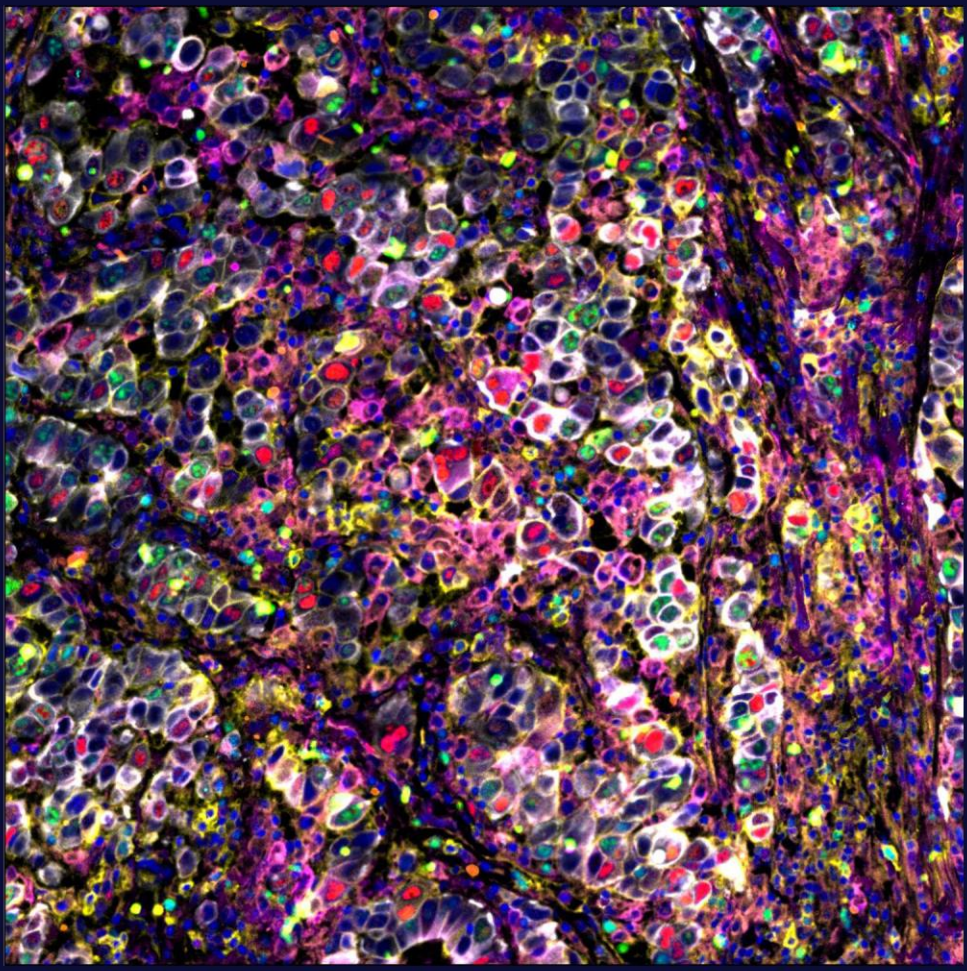
Abstracts of papers presented
at the LXXXIX Cold Spring Harbor Symposium
on Quantitative Biology

SENESCENCE & AGING

May 28–June 1, 2025



Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

Abstracts of papers presented
at the LXXXIX Cold Spring Harbor Symposium
on Quantitative Biology

SENESCENCE & AGING

May 28–June 1, 2025

Arranged by

Terri Grodzicker, David Stewart & Bruce Stillman
Cold Spring Harbor Laboratory

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MEETINGS & COURSES PROGRAM

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Cover: Multiplex IF image showing accumulation of senescent cancer cells in a human lung cancer post therapy.
Markers: Senescence: uPAR (in pink), P21 (red), P53 (orange), pSTAT1 (cyan), and MHCI (yellow); Tumor cells: PANCK (white); Cycling cells: KI67 (green).
Credit: Aveline Filliol, Matt Bott and Maria Skamagki (Lowe Lab).

SYMPOSIUM LXXXIX: SENESCENCE & AGING
Wednesday, May 28 – Sunday, June 1, 2025

Wednesday	7:30 pm – 10:00 pm	**1** Introduction
Thursday	9:00 am – 12:15 pm	**2** Organ and Tissue Aging
Thursday	2:00 pm – 5:00 pm	**3** Senescence and the Brain
Thursday	5:00 pm	*Wine and Cheese Party*
Thursday	7:30 pm – 10:30 pm	**Poster Session I**
Friday	9:00 am – 12:15 pm	**4** Cancer
Friday	1:45 pm – 4:45 pm	**5** Immune System and Aging
Friday	5:30 pm – 6:30 pm	**DORCAS CUMMINGS LECTURE**
Friday	6:30 pm	*Beach Picnic*
Saturday	9:00 am – 12:15 pm	**6** Metabolism
Saturday	2:00 pm – 4:30 pm	**7** Longevity, Lifespan and Regeneration
Saturday	4:30 pm – 6:30 pm	**Poster Session II**
Saturday	6:30 pm 7:30 pm	*Cocktails* *Banquet*
Sunday	9:30 am – 12:15 pm	**8** Hematopoiesis and Aging / Autophagy and Senescence

All times shown are US Eastern: Time Zone Converter

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

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WEDNESDAY, May 28—7:30 PM

SESSION 1 INTRODUCTION

Chairperson: **Bruce Stillman,** Cold Spring Harbor Laboratory, New York

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SESSION 2 ORGAN AND TISSUE AGING

Chairperson: **Elaine Fuchs,** Rockefeller University, New York, New York

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THURSDAY, May 29—2:00 PM

SESSION 3 SENESCENCE AND THE BRAIN

Chairperson: **Fred Gage,** Salk Institute, La Jolla, California

Enzymes regulating histone acetylation in senescence and brain aging
Shelley L. Berger.
Presenter affiliation: University of Pennsylvania, Penn Epigenetics Institute, Philadelphia, Pennsylvania. 17

THURSDAY, May 29—5:00 PM

Wine and Cheese Party

THURSDAY, May 29—7:30 PM

POSTER SESSION I

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FRIDAY, May 30—9:00 AM

SESSION 4 CANCER

Chairperson: **Ali Shilatifard,** Northwestern University, Chicago, Illinois

Targeting the interface between senescence and cancer
Scott W. Lowe.
Presenter affiliation: Memorial Sloan Kettering Cancer Center, New York, New York. 18

Enhancing immune-mediated killing of senescent cells
Jesus Gil.
Presenter affiliation: MRC Laboratory of Medical Sciences, London, United Kingdom; Institute of Clinical Sciences (ICS), London, United Kingdom. 19

Oncogene-induced senescence and tumor initiation
Masashi Narita.
Presenter affiliation: University of Cambridge, Cambridge, United Kingdom. 20

FRIDAY, May 30—1:45 PM

SESSION 5 IMMUNE SYSTEM AND AGING

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Igniting the flame—Role of cGAS in cellular senescence and inflammaging
Zhijian "James" Chen.
Presenter affiliation: University of Texas Southwestern Medical Center, Dallas, Texas. 26

Deconstructing aging with senolytic CAR T cells
Corina Amor.
Presenter affiliation: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 27

Clonal evolution in the aging hematopoietic
Margaret A. Goodell.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 28

Somatic retrotransposition in cellular senescence and aging
John M. Sedivy.
Presenter affiliation: Brown University Center on the Biology of Aging, Providence, Rhode Island. 29

Genome evolution of cells progressing through replicative crisis
Jan Karlseder, Tobias T. Schmidt, Marco R. Cosenza, Tessa Popey, Jesse Dixon, Jan O. Korbel.
Presenter affiliation: The Salk Institute for Biological Studies, La Jolla, California. 30

FRIDAY, May 30—5:30 PM

DORCAS CUMMINGS LECTURE

Scott Lowe
Memorial Sloan Kettering Cancer Center
Howard Hughes Medical Institute

"Zombie Cells at the Intersection of Cancer and Aging"

SESSION 6 METABOLISM

Chairperson: **Shelley Berger**, University of Pennsylvania, Philadelphia

SESSION 7 LONGEVITY, LIFESPAN AND REGENERATION

Chairperson: **Elly Tanaka,** Institute of Molecular Biotechnology, Vienna, Austria

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POSTER SESSION II

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SESSION 8 HEMATOPOIESIS AND AGING / AUTOPHAGY AND SENESCENCE

Chairperson: **Emmanuelle Passegué,** Columbia University Medical Center, New York, New York

POSTER SESSION I

POSTER SESSION II

A novel role for transcription factor CEH-62/GBX1 in restoration and recovery from adult reproductive diapause
Damini Sant, Kazuto Kawamura, Birgit Gerisch, Tim Nonninger, Jennifer Mak, Adam Antebi.
Presenter affiliation: Max Planck Institute for Biology of Ageing, Cologne, Germany. 178

Sex-dependent changes in AMPAR expression and Na, K-ATPase activity in the cerebellum and hippocampus of α-Klotho-Hypomorphic mice
Geovana Santos, Marina Cararo-Lopes, Isabela Possebom, Larissa Lima, Cristoforo Scavone, Elisa Kawamoto.
Presenter affiliation: University of São Paulo, São Paulo, Brazil. 179

A novel multi-modal platform for the accurate identification and isolation of senescent cells
Annarita Scaramozza, Xu Zhang, Nirvan Rouzbeh, Liz Murray, Shan Sabri, Shreya Deshmukh, Ria Gupta, Sina Moeinzadeh, Filiz G. Yasar, Yunmin Li, Teresa Ai, Pier Federico Gherardini, Tarun Khurana, Mostafa Ronaghi, Nathan K. LeBrasseur, Gary P. Schroth.
Presenter affiliation: Cellanome, Foster City, California. 180

Tissue aging—Effects of the apolipoprotein E genotype on adipose-tissue stem cells (ASC) physiology and differentiation
Fridtjof Seiffert, Annemarie Meinck, Silvia L. Monge Rodriguez, Christin Volk, Sohana Islam Khan, Alicia Toto Nienguesso, Samiya Al-Robaiy, Alexander Navarrete Santos, Anne Navarrete Santos, Juliane-Susanne Jung.
Presenter affiliation: Medical Faculty of Martin Luther University, Halle (Saale), Germany. 181

Role of chaperone-mediated autophagy in cellular senescence in aging
Rebecca Sereda, Kristen Lindenau, Antonio Diaz, Rabia R. Khawaja, Olaya Santiago-Fernández, Ronald Cutler, Jazmin Calyeca, Sylvére Durand, Fanny Aprahamian, Guido Kroemer, Toren Finkel, Simone Sidoli, Ana L. Mora, Mauricio Rojas, Susmita Kaushik, Ana Maria Cuervo.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York. 182

INTER-ORGAN COMMUNICATION MANAGEMENT (IOCoM): A NEW INTERVENTIONAL APPROACH TO UNDERSTAND THE MECHANISM OF MAMMALIAN AGING AND LONGEVITY CONTROL

Shin-ichiro Imai

Washington University School of Medicine, Department of Developmental Biology, Department of Medicine (Joint), St. Louis, MO

The importance of inter-organ communication has been recognized as a key mechanism for mammalian aging and longevity control. The hypothalamus has been demonstrated to function as a high-order control center for coordinating key inter-organ communication networks, and the mammalian NAD+-dependent protein deacetylase SIRT1 plays a crucial role in counteracting age-associated functional decline and promoting longevity in the hypothalamus. We have identified a specific neural population in the dorsomedial hypothalamus (DMH), named $DMH^{Ppp1r17}$ neurons, that counteracts aging and promotes longevity through the communication between the hypothalamus and white adipose tissue (WAT) (Tokizane et al., *Cell Metab*., 2024). $DMH^{Ppp1r17}$ neurons send a signal to WAT through the sympathetic nervous system (SNS), stimulating lipolysis and the secretion of extracellular nicotinamide phosphoribosyltransferase (eNAMPT), a key NAD+ biosynthetic enzyme, contained in extracellular vesicles (eNAMPT-EVs). eNAMPT-EVs travel through blood circulation and reach specific brain regions, stimulating NAD+ production in these regions. Interestingly, eNAMPT-EVs are able to improve cognitive flexibility in aged mice. Thus, the feedback loop between the hypothalamus and WAT, mediated by eNAMPT-EVs, plays a critical role in maintaining metabolic resilience, cognitive flexibility, and lifespan. On the other hand, nicotinamide mononucleotide (NMN), a key NAD+ intermediate, has been proven to show significant anti-aging effects in mice and has also been reported to show some beneficial effects in human clinical trials. Our previous work has demonstrated that a subset of neurons in the lateral hypothalamus (LH) that express *Slc12a8*, a specific NMN transporter, regulates skeletal muscle structure and function through the sympathetic nerve-β2 adrenergic receptor (β2AR) axis, counteracting age-associated sarcopenia and frailty (Ito et al., *Cell Re*p., 2022). In response to this signal from the hypothalamus, skeletal muscle secretes a myokine, which stimulates another specific subset of neurons in the hypothalamus and maintain their function. Thus, another feedback loop between the hypothalamus and skeletal muscle also plays an important role in mammalian aging and longevity control. These multi-layered feedback loops between the hypothalamus and peripheral tissues are the core machinery for mammalian aging and longevity control through systemic NAD+ regulation by eNAMPT-EVs and NMN/Slc12a8. Based on our current understanding of such inter-organ communication networks, we propose a novel concept for an effective anti-aging intervention termed Inter-Organ Communication Management (IOCoM). IOCoM aims to enhance the robustness of inter-organ communication networks, improving systemic resilience against internal and external perturbations and promoting longevity in mammals.

TRANSPOSABLE ELEMENTS AS A TRIGGER FOR SENESCENT PHENOTYPES IN iNEURONS

Fred Gage[1], Joseph Herdy[1], Lukas Karbacher[1,2], Jerome Mertens[2], Oliver Borongo[2], Dylan Reid[3], Larissa Traxler[2], Jessica Lagerwall[2], Emma Taylor[1], Vincent Hyunh[1]

[1]Salk Institute, Laboratory of Genetics, La Jolla, CA, [2]UCSD, Neuroscience, La Jolla, CA, [3]Ionis Pharmaceuticals, Carlsbad, CA

Alzheimer's disease (AD) is a severe neurodegenerative disorder that exclusively effects elderly people. Despite decades of research, AD remains a debilitating, progressive, and ultimately fatal dementia with no disease-modifying treatment options. This is partly due to the lack of human model systems that capture complex human genetics and human biological age. We have previously shown that induced neurons (iNs) directly converted from patient's fibroblast overcome these limitations by retaining neuron-specific hallmarks of aging and reflect unifying sporadic AD-related signatures. Using a multi-omic approach, we showed that AD iNs have an increased population of senescent cells that have impaired electrophysiological activity, metabolic reprogramming, and most critically the gain of inflammatory senescence associated secretory phenotype (SASP) that could activate human glia. Our data indicate that chemical or genetic ablation of this minority population of cells could effectively eliminate the neuroinflammatory signature in AD iNs, highlighting senescence as a functional target for therapeutic interventions in AD. However, it is still unknown through what mechanism neurons, which aren't a pro-inflammatory cell type, could gain this feature during senescence.

Transposons are repetitive DNA elements that represent approximately 45% of the human genome and are released from epigenetic silencing during aging. Late-life activation of the transposon long interspersed nuclear element 1 (LINE-1) has been linked to aging pathologies by increasing genomic mutagenesis and generating cytoplasmic DNA fragments that trigger a non-self immune response. Here, we provide evidence that LINE-1 derived cytoplasmic DNA underlies the initiation of a SASP in AD neurons. Using a Crispr and EdU labeling strategy, we have observed distinct cytoplasmic DNA in AD iNs that can be reduced by targeting LINE-1. In addition to LINE-1 knockdown, interventions that eliminate LINE-1 reverse transcription reduce SASP expression, suggesting a cytoplasmic DNA sensing mechanism. Our data point to LINE-1 as the mechanism for gain-of-function in inflammation in senescence neurons, and a targetable candidate for reducing late-life neuroinflammation in AD.

A COMPLEX TiME: HOW THE AGING TUMOR IMMUNE MICROENVIRONMENT GOVERNS TUMOR PROGRESSION

Ashani T Weeraratna[1,2]

[1]Johns Hopkins Bloomberg School of Public Health, Department of Biochemistry and Molecular Biology, Baltimore, MD, [2]Johns Hopkins School of Medicine, Department of Oncology, Baltimore, MD

Patients 65 and older account for 69% of all new cancer diagnoses. Systemic age-related changes- both secreted and biophysical- drive metastasis, immune cell recruitment, and changes in the vasculature. We have made the seminal discovery that aged fibroblasts secrete, or stop secreting, key molecules that affect multiple aspects of tumorigenesis in melanoma, pancreatic and ovarian cancer. Our current investigations include: the loss of molecules that maintain ECM integrity, resulting in changes in mechanotransduction and increased metastasis; the secretion of molecules that increase resistance to targeted therapy; the secretion of macromolecules such as lipids that are taken up by melanoma cells in the aged microenvironment, affecting tumor cell metabolism; changes in the aged immune tumor microenvironment; the secretion of non-canonical Wnt molecules that affect cell signaling leading to angiogenesis, metastasis, tumor dormancy and therapy resistance. Additionally, we are investigating the evolving role of the immune microenvironment during aging—examining changes at primary and distal tumor sites while mapping the systemic immune landscape. We are also attempting to understand the intersectionality of systemic host factors such as biological sex and age, and how those factors compound metastatic progression and responses to cancer therapy.

PERIARTERIOLAR NICHES BECOME INFLAMED IN AGING BONE MARROW, REMODELING THE STROMAL MICROENVIRONMENT AND DEPLETING LYMPHOID PROGENITORS

Sean J Morrison[1,2]

[1]Children's Research Institute, UT Southwestern, Dallas, TX, [2]Howard Hughes Medical Institute, UT Southwestern, Dallas, TX

In early postnatal and young adult bone marrow, Leptin receptor-expressing (LepR+) stromal cells and endothelial cells synthesize factors required for hematopoietic stem cell (HSC) maintenance, including Stem Cell Factor (SCF) and Cxcl12. However, little is known about how these stromal cells change during aging. We performed single-cell RNA sequencing of mouse bone marrow stromal cells at 2, 12, and 24 months of age. We identified five transcriptionally-distinct subsets of LepR+ cells, all of which expressed the highest levels of Scf and Cxcl12 in bone marrow throughout adult life. In aging bone marrow, SCF from LepR+ cells, but not endothelial cells, continued to be necessary for the maintenance of HSCs and early restricted progenitors. However, arteriolar endothelial cells and other periarteriolar cells expressed increasing levels of interferon during aging. This increased the numbers of periarteriolar Sca1+Cxcl9+LepR+ cells with an inflammatory gene signature and depleted lymphoid progenitors, at least some of which are also periarteriolar. The periarteriolar environment thus became particularly inflamed during aging, remodeling the stromal microenvironment and depleting lymphoid progenitors in an interferon-dependent manner.

Periarteriolar Sca1+Cxcl9+LepR+ cells represent a subset of LepR+ cells not described previously. These cells increased in number with age and were marked by interferon regulated gene expression. Treatment of old mice with antibodies that blocked type I and type II interferon signaling depleted Sca1+Cxcl9+LepR+ cells and increased the numbers of lymphoid progenitors. Increasing interferon expression thus contributes to the changes in bone marrow hematopoiesis during aging, remodeling the stroma and depleting lymphoid progenitors. These observations raise the possibility that inflammation is not uniform across aging bone marrow: it may preferentially affect certain microenvironments, including around arterioles, which serve as a niche for at least some lymphoid progenitors.

AGING SKIN STEM CELLS BEAR SCARS OF A LIFE OF STRESS

Elaine Fuchs[1,3], Christopher Cowley[1], Sairaj Sajjath[1], Luis Soto-Ugaldi[1,2], Lisa Polak[1], Dana Pe'er[2,3]

[1]The Rockefeller University, Mammalian Cell Biology & Development, New York, NY, [2]Memorial Sloan Kettering Cancer Center, Computational and Systems Biology, New York, NY, [3]Howard Hughes Medical Institute, Investigators, New York, NY

According to Webster, memory “is the power or process of reproducing or recalling what has been learned and retained especially through associative mechanisms.” It has generally been thought to be the privilege of the brain, and indeed as most neurons are long-lived, they have the capacity to store memories of their experiences and recall them months later. However many tissues of our body learn from their past experiences, and like memories that occur in the brain, tissue memories have both beneficial and maladaptive consequences. An excellent example of this is our barrier epithelial tissues such as the skin, lung and gut, which are the first line of defense between our body and the outside world. To survive in these harsh environments and continually generate and rejuvenate their tissues, these epithelia must maintain reservoirs of self-renewing stem cells that can produce tissue long-term. When the barrier has been breached, for example by wounding, the stem cells must not only repair the damaged tissue but also call to the immune system to help guard against pathogen entry. All the while the stem cells must protect themselves from a variety of assaults, including not only injury but also metabolic stresses that stimulate an inflammatory response. My laboratory discovered that stem cells harbor epigenetic memories of their stressful encounters within their chromatin and retain these memories until subsequent encounters, when they are unleashed with heightened robustness. The underlying mechanisms have tantalizing similarities to the memories we store in our brain, and suggest that perhaps all our tissues store memories to learn from their experiences. While evolutionarily advantageous in healing wounds and encountering subsequent pathogens, these epigenetic memories can also be maladaptive, contributing to age-related illnesses such as chronic inflammation and malignancy. Our focus is on dissecting the underlying mechanisms of long-lived epigenetic memories in the skin with the global objective of erasing the bad memories and keeping the good ones for the sake of optimal tissue fitness as we age.

NDRG1 AND RESILIENCE OF AGED STEM CELLS

Thomas A Rando

University of California, Los Angeles, Los Angeles, CA

Quiescent stem cells face the dual challenge of aging of having to survive over a lifetime, facing the challenges of chronological aging like those of all postmitotic cells, while have to preserve their inherent replicative potential underlying the fundamental roles of tissue homeostasis and repair. In studies of muscle stem cells (MuSCs), we have found that, with age, the cells lose resilience and exhibit a gradual reduction in cell number. Analysis of the transcriptome of aged MuSCs compared with young MuSCs reveal an upregulation of genes that are known to be involved in cell cycle regulation. We focused on one of the most highly upregulated genes, NDRG1, a known tumor suppression gene. Conditional knockout of NDRG1 in young mice resulted in negligible effects of MuSCs in a variety of functional assays of cell cycle entry, proliferation, and differentiation. However, deletion of NDRG1 in aged mice resulted in an acceleration of cell cycle entry ex vivo and an improvement in muscle regeneration in vivo. In parallel, NDRG1-deficient age MuSCs exhibited a decreased resilience to a variety of cell stressors compared to wild-type controls We propose that NDRG1 represents a regulator of a compensatory program to counter age-related loss of resilience. These data suggest that the pressure of cell survival on quiescent stem cells during the aging process results in the upregulation of survival pathways at the expense of regenerative potential. This selection process leads a survivorship bias that obscures causes versus consequences of stem cell aging

EPIGENETIC DYSREGULATION IN AGED MUSCLE STEM CELLS DRIVES FIBROTIC MESENCHYMAL PROGENITOR EXPANSION

Giulia Riparini[1], Morgan Mackenzie[1], Faiza Naz[2], Stphen Brooks[3], Anshu Deewan[4], Brittany Dulek[4], Shamima Islam[2], Kyung Dae Ko[1], Wanxia L Tsai[5], Massimo Gadina[5], Stefania Dell'Orso[2], Vittorio Sartorelli[1]

[1]National Institutes of Health (NIH), Laboratory of Muscle Stem Cells and Gene Regulation, National Institute of Arthritis and Musculoskeletal and Skin Diseas, Bethesda, MD, [2]NIAMS, NIH, Genomic Technology Section, Bethesda, MD, [3]NIAMS, NIH, Biodata Mining and Discovery Section, Bethesda, MD, [4]NIAID, NIH, Integrated Data Sciences Section, Research Technologies Branch, Bethesda, MD, [5]NIAMS, NIH, Translational Immunology Section, Bethesda, MD

Sarcopenia, the age-related decline in muscle mass, strength, and function, is marked by impaired muscle homeostasis, compromised regenerative potential of muscle stem cells (MuSCs), and fibrosis. Similar to inflammaging, fibroaging exacerbates regenerative dysfunction and impairs skeletal muscle performance. In this study, we report that aged MuSCs instruct fibro-adipogenic progenitors (FAPs) to proliferate and acquire a fibrogenic phenotype, independent of other cell types. Both Polycomb-deficient $Ezh2^{-/-}$ and aged mice exhibited defective regeneration, FAP expansion, fibrosis, and elevated levels of MuSC-secreted interleukin 6 (IL-6) and Spp1/Osteopontin.

Epigenetic erosion- characterized by the loss of stable epigenetic patterns during aging- leads to dysregulated gene expression. In aged MuSCs, erosion of the histone H3K27me3 repressive mark at the NF-kB gene correlated with its increased expression, enhancing chromatin recruitment to the IL-6 and Spp1 genes, and thereby activating their expression. Blocking IL-6 and Spp1 signaling in co-cultures or aged mice reduced FAP proliferation and muscle fibrosis. These findings highlight that epigenetic dysregulation of aged MuSCs contributes to aged-related muscle fibrosis. Rather than simply passively receiving inflammatory and pro-fibrotic signals, aged MuSCs actively promote fibrosis by driving the expansion of pro-fibrotic FAPs.

ADIPOCYTE SENESCENCE AND BREAST CANCER

Kirsty Spalding

Karolinska Institutet, Department of Cell and Molecular Biology, Stockholm, Sweden

Cellular senescence is emerging as a key driver of obesity-associated metabolic dysfunction. While adipocytes have long been considered post-mitotic, we recently identified a mechanism linking adipocyte hypertrophy to premature senescence. In obesity, fat cells enlarge and adopt a pro-inflammatory secretory profile that contributes to adipose tissue dysfunction. We demonstrate that mature human adipocytes can reactivate a cell cycle program in response to obesity and hyperinsulinemia, leading to increases in cell size, nuclear size, and DNA content. Chronic hyperinsulinemia, however, promotes the acquisition of a senescent phenotype, marked by a distinct transcriptomic and secretory profile. These findings suggest that adipocyte senescence is a critical and underexplored factor in obesity-related disease. The role of adipocyte senescence in humans and its potential contribution to breast cancer progression will be explored.

