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

THE 36TH ANNUAL AFAR GRANTEE CONFERENCE

GLENN WORKSHOP ON THE BIOLOGY OF AGING

May 31 – June 2, 2023

Program Book

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GLENN FOUNDATION FOR MEDICAL RESEARCH

THE PAUL F. GLENN/AFAR CONFERENCE ON THE BIOLOGY OF AGING
THE 36TH ANNUAL AFAR GRANTEE CONFERENCE

MAY 31 – JUNE 2, 2023
Ritz-Carlton Bacara
Santa Barbara, CA

#AFAR2023 @AFARorg

WEDNESDAY, MAY 31

3:30 – 4:00 p.m.	**Registration with light refreshment**	*Santa Ynez Terrace*
4:00 – 4:10 p.m.	**Welcome** **Stephanie Lederman** Executive Director American Federation for Aging Research **Steven N. Austad, PhD** Interim Chair, Board of Directors and Senior Scientific Director American Federation for Aging Research	*Santa Ynez*
4:10 – 5:10 p.m.	**Breakthroughs in Gerontology (BIG) Presentations** **Early life ROS as regulator of lifespan and age-associated diseases** Ursula Jakob, PhD **(2019 BIG awardee)** Professor University of Michigan **Interorgan communication in aging** Norbert Perrimon, PhD **(2019 BIG awardee)** Professor of Genetics Blavatnik Institute, Harvard Medical School	
5:10 – 5:40 p.m.	**The Rat is Back!** Steven N. Austad, PhD Distinguished Professor and Protective Life Endowed Chair in Healthy Aging Research, The University of Alabama at Birmingham Interim Chair, Board of Directors and Senior Scientific Director, American Federation for Aging Research	

5:40 – 6:00 p.m.	**Break**	
6:00 – 7:00 p.m.	**Reception**	*Rotunda Terrace*
7:00 – 7:30 p.m.	**Welcome and Introduction** **Mark Colllins** Glenn Foundation for Medical Research **Misinformation In and About Science** Carl T. Bergstrom, PhD Professor of Biology, University of Washington	*Santa Ynez*
7:30 – 9:00 p.m.	**Dinner**	*Rotunda*

THURSDAY, JUNE 1

7:30 – 9:00 a.m.	**Breakfast**	*Rotunda Terrace*
9:00 – 9:10 a.m.	**Meeting Overview and Goals** **Kevin Lee, PhD** Senior Scientific & Programmatic Advisor Glenn Foundation for Medical Research	*Santa Ynez*
9:10 – 9:40 a.m.	**Translating Discoveries from Aging Biology into New Therapies for Cancer Patients** Mina Sedrak, MD, MS Associate Professor City of Hope	
9:40 – 10:10 a.m.	**How Should We Test the Geroscience Hypothesis in Human Trials?** Jamie Justice, PhD Assistant Professor Wake Forest School of Medicine	
10:10 – 10:40 a.m.	**Break**	
10:40 – 11:10 a.m.	**Treatment Strategies to Prevent and Reverse Cognitive Decline Associated with Aging and Traumatic Brain Injury** Susanna Rosi, PhD Principal Investigator Altos Labs Bay Area Institute	
11:10 – 11:40 a.m.	**Transposable Element Activation in Neurodegenerative Tauopathies: From Bench to Bedside** Bess Frost, PhD Associate Professor Bartell Zachry Distinguished Professor for Research in Neurodegenerative Disorders, UT Health San Antonio	
11:40 a.m. – 12:00 p.m.	**Poster set-up in Ballroom A**	

12:00 – 1:30 p.m. **Lunch** *Rotunda Terrace*

1:30 – 2:30 p.m. **Panel Discussion - Past, Present, and Future of Aging**

Panelists:
Kristen Fortney, PhD, Co-Founder, CEO, BIOAGE
Jamie Justice, PhD, Wake Forest School of Medicine
Mina Sedrak, MD, MS, City of Hope
Eric Verdin, MD, President and CEO,
Professor, Buck Institute for Research on Aging

Moderator: Kevin Lee, PhD, Glenn Foundation for Medical Research

2:30 – 4:30 p.m. **Poster Session with Small Group Presentations** *Ballroom A*

2:30 – 3:00 pm: general viewing

3:00 – 4:30 pm: small group presentations:

3:00 – 3:30 pm
Group A, Bess Frost, moderator
Group D, Richard Miller, moderator

3:30 – 4:00 pm
Group B, Ursula Jakob, moderator
Group E, Steven Austad, moderator

4:00 – 4:30 pm
Group C, Jamie Justice, moderator
Group F, Gerald Shadel, moderator

Poster numbers and small group presentation assignments can be found in the program book. Each poster presenter has 2 minutes (think elevator pitch) to present their poster, with 3 minutes for questions before moving to the next poster. If your group is not presenting, please join another group.

4:30 – 6:00 p.m. **Free time**

6:00 – 8:00 p.m. **Dinner** *Rotunda Terrace*

FRIDAY, JUNE 2

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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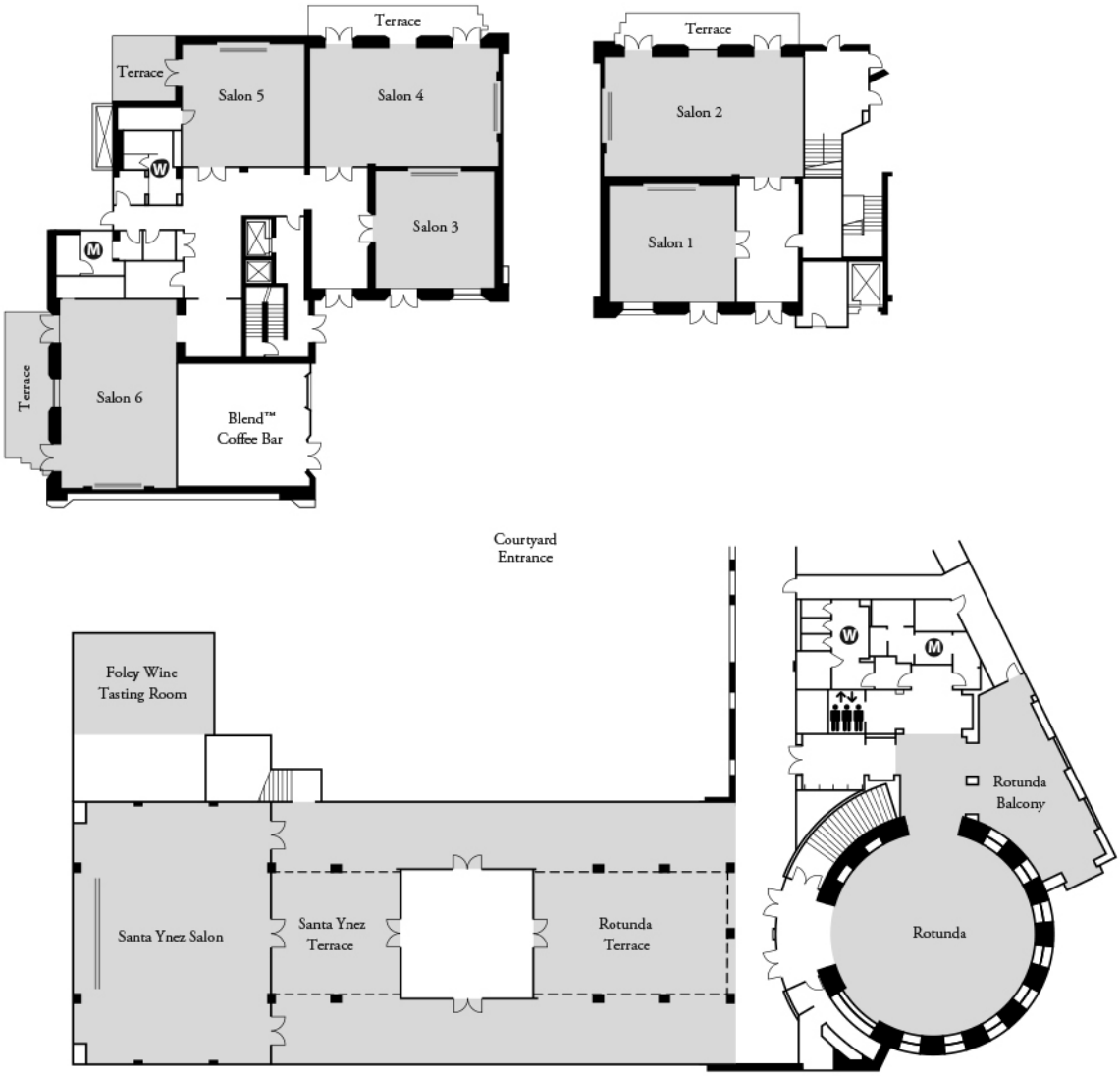
Rotunda, Santa Ynez Terrace & Salons



