-driven respiration and an augmented NADH-independent Complex II-driven respiration compared to controls. In conclusion, cardiac SLC25A51 deficiency leads to reduced mtNAD⁺ levels, hyperacetylation, impaired Complex I-driven respiration, cardiac hypertrophy and premature death. These data demonstrate that SLC25A51 has an essential role in maintaining cardiac function and supports that lower SLC25A51 expression could be an important mediator of cardiac aging.

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What makes clocks tick?

Decomposing adult epigenetic clocks to probe the early origins of epigenetic ageing

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Abstract

Background: DNA methylation (DNAm) at specific sites can be used to calculate ‘epigenetic clocks’, which in adulthood are used as indicators of age(ing). However, little is known about how these clock sites ‘behave’ during development and what factors influence their variability in early life. This knowledge could be used to optimize healthy aging well before the onset of age-related conditions.

Methods: Here, we leveraged data from two longitudinal population-based cohorts with repeatedly assessed DNAm (N=5,019 samples from 2,348 individuals) to characterize trajectories of adult clock sites from birth to early adulthood. We examined sites in first-generation clocks (trained on age: Horvath, Hannum, Weidner, and Zhang; N_{total}=967 sites) and second/third-generation clocks (trained on mortality or age-related indicators: PhenoAge, DunedinPACE, and TelomereClock; N_{total}=821 sites), together forming a set of 1721 unique clock sites. To probe potential genetic and early environmental influences on these sites, we also tested for enrichment of methylation quantitative trait loci (mQTLs in cord blood) and associations with prenatal exposures based on existing epigenome-wide association meta-analyses.

Results: Compared to non-clock sites, clock sites were more likely to show significant change ($p < 1 \times 10^{-07}$) in methylation levels across development (66%, enrichment $p = 3.07 \times 10^{-35}$), including non-linear changes (i.e. changing at different rates during different developmental periods; 32%, $p = 4.92 \times 10^{-63}$). Clock sites were more likely to differ between individuals already at birth (37%, $p = 1.55 \times 10^{-39}$), but not from later time points. In turn, individual differences at birth were highly predictive of individual differences in DNAm at age 18yrs. Furthermore, variability at birth in clock sites was strongly enriched for genetic influences (mQTLs; 43%, OR=3, $p = 3.90 \times 10^{-113}$) and also a range of prenatal exposures (OR=3, $p = 2.61 \times 10^{-11}$), including maternal hypertensive disorders, BMI, obesity, plasma folate levels, and smoking.

Discussion: We find that adult clock sites (i) diverge widely in their developmental trajectories, often showing non-linear change over time; (ii) are substantially more likely than non-clock sites to vary between individuals already from birth; and (iii) show enrichment for genetic effects and prenatal environmental exposures. These results suggests that age(ing)-related epigenetic processes might originate – and differ between individuals – already very early in development. Understanding what drives these differences may in future help us to devise better strategies to promote healthy ageing.

Restoring the ability of aged muscle to adapt to aerobic exercise with heterochronic plasma transfer.

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Exercise is less effective at improving aerobic capacity ($\dot{V}O_{2max}$) and muscle function in aged versus young or adult individuals. Improving the ability of aged skeletal muscle to adapt to exercise is an attractive strategy to reduce the loss of skeletal muscle mass and function. Prior studies using heterochronic plasma transfer (HPT) show that changing the aged systemic environment with the addition of “youthful factors” and/or “exercise factors” can rescue age-related declines in cellular and muscle function. We hypothesized that: 1) old (24-month) C57BL/6 mice undergoing HPT from young (OY, 5-months) C57BL/6 mice would have greater muscle adaptations to aerobic exercise compared to old sham controls (OC), and 2) these adaptations would be greater when using plasma from young, lifelong aerobically active (OYE, 5-months) C57BL/6 mice. To test these hypotheses, we assessed $\dot{V}O_{2max}$, grip strength, quantitative and kinetic mitochondrial proteomics, and high-resolution respirometry. We found a main effect of training where $\dot{V}O_{2max}$ was greater in all groups compared to baseline, but there was no effect of plasma. Muscle strength was greater in the OY and OYE compared to OC, but there was no difference between the OY and OYE. There were no differences in mitochondrial respiration or bulk mitochondrial fractional synthesis rates. However, there were differences in mitochondrial remodeling of individual proteins between OY and OYE indicating that the addition of exercise factors in plasma induced mitochondrial remodeling that is distinct from young plasma alone. Changing the systemic milieu does not produce an additive effect to exercise-induced increases in $\dot{V}O_{2max}$ or mitochondrial respiration but does cause improvements in muscle strength adaptations to exercise and differences in mitochondrial remodeling. This project was funded by the American Federation for Aging Research and a VA Pilot Award.

Big data-guided anti-aging drug development

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The three-dimensional (3-D) structure of chromatin undergoes significant alterations in various disease states and is a characteristic feature of the aging process. In diverse aging contexts, including premature aging disorders, cellular senescence, and normal aging, the nuclear lamina and the associated heterochromatin are frequently disrupted. Although these conserved structural changes have been documented for more than two decades, their impact on gene expression and their contribution to age-related degenerative processes remain poorly understood. Through extensive computational analysis and experimental validation, we demonstrate that genes lacking CpG islands (CGI- genes), which form heterochromatin when transcriptionally inactive, are globally misexpressed in aged nuclei with compromised chromatin architectures. Our findings reveal that CGI- gene misexpression is a common characteristic of mammalian aging and provides a molecular basis for various age-associated defects, including loss of cellular identity, increased transcriptional noise, and chronic inflammation associated with aging.