A REVISED MODEL OF TISSUE REGENERATION IN THE GUT EPITHELIUM

Claudia Capdevila[1,2,3,4], Hyeonjeong Lee[1,2,3,4], Jonathan Miller[1,2,3,4,5], Sierra Ball[1,2,3,4], Liang Cheng[1,2,3,4], Timothy C Wang[1,2,4], Arnold Han[1,3,4,6], Kelley S Yan[1,2,3,4]

[1]Columbia University Irving Medical Center, Department of Medicine, Division of Digestive & Liver Diseases, New York, NY, [2]Columbia University Irving Medical Center, Columbia Stem Cell Initiative, Department of Genetics & Development, New York, NY, [3]Columbia University Irving Medical Center, Herbert Irving Comprehensive Cancer Center, New York, NY, [4]Columbia University Irving Medical Center, Digestive & Liver Diseases Research Center, New York, NY, [5]Columbia University Irving Medical Center, Department of Pediatrics, New York, NY, [6]Columbia University Irving Medical Center, Department of Microbiology & Immunology, New York, NY

The mammalian gut epithelium undergoes robust and continuous regeneration as supported by stem and progenitor cells in the crypts. In the prevailing model, *Lgr5* + cells at the crypt base are the sole population of intestinal stem cells (ISCs) and colon stem cells (CSCs) that sustain homeostatic regeneration of these tissues. Aging is associated with impaired regeneration of the small intestine and colon epithelium. Here we show that functional stem/progenitor cell number is diminished during aging that is not fully explained by the Lgr5 stem cell model. Recently, we identified a novel population of *Fgfbp1*+ upper crypt ISCs, distinct from *Lgr5*+ cells at the base, that underlies homeostatic regeneration of the intestinal epithelium and give rise to the *Lgr5*+ cells. Here, we present a revised model of tissue regeneration that reconciles the *Lgr5*+ stem cell model with new fate mapping studies of *Fgfbp1*+ upper crypt stem cells in the intestine and colon. We further demonstrate a new previously unappreciated role of *Lgr5*+ cells in the colon. We also discuss our new studies of stem cell dynamics and how they are altered during aging.

SOMATIC EVOLUTION, CANCER, AND OUR INEVITABLE DECLINE WITH AGE - INEXTRICABLY LINKED

Edward J Evans, Jr.*[1], Emilia Lim*[2,3,4], Fabio Marongiu*[1,5], Ferriol Calvet*[6], Faiz Jabbar[2], Shi Biao Chia[1], Amy Briggs[1], Jonathan Kurche[1], Andrii I Rozhok[1], William Hill[2], Clare Weeden[2], Oriol Pich[2,3], Mariam Jamal-Hanjani[3], Nuria Lopez-Bigas#[6], Charles Swanton#[2,3], James DeGregori#[1]

[1]University of Colorado, Anschutz Medical Campus, Aurora, CO, [2]The Francis Crick Institute, London, United Kingdom, [3]University College London, Cancer Institute, London, United Kingdom, [4]University of British Columbia, Vancouver, Canada, [5]University of Cagliari, Cagliari, Italy, [6]Institute for Research in Biomedicine, IRB, Barcelona, Spain

Why do we get cancer? Why is cancer highly associated with old age? Indeed, why do we age at all? Of course, aging is associated with the accumulation of more mutations, and some of these mutations can contribute to cancer phenotypes. But we now understand that carcinogenesis is much more complex than originally appreciated. In particular, there are tissue environmental forces that both impede and promote cancer evolution. Just as organismal evolution is known to be driven by environmental changes, cellular (somatic) evolution in our bodies is similarly driven by changes in tissue environments, whether caused by the normal process of aging, by lifestyle choices or by extrinsic exposures. Environmental change promotes selection for new phenotypes that are adaptive to the new context. In our tissues, aging or insult-driven alterations in tissues drive selection for adaptive mutations, and some of these mutations can confer malignant phenotypes. We have been using mouse models of cancer initiation, mathematical models of cellular evolution, and analyses of human tissue samples to better understand the evolutionary forces that control somatic cell evolution and thus cancer risk. We have shown that aging and inflammation dependent changes in stem cells and their tissue environments dramatically dictate whether cancer-causing mutations are advantageous to stem cells in our tissues, starting the cells down the path to cancer. Importantly, recent studies from many labs have shown how as we age our tissues become dominated by clones bearing mutations in known cancer-associated mutations. We have recently shown that cigarette smoking can promote the expansion of some of these mutations. We propose a model whereby aging and contexts like smoking can promote selection for cells with adaptive mutations, which can contribute to not only cancer risk but also tissue aging. Thus, while young tissues impede selection for such adaptive mutations, old age is associated with a feed-forward loop of aging tissue-mediated selection for mutant clones that then increase tissue aging. Thus, understanding the forces controlling clonal selection as we age and due to lifetime exposures could be critical for controlling multiple diseases of old age.
* equal contributions; #co-corresponding

THE MECHANISMS AND MECHANICS OF THE AGING BODY

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Aging is closely linked to the induction of cell senescence, yet the underlying mechanisms remain poorly understood. A major challenge is identifying pathways that normally prevent senescence, become lost with aging, and play a functional role in counteracting the aging process. Here, we connect the structural and functional decline of aging tissues to the reduced activity of the key regulators of cellular mechanosignaling, YAP and TAZ. During physiological aging, YAP/TAZ activity diminishes in stromal cells, and experimentally mimicking this decline through genetic inactivation of YAP/TAZ accelerates aging. Conversely, maintaining YAP function rejuvenates aged cells and counteracts aging-related traits, whether caused by natural aging or accelerated by a mechanically defective extracellular matrix. The aging traits triggered by YAP/TAZ inactivation are preceded by the onset of tissue senescence. This occurs because YAP/TAZ mechanotransduction suppresses cGAS–STING signaling, and blocking STING prevents tissue senescence and premature aging-related degeneration following YAP/TAZ loss. Mechanistically, YAP/TAZ regulate cGAS–STING signaling by preserving nuclear envelope integrity, at least in part through direct transcriptional regulation of lamin B1 and ACTR2, the latter playing a role in constructing the peri-nuclear actin cap. These findings reveal that the decline of YAP/TAZ mechanotransduction drives aging by activating cGAS–STING signaling, a fundamental component of innate immunity. Therefore, enhancing YAP/TAZ mechanosignaling or inhibiting STING may offer promising strategies to reduce senescence-associated inflammation and promote healthy aging.

RE-ACTIVATION OF NEUROGENIC NICHES IN AGING BRAIN

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Loss of neurons in the adult nervous system has long been believed to be irreversible. Indeed, over a century ago, the great Spanish neurobiologist Ramon y Cajal recognized: “In the adult centers, the neural paths are something fixed and immutable: everything may die, nothing may be regenerated” and challenged the next generations to overcome this limitation. Over last decades, two regions of adult mammalian brain – the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (SGZ) – have been shown to harbor neural stem cells (NSCs). In developing brain, these NSCs are in an active proliferative state (“active NSCs”), producing both glia and neuron precursors that migrate and differentiate into corresponding functional, mature cells. Progressing from young to adult age, both the number of NSCs and their ability to generate neurons sharply decline. Identifying the genes and process(es) underlying age-dependent NSC-to-neuron differentiation in the mammalian brain could provide a means to reactivate dormant NSCs or to induce stem cell-like properties in other glial populations in aged brain.

To address Ramon y Cajal’s >100 year old challenge, we report development of a multimodal MERFISH platform which combines spatial transcriptomics with subcellular resolution on the scale of 2,085-genes, indirect immunofluorescence protein localization to enable lineage tracing, and EdU labeling to mark DNA replication. With it, we identify populations of NSCs lining one ventricular wall (in the SVZ) or within the subgranular zone (SGZ) that in aged mice have entered a quiescent state marked by reduced expression of neurogenic and cell-cycle genes. We demonstrate that transient suppression of RNA binding protein Polypyrimidine-Tract-Binding-Protein (Ptbp1) (with a therapeutically viable injection of an antisense oligonucleotide [ASO]) reactivates dormant NSCs in both neurogenic niches in aged mice, inducing their cell cycle re-entry, proliferation, and conversion into neurons via a canonical neurogenesis pathway. Some of these newly formed neurons migrate to the striatum and acquire transcriptomes characteristic of mature medium spiny neurons. Similar PTBP1-expressing stem cell-like cells are identified in aged human brain, supporting the reactivation of neurogenesis as a potential therapy against age-related neurodegeneration.

FROM OLD SKIN TO OLD BRAIN: DIRECT CONVERSION TO EXPLORE THE INTERFACE BETWEEN NEURONAL AGING AND DISEASE.

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Old age is the dominating risk factor for many neurodegenerative disorders, many of which exclusively affect people at older ages. Sporadic forms of Alzheimer's Disease (AD) for example represent the overwhelming majority of all cases, but most research on AD has been performed on genetic causes and their directly related pathways, also because we lacked models that can reflect complex human genetics and age in a neuronal context. Disease models based on patient-specific iPSCs represent an attractive solution to overcome limitations of animal models, but iPSC reprogramming results in cellular rejuvenation and thus yields phenotypically young neurons mirroring fetal development. By contrast, direct conversion of old patient fibroblasts into induced neurons (iNs) preserves endogenous signatures of aging and produces neurons that resemble the adult human brain on an epigenome- and transcriptome-wide scale. To control for the involvement of aging in neurodegeneration, we generate age-equivalent fibroblast-derived iNs from normal aging donors and patients cohorts. Patient-derived iNs show increased signs of cellular stress and damage, and enter a hypo-mature neuronal identity characterized by markers of stress, cell cycle, metabolic reprogramming, and de-differentiation. Using disease models based on iNs from donors along the spectrum from healthy aging, to mild cognitive impairment (MCI), AD, and other neurodegenerative disorders, we seek to understand the disease-driving mechanisms that lead to age-related neurodegeneration. In my presentation, I will discuss recent advances in utilizing human cell reprogramming disease models including iNs to study neurodegenerative disorders, focusing on how these models capture the complexities of aging in vitro, and I will highlight the latest developments in modeling human aging using human in vitro modeling approaches.

ACTIVATED NEUROGENESIS IMPROVES AMYLOID-B PATHOLOGY AND COGNITION IN ALZHEIMER'S DISEASE MODEL MICE

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Adult hippocampal neurogenesis declines with aging and neurological disorders, leading to cognitive impairment. We previously demonstrated that inducing Plagl2, a zinc finger transcription factor gene, and inhibiting Dyrk1a, a gene associated with Down syndrome, can functionally rejuvenate aged neural stem cells (NSCs), thereby promoting neurogenesis and improving cognition in aged mice. Here, we found that this treatment effectively activated neurogenesis, reduced amyloid-β deposition, and enhanced cognition in Alzheimer's disease model mice. Downstream of this treatment, many genes were globally upregulated or downregulated in the hippocampus. The upregulated genes include those involved in microglial activation for amyloid-β clearance, while the down-regulated genes include Prkag2 (encoding protein kinase AMP-activated non-catalytic subunit gamma 2) and Maml2 (encoding mastermind-like 2 co-activator). These results suggest that activating neurogenesis by inducing Plagl2 and anti-Dyrk1a activity can mitigate age-related neurological disorders, including Alzheimer's disease, by regulating a broad network of downstream genes, which may serve as promising therapeutic targets.

NEURON-AUTONOMOUS AND NON-AUTONOMOUS REGULATION OF MEMORY

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Memory loss is among the most debilitating symptoms of aging. Using the *C. elegans* model for the study of aging and longevity, we have developed assays to test learning and memory and their decline with age. Our studies have identified key, evolutionarily conserved regulators of cognitive aging and its decline, and we have discovered at least two mechanisms to reactivate functional memory with age; we have been able to replicate rescue of memory via activation of Gaq signaling in two-year old mice, the equivalent of 70-80+ yo humans. We also made the surprising discovery that the hypodermis, the skin- and liver-like metabolic tissue of the worm, can also influence the memory ability of *C. elegans*. Both the neuron-autonomous and non-autonomous signaling mechanisms impinge on CREB transcription factor function, pointing to a conserved mechanism of memory maintenance

MECHANISMS OF BRAIN AGING AND REJUVENATION

Anne Brunet

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Aging is associated with a decline in tissue function and the onset of a constellation of diseases. We are interested in understanding aging, with a particular focus on brain aging. Because aging is complex, we use organisms with diverse lifespans – the worm *C. elegans*, the African killifish, the mouse, and cells from mice and humans. We are interested in identifying epigenetic and metabolic pathways involved in delaying aging in response to external stimuli, including nutrients and the opposite sex. Our lab is also interested in using mouse models to address complex questions about mammalian aging, notably the regulation of regenerative neural stem cells and their progeny during aging. Finally, we are pioneering the naturally short-lived African killifish as a new model to identify principles underlying vertebrate aging and "suspended animation". We hope that these discoveries will identify new strategies to delay, suspend, or even reverse aspects of aging and age-related diseases.

ENZYMES REGULATING HISTONE ACETYLATION IN SENESCENCE AND BRAIN AGING

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Chromatin regulatory proteins are frequently mutated or overexpressed in human disease. Because they are enzymes and engage in protein-protein interactions, histone modifying and other chromatin proteins are outstanding targets for drug development. Our work focuses on elucidation of chromatin pathways, in particular, of acetylation-linked enzymes, that might be disease drivers, and as potential therapeutic approaches that might augment clinical treatment. In aging, we study senescence and diseases of aging-associated memory.

Our recent work in cellular senescence focuses on acetylation linked to metabolism. First, we have new findings of SIRT7, the NAD-dependent deacetylase, and its deacetylation of a transcription factor NUCKS1. Second, we have made an unanticipated connection of PDHX, a component of the Pyruvate Dehydrogenase Complex that produces acetyl-CoA, in moonlighting “solo” in the nucleus to regulate a histone acetyltransferase. Both of these pathways promote gene expression during cell senescence and in liver aging in the mouse model and thus represent potential therapeutic targets of inhibition.

We also investigate epigenetics in the context of Alzheimer’s disease, finding that human Alzheimer’s is not an accelerated form of normal aging, but rather is a distinct disease pathway. In the mouse model we study another metabolic enzyme producing acetyl-CoA, ACSS2, and show it is located in the nucleus (in hippocampus neurons) and has a key role in normal memory. We find in a mouse preclinical model that disruption of ACSS2 worsens pathology associated with Alzheimer’s disease, and increased ACSS2 levels is ameliorative to Alzheimer’s.

Taken together, our findings reveal numerous mechanisms and pathways in which acetylation regulates chromatin and transcription in senescence and in age-associated disease.

TARGETING THE INTERFACE BETWEEN SENESCENCE AND CANCER

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Cellular senescence is a mysterious process that has fascinated scientists since the late 1950s when Leonard Hayflick discovered that normal human cells grown in a Petri dish eventually stop dividing. Unlike cancer cells, which can replicate indefinitely, these "zombie cells" remain alive but permanently arrested—unable to divide yet still active. Over time, researchers have found that senescent cells play a paradoxical role in human health. In some cases, they help prevent cancer by halting the growth of precancerous cells and promoting tissue repair. However, as we age, the accumulation of these cells can lead to chronic inflammation and contribute to diseases such as fibrosis, neurodegeneration, and metabolic disorders like diabetes. This dual nature has sparked intense interest in harnessing senescence for therapeutic benefit. Scientists are exploring ways to either enhance its protective effects—helping the immune system clear cancer—or remove harmful senescent cells to combat aging-related diseases. Exciting breakthroughs in targeting senescence-specific molecules have demonstrated remarkable therapeutic potential in preclinical models, offering hope for new treatments that could extend healthspan and improve outcomes in a remarkable range of human diseases. In this lecture, I will discuss what we have learned about the process of senescence, the promise of emerging therapies, and what these "zombie cells" reveal about the intricate links between cancer and aging.

ENHANCING IMMUNE-MEDIATED KILLING OF SENESCENT CELLS

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Senescent cells are induced in response to oncogenic activation and tissue damage and are normally cleared by the immune system. When the immune surveillance of senescent cells is not effective, senescent cells linger, as happens in aged, cancerous and fibrotic tissues. This aberrant accumulation of senescent cells is associated with cancer and multiple age-related diseases. Recently, drugs that selectively kill senescent cells, termed senolytics, have proven beneficial in improving the outcomes of many of these pathologies. An alternative way to clear senescent cells is by potentiating their immune-mediated clearance. I will describe our screens to identify ways to enhance the elimination of senescent cells by Natural Killer (NK) cells. We identify that SMARCA4 regulates the secretion of immunomodulatory chemokines by senescent cells. A PROTAC targeting SMARCA4 increases the senescence-associated secretory phenotype (SASP), enhances NK-mediated killing of senescent cells and synergises with cisplatin to increase the infiltration of CD8 T cells and mature, activated NK cells in an immunocompetent model of ovarian cancer. Our results indicate that SMARCA4 inhibitors enhance NK-mediated surveillance of senescent cells and may represent senotherapeutic interventions for ovarian cancer and other senescence-associated diseases.

ONCOGENE-INDUCED SENESCENCE AND TUMOR INITIATION

Masashi Narita

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Oncogene-induced senescence (OIS) is a reactive cellular program triggered by excessive oncogenic RAS or downstream effectors. However, endogenous monoallelic expression of oncogenic RAS often fails to induce robust senescence, raising questions about whether physiological RAS-driven preneoplasia is uniformly characterized by senescence. A key challenge lies in the highly heterogeneous nature of senescence, both at the population and single-cell levels. Notably, increasing evidence suggests that oncogenic RAS levels gradually rise during tumor development, implicating intermediate cellular states. Here, we propose the concept of an OIS spectrum, where oncogenic dosage-dependent states transition between normal, ‘sub-OIS’ intermediates and full senescence. These sub-OIS states, often linked to fetal and progenitor factors, may play a critical role in tumor initiation.

PROSTATE CANCER MORTALITY AND SENESCENCE

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With over 299,000 new cases in the United States in 2024, Prostate cancer (PC) is the most common male cancer diagnosed. Approximately one in three men over the age of 50 shows histological evidence of the disease, however only one in ten will be diagnosed with clinically significant PC. While therapeutic options for localized PC are effective, metastatic PC is a yet incurable disease because standard of care anti-androgen therapies invariably result in deadly disease relapse. *PTEN/ TP53* loss is a most significant event of human lethal metastatic PC as summarized by the large scale cancer genome sequencing efforts and confirmed by two decades of functional PC modeling in mouse.
However, it has also become clear that in spite of this genetic predominance, terminal PC is in no way restricted to PI 3-kinase signaling. Instead, metastatic tumors have evolved to acquire a plasticity that allows for resistance to all known cancer treatment regimens.

To explore if genetic determinants are behind this evolution that separates indolent from lethal PC, we sequenced single nucleus genomes (SNGs) from 31 metastatic sites of patients who succumbed to the disease and compared them to SNGs of patients at first diagnosis, including analysis of regional disease progression across the organ. In parallel, we studied SNGs of metastatic progression in RapidCaP, a GEM model for lethal metastatic PC. Our conclusions from this analysis will be presented.

CELLULAR SENESCENCE AND SENOLYTICS: FROM DISCOVERY TO CLINICAL TRANSLATION

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Cellular senescence is a cell fate characterized by essentially irreversible replicative arrest in response to extrinsic and intrinsic stresses. Almost any cell type can become senescent. Senescent cells (SnCs) are metabolically active with a Warburg shift, resistant to apoptotic and necrotic cell death due to senescent cell anti-apoptotic pathways (SCAPs), and generally removed by innate and adaptive immune system components. SnCs acquire a senescence-associated secretory phenotype (SASP) that can include a range of proteins, peptides, reactive small molecules, lipids, carbohydrates, coding and non-coding nucleotides, and organelles or fragments of organelles including mitochondria and mitochondrial DNA. The SASP is highly and rapidly variable and depends on the cell type that became senescent, how long it has been senescent, host genetics, and the micro-environment the SnC is exposed to. The SASP can be pro-apoptotic against neighboring cells, interfere with stem and progenitor cell function, attract, activate, and anchor immune cells, be pro-growth, pro-fibrotic, and/or pro-coagulant, spread senescence in a paracrine/endocrine manner, and accelerate other fundamental aging process including increases in mTOR, ROS, and inflammation, decreased geroprotective factors, NAD, and immune, neural, special sense, heart, renal, lung, and skin function. Senescence is important during development, tissue remodeling, and limits tumorigenesis. However, pathologic accumulation of SnCs related to failed immune removal is implicated in a range of disorders and diseases across the lifespan. SnC removal by apoptosis-inducing "senolytic" agents (of which there are now >100) or SASP inhibitors (senomorphics) have indicated benefit in pre-clinical and clinical models of multiple conditions/disorders/diseases and the combinations of these diseases that often occurs within patients. The NIH-funded Translational Geroscience Network (TGN) is assisting with >85 clinical trials of gerotherapeutics, >20 of which are senolytics, for a range of indications across the lifespan. Similar networks are being established in Canada, Europe, and elsewhere. Efforts are being made across trials to harmonize data collection, questionnaires, blood, urine, biopsy, and other analytes, data processing, and sample storage conditions to allow comparisons and facilitate reverse translation from bedside to bench. Combining interventions targeting aging mechanisms with disease-specific drugs could result in more than additive benefits for currently difficult-to-treat or intractable diseases. Some of the trials have suggested early, promising results.

A NOVEL REDOX-ACTIVATED PAN-SENOLYTIC PREVENTS RAS-/BRAF-DRIVEN CANCERS, ATTENUATES AGING PHENOTYPES, AND EXTENDS LIFESPAN

Clemens A Schmitt (and colleagues)[1,2,3]

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While exerting important protective functions in tissue development, integrity, regeneration and tumor suppression as an acute cellular state switch in response to a variety of triggers, cellular senescence may turn into a pathogenic principle if senescent cells evade their clearance and persist for extended periods of time. Accordingly, senescent cells that accumulate during aging account for age-related pathologies, including organ fibrosis, cardiovascular diseases, severe virus infections, muscular decline, neurodegeneration, and cancer. These detrimental implications are attributed to chronic inflammation, immune dysregulation and extracellular matrix remodeling via the senescence-associated secretome, and stem-like reprogramming in senescent cells that might occasionally re-enter the cell-cycle. Especially gained stemness of previously senescent (pre-)malignant cells may underlie aggressive tumor manifestation, relapse or metastasis. Collectively, these harmful features provide a strong rationale for the selective therapeutic elimination of senescent cells.

We present here a novel redox-activatable agent, dubbed SenLyt, which preferentially kills senescent cells by exploiting senescence-characteristic redox features as its activating principle. SenLyt consistently removed cells in replicative, oncogene-, oncogene-inhibition-, therapy- and virus-induced senescence in preclinical cell culture and animal models, thus attenuating detrimental aging- or cancer-related phenotypes as a pan-senolytic. When equal pro-senescent triggers were applied to senescence-incapable model systems, the cytotoxic potential of SenLyt was limited. SenLyt, which was well-tolerated in all in vivo-settings tested, reduced the content of senescent cells in organs of aged animals, alleviated age-related pulmonary fibrosis, and partly restored physical strength of old mice when compared to a cohort of young animals. In an otherwise not specifically disease-prone cohort of aged mice, a short course of SenLyt significantly prolonged lifespan compared to mock treatment. Most strikingly, SenLyt delayed and reduced full-blown tumor formation in Ras- or Braf-driven mouse models by eliminating early senescent lesions, thereby establishing a novel system-wide cancer prevention strategy. In essence, SenLyt operates as a broadly active, well-tolerated and highly specific pan-senolytic based on its dual senescence-related mode of activation and killing.

TRANSCRIPTIONAL ELONGATION IN REGULATION OF CELLULAR AGING AND SENESCENCE

Ali Shilatifard

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Studies from our laboratory for the past thirty years have shown that transcriptional elongation control by RNA polymerase II (RNAPII) is a key regulatory step in controlling developmental gene expression and that misregulation of the elongation stage of transcription can result in pathogenesis of cancer and developmental disorders. This pathway is now being used as targeted therapeutics in cancer. We have also established distinct mechanistic roles for the essential elongation factors such as PAF1, NELF, SPT5, and SPT6 and the Super Elongation Complex (SEC) via acute depletion of each factor individually and studying them in cellular context. Here, I will show data that leverage these degron lines to explore the regulatory intersection of elongation control and mRNA processing. Integrating long- and short-read RNA-seq data to quantify transcript isoform usage at single-molecule resolution, we identify elongation factor-specific RNA processing regulons including a cellular senescence-enriched regulon shared by several elongation factors. We show that long-term depletion of Pol II elongation factors results in reversible growth arrest following early upregulation of a small group of genes, which include the senescence-associated genes CDKN1A (p21) and CCN2. We perform genetic suppressor screens to define the mechanistic insight and found that mRNA processing defects and the 3' extension of RNAPII occupancy past transcription end sites at genes induced by elongation factor depletion. Also, we demonstrate that genetic loss of specific Pol II elongation factors confers a growth advantage to aging human primary dermal fibroblasts. These findings establish the existence of novel Pol II elongation-dependent mechanisms regulating transcription termination-coupled processes, and links these to the complex phenomena of cellular senescence and aging.

CYCLIC DINUCLEOTIDE-MEDIATE INNATE IMMUNITY: BASIC MECHANISMS & TRANSLATIONAL IMPLICATIONS

Andrea Ablasser

Ecole Polytechnique Fédérale de Lausanne, Global Health Institute, Lausanne, Switzerland

The life of any organism depends on the ability of cells to detect and to respond to pathogens. In order to detect the immense variety of pathogenic entities, the innate immune system of mammals has evolved a range of distinct sensing strategies.
One major mechanism is based on the recognition of microbial DNA - an invariant and highly immunogenic pathogen-associated molecular pattern. Host cells, however, contain abundant sources of self-DNA. In the context of cellular damage or metabolic derangement, “out-of-the-context” self-DNA can elicit potentially damaging inflammatory responses. Our research focuses on the so-called cGAS-STING system - an evolutionary highly conserved innate DNA sensing system. On DNA binding, cGAS is activated to produce a second messenger cyclic dinucleotide (cyclic GMP-AMP), which stimulates the adaptor protein STING to induce innate immune responses. While this process was originally discovered as a crucial component of the immune defense against pathogens, recent work has elucidated a pathogenic role for innate DNA sensing in a variety of sterile inflammatory diseases. In this talk I will discuss recent findings on molecular regulation of DNA sensing and highlight opportunities for harnessing cGAS-STING for therapeutic purposes.

IGNITING THE FLAME – ROLE OF cGAS IN CELLULAR SENESCENCE AND INFLAMMAGING

Zhijian "James" Chen

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DNA entering the cytoplasm of mammalian cells is a danger signal that triggers a potent innate immune response, including the production of type-I interferons and inflammatory cytokines. cGAS is a DNA sensing enzyme that triggers the innate immune response to cytosolic DNA. Upon binding double-stranded DNA, cGAS catalyzes the conversion of GTP and ATP into cyclic GMP-AMP (cGAMP), which functions as a second messenger that activates the adaptor protein STING and the downstream pathway. The cGAS-STING pathway plays a critical role in immune defense, cellular senescence, autoimmune diseases and cancer. As such, this pathway must be tightly regulated. However, as animals age, accumulation of cellular damage, including damage to the genome and mitochondria, leads to senescence and inflammation. This process, known as inflammaging, is believed to be detrimental to health. I will discuss our recent work on the role of cGAS in inflammation and aging.