THE RITZ-CARLTON

BACARA, SANTA BARBARA

BEACHES & POOLS

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

GOLF

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

OUTDOOR ADVENTURE

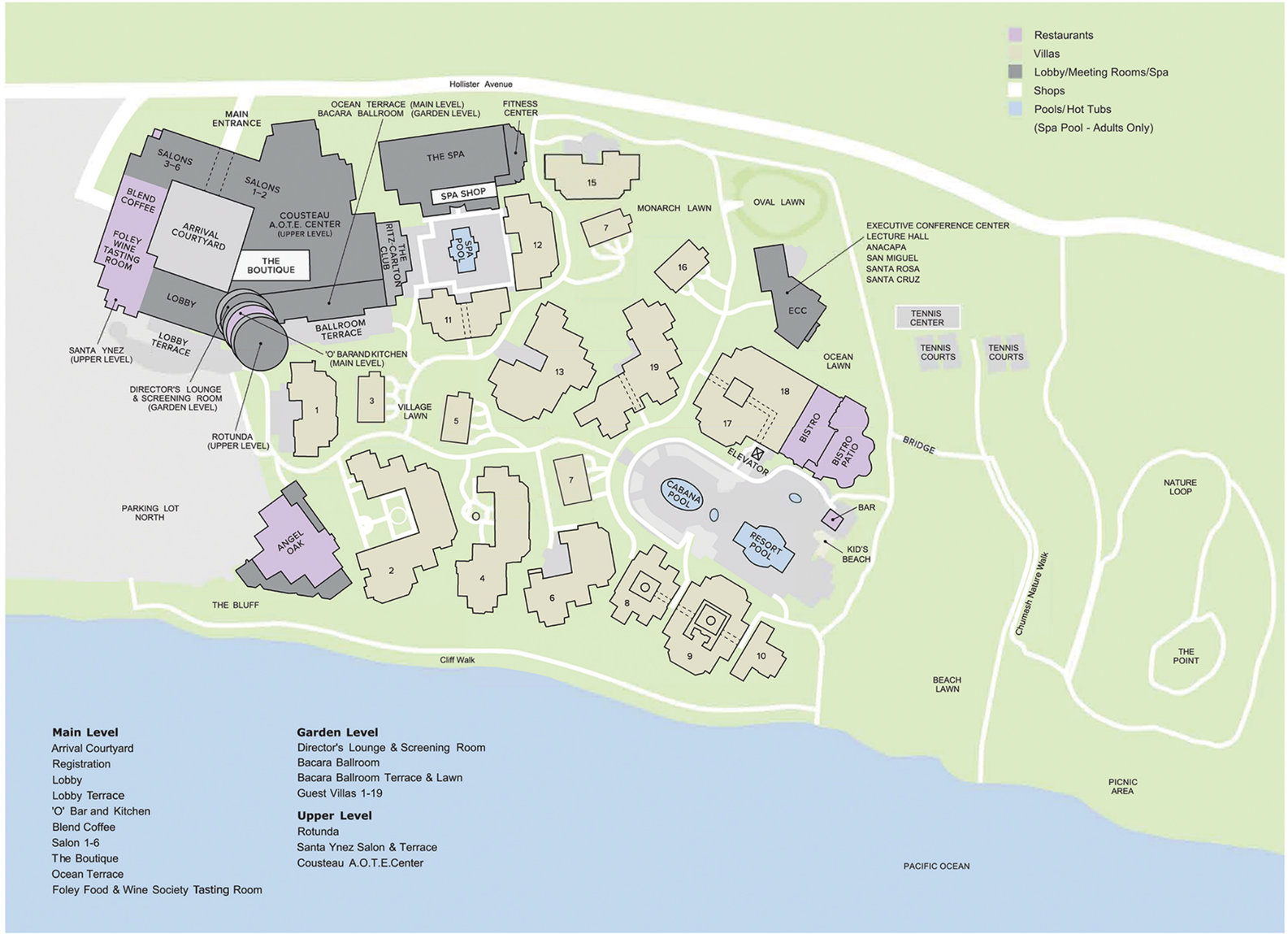
Santa Barbara offers horseback riding, biking, hiking and other activities. Pick up a hiking map. or book activities, including complimentary two-hour bike rentals at the front desk.

WINE COUNTRY

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

DINING GUIDE

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



Thursday, May 25, 2023
Datablitz

Group 1
Moderator: Darren Baker
Timekeeper: Odette van der Willik
12:00 - 1:30 pm Eastern Time
https://us02web.zoom.us/j/83254042015?pwd=Yk9WNHdpd1R0VjNodThaNm5XQ25Cdz09

Presenters

Brian Diekman	University of North Carolina at Chapel Hill
Madison Doolittle	Mayo Clinic
Angela Lombardi	Albert Einstein College of Medicine
Milos Marinkovic	UTHSCSA
Judith Simcox	University of Wisconsin Madison
George Sutphin	University of Arizona
Lei Zhang	University of Minnesota
Xu Zhang	Mayo Clinic

The academic equivalent of speed dating – a fast-track vehicle to understand research and possible synergies with others. Each session involves a very broad research theme, with grantees presenting their research in seven minutes or less – the time limit will be strictly enforced. Each presenter may show up to 6-7 slides, 7 minutes for presentation and 2 minutes for Q&A.

Thursday, May 25, 2023
Datablitz

Group 2
Moderator: Kevin Lee
Timekeeper: Hattie Herman
2:00 - 3:30 pm Eastern Time
https://us02web.zoom.us/j/83706303294?pwd=azRDMDhYZ2hBSEtaNXZ2bWxCcFA5Zz09

Presenters

Shailaja Allani	Florida Atlantic University
Suzanne Angeli	The University of Maine
Junyue Cao	The Rockefeller University
Cintia de Paiva	Baylor College of Medicine
Chatrawee Duangjan	University of Southern California
Maria Maryanovich	Albert Einstein College of Medicine
Laura Musselman	Binghamton University
Pradeep Ramalingam	Hackensack University Medical College

The academic equivalent of speed dating – a fast-track vehicle to understand research and possible synergies with others. Each session involves a very broad research theme, with grantees presenting their research in seven minutes or less – the time limit will be strictly enforced. Each presenter may show up to 6-7 slides, 7 minutes for presentation and 2 minutes for Q&A.

Thursday, May 25, 2023
Datablitz

First	Last	Institution	key words	email	Group
Shailaja	Allani	Florida Atlantic University	Oxidative damage, reactive oxygen species, methionine sulfoxide reductase	skesaraj@fau.edu	2
Suzanne	Angeli	The University of Maine	Mitochondrial unfolded protein response Mitochondrial permeability transition pore C. elegans	suzanne.angeli@maine.edu	2
Junyue	Cao	The Rockefeller University	Single-cell transcriptomics; Aging	jcao@rockefeller.edu	2
Cintia	de Paiva	Baylor College of Medicine	Aging/lacrimal gland/lipidomics/diversity outbred mice	cintiadp@bcm.edu	2
Brian	Diekman	University of North Carolina at Chapel Hill	senescence, DNA damage, chondrocytes, osteoarthritis, Sirtuin 6	bdiekman@email.unc.edu	1
Madison	Doolittle	Mayo Clinic	Senescence, skeleton, osteolineage	doolittle.madison@mayo.edu	1
Chatrawee	Duangjan	University of Southern California	insulin degrading enzyme (IDE), insulin clearance, WDR23, proteostasis, liver, hepatocytes, NRF2	duangjan@usc.edu	2
Angela	Lombardi	Albert Einstein College of Medicine	Diabetes; Ketones; Mitochondria; Autophagy.	angela.lombardi@einsteinmed.edu	1
Milos	Marinkovic	UTHSCSA	extracellular matrix, aging bone marrow stromal niche, bone density, CCN1/CYR61	marinkovic@uthscsa.edu	1
Maria	Maryanovich	Albert Einstein College of Medicine	HSCs, MSCs, HSC niche, aging, ROS, adrenergic signaling, NADPH oxidase	maria.marianovich@einsteinmed.edu	2

Thursday, May 25, 2023
Datablitz

First	Last	Institution	key words	email	Group
Laura	Musselman	Binghamton University	experimental evolution, GWAS, longevity, diet, physiology, Drosophila	lmusselm@binghamton.edu	2
Pradeep	Ramalingam	Hackensack University Medical College	hematopoietic stem cells, inflammaging, DNA damage, microenvironment, vascular niche	prr2008@alumni.weill.cornell.edu	2
Judith	Simcox	University of Wisconsin Madison	Ceramide, lipidomics, energy expenditure, adipose tissue,	jsimcox@wisc.edu	1
George	Sutphin	University of Arizona	aging, longevity, tryptophan-kynurenine, NAD, metabolism, stress response	sutphin@arizona.edu	1
Lei	Zhang	University of Minnesota, Institute On The Biolc	Senescence, senolytic, fisetin analogs, drug discovery	leizhang@umn.edu	1
Xu	Zhang	Mayo Clinic	Aging, Cellular senescence, single-cell RNA sequencing, skeletal muscle	zhang.xu@mayo.edu	1

Research Abstracts
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JF = Glenn and AFAR Grant for Junior Faculty
PD = Glenn Postdoctoral Fellowship Program
NSC = Nathan Shock Center Pilot Award

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Mechanistic investigations into p53-mediated immunosurveillance in solid tumor using *in situ* cell surface proteomics

Zeda Zhang[1,5], Charles Xu[2], Wei Luan[1], Janelle Simon[1,4], Yu-jui Ho[1,4], Changyu Zhu[1], Hsuan-An Chen[3], Qingwen Jiang[4], Namrata Udeshi[2], Steve Carr[2], Jiefu Li[5] & Scott Lowe[1,5]

[1]Cancer Biology and Genetics Program, Sloan Kettering Institute; [2]Broad Institute; [3]Rockefeller University; [4]Molecular Pharmacology Program, Sloan Kettering Institute; [5]Howard Hughes Medical Institute.

Patients with p53-deficient tumors often have inferior prognoses, yet an effective strategy for p53-deficient cancer is not widely available. p53-regulates a large portion of cell surface proteins (surfacesome) that play essential roles in cell growth, proliferation, differentiation, and death. During tumor progression, remodeling of the tumor cell surface plays a major role immune evasion, which creates a major hurdle to maximize cancer immunotherapy therapy. Previous research from our lab showed that re-activation of p53 in full-established tumor can trigger potent immune surveillance and clearance. Understanding how p53-mediated surface remodeling contributes to immune evasion and resistance to therapies will help develop new immunomodulatory agents for p53-deficient tumors. However, selectively profiling the tumor surfacesome *in vivo* in an unbiased and high-throughput manner remains technically challenging. I propose to leverage a novel proximity-dependent surface proteomics approach to selectively analyze cell surface activities in intact tumor tissues. We have found immunomodulatory cell surface molecules that have not been identified by RNA-seq before, indicating the potential of this new approach to uncover new biology.

POSTER 2

Title: Identification of a novel senolytic compound to enhance skeletal muscle health in older mice

Authors: Xu Zhang[1,2], Yan Er Ng[1], Lei Zhang[3], Yang Yang[4], Paul D. Robbins[3], Guangrong Zheng[5], Daohong Zhou[4], Nathan K. LeBrasseur[1,6]

Author Affiliations: [1]Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, USA; [2]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, 55905, USA; [3]Institute on the Biology of Aging and Metabolism, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA; [4]Department of Biochemistry & Structural Biology and Center for Innovative Drug Discovery, University of Texas Health Science Center at San Antonio, TX, USA; [5]Department of Medicinal Chemistry, College of Pharmacy, University of Florida, FL, USA. 6Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN, USA.

Background: Fibroadipogenic progenitor cells (FAPs) are critical for maintaining skeletal muscle health by regulating tissue repair and regeneration. Recently, we demonstrated a subpopulation of FAPs in skeletal muscle of old mice exhibit core features of senescence, including DNA damage, chromatin reorganization, and the activation of senescence-related genes, such as $p16^{Ink4a}$. However, their significance in skeletal muscle remains unclear.

Objectives: To 1) develop an efficient platform for screening senolytic compounds that selectively target senescent but not non-senescent primary FAPs *in vitro*; and 2) examine the effects of a lead senolytic candidate on the molecular phenotype of skeletal muscle and measures of muscle performance and physical function *in vivo* in older mice.