By leveraging a big data approach, we have discovered group of compounds to inhibit the misexpression of CGI- genes during the aging process. Our findings demonstrate that the pharmacochemical suppression of CGI- gene misexpression can effectively attenuate physiological deterioration in aged mice. Moreover, our data show that pharmacochemical inhibition of CGI- gene misexpression can alleviate the cardiac dysfunction in mice with nuclear architecture disruption in heart. Taken together, our results provide compelling evidence that CGI- gene misexpression is directly linked to age-related physiological decline, thus presenting a novel therapeutic target for interventions aimed at mitigating the physiological decline of aging.

Uncovering novel neuropeptide regulators of cognitive healthspan

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Over the past century, advances in healthcare and technology have extended human life expectancy; however, as the number of aged individuals increases globally, age-related health problems present a growing public health threat. One of the most prominent features of normal aging is cognitive decline, defined by a decreased ability to learn and remember. Without effective therapies to prevent or treat cognitive decline, millions of individuals will experience a diminished quality of life; therefore, it is critical to identify new ways to improve cognitive healthspan. The nematode *C. elegans* is an excellent model for studying this problem, given their ability to form molecularly conserved associative memories; the same mechanisms control learning and memory in worms and mammals. The worm is also short-lived, and rapidly exhibits age-related cognitive decline relative to other organisms (~ 1 week). Combined with the wealth of genetic tools available, *C. elegans* is an ideal system to rapidly uncover new pathways and molecules that control cognitive aging. Our previous work in this model discovered that mutants with a gain-of-function mutation in the highly conserved G_{αq} signaling pathway exhibit enhanced long-term memory (LTM) as young animals, and maintain the ability to learn and remember with age better than wild-type animals. We determined the site of action of the memory boosting effects of enhanced G_{αq} signaling in young and aged animals was a single sensory neuron pair, the AWC, and further established that G_{αq} signaling promotes memory in young adults by increasing AWC neuropeptide secretion. These results left us with several questions we sought to answer – Does increasing AWC neuropeptide secretion slow cognitive aging? If so, what peptides improve the ability to learn and remember with age? What are their receptors?

Here we have used genetic tools to increase neuropeptide secretion specifically from the AWC neuron and found that elevated AWC-specific neuropeptide release is sufficient to improve the ability to learn and remember with age. These beneficial effects on cognitive aging are observed without any detectable effects on lifespan, suggesting that AWC-secreted peptides are true healthspan-promoting factors. We have performed a targeted neuron-specific RNAi screen of AWC-expressed peptides to determine which are necessary for the improved cognitive aging phenotype we observed in gain-of-function G_{αq} signaling mutants. We find that members of each of the three major neuropeptide families in the worm (insulin-like peptide/ILP, FMRFamide-like peptides/FLP, and Neuropeptide-like/NLPs) are required for enhanced learning and memory. Excitingly, the putative receptors for these “pro-cognition” peptides are conserved between worms and mammals, where they are linked to learning and memory, and may present new druggable targets to slow cognitive aging. We find that the novel “pro-memory” ILP is required for learning and memory ability, and using a novel feeding-based approach, we report that administration of this ILP can boost memory ability in wild-type animals. We are currently examining if the effects of manipulating this ILP on both lifespan and cognitive aging, to determine if it is a novel neuronal healthspan regulator. Because many known pathways that slow cognitive aging are shared between species, our findings here have the potential to uncover novel therapeutic targets for the development of treatments for cognitive impairment.

HNF4- α activation in the liver mediates rejuvenating cognitive benefits of exercise in aging

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The growing elderly population has spurred interest in innovative treatments for age-related cognitive decline. Systemic interventions such as exercise have been shown to rejuvenate cognition in aged animals and promote cognitive benefits in elderly humans. We and others have shown that exercise prompts the liver to release blood factors that transfer the benefits of exercise to the aged brain, establishing a liver-to-brain rejuvenation axis. However, the molecular mechanisms through which the liver mediates the cognitive benefits of exercise are unknown. We performed bioinformatic analysis of both the blood plasma proteome and liver transcriptome of aged mice following exercise and identified enrichment for a master transcription factor, Hepatocyte nuclear factor 4 alpha (HNF4- α). We also observed that increased HNF4- α activation in the liver of exercised aged mice positively correlated with improved cognitive performance. Functionally, targeting *Hnf4a* in the liver using antisense oligonucleotides (ASO) mitigated the cognitive benefits of exercise in aged mice. Mechanistically, we posit HNF4- α downstream of AMP-activated protein kinase (AMPK). Targeting *Hnf4a* by ASO in the liver of aged mice blunts cognitive enhancements elicited by viral overexpression of a constitutively active form of liver-derived AMPK. To explore the rejuvenating potential of mimicking exercise-induced HNF4- α activation, we used a liver specific viral-mediated overexpression approach to increase a constitutively active form of *Hnf4a*^{S313D} in the liver and observed cognitive improvements in aged mice. Collectively, these studies will have significant translational potential, identifying HNF4- α as a master regulator of a liver-to-brain rejuvenation axis whose targeted activation can confer the benefits of exercise on cognitive function at old age.

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