DECONSTRUCTING AGING WITH SENOLYTIC CAR T CELLS

Corina Amor

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Cellular senescence, a stress response program characterized by stable cell cycle arrest and a proinflammatory secretome, is a key hallmark of the aging process. While in younger organisms senescent cells are effectively cleared by the immune system; in the elderly they accumulate in tissues and are thought to contribute to aging pathobiology. Yet how do they build up and how do they contribute to aging remains unclear. Two key limitations that have held the field back in answering these questions have been the lack of: 1) surface markers to isolate and characterize senescent cells from aged tissues and 2) specific somatic approaches to target them without the need to breed and age for years genetically engineered mouse models (GEMMs) or employ unspecific chemical approaches. In a departure from these strategies we developed the first cell-based senolytic therapy based on chimeric antigen receptor (CAR) T cells targeting uPAR, a cell-surface protein upregulated on senescent cells. Harnessing them, we here explore the profile and characteristics of senescent cells that accumulate during physiological aging and their functional impact on aging phenotypes. In addition, we explore the preventive and therapeutic potential of immune-based cell therapies for age-related pathologies.

CLONAL EVOLUTION IN THE AGING HEMATOPOIETIC

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As we age, all cells steadily accrue mutations. While most mutations are neutral or deleterious, a few have positive effects on cellular fitness, leading to improved survival or, for stem cells, more progeny. In the hematopoietic system, this leads to “clonal hematopoiesis” (CH) where a few variant stem cells contribute disproportionately to blood production over time. This loss of diversity in the blood, along with dominance by a subset of clones, is associated with increased all-cause mortality, hematologic malignancies, cardiovascular diseases, and other aging effects. From human data, about 30 genes have been shown to confer increased hematopoietic stem cell (HSC) fitness when mutated. These genes are implicated in diverse functions, including DNA repair, epigenetic regulation, and genome organization. However, we have a poor understanding of how these mutations converge on increased HSC fitness. We will discuss mouse models of clonal evolution, and the insights they offer into human aging-associated disease.

SOMATIC RETROTRANSPOSITION IN CELLULAR SENESCENCE AND AGING

John M Sedivy

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Large proportions of most genomes are comprised of repetitive sequences, the majority of which were generated by the activity of transposable elements (TEs) in the germline. All organisms have evolved mechanisms, such as transcriptional silencing, to repress the activity of their endogenous TEs. In humans retrotransposable elements (RTEs) are particularly prominent and occupy almost 40% of our genomes. Recent evidence suggests that RTEs can be derepressed in some contexts in our somatic tissues, for example during natural aging, cellular senescence, and in age-related diseases such as cancer and neurodegeneration. One contributing factor appears to be the widespread epigenetic changes that occur during aging, in particular the loss of heterochromatin that leads to a more permissive transcriptional environment. The expression of RTEs leads to two main effects: 1) genome instability and DNA damage, due to the retrotransposition process itself, and 2) the promotion of inflammation, due to the presence of RTE nucleic acids. The latter are recognized by the interferon system and activate innate immune pathways. Low grade persistent inflammation, well-known as “sterile inflammation” and more recently “inflammaging”, is a hallmark of aging and is believed to drive many age-related pathologies. We have shown that cellular senescence is accompanied by an increase in RTE transcription. More recently we have found that during natural aging of mouse tissues, and in particular with advanced age, several families of RTEs become active. I will discuss the molecular processes that may lead to the derepression of RTEs with age, as well as the consequences of their activation on the host. Treatment of aged mice with nucleoside reverse transcriptase inhibitor (NRTI) drugs, which were developed to treat HIV infection, has been shown to alleviate age-associated inflammation in several tissues. In human epidemiological studies, NRTI use has been associated with decreased incidence of type II diabetes, macular degeneration and Alzheimer’s disease. We suggest that somatic retrotransposition is a hitherto unappreciated aging process, that activation of RTEs is likely to be an important contributor to the progressive dysfunction of aging cells, and that controlling the sequelae of RTE deregulation provides therapeutic opportunities.

Research in the Sedivy lab relevant to this presentation was supported by NIH grants P01 AG051449, R01 AG016694 and R01AG078925.

GENOME EVOLUTION OF CELLS PROGRESSING THROUGH REPLICATIVE CRISIS

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Shortening of telomeres, the repetitive, protective ends of eukaryotic linear chromosomes, restrict the proliferation capacity of human somatic cells by inducing two distinct proliferation barriers replicative senescence and crisis. During malignant transformation, premalignant cells have to overcome these barriers and acquire a telomere maintenance mechanism (TMM) to achieve replicative immortality. Currently, replicative crisis, TMM acquisition and the early stages of carcinogenesis are poorly understood. Here, we model and analyze the early stages of tumorigenesis using an in vitro crisis escape model. Out of 81 million IMR90 E6E7 lung fibroblast crisis cells two distinct populations of post-crisis clones emerged: four mortal clones that succumb after proliferating initially, and four immortal clones that maintain their telomeres by telomerase. Single-cell DNA strand-sequencing of crisis cells revealed high genome instability characterized by complex structural variants (SVs). Unexpectedly, the emerging post-crisis clones showed relative few and simple SVs, suggesting that crisis has evolved to stringently counterselect against cells with high genomic instability. However, with accumulating divisions post crisis escape, the genomes of the immortal post-crisis clones evolve and amass additional genomic alterations, including tetraploidization events with subsequent losses and gains of individual chromosomes, as well as structural rearrangements in the telomerase promoter region. Thus, our data indicate that the highly rearranged genomes found in some cancers are not originating from crisis but are rather acquired after crisis escape and immortalization.

METFORMIN TARGETS MITOCHONDRIA TO IMPROVE HEALTHSPAN.

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Metformin, a commonly prescribed medication for type 2 diabetes, has gained attention as a potential geroprotective agent. However, the precise mechanisms underlying its effects on healthspan remain incompletely elucidated. Here, we present evidence suggesting that the drug's anti-cancer, anti-inflammatory, and anti-diabetic properties are mediated through the mild inhibition of mitochondrial complex I.

DECODING THE ROLE OF MITOCHONDRIA IN CELLULAR SENESCENCE

Manuel Serrano

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The importance of mitochondria in cellular senescence is well-established. However, the molecular details are poorly understood. I will present our most recent work on mitochondrial biology in cellular senescence. In particular on the role of cyclophilin D and the transition permeability pore (mPTP) to allow the exit of calcium and compensate for its elevated influx from the endoplasmic reticulum. Also, mitochondria play a key role in the SASP by releasing dsDNA and dsRNA into the cytosol. These, in turn, activate the cGAS/STING and the RIGI/MDA5/MAVS pathways, both converging on the activation of the interferon and NFkB pathways. This work has led to the identification of new senolytic and senomorphic treatments.

NUTRIENT REGULATION OF AGE-REVERSAL AND SENESCENCE IN WORMS

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Diapause is a long-lived state of resilience that allows organisms to outlast adversity, whose study has led to seminal insights into the biology of aging. C. elegans can endure months in a fasting-induced adult reproductive diapause (ARD), and upon refeeding, regenerate and reproduce. Interestingly, we observe that during their time fasting in ARD, animals gradually age, as measured by transcriptomic clocks, but remarkably rejuvenate their transcriptional age upon refeeding, suggesting that nutrient provision promotes restoration. Thus, while fasting is usually thought to promote anti-aging effects, our studies suggest that refeeding may also play an equally important role. Through genetic screens, we discover that hlh-30/TFEB transcription factor is a master regulator of ARD whose mutation results in loss of survivorship and reproductive capacity. Closer examination reveals that such mutants arrest in a novel senescent-like state during ARD and refeeding, resembling mammalian cellular senescence--a phenotype not previously noted before in worms. Germline stem cells are characterized by DNA damage, nucleolar expansion, cell cycle arrest, and mitochondrial dysfunction. On the organism level, we observe dysregulated immune and growth metabolic signatures, elevated SAβ-gal and accelerated aging. This senescent-like state is likely induced by a misalignment of nutrient availability with metabolism and growth signaling. Suppressor screens reveal that inhibition of systemic TGFβ signaling bypasses hlh-30 senescence, and restores survivorship and stem cell longevity, implicating this axis as an evolutionarily ancient regulator of metazoan senescence. Importantly, we demonstrate that TFEB's vital role is conserved in mouse embryonic and human cancer diapause. Further genetic dissection of ARD also identifies a fasting-induced epigenetic factor whose mutation impacts biological age, resilience and restoration, and whose mammalian counterparts show a strikingly similar regulation and physiological role. Thus, ARD offers a powerful model to study resilience, restoration and senescence in vivo, directly relevant to mechanisms of mammalian longevity.

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SIGNATURES OF AGING IN NONHUMAN PRIMATES

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Caloric restriction (CR) without malnutrition is an intervention with proven ability to delay aging in diverse species. Although there has been considerable public interest in applying this regimen to improve human health and longevity, its value as a window on the biology of aging is just as compelling. The Wisconsin National Primate Research Center initiated a study of Aging and Caloric restriction in rhesus monkeys more than 35 years ago and showed significant improvements in health and survival. This highly translational model is allowing us to gain insight into systemic and tissue specific changes occurring with age, how those changes relate to physiological outcomes, and how CR alters those signatures. Longitudinal profiling of circulating factors linked to metabolism has identified an inflection point in late middle age that is coincident with the onset of age-related diseases and conditions. Molecular profiling in brain, adipose, and skeletal muscle reveal the anticipated age-associated signatures of immune and inflammatory dysfunction, but in each case, these established aging signatures are coincident with changes in metabolism., Phenotypes of aging link to local tissue metabolism and the consistent impact of CR among tissues is to not simply oppose aging but to induce changes in metabolism. Our working hypothesis is that age-associated changes in metabolic capacity are not just a biomarker of aging but may also be causally involved in creating increased disease vulnerability.

DIETARY REGULATION OF REGENERATIVE STEM CELL PLASTICITY

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Intestinal epithelial cells acquire stem cell activity to promote regeneration in response to tissue damage, but it remains unclear whether such plasticity occurs under normal physiological conditions. Furthermore, this plasticity response is exploited by cancer cells, posing a challenge for developing regenerative therapeutics to treat diseases with intestinal epithelial degeneration. Here, we uncovered that a single dietary nutrient, arachidonic acid (AA), can induce stem cell plasticity under homeostatic conditions and boosts regenerative capacity of intestinal epithelium against various damaging insults without increasing risk of cancer. Dietary AA emulates the regenerative response to tissue injury by activating an adaptive and conserved regenerative signaling in mice and humans. Mechanistically, AA elicits epigenetic reprogramming by transcriptional regulators Creb1 and Yap1 to promote stem cell plasticity and regenerative capacity. A key target gene of this transcriptional circuit is S100a6, which is crucial for damage-induced regenerative response. These results unveil a nutrient-triggered epigenetic mechanism for inducing regenerative stem cell plasticity that is uncoupled from tumorigenesis and promotes resilience of epithelial tissues. Thus, such pro-regenerative dietary interventions have therapeutic potential for degenerative epithelial diseases.

IDENTIFYING METABOLIC DEPENDENCIES IN PANCREATIC CANCER

Alec Kimmelman

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Pancreatic cancers are highly resistant to currently available therapeutics which results in a 5-year survival rate of approximately 9%. We believe that this resistance points toward altered cell metabolic pathways. In this regard we have previously shown that that oncogenic Kras promotes a rewiring of pancreatic cancer metabolism allowing carbon sources to be utilized in a variety of biosynthetic pathways. Importantly, several of these metabolic pathways are critical for tumor growth and therefore represent potential therapeutic targets. Ongoing studies from our group are exploring targeting various aspects of metabolism as therapeutic approaches.

Additional studies from our group have demonstrated pancreatic cancers have elevated basal autophagy which is required for their continued growth. Importantly, inhibition of autophagy pharmacologically or genetically leads to decreased oxidative phosphorylation, a drop in ATP production, and ultimately growth inhibition. These findings have implicated autophagy as a key component of pancreatic cancer metabolism and have motivated the opening of multiple clinical trials assessing the efficacy of hydroxychloroquine as an autophagy inhibitor in pancreatic cancer, which have now shown clinical activity in pancreatic cancer patients. Recently, we have identified a novel selective autophagy pathway that degrades MHC-I and promotes immune evasion in pancreatic tumors. Ongoing work from our group seeks to understand the metabolic contributions that autophagy makes in pancreatic tumors. These and other aspects of pancreatic cancer metabolism will be discussed.

DIRECTING MATURATION AND AGE IN HUMAN PSC-DERIVED NEURONS

Lorenz Studer

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Human pluripotent stem cells (hPSCs) present a powerful tool for studying human disease and for developing novel cell-based therapies in regenerative medicine. Our group has developed strategies to coax human PSCs into many specific neuron types on demand and at scale. For some lineages, such as midbrain dopamine neurons, those efforts have recently translated into a first-in-human clinical trial using clinical grade, “off-the-shelf” dopamine neurons for treating patients with advanced Parkinson’s disease. However, despite such rapid progress, several key challenges remain for generating relevant hPSC-derived lineages such as for studying adult or late-onset nervous system disorders. One such challenge is the control of cellular maturation and age to drive hPSC-derived neurons from a predominantly fetal identity to an adult or aged-like state. We have recently described chemical and genetic strategies that converged on the identification of an epigenetic barrier of maturation, present in immature neurons and their immediate neural precursors, and that is linked to the highly protracted timing of human neuron maturation. I will present data from a recent genome wide CRISPR screen which identified additional regulators of human timing across multiple developmental. Finally, we have used CRISPR-based and in silico screening approaches to identity drivers to neuronal age and senescence that enable the induction of late-onset disease phenotypes in various models of neurodegenerative disorders such as Parkinson’s and Alzheimer’s disease. Those strategies bring us one step closer to the goal of directing both cell fate as well as maturation and age on demand in human PSC-derived cells.

MECHANISMS THAT INFLUENCE THE PLASTICITY OF AGING

Qijing Xie, Cynthia Kenyon

Calico Life Sciences, South San Francisco, CA

For newcomers, the talk will begin with a brief history of the field; specifically, the discovery that the rate of aging is plastic and subject to regulation by specific genes. Before the 1990s, most people thought that aging was a purely entropic, unregulated process. We wear out, like old cars. However, studies in the small roundworm C. elegans showed that dramatic changes in the rate of aging and lifespan could be produced by single-gene mutations; for example, in a gene called daf-2. Mutations that reduce the activity of daf-2, which encodes a homolog of the vertebrate insulin- and IGF1-receptor genes, double the lifespan of the animal. Interestingly, this doubling requires the activity of classical stress-resistance transcription factors (including daf-16/FOXO, hsf-1/HSF, skn-1/NRF2 and hlh-30/TFEB), which reprogram the genome to increase resiliency. The discovery of daf-2 and other longevity genes showed that the rate of aging was highly plastic. Even if we couldn't define "aging", we could study it by analyzing familiar signaling and transcriptional pathways. The insulin/IGF-1 regulation of aging is evolutionarily ancient and conserved—mutations in this network extend lifespan in flies and mice, and potentially in humans as well. Small dogs, which are IGF-1 mutants, live longer than larger dogs, and very large dogs, which have high IGF-1 levels, are short lived. I will describe ongoing clinical trials in large dogs that aim to increase healthspan and lifespan by decreasing IGF-1 levels.

In addition to its regulation by insulin/IGF-1 signaling, animal healthspan and lifespan can be increased in many other ways, including perturbations affecting mitochondrial biology, inflammation, the immune system, the vasculature and autophagy. The second part of the talk will describe recent adoptive cell transfer experiments in mice that highlight potential effects of inflammatory senescent cells on age-dependent metabolic and immunologic reprogramming.

PHEROMONES, AGING AND REJUVENATION IN ANTS

Claude Desplan[1], Luok Wen Yong[1], Francisco Carmona[1], Long Ding[1], Hua Yan[2], Danny Reinberg[3]

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Ants are social insects that live in colonies of morphologically and physiologically different individuals that are genetically similar. Ant colonies contain many workers (all females) that perform most tasks but do not lay eggs, while the queen is solely responsible for reproduction. Remarkably, queens live 10-40 times longer than their sister workers, in sharp contrast with most animals in which high reproduction leads to shortened lifespan. The jumping ant Harpegnathos exhibits a high degree of aging plasticity: In the absence of queen pheromones, the workers enter a dueling ritual and the winners become pseudo-queens, called gamergates. Gamergates dramatically change their behavior, produce eggs, reconfigure their brain and, most dramatically, have a 5X lifespan extension. Remarkably, when re-placed in the presence of a genuine queen, gamergates transition back into workers with an accompanying shortened lifespan. We established Harpegnathos as a model system that can be manipulated with CRISPR/Cas9, providing a unique opportunity to study the molecular mechanisms that control the response to pheromones, aging, as well as the crosstalk between aging and reproduction.

Gamergates have a highly elevated production of Insulin to support the high metabolism required for egg production, but this is accompanied by differential regulation of the two branches of the Insulin signaling pathway in target tissues. The MAPK branch of Insulin signaling pathway is activated in the gamergate fat body and ovary, but the AKT branch is not: Instead, an extracellular “anti-Insulin” protein, ImpL2, blocks AKT phosphorylation, thus allowing nuclear localization of FOXO in spite of the very high insulin. MAPK activity, which is required for lipid production of oogenesis is preserved while repression of the AKT branch contributes to the dramatically extended longevity in gamergates.

We are investigating how workers have a hugely amplified olfactory system to perceive pheromones, how this perception leads to caste transition, and how ImpL2 interacts with the downstream insulin pathways, leading to the specific inhibition of the AKT (but not MAPK) pathways.

The lack of queen pheromones leads to transition to gamergates and is associated with brain remodeling that is communicated to the rest of the body and delays aging. We have identified a circulating regulator responsible for controlling dueling, ovarian development and delayed aging in gamergates. We will describe how this factor exerts control over Juvenile Hormone and Ecdysone response.

TISSUE TURNOVER AND REGENERATION

Alejandro Sánchez Alvarado

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It is paradoxical that for many organisms (including humans), the apparent anatomical stability of their adult bodies is maintained by constant change. Despite the importance of tissue homeostasis and regeneration to human biology and health, relatively little is known about how these processes are regulated. As such, numerous questions remain unanswered, including: How do organ systems maintain their order and function while in a state of cell flux? How do animals control and coordinate the size and cell number of multiple organ systems? Does regeneration of body parts lost to injury invoke embryonic processes, generic patterning mechanisms, or unique circuitry comprised of well-established patterning genes? Answering any of these questions would set a baseline from which to try to enhance regenerative properties in multicellular organisms such as humans, particularly after injury.

One way to solve a complex problem is to reduce it to a simpler, easier to answer problem. Therefore, reducing the complexities of regeneration and tissue homeostasis to the study of comparatively simpler systems would allow for a systematic dissection and mechanistic understanding of these processes. Here, I will discuss how the use of single-cell and spatial transcriptomics is helping define the cellular and molecular environments that support pluripotency in the highly regenerative freshwater planarian *Schmidtea mediterranea* and regeneration of missing organs in the hemichordate *Ptychodera flava*. Our studies are beginning to shed light on the way adult animals regulate tissue homeostasis and the replacement of body parts lost to injury.

USING THE AXOLOTL TO UNDERSTAND ADAPTATIONS OF DEVELOPMENTAL PROGRAMS FOR REGENERATION

Elly Tanaka

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The axolotl limb is clearly homologous to mammalian limbs yet it shows the remarkable ability to regenerate. This trait relies on one hand, on cellular plasticity involving the dedifferentiation of fibroblasts into a multipotent connective tissue progenitor. On this other hand, regeneration is an ordered process that regenerates the correct amount of limb with the correct pattern. This aspect relies on stable cell identities, called positional memory that allow cells from different limb regions to launch the appropriate developmental program. I will discuss how we study the molecular basis of these phenomena

THE ROLE OF REPRODUCTION IN SHAPING VERTEBRATE LIFE HISTORY

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Classical evolutionary theories propose trade-offs among reproduction, damage repair, and lifespan. However, empirical evidence for these trade-offs in vertebrates remains limited, largely due to the experimental challenges posed by the long lifespans of traditional vertebrate models. To address this gap, we have recently developed a suite of genetic tools for the naturally short-lived turquoise killifish *(Nothobranchius furzeri),* enabling the identification of novel regulators of aging and disease. To investigate the role of reproduction in vertebrate aging, we genetically arrested germline development at distinct stages, revealing that germline ablation promotes longevity and enhances somatic repair in a sex-specific manner. We then explored whether an inverse model—one that accelerates vertebrate reproduction—could be experimentally induced. Strikingly, we identified *vestigial-like 3 (vgll3),* a transcription cofactor previously linked to age at maturity in humans and Atlantic salmon through GWAS studies, as an antagonistically pleiotropic gene in killifish. Disrupting two conserved vgll3 isoforms demonstrated that *vgll3* reduction, in an isoform- or dose-dependent manner, accelerates male growth and reproductive development. However, these early-life benefits come at a late-life cost, as older mutant males with a disrupted long isoform develop melanoma-like tumours and exhibit increased age-related mortality. These findings position *vgll3* as a key regulator of vertebrate life-history trade-offs, balancing early-life fitness with late-life disease risk. More broadly, our results suggest that the onset of reproduction may function as a rheostat for scaling vertebrate life-history trajectories.

MAPPING THE GENETIC ARCHITECTURE OF BRAIN AGING IN KILLIFISH

Dennis M de Bakker[1,2], Leonhard Kuhlmann[1], Lisa Adam[1], Danny Arends[3], Therese Dau[1], Emilio Cirri[1], Alessandro Ori[1], Robert Williams[4], Dario Riccardo Valenzano[1,2]

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Laboratory model organisms have demonstrated that single-gene mutations can dramatically impact aging and lifespan. However, such artificially induced mutations may not reflect how aging evolves in natural populations. Studies in wild vertebrates reveal a complex genetic architecture, where lifespan is shaped by numerous naturally occurring variants with varying effects. The turquoise killifish (*Nothobranchius furzeri*) has provided key insights into the polygenic basis of lifespan differences across populations and species, largely driven by the accumulation of slightly deleterious germline mutations due to drift and relaxed purifying selection on late-acting genes.

To uncover the genetic underpinnings of spontaneous brain aging in a natural vertebrate model, we conducted a forward-genetic study using a cross between two killifish species (*N. furzeri* and *N. kadleci*) with divergent neurodegenerative phenotypes. By integrating QTL mapping with brain transcriptomics and proteomics, we identify key genomic regions and molecular signatures associated with fast or slow brain aging. This approach reveals novel, naturally occurring variants and chromosomal changes that influence neurodegeneration, highlighting pathways distinct from those identified through traditional candidate-gene approaches.

Our findings demonstrate the power of unbiased genetic approaches, such as forward genetics, in uncovering the true complexity of aging evolution. By leveraging QTL mapping and genome-wide analyses, we move beyond reductionist single-gene paradigms to identify the naturally occurring genetic variation that shapes lifespan and aging in wild populations—offering a more realistic and comprehensive view of aging biology.

HEMATOPOIETIC STEM CELLS THROUGH THE AGES: A LIFETIME OF ADAPTATION TO ORGANISMAL DEMANDS

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Hematopoietic stem cells (HSC) are responsible for lifelong blood production, but their function deteriorates with age. Understanding the mechanisms underlying HSC aging is vital for developing interventions preventing or delaying hematopoietic deterioration. I will start by reviewing some of our previous work articulating a conceptual model whereas aging degrades the interactions between HSCs and their bone marrow niche environment thereby triggering a set of adaptive responses with both beneficial and damaging consequences. Understanding this complex array of co-regulations is key for developing effective anti-aging rejuvenation approaches. I will then detail recent work identifying nucleolar stress response engagement as a cytoprotective response providing HSC resilience during aging. We found previously that old HSCs accumulate nucleolar γH2AX histone marks as a consequence of replication stress. The nucleolus is the site of ribosome biogenesis that integrates responses to diverse cellular stressors via modulation of p53 signaling, and HSCs rely on the precise regulation of protein translation to maintain their regenerative potential. Here, we connect nucleolar γH2AX in old HSCs with induction of a cytoprotective nucleolar stress response (NSR). We show that triggering nucleolar stress in HSCs impairs protein translation and delays cell cycle progression, with the resulting engagement of the p53-mediated NSR essential for preserving HSC functionality and the residual regenerative potential of old HSCs. Moreover, we establish a connection between NSR engagement and maintenance of functional quiescence under stress, identifying an activation-resistant subset of old HSCs with enhanced NSR activity, decreased cell cycle activation threshold, and preserved stemness. These findings unveil a new stress-response mechanism that is essential for maintaining the functional output of old HSCs in their native aged environment, which could be harnessed for pro-regenerative interventions.

ALLEVIATE AGING-ASSOCIATED FUNCTIONAL DEFECTS BY TARGETING DEFECTIVE HEMATOPOIETIC STEM CELLS

Yi Zhang

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Aging is a process accompanied by functional decline in tissues and organs with great social and medical consequences. Developing effective anti-aging strategies is of great significance. As the source of all blood and immune cells, hematopoietic stem cells (HSCs) play important roles in healthy aging. Through comprehensive molecular and functional analyses, we identified a subset of HSCs in aged mice that exhibit "youthful" molecular profiles and functions, marked by low expression levels of CD150. Mechanistically, $CD150^{low}$ HSCs from old mice can effectively differentiate into downstream lineage cells but not their $CD150^{high}$ counterparts. Notably, transplantation of old $CD150^{low}$ HSCs attenuates aging phenotypes and prolongs the lifespan of elderly mice compared to those transplanted with unselected or $CD150^{high}$ HSCs. Importantly, reducing the dysfunctional $CD150^{high}$ HSCs can alleviate aging phenotypes in old recipient mice. Furthermore, a CRISPR-based in vivo screen identified genes whose up-regulation in old HSCs drives the myeloid biased differentiation, which partly accounts for their functional decline. Mechanistic study revealed that the factors contribute to the myeloid bias by affecting mitochondria function. Thus, our study demonstrates the presence of "youthful" HSCs in old mice, and that rejuvenation can be achieved by removing the dysfunctional HSCs or improving mitochondria function.

AGING AND DIVERSITY OF HEMATOPOIETIC STEM CELLS

Irving L Weissman

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Hematopoietic stem cells [HSC] are the only cells in the blood system that self-renew, form and replenish all blood cells throughout life. Therefore, heterogeneity and pathologies of the blood and immune system must derive from HSC.