Methods: We isolated primary FAPs from mouse skeletal muscle using magnetic-activated cell sorting and induced senescence with etoposide. We tested a panel of senolytic candidates and their derivatives, previously shown to target other senescent cells, for their ability to kill senescent FAPs using the IncuCyte live-cell imaging system. To assess *the in vivo* effect of a lead senolytic on skeletal muscle, we administered the drug to aged mice and examined the impact on the molecular phenotype, histological parameters of skeletal muscle, as well as muscle strength and physical function.

Results: We screened 26 compounds for their effects on proliferative and senescent FAPs and successfully identified seven compounds, including Navitoclax, PROTAC 753B, and 5 other novel PROTAC compounds, that selectively killed senescent FAPs but not non-senescent FAPs even at high doses *in vitro*. Mice administrated of 753B for 6 months had lower senescent marker expression, fewer denervated myofibers, and improved muscle strength compared to vehicle-treated mice.

Conclusion: Our findings suggest that senotherapeutic interventions targeting senescent FAPs could be a promising strategy to prevent or delay skeletal muscle aging. The developed platform for screening senolytic drugs using senescent FAPs could also be utilized for identifying effective senolytics for other cell types.

Keywords: Cellular Senescence, Fibroadipogenic progenitors (FAPs), Senotherapeutic interventions, Muscle strength

Targeting cellular senescence with novel senotherapeutics by design to extend healthspan

Lei Zhang
Institute on the Biology of Aging and Metabolism
University of Minnesota
lzhang@umn.edu

Senescent cells accumulate with age and contribute to aging and the pathogenesis of many age-related diseases. Drugs that induce apoptosis in senescent cells specifically, termed senolytics, have emerged as an effective therapeutic approach to improve aging phenotypes and associated co-morbidities. Despite the promising potential of this approach, only a handful of senolytics have been reported, including a natural flavonoid fisetin discovered by our group. Fisetin has been shown to reduce senescence, suppress age-related pathology, and extend healthspan in aged mice. However, its moderate potency and poor bioavailability may limit its effectiveness in clinical applications. By leveraging drug design, medicinal chemistry and high-content imaging analysis, we have successfully optimized the senolytic activity of fisetin, leading to the identification of two fisetin analogs (FAs), SR29384 and SR31133, with improved senolytic potency and enhanced physicochemical properties. The therapeutic potential of the two FAs was evaluated in wild type old C57Bl/6 mice. Acute treatment with the two FAs, especially SR29384, significantly decreased the expression of multiple senescence and SASP factors in different tissues including kidney, brain and lung. Moreover, chronic treatment of the *Ercc1*$^{-/\Delta}$ mouse model of accelerated aging with the best fisetin analog SR29384 resulted in an extension of healthspan and reduced senescence and SASP factors significantly in multiple tissues including kidney, liver, lung and brain. These novel fisetin analogs with improved senolytic activity and reduced toxicity in non-senescent cells have clinical potential to slow aging and reduce severity of age-related diseases driven by senescence.

Title: Understand the aging of antiviral CD8 T cells

Author: Guohua Lou[1], Ziang Zhu[2,3], Chen Yao[2], Tuoqi Wu[2]
[1]Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO, 80045, USA.
[2]Department of Immunology, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA.
[3]Immunology Ph.D. Program, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA.

Abstract

To begin to understand the molecular mechanism of age-associated changes in antiviral T cell response, we used a mouse model of murine hepatitis virus (MHV) infection. Aging increased mortality and decreased antiviral CD4 and CD8 T cell responses in MHV infected mice. Surprisingly, although aging increased terminally differentiated CD8 T cells at baseline, our single-cell RNA-seq analysis in aged mice after infection revealed a profound reduction in terminally differentiated effector CD8 T cells and an elevated gene-signature of T-cell exhaustion. Using *in vitro* activated CD8 T cells from young and aged mice, we showed that age-associated decline in T-cell expansion was primarily caused by TCR-triggered apoptosis and necroptosis pathways and was rescued by shortening TCR stimulation and enhancing IL2 signaling. In addition, we found that aging reduced baseline metabolic rate in aged CD8 T cells, and impeded the metabolic adaptation of CD8 T cells after activation. Thus, our results revealed the molecular mechanism underlying the defective CD8 T cell response against viruses.

POSTER 5

Title: Dihomo-gamma-linoleic acid promotes lytic death of senescent cells and improves age-related pathology in male mice

Authors. Samantha Jezak, Chisaka Kuehnemann, Ioannis Siokas, Bronwyn Mogck, and Christopher Wiley

Affiliation. Jean Mayer USDA Human Nutrition Center on Aging at Tufts University, 711 Washington St, Boston, MA 02111

Abstract. Age is the greatest risk factor for developing several chronic degenerative disease, and current projections indicate that by 2034 there will be more Americans over the age of 65 than under the age of 18. Thus, interventions that target the basic processes that drive aging are essential for addressing this unmet need. One such process is cellular senescence, a complex stress response consisting of a permanent proliferative arrest linked to the secretion of a combination of biological molecules collectively known as the senescence-associated secretory phenotype, or SASP. We recently showed that a class of oxygenated signaling lipids, the prostaglandins, are a part of the SASP and act to reinforce the proliferative arrest and promote select protein parts of the SASP. Here we show that elevating dihomo-gamma-linoleic acid (DGLA) - an endogenous polyunsaturated fatty acid - results in selective elimination of senescent cells by a cell death process that is neither apoptosis nor ferroptosis, but shows features of lytic cell death. This cell death process is dependent on prostaglandin synthase 2 (PTGS2/COX-2), thereby targeting a key mediator of senescence and the SASP. After aged mice were administered DGLA, males showed lower levels of senescent cells in fat and liver tissues, but females were less responsive. DGLA also improved several age-related conditions including inflammation foci and fibrosis in the liver and crown-like structures in adipose tissue. These findings were coupled to improvements in blood biomarkers of liver, kidney, and adipose function. Together, these data demonstrate that methods that elevate DGLA levels can improve age-related diseases via elimination of senescent cells by lytic death.

POSTER 6

The Effects of Kynurenine Pathway Manipulation on Metabolism and Healthspan in Mice

Reyhan Westbrook[1], Nick Milcik[1], Anne Le[4,5] Tae Chung[2,3], Taylor Bopp[1], Peter M. Abadir[1], Jeremy Walston[1],

[1]Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD
[2]Department of Physical Medicine and Rehabilitation, Johns Hopkins University School of
[3]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD
[4]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD
[5]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

The Kynurenine pathway (KP) is a conserved molecular pathway that is altered with inflammation, aging, and frailty in mammals, including humans. Genetic and pharmacological manipulation of this pathway is linked to lifespan in lower organisms. In this study, we sought to elucidate further the role of the KP in the etiological development of inflammation-related functional decline in mice. We also sought to determine if genetically and pharmacologically altering this pathway can affect healthspan in mammals. We used stable isotope resolved metabolomics to measure the aging-related changes in the metabolism of tryptophan in mice using a fluxomic approach. We traced the metabolism of $^{13}C_{11}$ tryptophan in the plasma, liver, kidney, skeletal muscle and brain of 3 month-old and 20 month-old C57Bl/6 mice to assess the changes in tryptophan metabolism that occur with aging. We also used 3 month-old and 20 month-old Interleukin 10 knock-out (IL-10 ko) mice (n=5 per group) to determine how chronic inflammation affects the rates of tryptophan metabolism. We saw elevated rates of tryptophan metabolism to kynurenines in plasma, liver and kidneys of older and chronically inflamed mice with the highest levels of kynurenine generation found in older IL-10 ko mice. Interestingly, we saw that while levels of kynurenine generated from tryptophan were elevated, production of NAD+ and nicotinamide were decreased with aging and with chronic inflammation in plasma and in tissues. Future studies will determine how gene expression, protein level and other physiological factors contribute to these findings. We also measured the effects of genetically and pharmacologically decreasing levels of kynurenines in mice on physiological, neuromuscular, and healthspan-related parameters using the Indoleamine 2,3 dioxygenase knock out (IDO ko) mouse and mice chronically treated with 1-methyl-L-tryptophan (1-MT). Both IDO ko mice and 1-MT treated mice had significantly reduced levels of kynurenines and nicotinamide. Simultaneously reducing levels of kynurenines while supplementing with nicotinamide restored levels of NAD+ and nicotinamide. *In vivo* nerve-evoked muscle contractility measurements showed reduced age-related decline in force in mice with suppressed levels of kynurenines and those with concomitant kynurenine pathway suppression and nicotinamide supplementation. Further, these mice had increased activity levels at older age and reduced frailty as measured by the frailty index. Together this data shows that kynurenine pathway manipulation may be a useful anti-aging therapeutic strategy.

POSTER 7

Impact of B12 deficiency on skeletal muscle mitochondrial DNA and function in advanced age

Thalacker-Mercer A.[1] and Field M.[2]
[1]Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham
[2]Division of Nutritional Sciences, Cornell University

Mitochondrial dysfunction, which is influenced by the maintenance of mitochondrial DNA, is a hallmark of aging that is linked to organ and organismal decline. Adequate cellular thymidylate (dTMP, or the “T” base in DNA) pools are essential for accurate DNA replication and for maintaining integrity of both the mitochondrial and nuclear genomes (mtDNA and nDNA, respectively). dTMP is unique among DNA bases in that it is dispensable for DNA synthesis, because DNA polymerases incorporate a uracil (or “U”) base into DNA when dTMP levels are low. Uracil misincorporation leads to activation of base-excision repair mechanisms, which in the continued absence of dTMP, lead to DNA double-strand breaks, stalled replication fork progression, and DNA instability. Folate and vitamin B12 (B12) are essential cofactors required for folate-mediated one-carbon metabolism (FOCM), which provides one-carbon groups for *de novo* biosynthesis of nucleotides (including dTMP). Our preliminary data suggest that mtDNA is more sensitive to impaired FOCM than is nDNA—*B12 deficiency, induced by genetic impairment or dietary deficiency of B12, led to a more than 50-fold increase in uracil misincorporation into the mtDNA* of mouse liver, while nDNA uracil levels remained unchanged. These observations are important because *B12 deficiency is common in older adults*. Furthermore, proteins encoded by genes in the mtDNA are essential for oxidative phosphorylation and cellular energy production; therefore, *B12 deficiency especially deleterious for cellular and organismal energetics*. Skeletal muscle represents a mitochondria dense tissue that is susceptible to mitochondrial dysfunction, impaired metabolism, and overall deterioration with advancing age. In fact, sarcopenia, the age-related loss of skeletal muscle mass, strength, and function, is one of the most common phenotypes of aging. Intriguingly, age-related skeletal muscle deterioration has been linked to B12 deficiency. However, *the direct link between skeletal muscle dysfunction and B12 deficiency has not been elucidated*. We demonstrated, through a pilot and feasibility grant from the Nathan Shock Center of Excellence, that genotype- or diet- induced low B12 status reduces electron transport chain, complex activity; we hypothesize that reduced complex activity is due to increased uracil incorporation into the mtDNA.