We prospectively isolated mouse, then human HSC, and showed in humans that purified autologous HSC can regenerate blood systems of metastatic breast cancer patients following high dose chemotherapy without re-introducing cancer cells. Mobilized peripheral blood [MPB] contains cancer cells in patients with metastases, as do CD34 enriched cells. For blood forming allotransplants [to repair genetically defective or autoimmune blood diseases], contamination of CD34 grafts with donor T cells causes graft vs host GvH] disease. Purified HSC do not cause GvH. Sadly, some BMT clinicians still call MPB and CD34 grafts ‘stem cell transplants’.

To replace genotoxic conditioning regimens, we developed non-toxic antibody-based conditioning, that allows allotransplants of purified donor HSC without GvH, and induce immunological tolerance to donor organs or cells.
Studies on HSC in aging revealed at least 2 subsets of HSC: those that produce balanced lymphoid-myeloid blood (balHSC), and those that make far more myeloid blood cells (myHSC). In young age balHSC dominate, while in aging the myHSC surpass balHSC in numbers, and in the elderly are the dominant type. The myHSC in addition to not generating new T and B cells with the diversity to recognize novel antigens, are also pro-inflammatory. Throughout life individual mice always have both balHSC and myHSC; current evidence favors them always to be separate HSC subsets rather than balHSC giving rise to myHSC during aging. In old mice, depleting myHSC with antibodies specific for epitopes expressed on the cell surface of myHSC but not balHSC leads to the return of abundant common lymphocyte progenitors and diverse naive T and B cells. These ‘rejuvenated’ old mice make adaptive T and B cell responses to antigens of novel of pathogens such as Friend retrovirus in mice, and protect them from fatal FV infections, a precedent perhaps for effective immunity in elderly populations for microbes introduced by trains, planes, cars, and boats.

AML, MPN, MDS, and myeloid blast crisis of CML are age-related blood diseases. For all of these, clones of HSC, in fact myHSC, are the site of sequential driver mutations that produce these premalignant and malignant neoplasms. We hypothesized that HSC must be the stage of blood cells that can accumulate such rare events. Studying single HSC in AML patients has confirmed this, and also revealed the surprising observation that the order of mutations leading to AML is not random; if there are for example 4 driver mutations per AML, of the potentially 256 combinations or orders, only one path leads to AML. We have proposed over 20 years ago that the same would be true of any tissue stem cell giving rise to that tissue's frank neoplasms.

T-CELL INTRINSIC AND EXTRINSIC FACTORS IMPACTING AGE-RELATED DEFECTS IN IMMUNITY TO INFECTION

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Vulnerability against new, previously not encountered infections is the most pronounced immune-related defect in older adults, responsible for millions of deaths annually. With primary infection, T cell immunity protects mammals against severe disease and death, but primary T cell responses are lower, slower and less diverse in older adults. There are several quantitative and qualitative reasons for this decline. A numerical decline in naïve T cells is one contributing factor. However, we found that the remaining naïve T cells (Tn) from the old organism can function on par with those from younger individuals, as long as they are activated in the young environment. By contrast, we mapped many defects in Tn cell maintenance and activation to the age-related changes in lymph node stroma, while others mapped to the age-related chances in circulating factors. We found that specific and discrete numeric and architectural defects in the old lymph nodes affect distinct stromal subpopulations, as evidenced by confocal microscopy and transcriptional analysis. A combination of cell subset loss, decline in extracellular matrix component synthesis and metabolic dysregulation at the level of cellular respiration in fibroblastic reticular cells (FRC) and lymphoid endothelial cells (LEC), as well as some less prominent defects in blood endothelial cells (BEC) all synergize to profoundly change structure and function of old stromal LN cells. Correction of stromal function in vitro and in vivo will be shown in discussing current and future T-cell rejuvenation treatments.

TISSUE BASED SENESCENCE-IMMUNE NETWORKS IN AGING

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The response to tissue damage and subsequent repair outcomes is a contributor to resilience with aging. The immune system is the guardian of tissue integrity and orchestrates multiple components of the repair response. While immune changes with aging have been studied in the context of infection and vaccine responses, less is understood about tissue-based immunological changes with aging that may impact repair. The recognition that the adaptive immune system, in addition to the innate, plays a role in the damage response introduces new mechanisms by which aging and systemic factors can influence local tissue repair. We found that the effector phenotype of T cells in wounds influences regenerative versus fibrotic repair outcomes in part through communication with the stroma. We further defined stromal senescence heterogeneity and phenotypes in muscle wounds of young and old mice using a senescence signature and transfer learning. T cell-pathological senescence communication networks that inhibit tissue repair in aging tissues led to excess fibrosis and scar-like tissue formation. Combination therapy targeting both immune and stromal cells restored tissue repair capacity, highlighting how immunotherapies may be an important tool for improved resilience with aging.

SELECTIVE AUTOPHAGY AND AGING: IMPACT ON CELLULAR FITNESS AND SENESCENCE

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We are interested in understanding the mechanisms and cellular consequences of age-dependent autophagy malfunction, a key driver of aging. We have found that a selective form of autophagy, chaperone-mediated autophagy (CMA), is markedly reduced with age in most organs and tissues. CMA plays a crucial role in the timely remodeling of a subset of the cellular proteome, enabling cells to adapt to an ever-changing extracellular environment. When this pathway fails with aging, these fine-tuned proteome adjustments are no longer possible, leading to disruptions in cellular homeostasis, energy balance, and stress adaptation. In this talk, I will discuss our recent advances in understanding the molecular mechanisms underlying CMA failure in aging and the impact that this CMA malfunction has on various cellular processes, including cell differentiation, functional remodeling, and senescence.

QUIESCENT CELL RE-ENTRY IS LIMITED BY MACROAUTOPHAGY-INDUCED LYSOSOMAL DAMAGE

Andrew Dillin, Andrew Murley

UC Berkeley/HHMI, MCB, Berkeley, CA

To maintain tissue homeostasis in animals, many cells are kept in a mitotically quiescent state until prompted to divide by appropriate signals. The re-activation of quiescent cells is perturbed with aging and may underly declining tissue homeostasis and resiliency. The unfolded protein response regulators IRE-1 and XBP-1 are required for re-activation of quiescent cells in developmentally arrested C. elegans. Utilizing a forward genetic screen in C. elegans, we discovered that macroautophagy targets protein aggregates to lysosomes in quiescent cells, leading to lysosome damage that prevents subsequent cell cycle re-entry in the absence of IRE-1/XBP-1 signaling. Genetic inhibition of autophagy and stimulation of lysosome biogenesis via the overexpression of HLH-30 (TFEB/TFE3) function synergistically to reduce lysosome damage in L1 arrest. Protein aggregates are also targeted to lysosomes by macroautophagy in quiescent cultured mammalian cells, causing lysosome damage that is associated with reduced cell cycle re-entry from quiescence. Our findings indicate that lysosome damage is a hallmark of quiescent cells, and that limiting lysosome damage in quiescent cells can stimulate their subsequent re-activation in response to growth cues.

α-SYNUCLEIN-INDUCED SENESCENCE IS A DRIVER AND THERAPEUTIC TARGET IN PARKINSON'S DISEASE.

Julio Aguado

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Cellular senescence, a state of irreversible cell cycle arrest accompanied by a pro-inflammatory secretory phenotype, is emerging as a key contributor to aging and age-related diseases, including neurodegenerative disorders. In the context of Parkinson's disease (PD), cellular senescence is increasingly recognized for its role in exacerbating neuroinflammation and neuronal dysfunction. Here, we report that preformed fibrils (PFF) of a-synuclein, a hallmark of PD, induce cellular senescence in vivo, providing both mechanistic insights and potential therapeutic avenues.

To investigate the role of a-synuclein-induced senescence in vivo, we injected PFF directly into the striatum of adult mice, creating a model of PD-like pathology. Transcriptomic and immunohistochemical analyses of the midbrain revealed significant upregulation of senescence-associated markers, including p16Ink4a and senescence-associated secretory phenotype (SASP) factors. These findings confirmed the induction of a senescence-like state in midbrain cells following a-synuclein PFF exposure.

Building on this model, we tested the efficacy of senolytic interventions aimed at selectively eliminating senescent cells. PFF-treated mice were administered senolytic agents and assessed for motor function, alongside transcriptomic and immunohistochemical evaluations. Remarkably, senolytic treatment improved motor performance in PFF-injected mice compared to vehicle-treated controls. Furthermore, transcriptomic analysis revealed a downregulation of pro-inflammatory and senescence-related pathways, while immunohistochemical studies showed reduced markers of senescence and neuroinflammation.

Our unpublished study underscores the important role of a-synuclein-induced senescence in PD pathogenesis and highlights the therapeutic potential of targeting senescent cells.

CHARACTERIZATION OF P-TYPE H^+-ATPASE PMA1 INHIBITORS THAT EXTEND CHRONOLOGICAL LIFESPAN IN FISSION YEAST

Hirofumi Aiba

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Fission yeast, a unicellular model organism for eukaryotes, is useful for studying cellular lifespan. Inhibition of the activity of Pma1, a widely conserved P-type proton exporting ATPase, has been shown to extend the chronological lifespan (CLS) in fission yeast Schizosaccharomyces pombe. To develop a specific inhibitor for Pma1 of S. pombe, we focused on Si01, a candidate inhibitor of Saccharomyces cerevisiae Pma1. First, we have established a synthetic method for Si01 and then investigated its Pma1 inhibitory activity and lifespan extension effect in fission yeast. Second, we also synthesized derivatives of Si01 and determined the minimum structure required for inhibition of S. pombe Pma1. Here we showed that the inhibitory activity of Pma1 correlates with the effect of lifespan extension. Si01 reduced the activity of purified Pma1 protein and extended the CLS of not only fission yeast but also budding yeast. These results provide a molecular basis for understanding the mechanism of Pma1 inhibition and the potential for developing molecules that regulate lifespan.

DEVELOPMENT OF AN NAMPT ACTIVATOR AS AN NAD+ BOOSTING AND HEALTHY AGING STRATEGY IN VIVO

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NAD+ is an essential metabolite required for multiple fundamental biological functions. Levels of NAD+ decline with age across various tissues and organisms, with the decrease being linked to multiple age-associated ailments. Cellular senescence is a process in which cells enter a stable state of growth arrest in response to stress. Accumulation of senescent cells in aged tissues compromises tissue structure and function through the persistent secretion of inflammatory mediators known as the SASP. To counteract these effects, therapeutic interventions that combine NAD+ augmentation with senolytic therapies represent promising approaches for preventing age-related disorders and promote healthy aging. Here, we demonstrate that senescent human IMR90 fibroblasts upregulate and retain high levels of intracellular nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD+ salvage pathway, without affecting NAD+ biosynthesis. To investigate the biological processes regulated by the NAMPT-NAD+ axis in senescence, we performed RNA sequencing of senescent cells after treatment with SBI-0802162, a novel NAMPT activator developed at Sanford Burnham Prebys. NAMPT activation in senescent cells led to upregulation of genes associated with stress response and inflammatory pathways, while simultaneously downregulating genes involved in cell cycle and oxidative phosphorylation. Viability assays revealed that NAMPT activation selectively reduced the survival of senescent cells, indicating its potential senolytic activity. We hypothesize that NAMPT activation in senescent cells leads to excessive accumulation of intracellular NAD+, triggering a stress response that ultimately results in cell death. Additionally, we report the characterization of the in vivo activity profile of SBI-0802162 in a novel combination strategy in mice and demonstrate that our intervention effectively suppresses age-associated inflammation across multiple tissues. Ongoing studies are investigating the molecular mechanisms underlying the potent anti-inflammatory effects of our intervention, as well as the potential long-term benefits of this NAD+ boosting and senolytic approach in age-related pathologies.

DISEASE-SPECIFIC SENESCENCE PHENOTYPES INDUCED BY BRAIN-DERIVED TAU OLIGOMERS FROM AD, DLB, AND PSP IN MOUSE PRIMARY ASTROCYTES

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Background: Cellular senescence is a state of irreversible growth arrest characterized by the secretion of pro-inflammatory factors, which contribute to tissue dysfunction and chronic inflammation. In the central nervous system, senescent astrocytes exacerbate neuroinflammation, promote tau pathology, and impair neuronal function, driving the progression of neurodegenerative diseases such as Alzheimer's Disease (AD), Dementia with Lewy Bodies (DLB), and Progressive Supranuclear Palsy (PSP). Despite the recognized role of astrocyte senescence in these disorders, the specific influence of brain-derived tau oligomers (BDTOs) on senescence induction in astrocytes remains unclear. This study addresses this gap by investigating whether BDTOs from AD, DLB, and PSP differentially induce senescence in astrocytes.

Methods: Primary astrocytes were isolated from WT (C57BL/6) mice and treated with BDTOs from AD, DLB, and PSP brains. Untreated astrocytes served as control. Cellular senescence was assessed using immunofluorescence and β-galactosidase staining to detect established senescence markers. Western blot and ELISA were used to quantify the secretion of senescence-associated secretory phenotype (SASP) markers, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). RNA sequencing will be conducted to analyze global gene expression changes and further elucidate the molecular pathways involved in BDTO-induced senescence.

Results: Treatment with BDTOs induced significant senescence in primary astrocytes, with distinct variations in the extent of the effect across the groups. Astrocytes treated with AD-derived BDTOs exhibited the highest levels of senescence, as evidenced by a pronounced increase in SASP markers. DLB-derived BDTOs induced senescence to a slightly lesser degree than AD, indicating a lower but still significant pathogenic potential. PSP-derived BDTOs induced the lowest levels of senescence, highlighting disease-specific differences in the impact of tau oligomers on astrocyte senescence.

Conclusion: The comparative analysis of BDTOs from AD, DLB, and PSP revealed the differential pathogenic potential of tau oligomers in driving astrocyte dysfunction and senescence, suggesting the existence of disease-specific tau polymorphs. Future studies will provide insights into the distinct or shared pathways by which tau oligomers contribute to neurodegenerative disease progression through astrocyte senescence. Results may offer insights into novel therapeutic strategies aimed at mitigating astrocyte-driven neurodegeneration.

ENLARGEMENT DRIVEN HEMATOPOIETIC STEM CELL DYSFUNCTION: MECHANISMS AND EVOLUTIONARY CONSERVATION

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Organismal aging is accompanied by reduced hematopoietic stem cell (HSC) function. Cellular enlargement (i.e., the cell's failure to maintain its optimal size) has emerged as a cause of this reduced HSC function during aging in mice. Importantly, reducing HSC size back to the optimal cell size rescues their function. However, the mechanism by which enlargement leads to HSC dysfunction is currently unclear. Furthermore, it is unknown whether this relation between increased size and reduced functionality holds true in human HSCs.

This research aims to discover the mechanisms behind enlargement induced HSC dysfunction and whether enlargement of human HSCs drives their dysfunction. We will assess the relationship between various pathways and cellular enlargement using immunofluorescence microscopy as well as knock-out mouse models. We will also study human HSCs using both in vitro and in vivo functional assays.

An understanding of how enlargement leads to stem cell dysfunction is essential to evaluate its potential as an aging factor and role in organismal aging. Furthermore, this study will determine whether stem cell enlargement is an aging hallmark preserved across species. If enlargement and dysfunction are connected in human HSCs, size reduction could be explored as a treatment to restore HSC function and therefore promote healthy aging.

OUABAIN-ACTIVATED NKA SIGNALING ATTENUATES OXIDATIVE STRESS IN PRIMARY CORTICAL NEURONS

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Oxidative stress corresponds, mainly, to an accumulation of reactive oxygen species, a product of external factors or physiological processes, principally cellular respiration. These free radicals have been proven to be involved in the aging process and impacts mainly the neural central system since it has the body's highest oxygen consumption. Because of that, oxidative stress has a vital role in the pathology of neurodegenerative diseases, such as Alzheimer's disease, where it is involved in beta-amyloid peptide production, epigenetic changes, and mitochondrial dysfunction. On the other hand, Ouabain is a cardiotonic steroid that acts as a binder to N+/K+ATPase activating signaling pathways linked to neuroprotection, such as NFkB transcription and neurotrophin production. In that context, considering the worldwide impact of Alzheimer's disease and Ouabain's neuroprotective potential, this work intends to explore this steroid's potential protection against oxidative stress. To achieve that, primary cortical neuronal cultures from Wistar rats were treated with Ouabain (10μM) or Vehicle for 2 hours. Then, cellular viability was analyzed using a Cytotox assay (n=3); oxidative response proteins (Keap1 and Ho1) synthesis was analyzed by Western Blot (n=3); and mitochondrial superoxide production by MitoSox assay (n=2). We first observed that Ouabain exposure did not change the neuronal viability ($p = 0,0975$). Next, we found out that Ouabain-treated cells presented a marked lower level of Keap1 ($74,14 \pm 6,268\%$ / $p = 0,0203$) compared to the Vehicle-treated ones ($100 \pm 5,382\%$). Cells treated with Ouabain also had a large decrease in HO1 levels ($72,11 \pm 5,286\%$ / $p = 0,0403$) compared to vehicle-treated cells ($100 \pm 9,302\%$). Finally, regarding superoxide production, Ouabain-treated cells had a lower production ($60.02 \pm 9.102\%$; $p = 0.0457$) compared to the control group ($100 \pm 14.98\%$). With these data, we can conclude that Ouabain can directly affect the oxidative response in cortical neuronal cells, attenuating it. Study supported by FAPESP 2023-01789-9; 2021/06009-6.

INTERPLAY BETWEEN SENESCENCE AND FERROPTOSIS IN OVARIAN AGING: A CONSERVED MECHANISM AND THERAPEUTIC TARGET

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Ovarian aging progresses more rapidly than most organs, limiting reproductive lifespan and influencing overall health. Cellular senescence, a key driver of ovarian decline, coexists with ferroptosis, a form of iron-dependent cell death. However, how these processes interact to shape ovarian biology remains poorly understood. We found that senescent stromal cells exhibit markers of ferroptosis, suggesting a mechanistic link between these pathways in ovarian aging. The accumulation of both senescence and ferroptosis with age highlights their potential contribution to follicle loss and tissue dysfunction. To investigate the molecular basis of this interplay, we leverage advanced multi-omics and spatial transcriptomics to uncover key transcription factors—Egr1, Fos, Atf3, and Rela—that regulate both senescence and ferroptosis in stromal cells. Further, we show that this process is evolutionarily conserved across mammals, underscoring its fundamental role in reproductive aging. Targeting ferroptosis represents a promising alternative strategy for protecting the ovarian reserve and delaying reproductive decline. Understanding the convergence of senescence and ferroptosis in ovarian aging holds the potential to unlock new therapeutic approaches to preserve female fertility and overall health.

THEOPHYLLINE-INDUCED HDAC ACTIVATION AS A NOVEL SENOMORPHIC STRATEGY: SUPPRESSION OF SASP IN SENESCENT CANCER CELLS

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Many chemotherapeutic agents used in cancer treatment cause DNA damage and trigger cellular senescence, which is characterized by the irreversible arrest of cell proliferation. Senescent cells secrete pro-inflammatory factors collectively known as the senescence-associated secretory phenotype (SASP), which can promote tumor progression through enhanced proliferation, metastasis, and immunosuppression. Therefore, inhibition of the secretory activity of senescent cells by senomorphic drugs gains important for cancer treatment. Recent studies have demonstrated that theophylline enhances histone deacetylase (HDAC) activity, leading to the suppression of inflammatory gene expression. Therefore, this study aimed to investigate the potential effect of theophylline on the secretory activity of senescent A549 cells induced by the chemotherapeutic agent doxorubicin. A549 cells were treated with doxorubicin, and the effect of theophylline on IL-6 and MMP-2 secretion, HDAC activation, and HDAC expression were evaluated in senescent cells. Our results showed that 4-day treatment with 300 nM doxorubicin induced senescence in A549 cells. While doxorubicin increased IL-6 and MMP-2 secretion in senescent cancer cells, treatment with theophylline inhibited this increase significantly. Additionally, theophylline induced the activation of HDAC in senescent A549 cells, suggesting that its suppression of secretory activity may be mediated through enhanced HDAC activity. These findings indicate that theophylline exhibits senomorphic activity and could potentially be used as an adjuvant therapy by eliminating the undesirable effects of SASP factors in cancer therapy.

REGULATION OF TRANSPOSABLE ELEMENTS IN AGING AND NEURODEGENERATION

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In the next decade, ~6.2 million and ~1.2 million Americans will be diagnosed with Alzheimer's disease (AD) and Parkinson's disease (PD), respectively. Despite the growing cases, we are still just grasping at molecular mechanisms behind these uncurable diseases. The immense complexity of the human brain underlies the challenge of understanding which brain cell types are most susceptible to malfunction. In addition, we lack fundamental understanding of genomic and biochemical events leading to these neurodegenerative disorders. Recent studies implicated transposable elements (TEs) in aging and neurodegenerative disorders, but the impact of brain TE RNA dynamics on these phenomena is not fully understood. Transposons are selfish invaders of animal genomes that are usually detrimental to animal fitness when they insert into and disrupt the expression of essential genes. All animal genomes are filled with transposons, and nearly 50% of all human DNA is comprised of transposon sequences. Most of these transposon sequences are silenced by small RNAs generated by the RNA interference (RNAi) pathway and packed into silent heterochromatin. However, an emerging hypothesis of animal aging and aging-related disorders is that transposon silencing is lost and transposon RNAs are expressed in a misregulated fashion. Strong expression of transposon RNAs in human cells are not fully understood as to whether they are aberrant mRNAs or unknown biomarkers of active gene expression programs. Since retrotransposons (LINE and SINEs) are the dominant class of human transposons and are implicated in aging and neurodegeneration, we want to determine how prominently might RNAi regulate retrotransposons by processing them into small RNAs during aging and neurodegeneration? To that end, we are using deep sequencing and bioinformatics approaches to determine how human transposon small RNA patterns change in brain samples from individuals with AD versus healthy controls.

DYSTROPHIC MICROGLIA ARE ASSOCIATED WITH A HAVCR2 VARIANT LINKED TO REDUCED ALZHEIMER'S RISK IN THE AGING HUMAN BRAIN

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The T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), encoded by HAVCR2, is an inhibitory receptor known to regulate immune cell activation and exhaustion. The Alzheimer's disease (AD) risk variant rs6891966 achieves genome-wide significance and is in robust linkage disequilibrium ($R^2 > 0.84$) with over 70 SNPs spanning the HAVCR1 and HAVCR2 genes. According to transcriptomic datasets, both TIM-1 (HAVCR1) and TIM-3 (HAVCR2) are selectively expressed by microglia in the human brain, with HAVCR2 expressed at approximately 25-fold higher levels than HAVCR1. To investigate how this AD-risk SNP may influence microglia and AD pathology, we evaluated postmortem brain tissue from 55 individuals in the UK-ADRC autopsy cohort with available genotyping and histopathological data. Cases represented a spectrum of pathology, including non-diseased controls, Braak NFT stages III–VI, limbic-predominant age-related TDP-43 encephalopathy (LATE-NC), and Lewy body disease (LBD). Microglial morphology (ramified, hypertrophic, and dystrophic) and total density were quantified in the hippocampus and neocortex. While total microglial density did not differ significantly by SNP status after adjusting for age, APOE ε4, and disease stage, carriers of the rs6891966 minor A allele consistently exhibited a reduced proportion of ramified microglia in regions such as CA1 and the frontal cortex. In contrast, the percentage of dystrophic microglia—often associated with degeneration or dysfunction—was significantly higher in carriers, particularly in the frontal and occipital cortices. These differences remained significant after full adjustment. Hypertrophic microglia showed region- and stage-specific trends but were not consistently associated with SNP status. Neuropathological assessment revealed region- and stage-specific differences in AD-related pathology. Notably, SNP carriers exhibited significantly greater frontal amyloid density in Braak NFT stage III/IV and higher hippocampal NFT burden among cognitively normal individuals, suggesting possible effects on early tau pathology. No significant differences in cognitive performance were observed between carriers and non-carriers across diagnostic groups. Together, these findings suggest that rs6891966 may influence AD risk by altering microglial functional states rather than cell numbers. The shift from homeostatic to activated and then to dysfunctional microglial morphologies in SNP carriers may contribute to the initiation or progression of AD-related pathology.

TARGETING SENESCENT CELLS WITH PRECISION: A NANOPARTICLE APPROACH TO COMBAT AGING AND ENHANCE ORGAN FITNESS

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Aging is characterized by a progressive decline in organ function, leading to an increased susceptibility for chronic disease and risks associated with acute bacterial or viral infection. Age-associated organ decline is mediated in part by the accumulation of senescent cells with age. Cellular senescence is a damage response program characterized by stable cell-cycle arrest and the secretion of pro-inflammatory cytokines and other tissue remodeling factors named the senescence-associated secretory phenotype (SASP). While senescence can promote wound healing and tumor suppression, during aging, however, the inappropriate accumulation of senescent cells results in a chronic pro-inflammatory environment that impairs organ function. “Senolytic” strategies that ablate senescent cells can ameliorate age-associated diseases in animal models. Nonetheless, conventional senolytic drugs have limited selectivity for senescent cells and, consequently, have dose-limiting toxicities that may make them unfavorable for treating older individuals. Here, we identified that p-selectin, a cell adhesion protein, is upregulated upon senescence induction in multiple mouse and human models. In addition, we observed that p-selectin is expressed in senescent cells of multiple murine aged tissues (heart, lung, liver, pancreas, kidney). Fucoidan nanoparticles, targeting p-selectin, specifically enrich in multiple aged organs and accumulate in p-selectin^{+} senescent cells of aged organs. P-selectin-targeting nanoparticles containing a senolytic drug (Navitoclax) profoundly improved age-associated fibrosis and organ function in multiple organs while reducing off-target toxicities when compared to their corresponding naked drugs. These results establish a novel senotherapeutic approach to specifically target p-selectin^{+} senescent cells from old tissues that serves as a broad and versatile therapeutic platform for treating senescent-associated pathologies with age.

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SENOLYTICS RESTORE HEMATOPOIETIC STEM CELL FUNCTION IN SICKLE CELL DISEASE

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Sickle cell disease (SCD) is an inherited anemia caused by β-globin gene mutations, resulting in abnormal hemoglobin polymerization driving systemic hemolysis, vaso-oclusion, inflammation, and organ damage. Hematopoietic stem and progenitor cell (HSPC) transplantation is the only potentially curative therapy, either by allogeneic or autologous transplantation following gene-editing of mobilized SCD HSPCs. However, the deleterious effects of SCD on the HSPC pool remain poorly understood and many individuals with SCD are unable to undergo gene-editing due to insufficient HSPC mobilization. Thus, we interrogated bone marrow HSPC fidelity and function in mice and individuals with SCD. We observed HSPC dysfunction including oxidative stress, DNA damage, extended cell cycle kinetics, increased cell size, and a 4-6 fold loss of long-term repopulating HSPCs in SCD mice by limiting dilution transplantation. Transcriptomic analysis of SCD mouse HSCs revealed downregulation of gene networks involved in histone/ribosome biogenesis and maintenance of p53 activity, signatures previously associated with cells transitioning to senescence, and consistently SCD mouse HSPCs display high levels of senescence-associated β-galactosidase (SA-β-gal) activity. We also detected increased DNA damage, SA-β-gal activity, and impaired ex vivo hematopoietic potential in HSPCs isolated from young individuals with SCD. Further we observed increased protein levels of the cell cycle inhibitors p16 and p21, which induce canonical senescence through retinoblastoma (Rb) protein hypo-phosphorylation, resulting in suppression of E2F, a master regulator of cell cycle and DNA synthesis/repair. Transcriptomic profiling of HSPCs from young individuals with SCD showed marked downregulation of E2F responsive gene networks. Importantly, p16 protein level was the only parameter inversely correlated with human SCD HSPC hematopoietic potential. Clearance of senescent HSPCs from mouse bone marrow using the senolytic BCL2/BCLXL inhibitor, ABT-263, can restore aging-related HSPC dysfunction. Thus, we tested if clearance of senescent HSPCs could restore function to the SCD HSPC pool, and found significantly increased HSPC numbers, reduced DNA damage, and restoration of hematopoietic repopulating activity in ABT-263 treated SCD mice. In total, our data reveals that SCD pathophysiology invokes senescence impairing HSPC function in mice and humans, and provides proof-of-concept for the use of senolytics to restore HSPC function during SCD.

NAD DEFICIENCY IN VIVO INDUCES CHANGES IN METABOLISM THAT MIMICS AGING IN A PARTIALLY REVERSIBLE MANNER.

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Nicotinamide adenine dinucleotide (NAD) is a critical nucleotide that regulates many cellular processes including metabolism, epigenetics, DNA repair and cell signaling. Importantly, NAD deficiency has been shown to cause Pellagra and may contribute to age-related metabolic dysfunction and several diseases states. However, the precise mechanisms by which NAD deficiency regulates metabolism in vivo are not fully understood. To investigate the effects of NAD deficiency in mice, we combined genetic and nutritional manipulations to induce moderate NAD deficiency in young mice.

Independent of the sex and age, our results show that mice with induced NAD deficiency develop distinct phenotypes within 4-6 months of niacin-free diet. NAD-deficient mice exhibited reduced weight, hunchback posture, browning/graying of the fur color, decreased exercise capacity, increased frailty scores, reduced lifespan, along with increased senescence burden, inflammation, and oxidative stress. Metabolomics and RNA seq analysis indicated that induction of NAD deficiency in young mice promoted metabolic changes and clustering closer to old mice than their control litter mattes. Importantly, most of these changes were partially or totally reversible by recovering NAD levels via dietary intervention. Finally, some features of the phenotype were partially prevented with senolytic drug treatment.

In conclusion, our data suggests that NAD decline may contribute to the metabolic dysfunction that occurs in aging or pathological conditions, and this dysfunction could be reversible via reconstitution of NAD levels.

INTEGRATIVE ANALYSIS OF PAN-CANCER DNA MUTATIONS AND EPIMUTATIONS

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Background: DNA methylation (DNAme) is a critical epigenetic mechanism that regulates cell differentiation and development. This process involves the addition of a methyl group to the fifth carbon of cytosine, forming 5-methylcytosine (5mC), a modification catalyzed by DNA methyltransferases. In tumorigenesis, global DNA methylation hypermutation is commonly observed, while locus-specific hypermutation plays a significant role in diagnosis, prognosis, and treatment. Tumor suppressor genes (TSGs) are often hypermethylated, whereas pericentromeric heterochromatin regions are typically hypomethylated. These alterations contribute to uncontrolled cell division, genomic instability, and tumor progression.
Methods: In this study, we present an integrative pan-cancer analysis of DNA methylation using TCGA data to compare tumor and normal tissues and explore organ-specific methylation patterns. Our goal is to uncover cancer-specific genomic features, identify potential diagnostic biomarkers, and discover tissue-specific methylation markers. We collected methylation array data from The Cancer Genome Atlas. It (TCGA) project and the Genotype-Tissue Expression (GTEx) consortium, and analyzed DNAme patterns across 24 cancer types and matching NAT, as well as across nine healthy organs. Using the Wilcoxon test and log-transformed FDR-adjusted p-values, we identified regions with significant methylation differences in: (1) normal organs, (2) tumor versus normal samples for each of the 24 cancer types, and (3) across different cancer types.
Results: We propose a novel algorithm for selecting hypermethylated and hypomethylated epimutations, normalized by adjacent normal tissue (NAT) and statistical measurements. Differentially methylated regions were then correlated with tumor mutational burden (TMB) and ranked to identify the most significantly altered cancers. Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) exhibited the highest levels of hypermethylation based on both standard deviation and mean difference criteria. In contrast, pheochromocytoma and paraganglioma (PCPG) consistently ranked among the top three for hypomethylation across various statistical measures.
Discussion: Our long-term objective is to develop a transformer-based deep learning model capable of classifying cancer types and predicting future epigenetic changes from methylation time series data.

MODELING HUMAN MIDBRAIN SENESCENCE: AN iPSC-BASED PLATFORM TO EXPLORE AGING AND AGE-RELATED NEURODEGENERATIVE DISORDERS.

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Aging represents the major risk factor for developing neurodegenerative disorders such as Parkinson's disease (PD). To study the impact of senescence in the context of the human midbrain, we used a procedure to age neuroepithelial stem cells (NESCs) and neural stem cells (NSCs), leveraging exhaustion through passaging. From these senescent stem cell cultures, we generated astrocytes and midbrain organoids that exhibit increased expression of proinflammatory cytokines and markers associated with cellular senescence (e.g., 53BP1, H2AX), increased expression of beta-galactosidase staining, and impaired mitophagy. One of the key advantages of this procedure is that it enables us to induce a senescent-like phenotype without relying on chemicals, radiation, or genetic modifications involving genes associated with premature aging (e.g. Progerin). We also used induced pluripotent stem cells (iPSCs) derived from PD patients carrying the LRRK2-G2019S mutation, which is responsible for a familial form of the pathology. We obtained astrocytes and midbrain organoids to evaluate the mentioned phenotypes in a pathological context. We observed that LRRK2-G2019S cultures showed a propensity to develop premature senescent phenotypes, such as positive staining for beta-galactosidase, without leveraging on stem cell exhaustion. However, when LRRK2-G2019S senescent stem cells were used to generate astrocytes and midbrain organoids, we observed an extensive pro-inflammatory profile.
These observations strongly suggest that our platform is suitable to study the interplay between senescence and pathological degeneration in the context of PD in a fully humanized and patient-specific model.

THE POLYCOMB PROTEIN BMI1 PROTECTS AGAINST PROGRESSIVE CEREBELLAR DEGENERATION AND ATAXIA

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Bmi1, a component of the Polycomb Repressive Complex 1, promotes stem cell function in multiple tissues through regulation of stem cell self-renewal, proliferation and differentiation. Germline deficiency of Bmi1 leads to postnatal defects in stem cell function in multiple tissues and adult neurological deficits, including ataxia and seizures. The cause of the neurological defects is unknown. Conditional deletion of *Bmi1* from neural cells early during development, using *Nestin-Cre*, led to adult-onset neurological phenotypes, including balance and gait defects, that worsened over time. To determine the cell types in which Bmi1 was required to prevent ataxia, conditional mouse models were used to delete *Bmi1* in specific neural cell types and mice were evaluated for the onset of adult neurological defects. Deletion of *Bmi1* in adult neural stem and progenitor cells did not cause neurological deficits nor did *Bmi1* deficiency in peripheral sensory neurons. Deletion of *Bmi1* in cerebellar Purkinje cells and molecular layer interneurons, using *Parvalbumin-Cre*, resulted in adult-onset neurological defects. The onset of neurological defects coincided with detection of cerebellum molecular layer thinning, a reduction in Purkinje cell dendrites and aberrant Purkinje dendrite arborization. Bmi1 negatively regulates the expression of the $p16^{Ink4a}$ and $p19^{ARF}$ tumor suppressors, but $p16^{Ink4a}$ and $p19^{ARF}$ deficiency only partially rescued neurological deficits. Thus, Bmi1 is required in cerebellar Purkinje cells and molecular layer interneurons to maintain adult cerebellar architecture and prevent adult-onset neurological defects and ataxia.

CHARACTERIZATION OF AGE-ASSOCIATED INFLAMMASOME ACTIVATION REVEALS TISSUE SPECIFIC DIFFERENCES IN TRANSCRIPTIONAL AND POST-TRANSLATIONAL INFLAMMATORY RESPONSES

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Aging is associated with systemic chronic, low-grade inflammation, termed 'inflammaging'. This pattern of inflammation is multifactorial and is driven by numerous inflammatory pathways, including the inflammasome. However, most studies to date have examined changes in the transcriptomes that are associated with aging and inflammaging, despite the fact that inflammasome activation is driven by a series of post-translational activation steps, culminating in the cleavage and activation of caspase-1. Here, we utilized transgenic mice expressing a caspase-1 biosensor to examine age-associated inflammasome activation in various organs and tissues to define these post-translational manifestations of inflammaging. Consistent with other studies, we observe increased inflammation, including inflammasome activation, in tissues. However, we note that the degree of inflammasome activation is not uniformly correlated with transcriptional changes commonly used as a surrogate for inflammasome activation in tissues. Furthermore, we used a skull thinning technique to monitor central nervous system inflammasome activation in vivo in aged mice and found that neuroinflammation is significantly amplified in aged mice in response to endotoxin challenge. Together, these data reveal that inflammaging is associated with both transcriptional and post-translational inflammatory pathways that are not uniform between tissues and establish new methodologies for measuring age-associated inflammasome activation in vivo and ex vivo.

BIOFABRICATION OF HUMAN 3D ORGAN-SPECIFIC MODELS FOR THE STUDY OF VASCULAR AGING

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Europe's aging population is increasing, with individuals over 65 projected to reach 28.9% by 2050. Aging is associated with a decline in health conditions, an increase in chronic illnesses, and growing healthcare expenditures. Among age-related diseases (ARDs), cardiovascular diseases remain the leading cause of mortality and morbidity. Endothelial dysfunction plays a key role in vascular aging and contributes to multiple ARDs. Heterochronic parabiosis experiments have shown that young blood can modulate aging hallmarks and improve cardiovascular and cognitive function. However, translating these findings to humans is challenging due to species-specific differences in aging. Developing human-relevant *in vitro* models that closely mimic endothelial aging could help bridge this gap and advance translational research.

We developed a custom high-throughput platform (up to 32 samples) to biofabricate 3D human organotypic microvascular models using human dermal microvascular endothelial cells (HDMECs) and dermal fibroblasts embedded in a fibrin hydrogel. To enhance physiological relevance, the model features mesoscale dimensions and incorporates organotypic co-culture and unidirectional hemodynamic stimuli, which help recover the *in vivo* endothelial transcriptomic phenotype. The platform enables automated hydrogel seeding, confocal imaging, and perfusability assessments. Serum samples from young (<35y/o) and old (>65y/o) healthy donors were collected, screened using an aptamer-based proteomic approach and are currently under investigation for their effect on the model's microvessels.

Optimized culture conditions supported the formation of self-assembled microvascular networks with physiologically relevant vessel diameters and lumen formation. Perfusability tests demonstrated flow of 70kDa-dextran through the lumens of the perfusable networks spanning the entire matrix. Exposure to old sera resulted in a loss of perfusability, suggesting functional impairment. We are currently validating aging markers (e.g., p16, H2AX, SIRT1) and microvascular structure by comparing our model to dermal biopsies from donors of different ages.

Our 3D microvascular model recapitulates key aspects of physiological microvessels in a high-throughput configuration and shows a promising differential response to young and old sera. Future work will include scaling out the platform to 96 samples, identifying how the serum from young and old donors affects the model at the transcriptomic and functional levels, and developing new organotypic models of human microvasculature, including cell types from different human tissues (e.g., blood-brain microvessels).

ENHANCED LIVER REGENERATION VIA TARGETED MRNA DELIVERY FOR PARTIAL *IN VIVO* REPROGRAMMING

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Recent studies suggest that injury-induced dedifferentiation, which leads to the formation of 'injury-responsive cells', contributes significantly to tissue repair across various organs, including the liver. Utilizing Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc: OSKM) for in vivo partial reprogramming generates 'injury-responsive cells' in the intestine, mirroring those derived from injury-induced dedifferentiation. Thus, the transgene induction of OSKM or viral delivery of Oct4, Sox2, and Klf4 shows promise in facilitating tissue regeneration in the intestine, liver, skeletal muscle, and retina. Herein, we demonstrated that transient OSKM induction produces two distinct liver progenitor-like cell populations. One of these populations resembles liver progenitor-like cells (LPLCs) generated by acute acetaminophen (APAP) injury without triggering immune responses. To explore in vivo reprogramming as a viable strategy for tissue regeneration, we employed lipid nanoparticles (LNP) carrying OSKM mRNA (OSKM mRNA-LNP) to stimulate LPLCs formation. Notably, the production of Sox9+ LPLCs, and OSKM-induced dedifferentiation, was closely correlated with successful tissue regeneration in the liver post APAP injury. Thus, the OSKM mRNA-LNP approach represents a promising therapeutic intervention for the repair of acute liver injuries.

TITRATION OF RAS ALTERS SENESCENT STATE AND INFLUENCES TUMOUR INITIATION

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Oncogenic RAS-induced senescence (OIS) is an autonomous tumour suppressor mechanism associated with premalignancy. Achieving this phenotype typically requires a high level of oncogenic stress, yet the phenotype provoked by lower oncogenic dosage remains unclear. In our recent publication, we describe oncogenic RAS dose escalation models, revealing a RAS dose-driven non-linear continuum. In the context of the pre-malignant liver, we identified different subtypes of tumour-initiating cells driven by distinct progenitor markers and translating to different types of liver tumours, mirroring subtypes seen in human disease. Subsequently, we have probed the non-cell autonomous response to this RAS dose, identifying unique subsets of immune cells that differentially interact with epithelial cells harbouring different levels of oncogenic signalling, and may non-cell autonomously contribute to tumour formation.

CASM AS A KEY REGULATOR OF THE SENESCENCE-CELL DEATH DECISION

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Cellular senescence is a hallmark of aging and a key driver of age-related diseases. While the triggers of senescence are well-studied, the mechanisms that sustain senescent cells remain poorly understood. Lysosomal expansion is a defining feature of senescence, marked by an increase in lysosome number and size, elevated senescence-associated β-galactosidase (SA-β-gal) activity, and accumulation of lipofuscin. Despite lysosomal expansion suggesting activation of TFEB and TFE3, master regulators of lysosomal biogenesis, their activation mechanism during senescence remains unclear—especially given the simultaneous activation of mTORC1, which typically inhibits TFEB.

Here, we identify a mTORC1-independent mechanism regulating TFEB activation during senescence, mediated by Conjugation of ATG8 on Single Membranes (CASM). We show that inhibition of CASM—via knockdown of ATG16L1, ATG12, or ATG5—prevents TFEB nuclear translocation and leads to cell death. These findings position CASM as a critical regulator of the senescence vs. cell death decision, highlighting lysosomal dynamics as a key determinant of senescent cell survival. Understanding this mechanism may provide novel therapeutic strategies for targeting senescent cells in aging and age-related diseases.

DISTINCT ROLES FOR NFκB SIGNALING IN HEMATOPOIETIC STEM CELLS AND THE BONE MARROW MILIEU DURING HEMATOPOIETIC AGING

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Hematopoietic aging is characterized by chronic inflammation associated with myeloid bias, HSC accumulation, and functional HSC impairment. Yet it remains unclear how inflammation promotes aging phenotypes. NFκB both responds to and directs inflammation, and we present an experimental model of elevated NFκB activity ("IκB–") to dissect its role in hematopoietic aging phenotypes. We found that while elevated NFκB activity is not sufficient for HSC accumulation, HSC-autonomous NFκB activity impairs their functionality, leading to reduced bone marrow reconstitution. In contrast, myeloid bias is driven by the IκB– proinflammatory bone marrow milieu through epigenomic and transcriptomic reprogramming of HSCs. A new scRNA-seq HSPC labeling framework enabled comparisons with aged murine and human HSC datasets, documenting an association between HSC-intrinsic NFκB activity and quiescence, but not myeloid bias. These findings delineate separate regulatory mechanisms that underlie the three hallmarks of hematopoietic aging, suggesting that they are specifically and independently therapeutically targetable.

STEAROYL-COENZYME A DESATURASE 2 EXPRESSION REFLECTS AGE-RELATED DECLINE IN MUSCLE STEM CELL ABUNDANCE AND FUNCTION

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Stearoyl-coenzyme A desaturase 2 (SCD2) is a Δ9-desaturase enzyme that converts saturated fatty acids to monounsaturated fatty acids, playing a crucial role in lipid metabolism and membrane fluidity. FAT-7, the homolog gene in *Caenorhabditis elegans* (*C. elegans*), has been shown to influence lifespan. Its deletion or inhibition reduces longevity, whereas overexpression or treatment with its metabolic products extends lifespan. In this study, we identified *fat-7* as a key gene differentially expressed between healthy and normal aging *C. elegans* populations. Furthermore, *fat-7* expression decreased with aging and inhibition of its expression exacerbated the age-related decline in exercise function.

To identify the murine homolog of *fat-7*, we analyzed skeletal muscle tissue from three independent cohorts of aged and young mice. Among candidate genes, *Scd2* was consistently downregulated in aged skeletal muscle across multiple datasets, leading us to focus on its role in aging. We further demonstrated that SCD2 expression significantly correlates with frailty index and physical performance in aged mice. Additionally, single-cell RNA sequencing (scRNA-seq) revealed that age-related *Scd2* downregulation in skeletal muscle is linked to reduced muscle stem cell (MuSC) abundance and diminished regenerative capacity. Furthermore, using the C2C12 myoblast cell line, we observed that *Scd2* expression is dynamically regulated during differentiation and that modulating its expression affects myogenic differentiation.

Taken together, our findings suggest that SCD2, the murine homolog of *C. elegans* FAT-7, is a potential biomarker reflecting MuSC status and aging-related functional decline. Moreover, targeting SCD2 expression may provide a promising strategy for preserving skeletal muscle mass and function during aging.

SENOMORPHIC EFFECT OF PARTIAL REPROGRAMMING

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Over the past few years, partial reprogramming via short, repetitive expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) has emerged as a promising approach for rejuvenation. The accumulation of cells undergoing senescence, an irreversible state of growth arrest, with time is considered one of the major contributors to aging. However, the impact of partial reprogramming on cellular senescence and its potential to reverse senescence phenotypes remains unclear.
Our study aimed to investigate the effects of OSKM expression on senescent cells. Our findings demonstrate that OSKM expression in senescent cells leads to a significant reduction in certain senescence markers, restores mitochondrial function, and reduces the senescence-associated secretory phenotype (SASP) without altering growth arrest. This effect of OSKM on the SASP is observed at the functional level, as shown by experiments with conditioned media from senescent cells with or without OSKM expression, as well as through in vivo analyses, both of which reveal a reduction in pro-inflammatory secretions and an enhancement of cellular health.
Furthermore, we have tested various chemical compounds that could replicate OSKM effects by similarly altering the senescent phenotype, reducing SASP, and restoring mitochondrial function, thus mimicking the rejuvenating effects of OSKM and opening possibilities for clinical translation.
In conclusion, our study reveals that OSKM expression exerts a senomorphic effect, modulating the senescence phenotype without inducing cell proliferation. These findings shed light on the complex interplay between partial reprogramming and senescence, providing valuable insights into the rejuvenation potential of partial reprogramming strategies.

SENESCENT CELLS DISRUPT ECM REMODELING IN RESPONSE TO EXERCISE

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Much like cars and homes, all tissues in the body require daily maintenance to ensure proper structure and function, a process called extracellular matrix (ECM) remodeling. This process is essential for repairing both daily damage and large-scale injuries, and it is performed primarily by resident cell types within each tissue. Our lab studies ECM maintenance in tendons, which facilitate movement by transmitting muscular contractions to bone. Tendons are also a highly dynamic ECM-rich tissue with sensitive and rapid remodeling responses to both chemical and mechanical stimuli. While we have previously established that the remodeling process is disrupted in aging, we are now focusing on why this occurs and how we can prevent or reverse it. Senescent cells, characterized by chronic growth arrest, altered metabolic activity and increased secretion of pro-inflammatory markers, have a high potential to play a role in disrupting tissue function. We recently developed an innovative microphysiologic model of senescence using intact tissue explants, permitting us to study the effect of senescence on physiologic tissue remodeling for the first time. In the present study, we focus on the ability of senescent cells to synthesize and remodel existing tissue structure in response to exercise. Control and induced senescent flexor tendon explants derived from male mice were stimulated with a 2-week cyclic adaptive mechanical loading protocols designed to increase matrix turnover and simulate exercise. The first week established a homeostatic baseline, after which half of the samples were subjected to an increase in loading for the next week. Samples were collected at day 7 (baseline) and day 14. As expected, young tendons exhibit robust expression of ECM-related genes and increases in protein synthesis with exercise. Interestingly, senescent explants exhibit no adaptive increases in most of the ECM genes, especially proteoglycans and MMPs. In some cases, senescent explants actively downregulate important ECM turnover genes, like TGF-β. Unfortunately, this lack of response could indicate inability to initiate repair mechanism for every day microtrauma, something we are actively exploring. Interestingly, exercise alone causes some features of senescent phenotype in young tendons, such as upregulation of p21 and downregulation of LMB1. However, this could be an adaptive mechanism to focus energy on matrix synthesis rather than replication or could be due to DNA damage and/or reactive oxygen species production associated with exercise. Senescent tendons, however, do not exhibit these exercise-induced changes but instead downregulate cell cycle markers like p16 and p19, suggesting a potential benefit for exercise that should be explored. Overall, this work suggests that the presence of senescent cells in tendon, even a small percentage, can have a large impact on the overall tissue response to exercise, thus leaving them vulnerable to injury and chronic degeneration. Given the critical role for ECM in determining cell fate, regulating cell behavior and providing mechanical integrity for all tissues, this further solidifies senescence as a target for treating age-related diseases of extracellular matrix dysfunction.

STRESS AND AGING INTERACTIONS INCREASE TRANSCRIPTIONAL HETEROGENEITY AND COMPROMISE FUNCTION OF THE HEMATOPOIETIC COMPARTMENT DURING SICKLE CELL DISEASE IN A MOUSE MODEL

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Sickle cell disease (SCD) is a genetic hemolytic blood disorder caused by mutation in the β-globin gene HBB, promoting hemoglobin polymerization in red blood cells (RBCs) in low oxygen, leading to hemolysis, vaso-occlusion, organ damage, stroke, reduced quality of life, and shortened life expectancy. Chronic systemic exposure to products of hemolysis and proliferative demands to maintain RBC numbers in SCD lead to DNA damage accumulation, oxidative stress, and chronic inflammation, compromising function of hematopoietic stem and progenitor cells (HSPCs) and their supporting bone marrow stromal cells (BMSCs). Previous work in our laboratory has found substantial functional deficits of HSPCs derived from both an SCD mouse model and human individuals with SCD. The stress-induced SCD cellular phenotypes in bone marrow are also hallmarks of aging, and indeed a recent report identified an association between SCD and accelerated epigenetic aging for some epigenetic clocks in blood mononuclear cells from individuals with SCD. While advances in care for SCD are improving survival to adulthood, little is known about interactions between SCD and aging at a molecular level in HSPCs and the supporting niche. To better understand the effects on HSPC and BMSC health and heterogeneity at molecular and cell-level resolution in SCD and with aging, we acquired a single-cell RNA-Seq dataset from SCD and non-SCD mice for both HSPC (c-Kit+) and BMSC pools, and across disease progression through aging: young (2 month), middle-age (6 month), and late middle-age (12 month) animals. Notably, substantial disease-associated death occurs by 12 months in the SCD animals. In total, more than a quarter-million cells were profiled across 48 total samples (n=4 per condition). We identified distinct SCD-specific RNA velocity vectors and differentiation trajectories across aging in both the HSPCs and bone marrow niche. Disease progression across aging in SCD was significantly associated with inflammatory signaling pathway activity in multiple HSPC cell types. Finally, we considered our HSPC and BMSC datasets together to predict inter-cellular signals dysregulated by SCD and particularly exacerbated by aging. Overall, this work provides insight into how SCD perturbs HSPC and BMSC heterogeneity, and how molecular consequences of disease progress with aging.

THE METABOLIC ENVIRONMENT SHAPES CELLULAR SENESCENCE: IMPLICATIONS FOR SENESCENCE IN VITRO MODELS AND TRANSLATION

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Metabolic interactions between cells and the resulting shared chemical environment are crucial factors influencing cellular physiology, survival, and drug response. In senescence, intercellular communication has been extensively studied at the protein level, primarily attributed to the senescence-associated secretory phenotype (SASP). However, the chemical alterations in senescent cells, signaling to neighboring cells, and their role in disease remain underexplored. Current in vitro studies often overlook the metabolic environment as a key modulator of cellular physiology and responses. Standard culture conditions, which are typically metabolite-rich, fail to capture the complexity of physiological chemical environments. These conditions suppress intracellular biosynthetic pathways, significantly altering gene expression and reducing the physiological relevance of in vitro models. We have shown that metabolite exchange between eukaryotic cells, particularly the sharing of amino acids, induces broad metabolic rewiring - affecting both the intracellular metabolome and the extracellular chemical space - with profound physiological consequences, including altered drug responses. Here, we systematically investigated how different culture media regarding chemical composition, standard and synthetic serum, dialyzed and non-dialyzed, affect senescence in human primary fibroblasts. By integrating phenotypic imaging of senescence markers with proteomics analysis, we observed that the cellular responses of senescent cells varied significantly depending on the chemical composition of the culture media. Additionally, we assessed protein turnover, which is known to be reduced in senescence, using SILAC (Stable Isotope Labeling by Amino Acids) in chemically distinct conditions. Gene set enrichment analysis (GSEA) revealed that the turnover of proteins from distinct gene ontologies was differentially affected depending on the metabolic composition of the culture media. These findings underscore the critical impact of the chemical environment on cellular senescence and highlight the limitations of conventional in vitro culture conditions. Next, we will profile the intracellular and extracellular chemical landscape of senescent cells in physiologically relevant culture conditions and dissect the chemical signaling that may contribute to paracrine senescence. This study may enhance in vitro senescence research by incorporating metabolic complexity, improving the physiological relevance of experimental models and their translational value.

P21+ SENESCENT MACROPHAGES FUEL INFLAMMATION AND DISEASE IN AGING AND METABOLIC DYSFUNCTION ASSOCIATED WITH STEATOHEPATITIS (MASH) LIVER.

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

NUTRIENT/mTOR SIGNALING IN REGULATION OF SENESCENCE-INDUCED BETA CELL DYSFUNCTION IN AGING AND DIABETES.