The tryptophan metabolite 3-hydroxyanthranilic acid extends lifespan and promotes stress resistance by activating NRF2/SKN-1.

Sutphin, George[1]; Dang, Hope[1]; Castro-Portuguez, Raul[1]; Espejo, Luis[1]; Backer, Grant[1]; Freitas, Samuel[1]; Spence, Erica[1]; Meyers, Jeremy[1]; Shuck, Karissa[1]; Corban, Caroline[2]; Liu, Teresa[2]; Bean, Shannon[2]; Sheehan, Susan[2]; Korstanje, Ron[2].

[1] University of Arizona, Tucson, AZ
[2] The Jackson Laboratory, Bar Harbor, ME

The metabolism of tryptophan by the kynurenine pathway is increasingly linked to aging and age-associated disease. Kynurenine pathway enzymes and metabolites influence a range of molecular processes critical to healthy aging, including regulation of inflammatory and immune responses, cellular redox homeostasis, and energy production. Aberrant kynurenine metabolism is observed during normal aging and has been implicated in a range of age-associated pathologies, including chronic inflammation, atherosclerosis, neurodegeneration, and cancer. In previous work, we and others identified three kynurenine pathway genes—*kynu-1*, *tdo-2*, and *acsd-1*—for which decreasing expression extends lifespan in invertebrate models. More recently we discovered that knockdown of *haao-1*, a fourth kynurenine pathway gene encoding the enzyme 3-hydroxyanthranilic acid dioxygenase (HAAO), extends lifespan by ~30% and delays age-associated decline in health in *Caenorhabditis elegans*. This lifespan extension is mediated by increased physiological levels of the HAAO substrate 3-hydroxyanthranilic acid (3HAA). *Haao* knockout mice or aging mice fed a diet supplemented with 3HAA are similarly long-lived. In *C. elegans*, 3HAA also promotes resistance to multiple forms of exogenous stress, including oxidative stress. The mechanism of action linking 3HAA to aging and stress resistance is complex and partially overlaps with multiple pathways previously implicated in aging. In recent work, we have identified activation of the Nrf2/SKN-1 oxidative stress response and alterations to iron homeostasis as key players in the benefits 3HAA. Ongoing work explores the relationship between 3HAA, Nrf2/SKN-1, and iron in *C. elegans* and mammalian aging, age-associated immune decline, and cancer. This works provides a foundation for more detailed examination of the molecular mechanisms underlying the benefits of 3HAA, and how these mechanisms interact with other interventions both within and beyond the kynurenine pathway. We anticipate that these findings will bolster growing interest in developing pharmacological strategies to target tryptophan metabolism to improve health aging. This work was supported by a Glenn Foundation for Medical Research and American Federation for Aging Research Grant for Junior Faculty, NIH P30AG038070, and NIH R35GM133588.

Leveraging gametogenesis-specific rejuvenation pathways to counteract cellular aging

Tina L. Sing[1], Katie Conlon[1], Stephanie H. Lu[1], Nicole Madrazo[1], Tianhao Yin[1], Katlin Morse[1], Juliet Barker[1], Ina Hollerer[1], Gloria A. Brar[1], Peter H. Sudmant[2], and Elçin Ünal[1]

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At the cellular level, aging manifests as an accumulation of conserved physiological defects that eventually cause functional decline, disease, and organismal death. Significant research into the causes of cellular aging has revealed a growing list of age-associated factors that are conserved from yeast to humans. These include the accumulation of misfolded protein aggregates, loss of lysosomal acidity, abnormal nucleolar morphology, and mitochondria dysfunction. Interestingly, the germ line contains inherent rejuvenation pathways that prevent age-associated damage from being passed onto progeny, which leads to lifespan resetting. Thus, there is strong incentive to understand how gametogenesis-specific rejuvenation genes can eliminate aging biomarkers and determine which of these pathways can be leveraged to counteract cellular aging in somatic cells. Gametogenesis is a highly regulated developmental program whereby diploid progenitor cells undergo cell division (meiosis) and differentiation to produce haploid gametes; however, the complete complement of meiotic genes involved in cellular rejuvenation remains largely unknown. Our lab and others are working to identify and understand how meiosis-specific mechanisms remove aging biomarkers to reset lifespan in *Saccharomyces cerevisiae* (reviewed in Sing et al., 2022). Excitingly, overexpression of the meiotic transcription factor, Ndt80, is sufficient to extend lifespan (Ünal et al., 2011) suggesting that at least a subset of gametogenesis-specific rejuvenation pathways can be used outside of their natural context.

Using mRNA isolated from different meiotic stages, we have constructed 5 inducible cDNA libraries to identify meiotic transcripts that can extend lifespan in budding yeast. We developed a screening pipeline to measure the effect of meiotic genes on competitive fitness in both young and old populations of budding yeast leading to the identification of 80 rejuvenation candidates. This includes genes with roles in different organelles (ex. mitochondria, endoplasmic reticulum, Golgi apparatus, and vacuole) and genes with diverse biological functions (ex. genome maintenance, RNA processing, cell cycle regulation, metabolism, and stress response). We believe that the diversity in this list is exciting because it may represent multiple pathways that converge to rejuvenate aging cells. In addition, we find a subset of candidates with unknown functions, which could signify understudied meiosis-specific genes or gene isoforms that can provide new unexplored targets for aging intervention. Finally, we find several rejuvenation candidates that are potential targets of Ndt80, which may facilitate nucleolar rejuvenation and lifespan extension that has been previously reported. Currently, we are using a microfluidic pedigree system to perform single-cell lifespan measurements in strains overexpressing each of the rejuvenation candidates identified in our screen. Future research will be focused on: (i) understanding how these genes promote cellular rejuvenation; (ii) determining if there is crosstalk between these genes; and (iii) optimizing gene dosing and synergy to promote longevity.

Keywords: aging, gametogenesis, meiosis, quality control, rejuvenation

Aging Induces Pyroptosis in Brown Adipose Tissue (BAT)

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Aging, Western diet and thermoneutral housing induce brown adipose tissue (BAT) involution, a process characterized by a reduction in BAT mass and function with increased lipid droplet size. We recently found that (1) an understudied longevity gene, syntaxin 4 (STX4), declines with age (2) brown adipocyte-specific knockout of STX4 results in pyroptotic activation of the caspase 1/4 pathway. Single-nucleus RNA sequencing (snRNA-seq) of aged mouse BAT identified a specific brown adipocyte population with distinctly low expression of uncoupling protein 1 ($UCP1^{low}$ cells), which were $Stx4^{low}$ and pyroptotic. Conversely, restoration of STX4 expression or pharmacological Casp1/4 inhibition protected mice from brown adipocyte pyroptosis and decline in thermogenic activity during aging. Mechanistically, aging reduced oxidative phosphorylation, glucose uptake and glycolysis in brown adipocytes, leading to reduced ATP levels, a known signal for pyroptosis. Taken together, these data provide a novel model of rapid brown adipocyte involution and demonstrate that physiological aging and BAT involution result from activation of pyroptotic signaling.

Targeting bone marrow inflammaging to preserve hematopoietic healthspan.

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Aging is associated with a decline in hematopoietic stem cell (HSC) fitness including a loss of their regenerative ability, and a myeloid-biased differentiation at the expense of lymphopoiesis. Consequently, older adults frequently manifest anemias, impaired immune responses towards infections and vaccines, and exhibit delayed recoveries following myelosuppressive therapies. While intrinsic defects within aged HSCs have been well-scrutinized, the mechanisms that drive the accrual of these defects remain poorly defined. Recent studies have confirmed that a chronic low-grade inflammatory stress within tissue-specific stem cell niches, plays a fundamental role in driving aging-associated stem cell dysfunction; a process termed ‘inflammaging’. It has been proposed that suppression of bone marrow (BM) inflammaging will have a positive impact on improving hematopoietic health span. However, the relevant cell types and molecular mechanisms that initiate BM inflammaging remain poorly defined. To this end, we have identified that during aging, endothelial cells (ECs) lining the vascular niches within the BM display chronic inflammation that impairs their HSC-supportive niche activity. Our findings demonstrate that elevated levels of Thrombospondin-1 (Thbs1) plays an essential role in driving inflammaging within the BM niche, and that deletion of Thbs1 is sufficient to preserve HSC fitness during aging. Our preliminary data indicates that interaction of Thbs1 with CD36/Fatty Acid Translocase plays a critical role in promoting BM inflammaging. Employing conditional mouse models, targeted metabolomics, and stem cell transplantation assays, our ongoing work is focused on elucidating whether aging-associated increase in Thbs1/CD36 activity instigates inflammaging within the BM, and whether blocking CD36 activity will preserve BM vascular function and hematopoietic healthspan during aging.

The role of IL-6 in promoting skeletal muscle and mitochondrial dysfunction in frailty

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The cytokine interleukin-6 (IL-6) has pleiotropic effects in skeletal muscle, inflammation and aging and is known to be increased in frail older adults as a part of chronic age-related inflammation. However, the direct effects of this chronic age-related inflammation, specifically IL-6, on aging skeletal muscle and skeletal muscle mitochondrial function has not been fully characterized, particularly as it relates to mitochondrial quality and skeletal muscle changes. The overall hypothesis for this project is that persistently elevated IL-6 levels beginning in middle aged mice promote a frailty-like phenotype, leading to adverse changes in skeletal muscle composition, which is mediated by altered mitochondrial quality. We tested this hypothesis using a mouse model with conditional expression of human IL-6 to better characterize the role of increased IL-6 in promoting frailty and age-related skeletal muscle mitochondrial dysregulation. These mice contain a tetracycline-induced human IL-6 element and constitutively expressed mitochondrial quality control element containing mCherry-GFP linked to a mitochondrial outer membrane protein (TetO-hIL6^{mitoQC}). Heterozygous female and male TetO-hIL6^{mitoQC} mice were induced at 7 months old, and physical assessments were performed at 2 months, 4 months and 6 months after induction. We found that after 6 months of induction, induced mice had decreased endurance on treadmill ($p = 0.05$) and rotorod testing ($p = 0.016$), and significant increases in frailty index ($p < 0.001$) compared to uninduced controls. Further characterization of gastrocnemius tissue in induced mice demonstrated increased expression of type 1 collagen but no significant changes to skeletal muscle fiber type when compared to uninduced controls. Additionally, mitoQC analysis of mitochondrial turnover in gastrocnemius demonstrated that when compared to controls, induced mice had lower numbers of intact mitochondria ($p = 0.005$) but a higher percentage of mitochondria cycling through mitolysosomes ($p = 0.04$). Gene expression of mitochondrial markers sirtuin 3 (SIRT3), Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha and Nicotinamide phosphoribosyltransferase (NAMPT) were also significantly lower in gastrocnemius of induced mice compared to uninduced controls. In summary, this work highlights the contribution of IL-6 to mitochondrial dysregulation and supports a causal role of IL-6 in physical decline and frailty.