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Type 2 diabetes (T2D) is an age-related disease characterized by insulin resistance and a decline in β-cell function and mass, and has been associated with increased senescence. Our previous work identified mTORC1 as a master regulator of β-cell growth, proliferation, and survival. mTORC1 signaling has been also associated with cell metabolism and aging. Our preliminary studies show that sustained mTORC1 activation in β-cell-specific TSC2-knockout mice ($\beta TSC2^{KO}$) exhibits improved glucose tolerance, hyperinsulinemia, increased β-cell mass and premature β-cell senescence at a young age, followed by a development of hyperglycemia at 9 month old and progression to diabetes by 12 months of age. Notably, islets from these mice exhibit close to 100% of β-gal+ β-cell population, increased p21, p16, pH2aX protein expression, and a SASP. To assess whether premature senescence in young $\beta TSC2^{KO}$ mice predisposes to β-cell failure under metabolic stress, we exposed 2 month old $\beta TSC2^{KO}$ mice to a high-fat diet (HFD). Feeding young $\beta TSC2^{KO}$ mice a HFD for 4 months accelerates diabetes development and β-cell failure at young age. Short-term treatment of HFD-fed $\beta TSC2^{KO}$ mice with a senolytic drug (ABT-263) improved glucose level and ABT-263 treatment in vitro induced increased cleaved-caspase 3 in $\beta TSC2^{KO}$ islets compared to controls.To gain mechanistic insight, RNA-sequencing was performed on 6-month-old control and $\beta TSC2^{KO}$ on regular chow and HFD conditions, and identified the suppressive and apoptotic, IGFBP3. In $\beta TSC2^{KO}$ mice, IGFBP3 progressively increases with age, paralleling hyperglycemia development. Treatment of young islets from control and $\beta TSC2^{KO}$ mice with exogenous IGFBP3, induced caspase 3/7 activity only in $\beta TSC2^{KO}$ islets, indicating increased apoptotic susceptibility to IGFBP3 in senescent cells. These studies demonstrate that sustained β-cell activation of mTORC1 in $\beta TSC2^{KO}$ mice results in diabetes and β-cell failure in old mice and this was associated with a striking increase in β-cell senescence. β-cell failure was accelerated by short-term feeding young $\beta TSC2^{KO}$ a HFD, highlighting the role of overactive mTORC1 during metabolic stress as a primary causative factor for senescence and diabetes and this was ameliorated by treatment with senolytics. Finally, RNAseq studies identified IGFBP3 as a mechanistic candidate for senescence-induced cell death.

DELETION OF PYHIN CYTOPLASMIC DNA SENSORS SHORTENS LIFESPAN THROUGH CANCER SUSCEPTIBILITY, BUT DECREASES AGE-RELATED FRAILTY IN MICE

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Chronic inflammation is a major contributor to aging and is recognized as a major hallmark of age-related decline. Persistent immune activation is implicated in many conditions of aging, such as arthritis, neurodegenerative disorders, and overall frailty. While the drivers of inflammation remain unclear, strong evidence ties chronic inflammation to accumulation of cytoplasmic DNA. Sources such as micronuclei or cDNA from transposable elements have been shown to accumulate with age, creating persistent immune activation that leads to cell death or senescence. The PYHIN (Pyrin and HIN domain-containing) protein family is central to the detection and response to cytoplasmic DNA, binding DNA in a sequence-independent manner to activate interferon signaling and inflammasome pathways. Interestingly, PYHIN proteins are absent in bats—a clade enriched in long-lived species whose unique immune system is linked to their extended healthspan and lifespan. To investigate the role of PYHIN proteins in age-related inflammation and mortality, we conducted a lifespan study on mice lacking all 13 mouse PYHIN proteins. PYHIN-deficient mice exhibited a reduced lifespan, possibly linked to an increased susceptibility to tumors. However, measurements of overall health and frailty with age suggest these mice are less frail, preserving their health at older ages. These findings suggest that while PYHIN proteins are essential for preventing aberrant cell growth that leads to cancer, but removing them can prevent age-related functional decline related to chronic inflammation.

REVITALIZING HEMATOPOIETIC STEM CELLS IN SICKLE CELL DISEASE THROUGH SENOTHERAPEUTIC INTERVENTION

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Sickle cell disease (SCD) is a widespread hematologic disorder treated with medications, blood transfusions, transplantations and, more recently, gene therapy. A major challenge in successful bone marrow (BM) transplantation following gene editing is the effective mobilization and collection of donor hematopoietic stem and progenitor cells (HSPCs), often requiring multiple cycles of mobilization and apheresis, with increased risk of side effects. Our previous study in SCD mice found reduced hematopoietic stem cell (HSC) frequency, loss of quiescence, lower repopulation potential, and premature senescence. This was further supported by increased DNA damage, senescence-associated beta-galactosidase, and P16/P21 levels in CD34+ cells (HSPCs) from SCD patients. In this context, we systematically evaluated FDA-approved senotherapeutic agent, Dasatinib and Quercetin (D+Q) that target increased survival signals in apoptotic resistant cells. D+Q was administered for 3 days every other week over a period of 10 weeks. D+Q treatment in young SCD mice, resulted in an almost two-fold increase in the frequency of phenotypic LT-HSCs, reaching levels similar to age-matched non-SCD mice. While BM cells from SCD mice displayed nearly a 5-fold reduction in colony forming units, D+Q treatment restored CFU levels to those of non-SCD controls, indicating improved hematopoietic progenitor and stem cell function. This improvement in colony forming units was observed across all hematopoietic progenitors. This was further corroborated by transplantation assay, which demonstrated a significant increase in the repopulation activity of BM cells ($p=0.029$). Our preliminary cytokine profiling displayed a trend of reduced SCD associated pro-inflammatory cytokines in both blood and BM lavage following D+Q treatment. Notably, there was a partial improvement in blood parameters, including higher hemoglobin (Hb), HCT and RBC count and a significant decrease in reticulocyte counts, suggesting a systemic effect of D+Q. These findings suggest that a combination of Dasatinib and Quercetin could enhance BM health, improving the quality and quantity of autologous HSCs in SCD, providing additional benefits for gene therapy and improving long-term outcomes for SCD patients.

AGING-DRIVEN METABOLIC DYSREGULATION AS A CONTRIBUTOR TO PARKINSON'S DISEASE PATHOGENESIS

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Aging is the strongest risk factor for Parkinson's disease (PD), a neurodegenerative disorder characterized by motor symptoms and α-Synuclein (αSyn) aggregation. This protein accumulation promotes metabolic dysfunction, contributing to disease mechanisms. Among the metabolic pathways perturbed in both aging and PD, sphingolipid metabolism plays a key role in neuronal function. Genetic variants affecting sphingolipid enzymes increase PD risk, yet the interplay between age-dependent sphingolipid alterations and PD pathogenesis remains poorly understood. Additionally, sphingolipid pathways are closely linked to other metabolic pathways, suggesting a broader metabolic signature in aging and disease. Here, we use fly and human postmortem brain metabolomics to investigate sphingolipid and other metabolic changes during aging and αSyn-induced neurodegeneration.

In control flies, we observe distinct metabolic trends such as acyl carnitines decreasing and sphingolipids increasing linearly with age. Notably, many of the acyl carnitines that decline with aging are also reduced in αSyn overexpressing flies, suggesting overlapping metabolic shifts. Aged flies exhibit widespread sphingolipid perturbations, affecting 61% of assayed species, with a marked reduction in ceramides. In contrast, αSyn flies display increased ceramide levels, backed up by changes in enzymatic RNA and protein levels. Our linear interaction models reveal that sphingolipids and other lipid groups, such as lysophospholipids, show age-disease interactions. Cross-species comparisons using human brains reveal that metabolites commonly perturbed in aged and αSyn flies, such as myo-inositol (lipid) and ergothioneine (amino acid derivative from diet), associate with cognitive and motor decline.

This study provides evidence that metabolism is dynamically modulated by aging, with implications for PD risk and progression. By disentangling age-dependent versus disease-specific metabolic shifts, we aim to refine our understanding of how aging shapes PD pathogenesis and identify potential metabolic targets for intervention.

SIRTUIN 6 (SIRT6) ACTIVATION TO REPAIR DNA DAMAGE IN CHONDROCYTES

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Aging is the largest risk factor for osteoarthritis (OA), and there is evidence that senescence of chondrocytes and other cell types in the joint may contribute to the development of OA. Previous studies have shown that DNA damage increase with age in chondrocytes from cadaveric donors such that older donors exhibit DNA damage comparable to the levels induced by a senescence-inducing dose of 10 Gy irradiation (IR) [1]. Sirtuin 6 (SIRT6), a nuclear NAD(+)-dependent deacetylase, plays a crucial role in DNA damage repair, including in joint tissue homeostasis [2]. However, its enzymatic activity declines with age in human chondrocytes [2]. Boosting SIRT6 activity with a small molecule activator (MDL-800) improves the rate of DNA damage repair after an irradiation challenge [3]. The current study determined the extent to which MDL-800 activates SIRT6 in end-stage OA cartilage obtained at total knee replacement.

Chondrocytes were isolated through enzymatic digestion and allowed to recover in monolayer culture before 24-hour treatment with 40 μM MDL-800 or DMSO vehicle control. Cells were harvested for either histone isolation and Western blot to assess acetylation of H3k9 (a SIRT6 target), or resuspended in low-melt agarose for the single-cell gel electrophoresis "comet" assay to assess DNA damage. MDL-800 increased the deacetylase activity of SIRT6 as indicated by a reduction in H3k9ac (normalized to total H3). Across chondrocytes from 9 donors, the extent of acetylation was significantly lower than the vehicle control ($p<0.001$, mean = 91.2% of DMSO). For these same donors, the percentage of DNA in the tail for ~200 cells per condition was averaged and normalized to the DMSO control for that donor. This measure showed a significant decrease in DNA damage with MDL-800 treatment ($p=0.005$, mean = 75.7% of DMSO).

The accumulation of DNA damage with age and during the development of OA may be responsible for an increased senescence burden. Enhancing DNA damage repair to limit the conversion to senescence is one possible strategy to prevent the secretion of catabolic mediators that accelerate cartilage degradation. The observed reduction in DNA damage with MDL-800, coupled with the reduced acetylation of H3k9, suggests that boosting SIRT6 activity may be a potential therapeutic avenue for OA.

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LIMITING CAP-DEPENDENT TRANSLATION INCREASES 20S PROTEASOMAL DEGRADATION AND PROTECTS THE PROTEOMIC INTEGRITY IN AUTOPHAGY-DEFICIENT SKELETAL MUSCLE.

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Postmitotic skeletal muscle critically depends on tightly regulated protein degradation to maintain proteomic stability. Disruptions in macroautophagy/autophagy-lysosomal or ubiquitin-proteasomal degradation pathways lead to the accumulation of damaged proteins, ultimately accelerating age-related muscle dysfunction. While in vitro studies have highlighted the complementary nature of these systems, their interplay at the organismal level remains poorly understood.
Here, we provide novel insights into this complex relationship in autophagy-deficient skeletal muscle. Our study demonstrates that, despite a compensatory increase in proteasome levels following autophagy impairment, 26S proteasome activity was not proportionally enhanced. This functional deficit was partially attributed to reduced ATP availability, which is essential for fueling the 26S proteasome.
Remarkably, we identified EIF4EBP1 activation as a key factor in restoring and even augmenting proteasomal function through dual mechanisms. First, genetic activation of EIF4EBP1 enhanced both ATP-dependent 26S proteasome and ATP-independent 20S proteasome activities, thereby expanding overall protein degradation capacity. Second, EIF4EBP1 activation induced muscle fiber transformation and increased mitochondrial biogenesis, leading to improved ATP production, which in turn supported 26S proteasome activation. Notably, the improved function of the 20S proteasome in EIF4EBP1-activated skeletal muscle was linked to an increased abundance of the immunoproteasome, a specialized subtype adapted to oxidative stress conditions. This dual action of EIF4EBP1 activation preserved proteomic integrity in autophagy-deficient skeletal muscle.
Our findings reveal a novel role for EIF4EBP1 in enhancing protein quality control, presenting a promising therapeutic strategy for autophagy-related muscular disorders and other conditions characterized by proteostatic imbalance.

TRANSCRIPTOMIC ENTROPY: A CONNECTION BETWEEN AGE-RELATED PROCESS AND CANCER PROGRESSION

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Aging and cancer share complex molecular mechanisms, yet distinguishing between causative factors and byproducts remains challenging. Here, we explored the role of transcriptomic entropy in these processes by applying Shannon Entropy to bulk RNA-sequencing data from thousands of human and mouse samples. We found that entropy changes during aging are highly tissue-specific, with some tissues showing increased entropy while others exhibit decreased or stable entropy levels. Transcriptomic entropy is associated with age-related processes, correlating positively with proliferation and cellular senescence in most tissues, and showing a tissue-specific correlation with stemness. Interestingly, while cellular reprogramming leads to increased transcriptomic entropy, calorie restriction does not alter entropy levels. In the context of cancer, we found that in general primary tumors have higher entropy compared to normal tissue, with further increases observed in metastatic stages. Elevated entropy levels were linked to poorer survival outcomes across various cancer types, suggesting its utility as a prognostic marker. Transcriptomic entropy increases post-relapse in cancer patients, while tumors that respond to treatment show a decrease in entropy over time. Additionally, differential expression analysis highlighted that genes associated with entropy are enriched in developmental processes and depleted in metabolic pathways, hinting at a connection between transcriptomic entropy and oncogenesis. Our study identifies transcriptomic entropy as a critical factor influencing both aging and cancer progression, providing new insights into this complex relationship.

CASEIN KINASE MEDIATED SILENCING OF LIPID CATABOLISM DETERMINES LONGEVITY IN RESPONSE TO INTERMITTENT FASTING

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Oscillations between lipid anabolism and catabolism allow long-term preservation of cellular health amid systemic metabolic fluctuations. As a conserved aging determinant, intermittent fasting can improve disease outcomes and extend lifespan expectancy. Yet, the relative importance of activating lipid catabolism versus its attenuation in fasting-induced longevity remains unclear. The robust adaptability of soil-dwelling worms, C. elegans, to variable nutrient availability provides an excellent means to better understand how metabolic transitions alter aging trajectories. While lipid breakdown triggered by fasting was not needed for lifespan extension through intermittent fasting, suppressing this catabolic response upon nutrient replenishment was necessary to achieve the physiological benefits of fasting. The fasting-responsive nuclear hormone receptor, NHR-49, is pivotal in activating lipid catabolism through β-oxidation. Unlike traditional ligand-regulated nuclear hormone receptors, NHR-49 employs a unique regulatory mechanism that bypasses ligand binding, instead relying on cofactors to mediate its transcriptional attenuation and turnover. We identify casein kinase 1 alpha 1 (KIN-19) as a central regulator of metabolic plasticity and fasting-induced longevity, which attenuates β-oxidation via primed phosphorylation of NHR-49. Overall, cooperative, ligand-independent silencing of this conserved nuclear hormone receptor promotes longevity associated with intermittent fasting.

SENESCENT CELLS DEPOSIT INTRACELLULAR CONTENTS THROUGH ADHESION-DEPENDENT FRAGMENTATION

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Cellular senescence, a complex state of stable arrest and enhanced secretion, is intricately linked to aging and disease. Here we identify a new feature of senescent cells, demonstrating that they break off large fragments of themselves through cell-to-cell adhesion. We term these senescent-cell adhesion fragments (SCAFs). We find SCAFs in all senescent cell types examined, as well as in senescent-cells bearing tissues in-vivo. SCAFs contain a variety of cell's organelles, but lack nuclear material. Dynamic analysis reveals SCAFs ultimately rupture and release their contents extracellularly, comprising a newly identified way of intracellular content release without killing the cell. Protein profiling identifies that SCAFs contain a complex proteome including damage-associated molecular patterns (DAMPs), capable of inducing pro-inflammatory cytokine expression in immune cells. SCAF formation inhibition studies reveal that SCAF formation is a contributor to senescent cell survival by limiting the accumulation of damaged organelles in the cell of origin. Furthermore, treatment of human fibroblasts with SCAFs reveals activation of signatures related to wound healing and cancer. In-vitro functional validation of these signatures shows that SCAFs promote proliferation, migration and invasion. Altogether, this identifies a new mechanism by which senescent cells communicate with their environment, with particular relevance for cancer and inflammaging.

AGING-RELATED FUNCTIONS OF THE AUTOPHAGY PROTEIN ATG16 AND ITS WD40 DOMAIN

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Aging is a primary risk factor for many disorders, including neurodegenerative diseases, and understanding the molecular mechanisms of aging could lead to prevention of such illnesses. One prominent hallmark of aging is macroautophagy (hereafter autophagy). During autophagy, cytosolic cargos are sequestered into double-membrane vesicles called autophagosomes, which are marked by an autophagy protein ATG8 and fuse with lysosomes to ensure cargo degradation. Decline of such lysosomal degradation via autophagy is linked to aging. At least in the nematode C. elegans, conserved longevity paradigms, such as reduced insulin signaling, germline removal, and dietary restriction, require both autophagy and lysosomal genes for their lifespan extension, suggesting the standing paradigm that autophagy involving lysosomal degradation, i.e., 'conventional' autophagy, is key to longevity. Interestingly, growing evidence is emerging that autophagy proteins, especially ones related to early steps of autophagosome formation, can have additional cellular functions beyond ensuring lysosomal degradation. However, it remains unclear whether non-canonical autophagy is important for aging.

A known molecular handle to regulate such non-canonical pathways (sometimes dubbed 'non-canonical autophagy') is the early-acting autophagy protein ATG16 and its C-terminal WD40 domain, which is dispensable for conventional autophagy, at least in mammalian cell cultures. The ATG16 WD40 domain, instead, has been found to attach ATG8 onto single-membrane vesicles. These vesicles can be used as vehicles for inter-cellular signaling, such as phagocytosis, endocytosis and secretion. To this end, I am investigating the importance of the ATG16 WD40 domain in organismal and cellular aging by using a powerful combination of genetic and cytological approaches in C. elegans and human cell culture models. Specifically, I have created C. elegans longevity mutants with reduced insulin signaling, germline removal, or dietary restriction with atg-16.2 (C. elegans ATG16 homolog) WD40 domain mutations, and I am analyzing these double mutants. My preliminary results show a specific requirement of the WD40 domain in some, but not all longevity paradigms. In parallel, I am addressing if the ATG16 WD40 domain plays a role in cellular senescence analyzing human cell cultures with proteomic approaches. I will discuss my ongoing work along with future directions, including efforts to identify molecular interactors of the ATG16 WD40 domain in vivo.

These studies are important because they may uncover novel, unappreciated roles for autophagy genes in aging, specifically mediated by ATG16 and its WD40 domain. Such molecular insights may lead to new entry points for combating aging-related diseases.

HARNESSING LYSOSOMAL pH AS A FAST, HIGH-THROUGHPUT APPROACH FOR SENESCENCE ASSESSMENT

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Cellular senescence is a major driver of aging and age-related diseases, associated with irreversible growth arrest and a pro-inflammatory secretome. Conventional assays, such as senescence-associated β-galactosidase staining, can be time-consuming and insufficiently sensitive. Here, we present a rapid, fluorescence-based method using LysoTracker—a lysosomal pH probe—as a potential surrogate indicator for senescence. Because senescent cells commonly exhibit altered lysosomal characteristics and increased lysosomal mass, LysoTracker signal intensity may correlate with traditional senescence markers. By quantifying this fluorescence readout in parallel with established assays, our approach offers a fast, quantitative, and high-throughput means to evaluate lysosomal acidity, which in turn can reflect aspects of the senescence phenotype. This streamlined technique not only expedites senescence detection but also holds promise for efficient screening of senolytics and anti-aging research.

FIBULIN-5, A MATRICELLULAR PROTEIN, MAINTAINS EPIDERMAL STEM CELL HETEROGENEITY BY CONTROLLING THE BIOMECHANICAL ENVIRONMENT DURING SKIN AGING

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The extracellular matrix (ECM) regulates the dynamic interactions between cells and their extracellular environment by translating mechanical cues into biochemical signals that maintain organ functions. Fibulin-5 (gene symbol: Fbln5) is a multifunctional ECM protein essential for the formation of elastic fibers and the regulation of cellular functions through integrin binding. Fibulin-5 expression decreases with aging in human skin, but its functional importance remains unknown. To address the roles of fibulin-5 in regulating both the biochemical and mechanical environments crucial for epidermal stem cells during skin aging, Fbln5 knockout (KO) mice were examined for change in their cellular and molecular phenotypes. Loss of Fbln5 in mice results in early impairments of epidermal stem cell properties and ECM integrity, similar to the chronological aging of the skin. Fibulin-5-dependent microenvironmental abnormalities reduced signaling factors essential for epidermal stem cell regulation, such as YAP and canonical Wnt. In human primary epidermal stem cells, activation of b-catenin downstream of the YAP induces a fast-cycling epidermal stem cell marker that is present at a young age. These findings highlight the important role of Fibulin-5-mediated crosstalk between the ECM and signaling pathways in regulating the balance of the epidermal stem cell populations during skin aging.

GENETIC REGULATION OF MACROPHAGE SENESCENCE AND THE PROTECTIVE EFFECT OF IPA AGAINST RADIATION-INDUCED SENESCENCE

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Senescence, a hallmark of aging, contributes to the progression of atherosclerosis, a major cause of cardiovascular disease. Atherosclerosis, a chronic inflammatory condition, is exacerbated by inflammaging. Senescent immune cells in the vessel wall release proinflammatory mediators, accelerating disease progression. Senescent macrophages polarize into M1 phenotypes and express inflammatory factors, promoting plaque progression and potentially leading to rupture and thrombosis. We hypothesize that genetic variation influences macrophage senescence in atherosclerosis.To investigate this, bone marrow was extracted from genetically diverse mouse strains, macrophages were cultured, and senescence was induced by irradiation. Gene expression was analyzed using qPCR and β-Galactosidase assays. Preliminary results revealed variation in senescence markers and possible sex-specific differences. These findings suggest genetic regulation of macrophage senescence and its relevance to atherosclerosis. Future work will use HMDP and molecular mapping to identify key genes driving this process. We also examined the effect of the microbiome-derived metabolite indole-3-propionic acid (IPA) on macrophage senescence. Bone marrow was isolated from C57BL/6 mice, and macrophages were cultured. Cells were treated with IPA or left untreated. After 24 hours, radiation was applied to induce senescence, and IPA-containing media was refreshed daily. RNA was extracted 10 days post-radiation, and senescence marker expression was assessed. qPCR results showed that radiation significantly increased macrophage-related senescence markers, which were reduced by IPA treatment, suggesting a protective effect of IPA against radiation-induced senescence.Together, these findings support the therapeutic potential of IPA in mitigating macrophage senescence and establish a foundation for identifying genetic and microbial factors regulating senescence pathways in age-related vascular diseases

DISSECTING AND TARGETING METABOLISM-INDUCED CHROMATIN CHANGES IN AGED HEMATOPOIETIC STEM CELLS

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Aging has been extensively studied in model organisms, yet the molecular mechanisms driving human hematopoietic stem and progenitor cell (HSPC) aging remain poorly understood. To investigate how metabolism influences the epigenetic landscape of aging HSPCs, we integrated metabolomic profile with ATACseq and RNAseq in young and aged HSPCs. We found that aged HSPCs at steady state upregulate key metabolic pathways, including the TCA cycle and nucleotide synthesis, alongside increased chromatin accessibility. Notably, these chromatin alterations could not be fully explained by transcriptional changes in epigenetic regulators alone. Key discovery was the elevated αKG:succinate ratio in aged HSPCs, we hypothesize that this metabolic shift could drives epigenetic remodelling, contributing to age-related dysfunction. Supporting this, exogenous succinate treatment in aged HSPCs reduced chromatin accessibility, as shown by ATAC-seq and increased levels of the repressive histone mark. Accessibility decreased in genes involved in the TCA cycle, oxidative phosphorylation, and inflammation, which correlated with lower gene expression in RNA-seq.

To further validate these metabolic effects, Seahorse mitochondrial stress tests showed that succinate treatment decreased oxygen consumption rate (OCR) and increased extracellular acidification rate (ECAR), reinforcing a metabolic shift observed in ATAC-seq and RNA-seq data. Additionally, alkaline comet assays revealed that succinate reduced DNA damage burden in aged HSPCs. Functionally, these metabolic and epigenetic changes enhanced clonogenic capacity in vitro, as assessed by bulk colony-forming and single-cell differentiation assays for myeloid, erythroid, and megakaryocytic lineages.

To determine whether succinate treatment could restore engraftment capacity, we treated aged HSPCs prior transplantation into immunocompromised mice. Remarkably, succinate-treated aged HSPCs regained engraftment ability to levels comparable to young HSPCs. Conversely, young HSPCs treated with αKG exhibited impaired engraftment, mimicking the effects of aging.

Our study identifies a key metabolic-epigenetic axis that regulates chromatin accessibility in aging HSPCs. By targeting this axis, we highlight the potential of metabolic interventions to restore aged HSPC function, opening new avenues for regenerative therapies in haematopoiesis and aging.

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AN INCREASE IN CELLULAR SENESCENCE MARKERS IN ASTROCYTES EXACERBATES ALZHEIMER'S DISEASE-LIKE PATHOLOGY IN A MOUSE MODEL

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Alzheimer's disease (AD) is one of the most common forms of dementia worldwide. It is a neurodegenerative disorder characterized by the accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles, with aging being a primary risk factor. Recent studies highlighted the critical role of glia in this process, specifically of astrocytes in AD pathogenesis. Astrocytes play an essential role in orchestrating brain metabolic and inflammatory activity. Pathological changes within astrocytes were reported in different neurological pathologies, including AD. Cellular senescence is one of the factors associated with aging and may affect glial cell activity. We aim to assess early changes within astrocytes that link to CS and to follow potential pathways that link to their failure to support brain activity. We identified time-dependent expression in CS markers within astrocytes during disease progression that correlates with their impairment in clearing amyloid beta plaques or uptake of neurotoxic oligomers. Furthermore, senescent astrocytes exhibit impaired neuronal support and altered metabolic activity, possibly contributing to disease progression. Targeting pathways affiliated with CS in astrocytes may deepen our understanding of disease progression and offer new therapeutic intervention avenues.

DEFINING THE ROLE OF THE SEROTONIN 2A RECEPTOR (5-HT2AR) IN SENESCENT RETINAL PIGMENTED EPITHELIUM (RPE) ASSOCIATED PROGRESSION OF AGE-RELATED MACULAR DEGENERATION (AMD).