Gene association scans for longevity in *Drosophila* and human patients

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Long-lived *Drosophila melanogaster* populations were generated by experimental evolution, where four control populations were maintained on a control diet, and four paired populations were selected for longevity on a high-sugar (HS) diet. The HS diet exacerbates aging-associated pathophysiology in these flies and also reduces their lifespan, so the selective pressure is expected to be stronger in HS-fed populations. We observed significant increases in survival in all eight populations after experimental evolution – increases that were associated with significant changes in allele frequencies. How these genes and associated pathways regulate the lifespan and healthspan is the focus of current research efforts. Perhaps surprisingly, there is little overlap at the gene level across the four population pairs, although pathways in neural development and function were overrepresented across populations.

Two neuronally-controlled traits that are associated with aging are feeding and physical activity. Older animals - both flies and humans - exhibit reduced physical activity, and impairments in gait are risk factors for other phenotypic aging traits. With respect to feeding, dietary restriction is associated with increased longevity, whereas overnutrition reduces the lifespan and healthspan across taxa. Therefore, we analyzed feeding and climbing ability in long-lived *Drosophila* with an emphasis on comparing paired control and HS-diet-selected populations. One exceptional population pair, E and F, are the focus of this poster. Both feeding and climbing ability are candidate mechanisms underlying the increased late-life fitness of HS-fed population (pop) E compared to its control, pop F.

Pops E and F exhibited diet-dependent and sex-dependent aging pathophysiology traits that were linked to differences in allele frequency and gene expression. One gene with numerous high-significance single nucleotide polymorphisms (SNPs), encoding the muscarinic acetylcholine receptor mAChR-a, was reduced in HS-selected pop E males and seems to act via control of feeding, since RNAi targeting this receptor led to reduced feeding in HS-fed males. Another gene, encoding the receptor tyrosine kinase Sevenless, was increased after selection on HS, predominantly in pop E females. SNPs in *sev* and its human ortholog *ROS1* were found to be correlated with aging traits. Based on the overlaps between orthologous gene regions that track with gait and physical activity, we hypothesize that Sev/ROS1 regulates muscle function in a manner that benefits those who overeat. Overall, our evolve-and-resequence experiment represents a novel paradigm in which to identify genes that influence the lifespan and healthspan.

Reactive oxygen species (ROS) as neural signal transducers in the aging HSC niche.

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Mammalian aging is associated with reduced tissue regeneration due to the declining function of tissue-specific stem cells. In the blood system, hematopoietic stem cell (HSC) aging is accompanied by an expansion of myeloid-biased HSCs with declined long-term self-renewal, contributing to the development of hematologic malignancies. HSCs are tightly linked with their microenvironment in the bone marrow (BM) for normal function, and HSC aging was shown to depend on the deterioration of their niche. Previous studies have uncovered a candidate perivascular niche cell marked by Nestin-GFP expression. These Nestin-marked stromal cells contain all BM mesenchymal stem cell (MSC) activity and are highly enriched for HSC maintenance and retention factors. Whole-mount confocal immunofluorescence imaging revealed distinct BM Nestin^{+} subsets, where the periarteriolar Nestin-GFP^{bright} and Myh11^{+} (periMSC) subtype is abundantly innervated by nerves from the sympathetic nervous system (SNS) and maintains HSC quiescence. In contrast, the reticular Nestin-GFP^{dim} and leptin receptor (Lepr) expressing MSC subset (reticMSCs) is homogeneously distributed with sinusoidal vasculature, denuded from innervation, and contributes to HSC maintenance.
We have previously shown that the SNS plays a central role in protecting HSCs from aging and that disruption of SNS signals in young mice leads to the expansion of poorly functional reticMSCs and the appearance of intrinsic aging-like phenotypes in HSCs. However, to fully understand the influences of the aging microenvironment on hematopoiesis, we need to address the mechanisms of neural signal transduction within the niche and whether their disruption leads to niche and HSC aging. Preliminary data supporting this application have identified key mechanisms downstream of the SNS in mediating SNS signaling and rely on our finding that perivascular MSC subsets in the BM form a network interconnected by extensive projections from the stromal cell membranes, allowing neural signal transduction that depends on connexin gap junctions (CxGJs). Furthermore, we have found that NADPH oxidase (NOX)-derived reactive oxygen species (ROS) in reticMSCs oscillate in a circadian manner and are diminished following sympathectomy or aging. In addition, disruption of NOX, specifically in periMSCs, disrupted reticMSC ROS oscillations and expanded both reticMSCs and HSCs in the BM. Based on these findings, we hypothesize that NOX-derived ROS originating from periMSCs transmit SNS signals via CxGJs to reticMSCs to elaborate neural signals beyond innervated niches and that disruption of ROS transmission can facilitate HSC dysfunction and hematopoietic aging.

The aging bone marrow stromal niche is depleted of Cyr61/CCN1, a key extracellular regulator of skeletal MSCs

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Aging-related skeletal degeneration involves declining function of bone mesenchymal stem cells (MSCs). The fate of MSCs is controlled by complex networks of regulatory cues in the local microenvironment (niche). However, changes in the aging bone marrow (BM) niche remain largely undefined. To address this gap, we produced ECMs *ex vivo* using BM stromal cells harvested from “young” (≤25 y/o) and “aged” (≥60 y/o) donors. Relative to aged extracellular matrix (aECM), young ECM (yECM) exhibited greater fibrillar organization and mechanical stiffness. Proteomic analyses indicated that Cyr61/CCN1, a matricellular protein containing binding motifs for osteogenic growth factors, BMP-2 and IGF-1, was notably absent in aECM. Moreover, MSCs on yECM demonstrated significantly higher responsiveness to both growth factors. In osteoblast-like cells, Cyr61 binds integrin $\alpha_V\beta_3$, to promote proliferation, differentiation and BMP-2 expression. Knock-down of Cyr61 in yECM abrogated responsiveness to both BMP-2 and IGF-1, suggesting a significant role for *ECM-bound* Cyr61 in regulating osteogenesis. More importantly, replenishing Cyr61 in aECM restored responsiveness of cultured MSCs to both growth factors. Analysis of L4-L5 vertebral bodies from “young” (9-11 m/o) and “aged” (21-33 m/o) mice showed a correlation between lower BMD and relative deficiency of Cyr61. Our study proposes a novel role for ECM-bound Cyr61 in regulating skeletal MSCs and demonstrates employing cell-derived matrices for studying aging of tissue-specific progenitor niches.

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Title: Novel Therapeutic Strategies in Age Associated Beta Cell Dysfunction
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Rationale: Alterations of glucose homeostasis and insulin secretory capacity increase with age and represent leading causes of morbidity and mortality, mainly linked to both the complications associated with type 2 diabetes and the elevated risk for several other age-related diseases. In order to prevent and/or improve diabetes-associated ailments of the pancreatic beta cell during aging we propose to leverage the therapeutic properties of ketone bodies. Ketones have long been known to improve survival of nerve cells and, interestingly, despite arising from different germ cell lines, pancreatic beta cells share significant common genetic and physiological characteristics with neurons. Of note, although excessive production of ketone bodies leads to life-threatening ketoacidosis in diabetic patients under glucose lowering treatment, emerging lines of evidence suggest that modest levels of ketones play adaptive and beneficial roles. Specifically, a functional role as key signaling molecule is increasingly acknowledged for beta-hydroxybutyrate (BHB), the most abundant ketone circulating in the human body. However, therapeutic applications of BHB have never been considered in age-related pancreatic beta cell dysfunction.
Hypothesis: In view of the known 1) anti-aging effects of ketone bodies, 2) role of mitochondria in beta cell homeostasis, and 3) role of autophagy in aging processes, our overarching hypothesis is that beta-cell dysfunction in old mice can be prevented or delayed by low levels of BHB, as a physiological attempt to adapt to a pathological condition.
Methods: The effects of BHB on metabolic fitness have been tested: 1) *in vivo* in 18-month-old C57BL/6J mice fed with a standard or BHB-enriched diet for 6 months; 2) *ex vivo* in murine islets isolated from aging mice fed with standard or BHB diet 3) *ex vivo* in islets isolated from brain-dead aging donors treated or not with BHB. The effect of BHB on apoptosis, ROS production, mitochondrial health and selective autophagy mechanisms have been also evaluated in both murine and human islets during aging.
Summary of Results: In our preliminary studies, we found that 18-month-old C57BL/6J mice fed with a BHB-enriched diet for 6 months displayed a significantly ameliorated response to GTT compared to sex and age-matched mice fed with standard diet. Furthermore, BHB treatment increased islet yield and protected pancreatic islets from apoptosis and oxidative stress, as observed by a significant decrease of TUNEL positive nuclei and of ROS production in islets isolated from old mice fed with a BHB-enriched diet. Considering that the loss of mitochondrial integrity is one of the main mechanisms underlying the progressive age induced-beta cell failure we explored the *ex vivo* effect of BHB on murine and human islets with a particular emphasis on their mitochondria in the setting of aging. We observed an impaired glucose stimulated insulin secretion in islets isolated from old mice fed with standard diet and from aging donors that was significantly reduced by BHB in both systems. In line with these results, BHB preserved mitochondrial membrane potential, attenuated the impaired mitochondrial respiration and mitochondrial fragmentation and rescued mitochondrial biogenesis during aging. Finally, we saw a significant increase in mitophagy (a selective form of autophagy that modulates clearing non-functional mitochondria) in aging murine and human islets after treatment with BHB, compared with control islets; confirming that BHB has a pivotal role in the modulation of mitochondrial fitness.
Conclusions: In this study we produced for the first time compelling evidence that mitochondrial alterations represent a targetable molecular signature in aging beta cells and that ketone bodies are potentially useful to address age-related beta cell dysfunction. Indeed, the BHB-mediated pathway may act synergistically with pharmacotherapy and might emerge as a glucose-lowering agent able to suppress the vicious cycle of hyperglycemia, ultimately improving the life of elderly people living with diabetes.

The pathophysiological role of Succinate Dehydrogenase deficiency in β-cell aging and diabetes

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Aging is a major risk factor for Type 2 Diabetes (T2D), a disease of glucose homeostasis characterized by progressive pancreatic β-cell failure. Mitochondrial dysfunction is a central contributor to aging and T2D pathogenesis, but much is unknown about the underlying mechanisms in β-cells. Succinate Dehydrogenase (SDH) is a mitochondrial enzyme that 1) catalyzes the oxidation of succinate to fumarate in the tricarboxylic acid (TCA) cycle and 2) participates in the electron transport chain (ETC) as complex II. Interestingly, analysis of human pancreas samples demonstrated a diabetes-associated reduction in SDH subunit B (SDHB) expression in β-cells. Spurred by these data, we generated and characterized a novel diabetes model of mitochondrial dysfunction: transgenic mice with a β-cell-specific deletion of SDHB, a key mitochondrial protein. We found that SDHB knockout mice ($SDHB^{\beta KO}$) have impaired glucose-stimulated ATP generation and insulin secretion, culminating in insulinopenic diabetes, a phenotype akin to age-related diabetes. Mechanistically we found that excess succinate, in the context of SDH dysfunction, aberrantly activated mTORC1 signaling and engendered a senescent-like phenotype, including dysregulated autophagy and up-regulated lipid metabolism. Based on these observations, we hypothesized that age-related SDH deficiency in β-cells aberrantly activates mTOR signaling, which drives premature senescence and β-cell aging. First, we found that, compared to young donors (<45 years), human islets from aged donors (>65 years) had impaired glucose-stimulated mitochondrial respiration and a significant reduction in spare reserve capacity, implicating SDH disruption. Similarly, we found that islets from aged C57BL/6 (B6) mice (18 mo) had a 50% reduction in SDH activity and an aberrant accumulation of succinate. Mimicking the hallmarks of aging and senescence, aged B6 islets had an inappropriate mTORC1 activation, and mTORC1 inhibition with rapamycin (an “anti-aging” compound) rescued the insulin secretion defect. Notably, in other metabolic tissues, reduced SDH activity results in the build-up of succinate, triggering a stress response via paracrine succinate receptor, SUCNR1, signaling. To delineate the mechanism by which SDH dysfunction affects mTORC1, we evaluated the effects of SDH inhibition (3-nitropropionic acid; 3-NPA) on immortalized R7T1 β-cells and found that 3-NPA-treated β-cells had increased succinate release into the medium, and potentially activated mTORC1 via SUCNR1. Taken together, these results demonstrate a surprisingly necessary role for the SDH complex in age-related β-cell mitochondrial function and introduce a pathogenic mechanism of β-cell aging that pertains to the increased prevalence of T2D in the aged population.

Title: Reversing a persistent “nighttime state” that limits memory in aging mice

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Abstract: Aging is accompanied by impairments in both memory and circadian rhythmicity, but it is unclear how these declines are connected. Recent work from our lab and others suggests that clock genes may functionally link the central circadian system with peripheral processes, like memory formation, providing a possible mechanism that, if impaired across the aging brain, could contribute to impairments in both systems. Although clock genes are best known for their roles in maintaining circadian rhythms in the suprachiasmatic nucleus (SCN), they are also expressed in other brain structures, including those critical for memory, like the dorsal hippocampus and retrosplenial cortex. Here, we first determined that spatial memory consolidation is best during the day (noon/ZT5) and worst at night (midnight/ZT17) in young (3-m.o.) mice. Next, using RNA-seq, we identified the clock gene *Per1* as a potential mechanism that might support robust memory during the day but only weak memory at night. To determine whether *Per1* might contribute to age-related memory impairments, we next compared *Per1* expression in the hippocampus of young and old mice and found that old mice with severe memory impairments fail to show learning-induced increases in local *Per1*. Further, bidirectional manipulation of *Per1* in the hippocampus modulated memory; reduction of *Per1* in young mice impairs memory whereas overexpression in old mice can ameliorate age-related memory impairments. In a second memory-relevant brain region, the retrosplenial cortex, a similar pattern was observed, with dampened *Per1* expression in the old brain compared to the young brain. Interestingly, in the brain’s central clock, the SCN, learning-induced increases in *Per1* were also impaired in the old brain. Finally, to test whether *Per1* supports memory in the retrosplenial cortex and SCN, we used viral CRISPRi to locally inhibit *Per1*. In the retrosplenial cortex, knockdown impaired memory formation, but in the SCN, the same knockdown had no impact on memory, suggesting *Per1* is selectively capable of supporting memory in memory-relevant brain regions. Together, our research suggests that the clock gene *Per1* may function locally within memory-relevant brain regions to support diurnal oscillations in memory in addition to its well-characterized role in the central circadian system. Further, our data are consistent with the idea that repression of *Per1* across the aging brain could contribute to impairments in both memory and the circadian clock, with reduced *Per1* mimicking a persistent “nighttime state” in the old brain.

Intergenerational effect of maternal age on offspring metabolome

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Advanced maternal age is associated with a decline in offspring health, lifespan, and stress resistance in a wide range of species, including humans. This is a phenomenon known as “maternal age effects.” However, the molecular and cellular mechanisms causing maternal age effects are unknown. *Brachionus manjavacas*, an aquatic invertebrate rotifer, has a two-week lifespan and a high maternal reproductive investment, which makes it a useful model to study aging and maternal age effects. In this study, we found that maternal age affects offspring lifespan, reproduction, and stress resistance in rotifers. Although old-mother offspring have a longer lifespan, they also have lower fecundity and are more sensitive to heat stress in early life and to rotenone in late life compared to young-mother offspring. To try to understand the mechanism causing intergenerational effects of maternal age, we compared the metabolomes of young, middle-aged, and old females; and of offspring born to those mothers. We found that many mitochondria-associated metabolites decrease not only with age, but also with increasing maternal age. In particular, most metabolites in glutathione metabolism and arginine biosynthesis pathways are lower in the early life of old-mother offspring compared to young-mother offspring. Interestingly, spermidine, which links the glutathione and arginine pathways, is higher in old-mother offspring. As spermidine is reported as a geroprotector and autophagy inducer, it may contribute to the longer lifespan of old-mother offspring. These findings will provide targets for future mechanistic investigation of maternal and metabolic effects on offspring health and lifespan.

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Exploring the mechanisms by which metformin attenuates several health benefits of exercise

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The multi-morbidity of aging limits the value of targeting any single age associated disease. Therefore, there is a need to identify treatments to intervene on fundamental mechanisms of aging to prevent the onset or delay the progression of multiple age-related conditions. Regular exercise is the current gold standard intervention to maintain and extend human health span. Alternatively, metformin decreases risk of morbidities and mortality in humans with hyperglycemia and type 2 diabetes. Despite exercise and metformin individually improving several molecular, cellular and physiological hallmarks of aging, we and others have shown that adding metformin to exercise training, diminishes the exercise-mediated increase in skeletal muscle mitochondrial respiration, insulin sensitivity, cardiorespiratory fitness, and skeletal muscle mass and function after exercise training. It remains far from understood how metformin and exercise interact. The goal of this pilot project was to explore the mechanisms by which metformin may antagonize exercise adaptations. To achieve this goal, we performed an 8-week progressive aerobic treadmill exercise training (AET, 5d/week, 60 min/day, 70-80% max workload) program in 8-month-old male C57BL6/J mice with (AET+MET) or without metformin (300mg/kg/day) in the drinking water and sedentary control (SED) (n=7-9/group). AET increased running capacity and glucose tolerance, but this was not apparent in the AET+MET group. Using high resolution respirometry with permeabilized muscle fibers, metformin prevented the increase in complex I (CI) and complex I+II (CI+II) linked respiration after AET. Additional analyses of site-specific hydrogen peroxide (H_2O_2) emissions in isolated skeletal muscle mitochondria revealed AET+MET increased H_2O_2 emissions at the flavin site of complex I (I_F) versus AET while there was no differences between groups at the quinol site in complex I (I_Q). Next, we performed RNA sequencing to understand pathways in skeletal muscle that may be associated with the inhibitory effects of metformin on skeletal muscle mitochondrial respiration, glucose metabolism, and mouse running performance. Principal component analysis revealed a distinct clustering of the muscle transcriptome where exercise and metformin each account for ~25-30% of the variance. Globally the number of differentially expressed upregulated (AET: 139 genes vs. AET+MET: 45) and downregulated (AET: 138 genes vs. AET+MET: 108) genes by aerobic exercise was attenuated by metformin. While there were no statistically different enriched pathways between AET vs. AET+MET, there were several pathways differentially enriched between AET and AET+MET vs. SED. Compared to SED, AET largely increased anti-viral and cell adhesion pathways while suppressing PPAR signaling and fatty acid degradation pathways. Conversely, AET+MET suppressed TCA cycle, OXPHOS, branched chain amino acid degradation, and carbon metabolism compared to SED. Collectively, our data in adult mice largely recapitulate the inhibitory effect of metformin on whole body cardiometabolic and skeletal muscle mitochondrial adaptations to exercise in humans. Further our data indicate metformin suppresses key metabolic processes in skeletal muscle that may contribute to how metformin attenuates several health benefits of exercise.

Hepatic WDR23 proteostasis mediates insulin clearance by regulating insulin degrading enzyme activity

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The clearance of insulin from circulation is a critical facet of metabolic homeostasis. Insulin is effectively cleared in the liver in part through the activity of the insulin degrading enzyme (IDE). WDR23 is a substrate receptor of the Cul4-ubiquitin ligase complex and acts as a sophisticated regulator of protein activation and turnover. Here we establish hepatic WDR23 in the regulation of insulin metabolism by regulating the abundance of IDE. An unbiased proteomic analysis of liver tissue of mice lacking Wdr23 revealed a significant increase in the steady state levels of the insulin degrading enzyme (IDE) which accompanied a diminished sensitivity to insulin stimulation, and enhanced IDE activity. A comparative assessment of the transcriptome of isolated livers from animals with and without WDR23 reveals significant changes in the transcriptional targets that respond to insulin and glucose receptor signaling. Furthermore, the phosphorylation state of regulatory factors in the insulin signaling cascade, IRS-1, AKT, MAPK and mTOR were dysregulated in Wdr23KO mice. These findings are recapitulated in cultured human cell models with genetic ablation of Wdr23 revealing a conserved role for WDR23 from mice to humans. Mechanistically, we uncovered a role for the cytoprotective transcription factor NRF2, a direct target of WDR23-Cul4 proteostasis, that mediates the enhanced transcriptional expression of IDE when WDR23 is ablated. Our study reveals WDR23 as a new biomarker of insulin metabolism and diabetes.