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Background: AMD is the leading cause of blindness in elderly, predicted to burden 300 million people by 2040. Dry AMD accounts for 90% of AMD cases and is characterized by the buildup of hydrophobic waste (drusen deposition). There are currently no effective treatments. AMD ensues through cellular aging of the RPE, disrupting the blood-retinal barrier (BRB) via cytoskeletal dysregulation, the loss of tight junctions, lysosomal dysfunction, decreased antioxidants, oxidative stress, and senescence. Diabetes-associated, glucose intolerance synergistically contributes to aging making diabetics prone to AMD. Though AMD pathology is well-described, underlying mechanisms of disease progression are ill-defined. We recently found an age-related decline of 5-HT2AR expression in the retina. Serotonin, partially through 5-HT2AR, modulates the cytoskeleton, phagocytosis/autophagy, metabolism, and inflammation, all factors relevant to AMD progression. However, the role of 5-HT2AR in AMD progression is unknown. Thus, we investigated the role of 5-HT2AR in age-related pathogenesis of the RPE.
Methods: Morphological and functional consequences of 5-HT2AR modulation were assessed in ARPE-19 via pharmacological agonism/antagonism (R-DOI/M100,907) or 5-HT2AR knockouts. Factors contributing to AMD pathology, including the cytoskeleton, junctions, lysosomes, antioxidants, oxidative stress, and lipids were evaluated by fluorescent microscopy, flow cytometry, and western blotting. Senescence was quantified by staining senescence-associated β-galactosidase (SA-β-Gal).
Results: 5-HT2AR knockouts in RPE exhibit pathological features reminiscent of AMD, including cytoskeletal stress fiber formation, the loss of occludin tight junctions, and increased monolayer permeability. Mirroring dry AMD, knockouts have increased lysosomal biomass and lipid accumulation. Consequential to low levels of antioxidant SOD enzymes, increased oxidized lipids and intracellular H_2O_2 were found in 5-HT2AR knockouts. An 8-fold increase in SA-β-Gal positive cells was also observed in 5-HT2AR knockouts. Intriguingly, the 5-HT2AR agonist, R-DOI, protected against diabetic glucose-induced pathology by preventing the loss of tight junctions, decreasing reactive oxygen species, and inhibiting senescence.
Conclusions: Our data suggests that age-related loss of 5-HT2AR activity promotes cellular aging and pathophysiological processes in AMD progression. These findings may be applicable beyond the eye as analogous processes are involved in neurodegenerative disease development and 5-HT2AR expression similarly decreases in the aging brain. Moreover, 5-HT2AR agonists possess a unique pharmacological feature, the capacity to permeate both retinal and brain barriers. This feature potentially makes 5-HT2AR agonists a novel therapeutic strategy for remediating diabetic glucose-induced and age-related pathologies.

MICROGLIA AND EXTRACELLULAR MATRIX CONTRIBUTIONS TO COGNITIVE AGING IN MICE

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Healthy brain aging is not simply a lack of change in neurobiological function, but rather an active process that engages endogenous mechanisms of plasticity to protect network function despite cellular and molecular alterations that accompany aging. Even in the absence of overt pathology, synapse loss and changes in synapse plasticity are hallmark features of brain aging, and preserved synapse status is linked to better cognitive outcomes. Both microglia and the extracellular matrix (ECM) can regulate synapse function throughout development and in early adulthood and both of these neuron-extrinsic factors have been linked to cognitive abilities in aging animals. However, the ways in which microglia and the ECM interact to shape synapse and neuronal function in the aging brain are largely unknown. In this study we combine sophisticated behavioral-, imaging-, and proteomic approaches to identify microglial and ECM phenotypes that promote cognitive resilience in aging mice as well as those associated with cognitive decline. Using ECM-optimized proteomic workflows, we discovered striking regional differences in aging-induced ECM remodeling, resulting in excess ECM abundance that is aligned with synapse protein abundance across key basal ganglia nuclei. Using high-resolution imaging, we demonstrate that excess ECM deposition in the ventral tegmental area (VTA) of aging mice is tightly correlated with microglial aging phenotypes in this brain region. Moreover, VTA microglial and ECM status aligned with local excitatory synapse numbers as well as deficits in reward-based learning in aging mice. Finally, to further probe relationships between microglia, the ECM, and cognitive aging outcomes, we used a battery of cognitive tests to classify young-adult and late-middle aged mice and into average young, aging impaired, and aging unimpaired groups using unsupervised clustering approaches. Microglia and the ECM were isolated from these animals using CD11b magnetic microbeads and tissue fractionation approaches, respectively, and these samples underwent proteomic analysis. Here we highlight key proteins and pathways associated with the preservation of cognitive behaviors in aging mice. Together, this study provides foundational observations that implicate microglia-ECM interactions in the regulation of synapse function and cognition across the lifespan.

ARACHIDONIC ACID CONVERTING ENZYMES ARE TARGETS FOR NOVEL SENOLYTICS

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With age, senescent cells accumulate in various tissues, which is causatively linked to a state of low-grade chronic inflammation, aging and age-related diseases. As a possible therapy for age-related diseases, the pharmacological elimination of senescent cells using senolytics was proven to be efficient in numerous preclinical disease models and first clinical pilot studies are ongoing. While the herein used first generation senolytics, targeting senescent cell anti-apoptotic pathways (SCAPs), are mainly repurposed drugs, we recently discovered an alternative target pathway for the development of novel senolytic compounds that is based on an altered lipid metabolism in senescent cells.
We found that the enzymatic phospholipase A2 activity as well as the resulting metabolite arachidonic acid are significantly upregulated intracellularly in senescent cells. Since high levels of arachidonic acid can induce apoptosis, we tested, if inhibition of enzymes that use arachidonic acid as a substrate would increase its levels further to cytotoxic dosis specifically in senescent cells. Indeed, a range of various cell types were selectively eliminated in vitro when senescent with therapeutic indices of up to 700-fold difference as compared to quiescent control cells. In vivo, the treatment of 26 months old C57BL6 mice resulted in decreased mRNA levels of p21 and SAbetaGal staining in various tissues as well as markedly improved neuromuscular functionality, frailty index score, as well as an around 40% increase in life span starting from treatment at the age of 27 months. In addition, we identified circulating miRNAs in plasma of mice that correlate with tissue levels of senescence marker genes in order to establish a companion diagnostic enabling minimally invasive monitoring of the senescent cell load.
In summary, we here provide a promising novel strategy to assess the senescent cell load based on circulating miRNAs and then to eliminate senescent cells for counteracting functional decline and diseases associated with aging targeting the arachidonic acid metabolism.

REGENERATION LEADS TO GLOBAL TISSUE REJUVENATION IN AGING SEXUAL PLANARIANS

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The possibility of reversing the adverse impacts of aging could significantly reduce age-related diseases and improve quality of life in older populations. Here, we report that the sexual lineage of the planarian, Schmidtea mediterranea, exhibits physiological decline within 18 months of birth, including altered tissue architecture, impaired fertility and motility, and increased oxidative stress. Single cell profiling of young and older planarian heads uncovered loss of neurons and muscle, increase of glia, and revealed minimal changes in somatic pluripotent stem cells, along with molecular signatures of aging across tissues. Remarkably, amputation followed by regeneration of lost tissues in older planarians led to reversal of these age-associated changes in tissues both proximal and distal to the injury at physiological, cellular and molecular levels. Our work suggests mechanisms of rejuvenation in both new and old tissues concurring with planarian regeneration, which may provide valuable insights for anti-aging interventions.

IMPACT OF SENESCENCE AND APOE ALLELES IN HUMAN CEREBRAL ORGANOIDS

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Numerous studies report that Apolipoprotein E isoforms APOE2, APOE3, and APOE4, confer different protective or pathogenic effects in terms of AD and longevity. This finding may be due in part to the response to cellular senescence, or the permanent termination of the cell cycle without cell death. Though cellular senescence benefits processes such as wound healing, when prolonged it can become maladaptive and lead to tissue dysfunction and aging. Our preliminary work shows that APOE4, the allele linked with increased AD risk and shorter lifespan, correlates with increased susceptibility to senescence, whereas APOE2 shows protection against AD and senescence. Therefore, we aim to elucidate the protective mechanism of APOE2 in senescence. To address this link, we utilized irradiation (IR) to induce senescence in cerebral organoids generated from isogenic APOE2, APOE3, and APOE4 human induced pluripotent stem cells (hiPSCs) and assessed histological, proteomic, and transcriptomic changes in cell lysates and conditioned media.

Through immunohistochemistry, we confirmed the success of senescence induction through decreased histone methylation H3K9me3, depletion of nuclear Lamin B1, and increased γH2AX nuclear foci. At the proteome and secretome levels, IR and control group organoids clustered separately under supervised clustering regardless of APOE genotype supporting senescence-induced changes. Additionally, each APOE genotype showed around 300 proteins significantly altered by irradiation, but the identity of these proteins differed across genotypes. Transcriptomics also indicated changes to cell population proportions in response to irradiation that varied dependent on APOE genotype. Further analysis of this data will reveal key pathways that regulate this relationship. Together, these data support the possibility that cells with different APOE genotypes respond to senescence through distinct mechanisms, identifying novel directions for aging research.

IMPROVEMENT OF DRY EYE BY CD38 INHIBITION AND NMN SUPPLY THROUGH LOCAL INTRACRINE REACTIVATION

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Meibomian glands, located within the tarsal plates of the eyelids, produce and secrete oil onto the ocular surface, contributing to the tear film stability. Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by glandular tissue atrophy, and the main cause of evaporative dry eye with incidence increasing with age. However, no causal therapy exists owing to the sparseness of the knowledge regarding the molecular mechanism of MGD pathogenesis by aging. Previously, we showed that tissue-intrinsic steroidogenesis (i.e., intracrine activity) via the enzyme 3β-HSD is essential for maintaining normal meibomian gland homeostasis (Sasaki et al, Nature Aging, 2022). Genetic ablation of 3β-HSD nullified local steroidogenesis and led to atrophy of the meibomian gland. This meibomian 3β-HSD activity was reduced significantly with age. Importantly, this reduction of enzymatic activity is ascribed to the reduction of the bioavailability of the coenzyme nicotinamide adenine dinucleotide (NAD), which is required for 3β-HSD to exert its dehydrogenase activity. Accordingly, topical eye drop application of nicotinamide mononucleotide (NMN) ameliorated dry-eye phenotype through reactivation of meibomian gland 3β-HSD activity. However, the molecular mechanism causing NAD depletion in the meibomian gland remains an unsolved issue of medical importance. NAD biosynthesis primarily relies on the salvage pathway based on the precursors nicotinamide (NAM) and NMN, and its net levels are regulated by the balance between synthesis and degradation. In this study, we found that CD38, an NAD/NMN consumption enzyme, is a critical contributor to the age-associated decline of NAD in the meibomian gland tissue. We immunohistochemically observed that CD38 positive cells intensely accumulated around the meibomian gland acini in aged mice. We provide evidence supporting this CD38 model by showing data demonstrating the effects of CD38 inhibition on the meibomian gland morphology, transcriptome, the level of NAD and its downstream meibomian 3β-HSD activity, etc. A synergetic effect of CD38 inhibition and NMN supply is therefore worth consideration in developing MGD therapy.

METABOLIC REWIRING IN SENESCENT CELLS: A FLUX BALANCE ANALYSIS ACROSS DIVERSE INDUCTION MODALITIES

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Cellular senescence is a heterogeneous and multifaceted state triggered by a range of intrinsic and extrinsic stressors, yet it is increasingly appreciated that metabolic remodeling is a core feature across senescent phenotypes. To systematically characterize these alterations, we performed genome-scale metabolic modeling using flux balance analysis (FBA) across multiple senescent cell types, each induced by distinct triggers including DNA damage, oncogene activation, and replicative exhaustion. Our integrative analysis reveals that senescence leads to widespread metabolic shifts, impacting central carbon metabolism, amino acid biosynthesis, and lipid metabolism. Notably, while global flux distributions are perturbed, specific pathways—including nucleotide synthesis and redox metabolism—exhibit consistent and disproportionate changes across senescence types. These findings suggest that, despite the diversity in senescence induction, there exists a convergent metabolic signature that may be exploitable for therapeutic targeting. Our study provides a systems-level view of metabolic dysfunction in senescence and highlights the utility of computational modeling to disentangle its complex metabolic landscape.

MALDI MASS SPECTROMETRY IMAGING: SPATIAL METABOLOMICS FOR BIOMEDICAL RESEARCH

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Optical imaging has revolutionized biology by enabling exploration of cellular and molecular processes in unprecedented detail. However, a key challenge remains in its limitation of visualize multiple molecules simultaneously. Small metabolites, in particular, are difficult to visualize using conventional probe-based optical imaging techniques. In contrast, mass spectrometry imaging (MSI), such as MALDI-MSI (matrix-assisted laser desorption ionization), offers a unique ability to profile a wide range of molecules, including small metabolites, lipids, and peptides, in situ without the need for labeling. Over the past few years, we have focused on enhancing the capabilities of MALDI MSI through innovations in sample preparation, matrix modification, and data analysis. This has facilitated the discovery of key metabolic regulations in neural development, including neuron-glia interactions and the brain-gut axis, as well as in neurological diseases such as neurodegeneration and brain tumors.

Despite these advancements, the application of MALDI MSI has been hindered by three technical challenges: low resolution, 2D surface imaging, and the inability to analyze living samples. To overcome these limitations, we explored the potential of combining the high-resolution 3D live imaging capabilities of optical imaging with the multiplexing power of mass spectrometry imaging through machine learning. Notably, preliminary studies comparing infrared microscopy with mass spectrometry imaging have revealed highly analogous latent space distributions (>90%) for a large number of specific molecular species (>100 metabolites). This discovery has prompted us to develop an AI-driven deep learning model to fuse MALDI MS imaging with biological vibrational spectroscopy, such as FTIR and Raman imaging, to build 3D metabolomic atlas and achieve 4D metabolic imaging eventually. In the long run, our goal is to provide a powerful, label-free, super-multiplex metabolic imaging tool combined with AI models to support a broad range of biomedical research.

CHARACTERIZATION OF A NOVEL FUNCTION FOR ELOA AS AN ELONGATION FACTOR REGULATING CELLULAR SENESCENC

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Cellular senescence is a typically irreversible process characterized by cell cycle arrest, morphological changes, and secretion of inflammatory factors. Physiologically, cellular senescence contributes to tissue dysfunction, neoplastic transformation, and age-related diseases. Many transcription factors related to RNA Polymerase II (RNAPII) have been reported to be involved in the induction of cellular senescence. However, the underlying mechanisms (and potential targets for therapeutic interventions) remain largely elusive. Here, by combining next-generation sequencing and biochemical assays, we demonstrate that depletion of the transcription factor SPT6 promotes cellular senescence-associated gene expression phenotypes, including suspended proliferation and robustly detectable β-galactosidase activity. Through a genome-wide CRISPR screen, we identified the transcriptional elongation factor ELOA as a regulator of cellular senescence. Interestingly, loss of ELOA rescued the proliferation defect caused by SPT6 depletion. ELOA knockout also led to downregulation of the senescence entry marker p21, suggesting a direct role for ELOA in the transcriptional regulation of cellular senescence. Overall, our results shed light on ELOA as a potential therapeutic target in future approaches to limit or reverse cellular senescence.

INFLAMMATION-INDUCED LYSOSOMAL DYSFUNCTION IN HUMAN iPSC-DERIVED MICROGLIA IS EXACERBATED BY *APOE* 4/4 GENOTYPE

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The ε4 isoform of apolipoprotein E (ApoE) is the most significant genetic risk factor for Alzheimer's disease. Glial cells are the main source of ApoE in the brain, and in microglia, the ε4 isoform of ApoE has been shown to impair mitochondrial metabolism and the uptake of lipids and Aβ42. Here, we show that human induced pluripotent stem cell (iPSC)-derived microglia (iMGs) with the *APOE*4/4 genotype exhibit reduced basal level pinocytosis and an overall downregulation of lysosomal proteins as compared to *APOE*3/3 iMGs. Inflammatory stimulation with a combination of LPS and IFNγ or Aβ42 induced mTORC1 activity, increased pinocytosis, and blocked autophagic flux, leading to the accumulation of sequestosome 1 (p62) in both *APOE*4/4 and *APOE*3/3 iMGs. Exposure to Aβ42 furthermore caused lysosomal membrane permeabilization, which was significantly stronger in *APOE*4/4 iMGs and positively correlated with the secretion of the proinflammatory chemokine IL-8. Metabolomics analysis indicated a dysregulation in amino acid metabolism, primarily L-glutamine, in *APOE*4/4 iMGs. Overall, our results suggest that inflammation-induced metabolic reprogramming places lysosomes under substantial stress. Lysosomal stress is more detrimental in *APOE*4/4 microglia, which exhibit defects in lysosomal biogenesis.

LONG-TERM PLASMA TRANSFER FROM CALORIE-RESTRICTED YOUNG DONORS ENHANCES LEAN MASS RETENTION AND PHYSICAL PERFORMANCE IN AGED RECIPIENTS

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Caloric restriction (CR) remains as the most successful method for extending lifespan across different species, yet the underlying mechanisms behind CR's beneficial effects remain mostly unknown. However, current theories, supported by work in our lab and others, demonstrate that many of these effects are achieved during CR through the release of CR-specific secreted factors that support health maintenance, physiological homeostasis, and physical function. Therefore, our research aims to determine if plasma derived from calorie-restricted young mice can prove beneficial to aged mice, in a superior manner to those observed with *ad libitum* (*ad lib*) fed young plasma alone. Specifically, we focused on the preservation of body composition and physical function, which serve as important indicators of overall health. We tested this by treating 18-month-old mice with isochronic (old – old), or heterochronic (young - old) plasma derived from either *ad lib* fed young mice or those on a CR diet. Treatments were administered through an innovative catheter system that allowed for plasma delivery three times per week for eight months. Notably, aged mice that received CR plasma retained more lean mass and had increased grip strength in comparison to mice treated with plasma from young *ad lib* fed animals. Additionally, the CR treated mice exhibited improved metabolic function and cognitive performance, as well as molecular-level rejuvenation across multiple tissues as evidenced by reductions in senescence and beneficial epigenetic remodeling. In these studies, CR plasma has demonstrated remarkable potential as a powerful therapeutic agent against age-related deterioration by extending both lifespan and healthspan as well as suspending aging hallmarks. These data provide strong evidence that the beneficial effects of CR are transferrable via plasma and that CR-induced secreted factors may be crucial for creating new therapeutic approaches that support healthy aging through the preservation of body composition and functionality.

YOUNG MICROBIOME TRANSPLANTATION ENHANCES RECOVERY AFTER MYOCARDIAL INFARCTION

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Background: The interplay between aging, gut microbiota, and cardiac repair post-myocardial infarction (MI) is complex and underexplored. Understanding this relationship is vital for developing new cardiovascular therapies for the elderly.

Methods: Aged mice had their native microbiome replaced with a young one before MI induction. Cardiac function was assessed via echocardiography and histology. For human relevance, gut microbiota and plasma metabolites in ST-elevation myocardial infarction (STEMI) patients of different ages were analyzed using 16S V3-V4 next-generation sequencing and liquid chromatography-mass spectrometry, focusing on age-related changes.

Results: Transplantation of a young microbiome into aged mice led to improved cardiac repair post-MI, indicated by reduced infarct size and preserved ejection fraction. In STEMI patients, age-related changes in gut microbiota and metabolic profiles were observed, supporting the significant role of gut microbiota in cardiovascular health, particularly in aging contexts.

Conclusions: This study highlights the pivotal role of gut microbiota in cardiovascular health and suggests microbiome transplantation as a potential therapeutic approach for elderly patients with cardiovascular diseases. Our findings offer a new perspective on the role of gut-heart axis in aging and cardiac recovery, proposing innovative strategies for future cardiovascular disease treatments.

HIGH RESOLUTION SPATIAL PROTEOGENOMIC PROFILING OF SA β-GAL POSITIVE NEURONS IN THE BRAIN PROVIDES INSIGHTS INTO THEIR TRUE CELL STATE.

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Cellular senescence is a change in cell fate in which cells become resistant to apoptosis, a phenomenon observed in neurons with neurofibrillary tangles (NFTs). Our team identified an accumulation of senescent neurons bearing NFTs in the tau transgenic rTg4510 mouse model and demonstrated their clearance with the treatment of senolytics, Dasatinib and Quercetin. However, identifying senescent cells, particularly neurons, remains challenging due to their complex phenotype.

One commonly used biomarker of classic cellular senescence is senescence-associated beta-galactosidase (SA β-gal). SA β-gal has shown limitations in reliably identifying neurescence (neuronal senescence) as it has been observed in neurons across the lifespan, even in young, healthy animals. Additionally, it has failed to identify senescent neurons and even inversely correlated with other markers of senescence in hippocampal neurons with NFTs and cortical neurons treated with the chemotherapy drug doxorubicin in cell culture. To study neurescence effectively, it is critical to determine whether the biomarkers being utilized are accurately identifying them.

SA β-gal is traditionally detected using an enzymatic assay performed at pH 6, whereby β-galactosidase in senescent cells retain activity, and compared to results from the assay run at physiological pH 4 as a control. A fluorescent version of this assay, SPiDER β-gal, was developed to enable co-staining of SA β-gal with other markers, offering more versatility in downstream assays.

In this study, we tested the utility of SPiDER β-gal to identify senescent cells in mouse brains. We analyzed young (5-month-old) and aged (25-month-old) wild-type (WT) fresh frozen sagittal mouse brain sections. We compared serial sections stained with either traditional X-gal or SPiDER β-gal. SA β-gal positive neurons were quantified across various brain regions. Additionally, we performed spatial proteogenomics using the GeoMx Digital Spatial Profiling (DSP) platform on SPiDER β-gal tissues. We measured the expression of 76 proteins relevant to neuronal health and senescence and the whole transcriptome. The Region of Interest (ROI) selection strategy consisted of selecting SPiDER β-gal positive neurons and neighboring negative neurons across various brain regions. Here we will discuss the overlap between SA β-gal, a classic marker of senescence, and SPiDER β-gal, a newer fluorescent alternative. Additionally, we will present findings from the spatial proteogenomic experiment and discuss the molecular features of SPiDER β-gal positive brain cells. Ultimately, this study provides valuable insights into the specificity of these biomarkers in detecting senescent neurons across various brain regions.

SENESCENCE ACCELERATED IN *APOE4* ASTROCYTES BY CHOLESTEROL TRANSPORTER ABCA1

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Alzheimer's disease (AD) is a progressive neurodegenerative disease comprising 60-70% of dementia cases. The strongest risk factors for late-onset AD are aging and *APOE* e4 genotype. Aging and *APOE4* are associated with increased cellular senescence, a cellular state defined by loss of proliferative ability and a secretory phenotype. Senescence in Alzheimer's disease has not been well-studied, particularly with regards to lipid metabolic dysfunction. Lipids, which accumulate more in AD and *APOE4*, have been described as important players in senescence. The pathways and mechanisms that may drive a senescent phenotype through lipid modulation is a large gap in our understanding of AD senescence. Past research in our lab has identified ATP-binding cassette transporter (ABCA1), a cholesterol transporter, as a potential driver of senescence through its effects on lipid accumulation, causing a buildup of lipids, particularly, oxidized cholesterol, in lysosomes. In AD and *APOE4*, ABCA1 appears to be trapped at lysosomes, which increases intracellular cholesterol accumulation and promotes inflammation and other key pathways involved in senescence. This work proposes that in *APOE4*, senescence is accelerated via lysosomal ABCA1 and cholesterol accumulation. Considering the difficulties of obtaining and maintaining primary human brain cells, induced pluripotent stem cells (iPSCs) allow for investigative studies on brain cells. In human iPSC-derived astrocytes (iAs) of *APOE3* and *APOE4* genotypes, I have first established a senescent phenotype via DNA damage. Delving further into a potential mechanism, I will assess whether a senescent phenotype can be reduced via pharmacological and genetic inhibition of ABCA1. I will also examine the potential crosstalk between senescent astrocytes and neurons, assessing neuronal senescence and dysfunction. The goal of this work is to examine the role of ABCA1 in cellular senescence with a focus on *APOE4*, furthering our understanding of how *APOE* genotype and cholesterol-related pathways may influence a senescent phenotype. Outcomes of this work may advance our understanding of how senescence is accelerated in AD astrocytes, as well as identify therapeutic targets to alleviate this process.

METABOLIC REPROGRAMMING CONFERS LIPID-BASED FERROPTOSIS RESISTANCE IN SENESCENCE-LIKE GASTRIC CANCER CELLS UNDER GLUTAMINE DEPRIVATION

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Senescence-like cancer cells exhibit low proliferative capacity but simultaneously display high metastatic and invasive potential, contributing to therapeutic resistance and tumor persistence. These cells are known to adopt various survival strategies based on metabolic flexibility, allowing them to adapt to external stress conditions. Glutamine plays a critical role in cellular survival by supporting tricarboxylic acid (TCA) cycle anaplerosis, maintaining redox homeostasis through NADPH production, and contributing to antioxidant defense via glutathione (GSH) synthesis. While glutamine deprivation typically imposes metabolic stress on cells, the senescence-like metastatic gastric cancer cells analyzed in this study maintained high viability even under glutamine-depleted conditions. Notably, these cells exhibited lipid droplet accumulation, which, as reported in recent studies, functions as a protective mechanism to evade ferroptosis. In addition, mitochondrial relocalization around lipid droplets was observed, suggesting a structural adaptation to promote efficient fatty acid oxidation for energy and redox balance. Bulk mRNA-seq analysis revealed significant upregulation of genes involved in lipid droplet biogenesis, fatty acid oxidation, and ferroptosis suppression under glutamine-deprived conditions. In contrast, non-senescent-like cancer cells rapidly underwent cell death without similar metabolic or structural changes. These findings suggest that senescence-like gastric cancer cells employ a unique metabolic reprogramming strategy that supports survival under nutrient stress by rerouting fatty acid metabolism to suppress ferroptosis. Furthermore, combinatorial treatment with a glutaminase (GLS) inhibitor and a diacylglycerol acyltransferase (DGAT) inhibitor, which blocks lipid droplet formation, may disrupt this adaptive metabolism and effectively induce ferroptosis. Collectively, this study highlights a distinct metabolic vulnerability of senescence-like cancer cells, offering a potential therapeutic strategy for treating highly aggressive, therapy-resistant gastric cancers.

THE LONG AND SHORT OF IT: AGE-DEPENDENT CHANGES IN RNAPII-MEDIATED TRANSCRIPTION OF GENES WITH DIFFERENT LENGTH

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Aging involves a gradual decline in molecular, cellular, and physiological functions, leading to diminished vitality and the onset of age-related diseases. Despite advances in omics technologies, the functional status of basal transcription processes in aging remains insufficiently understood. To address this, we conducted a comprehensive transcriptomic analysis of the brains and other tissues of young (3 months) and old (24 months) mice using bulk short read and long-read RNA sequencing.