BCL-2 Expression is Required to Define Senescent p16+ Cells in the Aged Murine Skeleton

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Cellular senescence contributes to age-related bone loss, yet the cells that become senescent *in vivo* remain poorly understood. This is due to heterogeneity of both skeletal cell types and senescent cell signatures that present technical challenges in delineating the cell types driving this aging phenotype. To address this, we developed and validated a mass cytometry (CyTOF) panel to define, characterize, and profile skeletal senescence at the single-cell level in mice. We enzymatically digested femurs and tibias from cohorts (n=12-15) of young (6-month) and old (24-month) mice to generate single-cell suspensions, which were then purified for mesenchymal cells (Lin-CD45-) before CyTOF analysis. We found that cells positive for the senescence marker p16 were diverse in their expression of senescence-associated markers, while also containing non-senescent (Ki67+) subpopulations. Within p16+ cells, we found a subset positive for the apoptosis resistance marker BCL-2 that was non-proliferative (Ki67-) and expressed inflammatory and DNA damage markers at a high level. In addition to CyTOF, we also identified these p16+Ki67-BCL2+ (“p16KB”) cells using scRNA-seq, which revealed that this was a highly secretory cell population that was enriched in senescence-associated secretory phenotype (SASP) gene expression, applied with the SenMayo geneset. Altogether, these p16KB cells fulfilled major criteria for senescence through a multiparametric approach. In our skeletal cell clusters, we found that both p16+ and p16KB cells were upregulated in an osteolineage population defined by high expression of CD24. We found this population to be the predominant cluster that was cleared through p16+ senescent cell clearance in aged *INK-ATTAC* mice. Additionally, we found that CD24 osteolineage cells were also cleared in the bones of aged wild-type (C57BL/6N) mice treated with the senolytic combination of Dasatinib and Quercetin. Upon isolation, CD24+ mesenchymal cells from the bones of aged mice exhibit drastically reduced colony formation efficiency and spontaneous senescence-associated β-galactosidase expression, consistent with functional outcomes of senescence. In summary, used a multiparametric approach to characterize senescent cells in the bone microenvironment, revealing a senescent osteolineage population in the murine skeleton that is targeted by senolytic therapy.

Title: The senescence-associated secretory phenotype of primary human chondrocytes

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Nathan Shock Center Collaborators: Judy Campisi and Birgit Schilling, The Buck Institute

Background, Significance, and Rationale

Aging is the leading risk factor for osteoarthritis (OA), due in part to age-related changes at both the tissue and cellular levels. At the tissue level, the loss of glycosaminoglycans (GAGs) and accumulation of advanced glycation end products (AGEs) results in a less resilient and more catabolic response to mechanical loading. At the cellular level, a subset of chondrocytes undergo senescence with aging, injury, and OA. The senescence-associated secretory phenotype (SASP) produced by these cells disrupts the overall function of the tissue; clearing senescent chondrocytes after joint injury increases matrix synthesis and decreases inflammatory gene expression. The goal of this Nathan Shock Center pilot project was to use our established systems and the proteomics expertise of the Cellular Senescence and Beyond Core (CSBC) at the Buck Institute to define the SASP of primary human chondrocytes.

Methods

Cartilage explants were harvested from cadaveric tissue donors without a clinical history of OA or macroscopic cartilage damage. We used our established protocol of DNA damage (10 Gy irradiation) and mitogenic stimulation (1 ng/ml TGF-β1 and 5 ng/ml bFGF) to induce a robust senescence phenotype[1]. These cells were then digested from the explant and transferred to monolayer culture under standard 10% serum conditions (all groups) as part of a senescence maturation phase. This period of monolayer culture also facilitates the collection of SASP factors in conditioned media. After 24 hours in serum-free conditions, the media was harvested for shipment to the CSBC for proteomic analysis.

Results

There were 62 proteins that were significantly upregulated in senescence-inducing conditions as compared to control (Q value <0.05, absolute log2ratio > 0.58). These proteins included previously identified SASP factors from other cell types and senescence induction conditions (MMP2, IGFBP2, SERPINA1, etc.) as well as factors that may be particularly enriched in chondrocytes. One such example is cartilage oligomeric matrix protein (COMP), which had a log2ratio of 4.24 and a Q value of 1.3e-9. COMP is of particular interest, as the presence of COMP at baseline and after 3 years of follow-up were both predictive of higher OA progression[2].

Future directions and long-term collaboration

Excessive mechanical loading of cartilage can lead to matrix degradation and cellular dysfunction, but whether specific loading patterns (or distinct tissue properties that emerge during aging) lead to increased levels of cellular senescence remain unclear. Further, the SASP may be influenced by the type of DNA damage and therefore comparisons of excessive loading and irradiation are warranted for a more complete understanding of how the chondrocyte SASP may contribute to OA.

References

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Effects of calorie restriction on in the aged lacrimal gland

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Purpose: Aging is a significant risk factor for dry eye disease, a tear-dysfunctional disease affecting millions worldwide. The lacrimal gland (LG) secretes growth factors and the aqueous part of the tear film. Calorie restriction (CR) has been shown to reduce the expression of inflammation and increase the life span in many organisms. This study investigated the effects of CR on the inflammation and lipid composition of aged LGs.

Methods: Female C57BL/6J (B6) mice of different ages were used to investigate the time course of inflammatory changes in the LG. Additional groups of B6 mice were subjected to two CR schemes: 1) preventive, from 6 months (6M) to 11M, and 2) curative, from 15M to 21M. Using Mass Spec, LGs were excised for histology, bulk RNA sequencing, or lipidomic analysis. H&E-stained LGs were scanned, photographed, and image analysis was used to calculate the focus score/4mm^2. The inflammatory foci (>50 infiltrating cells) were counted under a 10X microscope lens.

Results: Aged LGs have significantly increased inflammatory markers such as *Tnf* and *Ifng*. *Ctss*, *Ciita*, and *Il12* expression peaked at 12M and remained significantly elevated at 24M. The two CR schemes significantly blunted the age-related dry eye phenotype compared to the ad-libitum group. Bulk RNA sequencing of LGs subjected to the preventive CR showed an up modulation of circadian rhythm and increased genes related to secretory function and lipid metabolism. Downregulated pathways included genes involved in immune activation, B-cell receptor signaling, adaptive immune system, and extracellular matrix remodeling. Individual PCR validated these changes, as CR significantly decreased *Tnf* (46%), *Il1b* (46%), *Ifng* (60%), *Ciita* (64%), *Ctss* (46%), *Il12* (81%), *Cd4* (65%), *Cd19* (84%), and *Glycam1* (95%) (all $p<0.05$) while decreasing inflammasome-related genes. Untargeted lipidomics showed that CR reversed many age-related changes in lipids, including the decrease in sphingomyelin and phosphatidylcholine.

Conclusions: Our results indicate that CR can ameliorate the increase in age-related cytokine production and decrease LG inflammation. Further studies are needed to evaluate the mechanism of CR-mediated protection.

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Role of the PI3K-mTOR pathway in glucose-stimulated insulin secretion from pancreatic β cells

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Dysregulation of mechanistic target of rapamycin complex 1 (mTORC1) signaling has been linked to several metabolic diseases, including obesity and type 2 diabetes. mTORC1 senses local (nutrients, stresses) and systemic (growth factors) signals to control cell and tissue growth as well as systemic metabolism. Cell culture studies have found that insulin and growth factors activate mTORC1, in part, via PI3K signaling and the Akt-mediated phosphorylation of TSC2, which is the core functional component of the tuberous sclerosis complex (TSC) protein complex, an essential inhibitor of mTORC1. Akt-dependent phosphorylation of TSC2 inhibits the TSC complex to activate mTORC1. However, the physiological role of this regulation in vivo is unknown.

We have generated a novel conditional mouse model that expresses a TSC2 mutant lacking the 5 known Akt-regulatory sites (TSC2-5A) to specifically disconnect PI3K-Akt signaling from mTORC1 regulation, while retaining normal Akt activation and mTORC1 control by other upstream signals. Surprisingly, mice with whole-body expression of the TSC2-5A allele fully complement the embryonic lethality of *Tsc2*$^{-/-}$ mice, indicating that these phosphorylation sites are dispensable for normal mammalian development. However, relative to littermate TSC2-WT mice, TSC2-5A mice are strongly glucose intolerant, while maintaining insulin sensitivity, suggesting a defect in glucose-stimulated insulin secretion. This metabolic phenotype is associated with reduced mTORC1 activation in primary pancreatic islets isolated from TSC2-5A mice. Previous studies using mouse models with chronic activation or inhibition of mTORC1 in β-cells concluded that its primary role is in controlling β-cell mass and insulin production. However, β-cell mass, islet size and insulin content were unaffected in the TSC2-5A pancreas. Instead, we found that TSC2-5A β-cells are unable to acutely secrete insulin in response to glucose stimulation. Interestingly, TSC2-5A islets can secrete insulin to a level comparable to TSC2-WT when treated with KCl, demonstrating that insulin levels and the secretory machinery are intact. These findings provide novel evidence that the Akt-mediated activation of mTORC1 in pancreatic β-cells is required for proper glucose sensing and metabolism to trigger insulin release.

RNA virus-mediated changes in organismal oxygen consumption rate in young and old *Drosophila melanogaste*r males

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Aging is accompanied by increased susceptibility to infections including with viral pathogens resulting in higher morbidity and mortality among the elderly. Significant changes in host metabolism can take place following virus infection. Efficient immune responses are energetically costly, and viruses divert host molecular resources to promote their own replication. Virus-induced metabolic reprogramming could impact infection outcomes, however, how this is affected by aging and impacts organismal survival remains poorly understood. RNA virus infection of *Drosophila* with Flock House virus (FHV) is an effective model to study antiviral responses with age, where older flies die faster than younger flies due to impaired disease tolerance. Using this aged host-virus model, we conducted longitudinal, single-fly respirometry studies to determine if metabolism impacts infection outcomes. Analysis using linear mixed models on Oxygen Consumption Rate (OCR) following the first 72-hours post-infection showed that FHV modulates respiration, but age has no significant effect on OCR. However, the longitudinal assessment revealed that OCR in young flies progressively and significantly decreases, while OCR in aged flies remains constant throughout the three days of the experiment. Furthermore, we found that the OCR signature at 24-hours varied in response to both experimental treatment and survival status at 48- and 72-hours. FHV-injected flies that died prior to 48- or 72-hours measurements had a significantly lower OCR compared to survivors at 48-hours. Our findings suggest the host's metabolic profile could influence the outcome of viral infections.

Abstract title: Cardiac aging and heart failure with preserved ejection fraction induce distinct proteomic and phosphoproteomic remodeling in murine hearts.

Authors: Ying Ann Chiao, Kamil Kobak, Mario Leutert, Ricard A Rodriguez-Mias and Judit Villen

Heart failure with preserved ejection fraction (HFpEF) is an emerging health problem with high morbidity and mortality and accounts for 50% of all heart failure cases. Currently, there is a lack of effective, evidence-based treatments for HFpEF and a better understanding of its molecular mechanisms is critical for development of new HFpEF therapies. The prevalence of HFpEF increases sharply with age but despite this strong association with advanced age, the impacts of aging on HFpEF pathogenesis have not been established. The objective of the study is to determine the impacts of aging on cardiac proteomic and phosphoproteomic remodeling during HFpEF.

We employed a 5-week HFpEF-inducing regimen with a combination of metabolic (by high fat diet) and hypertensive (by L-NAME) stress, to induce HFpEF development in young and old mice. LC-MS/MS was performed on cardiac tissue samples before and after phosphopeptide enrichment to compare cardiac proteome and phosphoproteome of 1) young control, 2) young HFpEF, 3) old control and 4) old HFpEF mice. Our proteomic analysis showed that while aging induced a drastic proteomic remodeling in the heart, HFpEF development induced modest cardiac proteomic changes at both young and old ages. This suggests that HFpEF and aging mediate cardiac dysfunction through different mechanisms at proteomic levels. Our phosphoproteomic analysis found that while both aging and HFpEF development were accompanied by a significant remodeling of cardiac phosphoproteome, the phosphoproteomic changes induced by aging were different from the changes induced by HFpEF development. Interestingly, HFpEF development induced differential phosphoproteomic changes at the two age groups. For example, young HFpEF mice exhibited reduced levels of phosphorylation of T172 of AMP-activated protein kinase (AMPK2) compared to young control mice but old HFpEF mice exhibited increased T172 phosphorylation of AMPK2 compared to old control mice. The differential HFpEF-induced phosphoproteomic remodeling in the two age groups suggests that certain molecular mechanisms of HFpEF could be age-dependent. Together, our results support the importance to investigate the specific impacts of aging on HFpEF development and to identify molecular mechanisms of HFpEF using old HFpEF models.

The study is supported by Oklahoma Center for Adult Stem Cell Research (OCASCR) and a pilot project award from University of Washington Nathan Shock Center.

B cells promote inflammation and metabolic dysfunction in aged adipose tissue during endotoxemia and sepsis

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Sepsis is a life-threatening, systemic response to infection and is 13-times more likely to occur in individuals over 65, leading to hospitalization, increased mortality, and chronic repercussions. Why older persons are more susceptible to sepsis is poorly defined, although hyperactivation of the immune system is an underlying feature. Visceral white adipose tissue (vWAT) is the organ that ages first, including tissue expansion and increased immune cell activation. Immune cells inhibit stimulated lipolysis, a metabolic pathway essential for maintaining energy homeostasis. Lipolysis is also necessary to keep inflammation in check in mice challenged with lipopolysaccharide (LPS), a bacterial pathogen-associated molecular pattern from gram-negative bacteria. We investigated the contribution of aged B cells to metabolic dysfunction and inflammation during endotoxemia in vWAT. Old vWAT exhibited elevated inflammation and altered activation of resident B cells during LPS-induced endotoxemia. LPS induced vWAT lipolysis in young mice, but not in old mice. Diet induced obesity resulted in elevated LPS-induced inflammatory cytokines and B cells that failed to become activated by LPS. Deficiency of total B cells improved the homeostatic balance of immune cells within vWAT microenvironment, resulting in increased lipolysis and reduced *Tnfα* during endotoxemia in old mice. In contrast, B2 B cell depletion was insufficient to increase LPS-induced lipolysis or reduce inflammatory cytokines in vWAT from old mice, suggesting a role for B1 B cells. B1 B cells from young and old vWAT, but not primary lymphoid organs, were differentially activated by LPS or septic challenge, indicating age-associated dysfunction that is localized to vWAT. Our results demonstrate that endotoxemia results in immune and metabolic activation within the vWAT, both of which are dysfunctional during aging. These data reveal that B cell subsets may differentially impair lipolysis and exacerbate the inflammatory response in vWAT following pathogenic challenge.

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Aberrant endoplasmic reticulum dynamics as a driver of age-onset dysfunction

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The endoplasmic reticulum (ER) acts as a central hub for several critical processes that are dysregulated in aging, including protein homeostasis, lipid synthesis, calcium signaling and inter-organelle communication. To perform these diverse roles, structurally distinct ER subdomains associate with specific functions, including for example rough ER sheets with protein folding and secretion, or smooth ER tubules with lipid and membrane synthesis. Furthermore, specialized cell types employ conserved ER-shaping proteins to promote highly specific and often polarized ER network morphologies that are optimized for the predominant function(s) of the cell. Yet, despite the established links between several ER functions and age-related pathogenesis, we know surprisingly little about how ER structure-function evolves during aging or in contexts of age-related disease. To address this gap, we have combined live-imaging of native-labeled ER proteins and 3D volumetric electron microscopy (FIB-SEM) in *C. elegans*. We demonstrate that ER organization is extremely dynamic during aging. Virtually every tissue exhibits significant changes in the morphology or protein composition of the ER—and in most cases both changes occur. In our current work, we have focused on the intestine and epidermis of the worm given their central roles in these animals' metabolic functions. In these tissues, we observe a dramatic reduction in total ER content, but an especially pronounced loss of well-organized rough ER sheets and key components of the translocon, consistent with age-dependent failures in proteostasis. In contrast, lipid droplets and lipid-associated vesicles increase in abundance and associate physically with the aged ER, indicative of an overarching shift in roles of the ER from protein secretion to lipid metabolism. Intriguingly, the ER remodeling occurs as a very early event in the aging process, preceding other established hallmarks such as the rapid decline in protein homeostasis and altered mitochondrial dynamics. Altogether, our work suggests that maladaptive changes in ER structure-function act as an early trigger of metabolic and functional decline, not only of the ER but also through its impacts on neighboring organelle networks.

Unraveling circadian aging: The interplay of age and high-fat diet in circadian desynchrony

2023 AFAR Grantee Meeting Abstract

Keisuke Fukumura, Annika Barber

Daily rhythms of activity are coordinated by a master circadian clock in the brain, which serves as a pacemaker for clocks in every tissue of the body. These molecular clocks serve to keep organisms in sync with the environment and to coordinate the timing of physiological activity across organ systems. Aging and nutritional stress both contribute to the breakdown of circadian rhythms of behavior and metabolism. Deterioration of circadian physiology contributes to the onset and progression of common pathologies of aging including metabolic syndrome, diabetes, and neurodegenerative disorders. While the consequences of age and dietary stress on circadian physiology have each been studied individually, the potential synergistic effects have not been evaluated. We used the *Drosophila* model to examine the hypothesis that a high-fat diet accelerates aging circadian phenotypes. After first identifying an appropriate HFD condition, we found that aging on a standard diet suppressed rhythmic oscillation of the clock mRNA *timeless* in fly heads. On the other hand, aging on HFD suppressed rhythmic oscillation of the clock mRNA *period*, while *timeless* transcription remained robustly rhythmic. This suggests that HFD may have unique effects on circadian aging, protecting some rhythms while dampening others. Ongoing work will investigate the role of aging and HFD in deterioration of circadian rest:activity rhythms, and examine how the coincidence of age and dietary stress affects the phase relationships between clock neuron groups in the brain, and between central and peripheral clocks. To test the mechanistic potential of these observations, we will genetically induce phase desynchrony, or different clock speeds between different tissues and examine the effects on survival, circadian metabolism and circadian rest:activity behavior. Our findings thus far illustrate that “circadian aging” in a complex phenomenon that must consider factors affecting peripheral clocks, such as diet.

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 $G_{\alpha q}$ 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 $G_{\alpha q}$ signaling in young and aged animals was a single sensory neuron pair, the AWC, and further established that $G_{\alpha q}$ 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 $G_{\alpha q}$ signaling mutants. We find that members of each of the three major neuropeptide families in the worm (insulin-like peptide/INS, 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. In ongoing work, we are examining the roles of these receptors in associative behaviors and their neuronal sites where they exert their effects on learning and memory. We are also examining if peptide administration using a novel feeding-based approach can slow cognitive aging in the worm. 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.

The MSR enzymes and Alzheimer's Disease

Shailaja Allani, Santiago Matos, Mariana Berto Udo, Miguel Lopez-Toledano, Hung Wen Lin, Herbert Weissbach

Alzheimer's disease (AD), an age-related neurodegenerative disorder, is characterized by progressive loss of memory and impairment of cognitive function. According to Alzheimer's association's recent reports, it is the seventh most leading cause of death during the COVID-19 pandemic in the US. Oxidative damage and mitochondrial dysfunction have been shown as major contributing factors for accumulation of amyloid beta (Aβ) plaques. Another important factor in AD progression may be the oxidation of methionine at position 35 in Aβ (1-42) and several studies have indicated that oxidized Met^{35} accelerates neurotoxicity of Aβ.
The Methionine sulfoxide reductase (MSR) family of enzymes, comprised of MSRA, and 3 MSRB species (MSRB1, MSRB2, MSRB3) catalyze the reduction of protein bound methionine sulfoxide residues to methionine which can restore the function of proteins in which methionine is at the active site. In addition, the MSR system permits all exposed methionine residues in proteins to function as catalytic antioxidants. In this study the potential role of Met^{35} and the MSR system in the pathogenesis of AD is investigated. Our results show that oxidized Met^{35} forms high molecular weight fibrillar aggregates, indicating that Aβ aggregation is influenced by the oxidation state of Met^{35}. It is also shown that protein levels of MSRA are decreased in the hippocampus of AD transgenic female mice as compared to the wildtype mice, and that the enzymatic activity of both MSRA and MSRB2 are decreased in the hippocampus when compared to the cortex in female 3xTg-AD mice. It is interesting to note that these changes in MSR activity were not detected at the RNA level as evidenced by RNA-seq analysis obtained from the female 3xTg-AD mice. Unexpectedly, preliminary aging results indicate a small but a significant difference in the protein levels of MSRA and MSRB2 in both the cortex and hippocampus in male and female 3xTg-AD mice; in males the protein levels of these enzymes actually increased in the older 3xTg-AD mice, whereas in older females the protein levels of these enzymes decreased compared to the younger mice. Our data show a potential role of the MSR enzyme system to attenuate oxidative damage and suggest that activation of the MSR system is a potential therapeutic target for AD treatment.

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