We developed a novel algorithm capable of distinguishing old tissues from young based on intronic coverage in large and extra-large exons. Contrary to recently reported changes in the slope of the intronic coverage, indicating difference in the speed of elongation, we did not observe that phenomenon but rather a stable change in the amplitude of coverage over large and extra-large introns, which globally decreased with aging especially in tissues with low regenerative ability, like brain.

Our analysis revealed that the aged brain exhibited an enriched representation of genes connected to phagosome and microglia phagocytosis, synapse pruning, immune response, and neutrophil activation. In contrast, downregulated genes in the aged brain were associated with processes such as nervous system development, neuron projection, synapse organization, and gliogenesis.

We validated these findings using published human RNA-seq data and observed similar characteristics in the aging human brain, corroborating our mouse model results. Furthermore, significant changes were noted in the spliceosome during aging, correlating with short-read RNA-seq data and showing significant downregulation of genes and isoforms in the aged brain. Additionally, the aged brain showed significant overrepresentation of mono-exonic isoforms and novel intron retention isoforms. Our biochemical studies showed a significant decrease in the association of Med23, a mediator complex subunit, with RNAPII in aged mice, suggesting an altered transcriptional machinery.

Our findings underscore the importance of studying mRNA splicing events and transcriptional dynamics in the context of aging and provide a robust framework for identifying age-related transcriptomic changes. The observed reduction in mRNA dynamics and the altered regulatory role of RNA-binding proteins in the aged brain suggest a reshaping of the transcriptome with aging, driven by changes in splicing and mRNA processing.

RAS-DRIVEN CHROMATIN REWIRING IN SLOW-CYCLING CELLS

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Oncogene-induced senescence (OIS) is a tumour-suppressive state induced in response to high oncogenic activity. We recently highlighted the importance of RAS levels, both *in vivo* and *in vitro*, with striking effects on senescence clearance and tumour-initiating potential which vary between low- and high-RAS contexts. We and others characterised in depth OIS in fibroblast models, marked by stable cell cycle arrest and inflammatory senescence-associated secretory phenotype (SASP). In contrast, the RAS-titratable system we developed using hTERT-retinal epithelial cells (RPE1) shows a 'slow-cycling' phenotype with no clonal expansion: highly distinct from OIS, despite SASP-like induction of the pro-inflammatory factors. In these cells, RPE identity markers are primarily reduced and, instead, several neural progenitor genes are upregulated. A similar alteration in cell identity markers was also observed in the 'sub-OIS' cells in hepatocytes *in vivo*, which were linked to increased tumorigenesis. Moreover, unlike OIS fibroblasts, RAS-induced slow-cycling RPE1 cells exhibited minimal persistent DNA damage, low metabolic burden, and reduced biosynthetic MYC signalling. Using 3D epigenetic characterisation, we identified differential accessibility at known binding sites of neuronal transcription factors, such as NeuroD1. This was accompanied by changes in chromatin loops and enhancer-promoter interactions at those loci, reinforcing the feature of cell-identity shift. We propose this model as a unique opportunity to study a slow-cycling ecosystem *in vitro*.

THERAPEUTIC POTENTIAL OF USP14 AND UCHL5 MEDIATED ERRα REGULATION TO CONTROL MITOCHONDRIAL FUNCTION IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC), majority subtype of pancreatic cancer, is lethal disease showing a five-year survival rate of about 10%. The rapid proliferation and metabolic characteristic of PDAC require an increased protein turnover rate, leading proteotoxic stress. These conditions underscore the need for enhanced proteasome function in PDAC cells, making it a promising therapeutic target.
Two proteasomal deubiquitinases, ubiquitin-specific peptidase 14 (USP14) and ubiquitin carboxyl-terminal hydrolase isozyme L5 (UCHL5), act as ubiquitin trimmers on substrates. Analysis of published PDAC data revealed that aggressive ductal cells exhibited high expression levels of USP14 and UCHL5, correlating with poor patient survival rates. This observation was further validated through immunohistochemistry assays, suggesting that targeting these deubiquitinases could be a promising therapeutic strategy.
To address USP14 and UCHL5 as potential therapeutic targets, this study investigated what phenotypic changes of PDAC cell lines exhibited after treatment with b-AP15, a specific dual inhibitor of USP14 and UCHL5. As expected, b-AP15 treatment showed significant proliferation inhibition in PDAC cell lines and even xenograft models, suggesting potential therapeutic efficacy. To elucidate mechanisms of cellular phenotypic changes, transcriptomics and proteomics were employed. These comprehensive analyses revealed that a critical impact on mitochondrial function in PDAC cells. Importantly, the data indicated a inhibition of oxidative phosphorylation through estrogen-related receptor alpha autophagical degratation. These findings suggest the potential of b -AP15 as a therapeutic strategy targeting PDAC.

SUBTYPE-SPECIFIC IMMUNOTHROMBOTIC PROFILES IN ISCHEMIC STROKE THROMBI

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Ischemic stroke is a leading cause of disability and mortality, particularly in the aging population. It comprises distinct etiologic subtypes, and accurate classification is essential for appropriate secondary prevention and therapeutic strategies. Notably, large artery atherosclerosis (LAA) and cardioembolic (CE) subtypes are associated with a high risk of recurrence, yet their underlying pathological distinctions remain poorly defined.

Immunothrombosis, which represents the interplay between inflammation and coagulation, facilitates protective intravascular clot formation during infection or injury. However, its dysregulation contributes to thrombotic complications in cardiovascular diseases, such as myocardial infarction. To investigate subtype-specific differences in immunothrombotic activity and potential influences of immune aging in ischemic stroke, we performed immunohistochemistry and spatial transcriptomic profiling on thrombi retrieved from patients with LAA and CE stroke.
Using differential expression and pathway enrichment analyses, we identified aged neutrophil signatures with increased NET formation in CE thrombi. In contrast, LAA thrombi were enriched for aging-related pathways, including oxidative phosphorylation.

Collectively, we observed subtype-specific immune characteristics, including aged neutrophils in CE thrombi and increased mitochondrial oxidative phosphorylation in macrophages from LAA thrombi, consistent with metabolic changes associated with immune aging. These findings suggest that aging-associated immune changes, such as neutrophil senescence and macrophage metabolic reprogramming, may contribute to the pathophysiological differences between stroke subtypes.

ApoE KNOCKOUT DECREASES ADIPOGENIC DIFFERENTIATION POTENTIAL AND ALTERS ADIPOGENIC MARKERS IN RABBIT MESENCHYMAL STEM/STROMAL CELLS (ASC)

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Mesenchymal stem cells (MSCs) are affected by molecular and cellular aging mechanisms. These adult stem cells have the ability to self-renew and can differentiate into several cell types, e.g. fibroblasts and adipocytes. Our hypothesis is that metabolic factors (endogenous and exogenous) influence the aging process of MSCs in the adipose tissue niche. In this context, we consider apolipoprotein E (ApoE) as a critical factor for lipid and cholesterol metabolism. The rabbit is a valuable animal model for a variety of biomedical research areas such as embryology, organogenesis, and the modelling of diabetes, obesity, and cardiovascular diseases. Therefore, rabbit adipose-derived mesenchymal stem / stromal cells (ASCs) represent a promising tool for studying ApoE-dependent mechanisms of age-related diseases in detail.
In this study, ASCs were isolated from subcutaneous adipose tissue of female NZW (ZIKA hybrid) wild type and ApoE-knockout rabbits. Primary ASCs were successfully isolated, propagated, and characterized as described previously (Jung et al. 2019). We analyzed the impact of an ApoE mutation on fat cell differentiation, using an adipogenic differentiation protocol for wild type and ApoE-knockout ASCs at passage 4 and passage 20 by MesenCult™ Adipogenic Differentiation Kit (STEMCELL Technologies) over 21 days. The adipogenic differentiation efficiency of the ApoE-knockout ASCs was reduced during in vitro aging (passage 20) to the adipogenic differentiation of ASCs of wild type rabbits. In contrast, ASCs isolated from subcutaneous fat tissue from old wild types (>108 wks) had a higher adipogenic differentiation potential compared to young wild type ASCs. In addition, the mRNA expression of the adipogenic markers PPARG, CEBPA, SOX9, WNT10B, DLK1 and the adipocyte markers ADIPOQ, LEP and FABP4 was determined by qPCR at three different time points of adipogenic differentiation. The mRNA amount of PPARG, CEBPA, DLK1, ADIPOQ, and FABP4 decreased, whereas WNT10B and LEP increased in ApoE-knockout ASCs compared to wild type ASCs.
Together, we successfully established primary rabbit knockout ASC cultures, which provide a promising molecular model to investigate the mechanism of ApoE- and metabolism induced aging.

(Supported by DFG GZ JU 31463-1; DFG GRK 2155 ProMoAge and Wilhelm Roux Programme, MLU Faculty of Medicine) *- Jung JS, Volk C, Marga C, Navarrete Santos A, Jung M, Rujescu D, Navarrete Santos A (2019) Adipose-Derived Stem / Stromal Cells (ASCs) Recapitulate Aging Biomarkers And Show Reduced Stem Cell Plasticity Affecting Their Adipogenic Differentiation Capacity. Cell Reprogram; 21(4):187–199. doi:10.1089/cell.2019.0010*

PAI-1 INHIBITION MIMICS TRANSCRIPTOMIC LANDSCAPE OF CALORIC RESTRICTION IN MOUSE LIVER.

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Caloric restriction (CR) extends lifespan in mammals but the stringent 20% reduction in food intake is challenging to sustain; therefore, validating pharmacological mimics is of high interest. We previously linked a *SERPINE1* mutation in an Amish cohort to a 10-year lifespan increase and improved metabolism. *SERPINE1* encodes for Plasminogen Activator Inhibitor-1 (PAI-1) which is a clinical predictor of vascular stiffness, hypertension, and diastolic dysfunction. Moreover, PAI-1 amplifies accumulation of senescent cells as a component of the senescence associated secretory phenotype (SASP). Here, we compared hepatic transcriptomic profiles in mice after 8 weeks of CR, rapamycin, metformin, or the PAI-1 inhibitor TM5614 versus an ad libitum control. CR induced extensive changes (1087 genes downregulated, 1145 upregulated), while TM5614 showed the closest mimicry (211 down, 218 up), sharing ~50% of CR's differentially expressed genes, including downregulated *Il11ra1* and upregulated *Per2*, tied to longevity and metabolism. Rapamycin and metformin showed minimal overlap with CR. Gene Ontology analysis revealed CR and TM5614 downregulated metabolic pathways, and both countered gene-length-dependent transcription decline, an emerging measure of an aging transcriptomic landscape. TM5614 presents as a CR mimic, offering an alternative to CR to extend healthspan.

SODIUM-GLUCOSE COTRANSPORTER-2 INHIBITOR ALLEVIATES AGE-ASSOCIATED PHENOTYPE IN AGING MICE

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Objective: With the expanding aging population, there is an increasing emphasis on promoting healthy aging. We hypothesize that the Sodium-glucose cotransporter-2 inhibitor (SGLT2i) could serve as a potential senolytic therapy for age-related diseases.

Methods: Old mice aged 19-24 months were administered the SGLT2 inhibitor empagliflozin at 10 mg/kg (i.p.) for the first 3 days followed by 20 mg/kg (i.p.) daily for 4 weeks while maintained on a high-fat diet. Young mice aged 2-4 months were used as a control group matched with the old mice. Metabolic rates were assessed using a metabolic chamber system, including activity monitoring. Anxiety levels were evaluated using the open field test. Tissues and blood samples were collected at the study's conclusion for further analysis.

Results: Old mice treated with empagliflozin exhibited lower body weight gain compared to the aged vehicle control group, with no significant difference in food intake. In the liver, empagliflozin-treated mice showed significant reductions in senescence markers such as p53, p21, p16, and beta-galactosidase levels. This reduction correlated with decreased hepatic lipid droplets and a decline in pro-inflammatory macrophages. Similarly, empagliflozin treatment led to reduced levels of senescence markers, fat droplet size, and macrophage infiltration in epididymal fat. Metabolic rates, including oxygen consumption, carbon dioxide production, and activity levels, were notably enhanced in old mice treated with empagliflozin. Moreover, anxiety levels were significantly reduced and comparable to those observed in young control mice. Furthermore, empagliflozin treatment in old mice resulted in decreased levels of senescence markers (p21, p16) and inflammatory cytokines in the hippocampus and hindbrain regions.

Conclusion: These findings suggest that empagliflozin holds promise as a therapeutic agent for ameliorating age-associated pathologies.

LANATOSIDE C, A NOVEL SENOLYTIC, AMELIORATES ATHEROSCLEROSIS IN MICE

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Cellular senescence, a state of irreversible cell cycle arrest, contributes to aging and age-related diseases. Senolytics targeting cellular senescence could be applied to the prevention and treatment of age-related diseases. In this study, we identified lanatoside C (Lana C) as a senolytic compound. Lana C, a cardiac glycoside used for the treatment of cardiovascular diseases, is known to inhibit the transmembrane protein sodium-potassium adenosine triphosphatase (Na+/K+-ATPase). We found that Lana C depolarized and acidified senescent human umbilical vein endothelial cells (HUVECs), making them susceptible to apoptosis. The senolytic activity of Lana C was inhibited by potassium chloride (KCl) and Z-VAD-FMK (ZVF), a widely used pan-caspase inhibitor. Additionally, Lana C significantly ameliorated the senescence burden and the formation of atherosclerotic lesions in apolipoprotein E (ApoE-/-) or low-density lipoprotein receptor (Ldlr-/-) knockout mice. These results suggest that Lana C could be a promising senolytic for age-related diseases.

INVESTIGATION AND REGIONAL MAPPING OF THE MOLECULAR SIGNATURES UNDERLYING MEMORY FORMATION AND AGE-RELATED MEMORY LOSS

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Long-term memory (LTM) is a learning-dependent process occurring in the hippocampus and requiring activation of gene expression and *de novo* synthesis of molecular components critical for synapse strengthening and new synapse formation, processes actively modulated by glial cells. Despite foundational knowledge, our understanding of the regulatory mechanisms of LTM and how aging impacts them leading to memory decline remains limited. Here, we used snRNA-Seq to investigate both baseline and learning-induced molecular mechanisms in the aging mouse hippocampus in a cell-type specific manner. To assess memory performance of aged mice and correlate learning-induced molecular changes with memory loss severity, we developed a behavioral paradigm consisting of two sequential novel object recognition (NOR) tasks. The first NOR task evaluated LTM, revealing significantly lower memory performance in aged (23 mo) mice compared to young (5 mo) animals. Notably, the aged mice were distinguished into two groups: those with severe memory deficits (~80% of animals; aged-severe), and those with moderate memory deficits (~20%; aged-moderate). One week later, a subset of animals underwent a second NOR task, and hippocampi were isolated at one and five hours post-training, the critical time window for learning-induced transcriptomic changes associated with LTM. The remaining animals did not undergo the second training and served as baseline controls. We analyzed three FACS-enriched populations for each time point: activated neurons (cFOS+ and/or ARC+), non-activated neurons, and non-neuronal (NeuN-) populations. We present a transcriptomic atlas of the hippocampus, both at baseline and after learning, comprising ~ 0.7 million transcriptomes from young and aged mice. High-resolution cell clustering identified ~80 neuronal and non-neuronal clusters, uncovered age-associated molecular signatures at baseline, and provided a list of cell-specific and shared across cell types differentially expressed genes (DEGs). Moreover, examination of enriched activated neuronal populations upon learning revealed time-dependent and cell type-specific upregulation of activity-dependent markers, including immediate-early and synaptic plasticity genes. Notably, aged mice with severe memory deficits showed reduced responses of these markers post-learning, compared to the aged-moderate and young groups. In addition, we report a novel set of cell-type specific learning-induced signatures shared in young and aged-moderate but absent in the aged-severe mice. Finally, analysis of non-neuronal DEGs showed upregulation of genes linked to immune function and inflammation in the aged groups, while neuron-glial interaction analysis revealed neuroinflammatory responses potentially impacting learning-dependent pathways.

ROGUE mtDNA: SELFISH MUTATIONS ACCELERATE AGE-ASSOCIATED EROSION OF mtDNA INTEGRITY IN MAMMALS

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Mutations in the mitochondrial genome (mtDNA) accumulate progressively in somatic tissues during aging, contributing to various age-related conditions. The high copy number of mtDNA protects cells from the impact of newly arising recessive mutations; however, when intracellular levels of mutant mtDNA exceed a critical threshold, pathological phenotypes emerge. The mechanisms driving the intracellular expansion of mtDNA mutations have remained unknown. Here, using single-cell DNA sequencing, we found that specific mutant alleles located within the mtDNA replication control region were repeatedly present at high abundance in hepatocytes of aged mice and humans. These alleles confer a replication advantage (drive), resulting in selective amplification of the affected genome along with a broad spectrum of linked passenger mutations, some of which are deleterious. Notably, we found that the most prevalent mtDNA disease variant in humans, 3243A>G, behaved as such a driver, suggesting that a combination of replicative drive and functional defect underlies its prevalence in disease. We conclude that replicative drive, by accelerating the rise in abundance of linked deleterious mtDNA mutations, promotes age-associated erosion of mtDNA, and influences the transmission and progression of mitochondrial diseases.

AGED BONE DEFECT REPAIR IS SENSITIVE TO DELIVERY TIMING OF SENOLYTICS AND SENOMORPHICS

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Bone repair is challenging for aged women due to several factors including lower bone mineral density and cellular dysfunction of immune cells and bone cells, including osteoprogenitors and osteoclast precursors. The normal balance of bone building osteoblasts and bone resorbing osteoclast activity is tipped towards resorption by the combination of cellular dysfunction, accumulation of senescent cells and chronic inflammation that occurs with aging. Previous studies showed that targeted systemic elimination of senescent cells had beneficial effects on bone density in old mice.

Bone grafts assist with healing of bone injuries and are used as a scaffold for localized delivery of drugs. Localized delivery of senolytics and senomorphics may help reduce their off-target effects on the normal function of macrophages and bone progenitor cells that express markers of senescence or the SASP during their phenotype switching and differentiation. Since macrophages and progenitor cells must actively participate in a concerted manner during the first week of bone repair, we hypothesized delivery timing of senolytics or senomorphics could impact bone repair. We utilized bone-like calcium phosphate coatings on bone graft substitutes for localized delivery of the senolytic, ABT-263, or the senomorphic, Ruxolitinib (Rux). We tested the effects of early (immediate) and late (after 7 days) delivery of these two compounds in separate studies. The timing effect was evaluated (a) *in vitro* with chemically-aged MC3T3-E1 mouse osteoprogenitor cells, bone marrow derived macrophages or osteoclast precursors and (b) *in vivo* with calvarial bone defect studies in aged mice >20 months old. *In vivo* bone regeneration was measured by micro-CT analysis and histological studies (bone mineralization labels, and ALP and TRAP staining).

In vitro studies demonstrated a reduction of senescent cells and their SASP, a restoration of more youthful macrophage phenotype transitions, and improved *in vitro* mineralization as assessed by key osteogenic and inflammatory genes for delayed delivery of both of the compounds tested. Interestingly, only delayed delivery timing of both the senolytic and the senomorphic was optimal to enhance *in vivo* bone repair of aged mouse bone calvarial defects, while immediate delivery impaired bone healing. We broadly conclude that, in a similar manner to anti-inflammatory drugs, senolytics and senomorphics should be given up to one week after bone injury in order to maximize aged bone repair.

PARKINSON'S DISEASE RISK FACTOR FAM49B: A NOVEL REGULATOR OF MICROGLIA ACTIVITY IN AGE-ASSOCIATED NEURODEGENERATION

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Parkinson's disease (PD) is the second most common age-related neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra. While various gene mutations are known to contribute to PD, age remains the most significant risk factor. A major consequence of aging is the increasing neuroinflammation, which has been considered to be a major contributor of neuronal loss in neurodegenerative diseases. Emerging evidence suggests that neuroinflammation and microglial activity play crucial roles in the progression of the PD pathology. Through computational analysis of human gene expression of PD patients, we identified Family with Sequence Similarity 49, Member B (FAM49B) – a known genetic risk factor for PD – to decrease in expression with age in human brain samples. Furthermore, our analysis revealed a functional role of FAM49B specifically in microglia, suggesting that its decline in expression may contribute to dysregulated microglial function and increased neuroinflammation in PD patients. While current studies showed that FAM49B plays a role in cytoskeleton dynamics and mitochondrial function, its exact role in microglia cells remains unclear. In this study, we demonstrate that knockout of FAM49B in HMC3 microglial cells not only disrupts the cytoskeleton dynamics and mitochondrial function but also leads to dysregulation in cell migration and, most importantly, its inflammatory responses. These microglial alterations have the potential to contribute to exacerbated neuroinflammation, neuronal vulnerability, and the loss of dopaminergic neurons observed in PD patients. Taken together, these results provide new insights into the molecular mechanisms underlying PD progression. Understanding how FAM49B regulates microglial behavior could provide a new basis for therapeutic strategies targeting disease progression in PD patients.

DISRUPTION OF GERMINAL CENTER DYNAMICS WITHIN THE AGED LYMPH NODE MICROENVIRONMENT

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Weakened germinal center responses by the aged immune system result in diminished immunity against pathogens and reduced efficacy of vaccines. Prolonged contacts between activated B cells and CD4+ T cells are crucial to germinal center formation and T follicular helper cell (Tfh) differentiation, but it is unclear how aging impacts the quality of this interaction. Our previous work using 2-photon microscopy has shown that T cell motility within aged mouse lymph nodes was impaired with age due to increased fibrosis within the microenvironment. Peptide immunization confirmed that aged mice have decreased expansion of antigen-specific germinal center B cells and reduced antibody titers. Furthermore, aging was associated with decreased CD4+ T conventional cells and accumulated Tfh cells and regulatory T cells, even in naïve mice. Despite increased numbers, aged Tfh had reduced expression of the master transcription factor BCL6 and increased expression of purinergic modifier CD39, as well as indications of decreased metabolic function. Single-cell transcriptomic analysis also revealed expansion of CD4+ T cell subsets with natural killer cell signatures within aged, immunized mice. To determine the contribution of cell-extrinsic influences on antigen-specific Tfh induction, young, antigen-specific B and CD4+ T cells were adoptively transferred into aged hosts prior to peptide immunization. Transferred young cells in aged hosts had reduced expansion and differentiation into germinal center B cell and Tfh and reduced antigen-specific antibody titers when compared to those in young hosts. Transferred CD4+ T cells in aged hosts differentiated into Tfh cells with reduced BCL6, reduced PD-1, and increased CD39 expression. These results highlight the role of the aging lymphoid microenvironment in modulating CD4+ T cell differentiation, which contributes to impaired establishment and maintenance of germinal centers. Ongoing work uses live-cell 2-photon microscopy to quantify differences in CD4+ T cell priming by dendritic cells and in CD4+ T cell interactions with B cells within aged lymph nodes following immunization.

NUCLEOTIDE IMBALANCE INDUCES MITOCHONDRIAL DNA-DEPENDENT cGAS-STING SIGNALING IN SENESCENCE

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The metabolic and signaling functions of mitochondria ensure cell survival but when dysregulated are associated with inflammation, cell death and disease. Imbalanced nucleotide synthesis can induce mitochondrial DNA (mtDNA) release into the cytosol and cGAS-STING-dependent innate immune signaling. We show that an increased cellular ratio of ribonucleotides to deoxyribonucleotides leads to increased ribonucleotide incorporation into mtDNA causing replication stress and mtDNA release in mice, which increases with age. Reduced deoxyribonucleotide synthesis and ongoing mtDNA replication in senescence render cell-cycle arrested senescent cells susceptible to ribonucleotide incorporation, triggering mtDNA release into the cytosol and the mtDNA-dependent senescence-associated secretory phenotype (SASP). Our results support a critical role of mtDNA-dependent inflammation during the ageing process.

SENESCENT ENDOTHELIAL CELLS CONTROL T CELL IMMUNITY IN THE LUNG MICROENVIRONMENT DURING LATE-STAGE CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Chronic Obstructive Pulmonary Disease (COPD) is a leading global cause of mortality, frequently accompanied by T cell dysfunction and cellular senescence. This progressive condition is classified into mild, moderate, and severe stages, each presenting distinct clinical outcomes. Although COPD has been extensively studied, the mechanisms driving stage-specific immune alterations remain poorly understood.

This study integrated single-cell transcriptomic profiling of lung tissues with population-scale plasma proteomic data from the UK Biobank to investigate COPD across all stages. This approach enabled high-resolution analysis of cellular dynamics and intercellular communication during disease progression.

The analysis revealed a marked increase in endothelial cell senescence in severe COPD, as a key driver of immune dysregulation. These senescent cells exhibited a distinct senescence-associated secretory phenotype (SASP), featuring FGF and CXCL10, which interacted with naïve and cytotoxic T cells. These signals promoted unchecked proliferation of naïve T cells and impaired cytotoxic T cell function, indicating a decline in effective T cell immunity. The accumulation of senescent cells and their inflammatory secretions reflects pathological remodeling of the immune landscape, with reduced surveillance and persistent inflammation. Mechanistically, this senescence appears driven by Myc-induced transcriptional programs, potentially triggered by loss of TGF-β signaling.

Cellular senescence emerges as an active contributor to immune dysfunction in severe COPD, with effects beyond its conventional role in aging. By identifying SASP-mediated signaling as a critical feature of late-stage disease, this study highlights stage-specific therapeutic opportunities targeting senescent cells and their inflammatory outputs. Continued investigation across COPD severity is essential to develop tailored interventions that restore immune balance and improve patient outcomes.

THE miR-30-5p/TIA-1 AXIS DIRECTS CELLULAR SENESCENCE BY REGULATING MITOCHONDRIAL DYNAMICS

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Senescent cells exhibit a diverse spectrum of changes in their morphology, proliferative capacity, senescence-associated secretory phenotype (SASP) production, and mitochondrial homeostasis. These cells often manifest with elongated mitochondria, a hallmark of cellular senescence. However, the precise regulatory mechanisms orchestrating this phenomenon remain predominantly unexplored. In this study, we provide compelling evidence for decreases in TIA-1, a pivotal regulator of mitochondrial dynamics, in models of both replicative senescence and ionizing radiation (IR)-induced senescence. The downregulation of TIA-1 was determined to trigger mitochondrial elongation and enhance the expression of senescence-associated β-galactosidase, a marker of cellular senescence, in human foreskin fibroblast HS27 cells and human keratinocyte HaCaT cells. Conversely, the overexpression of TIA-1 mitigated IR-induced cellular senescence. Notably, we identified the miR-30-5p family as a novel factor regulating TIA-1 expression. Augmented expression of the miR-30-5p family was responsible for driving mitochondrial elongation and promoting cellular senescence in response to IR. Taken together, our findings underscore the significance of the miR-30-5p/TIA-1 axis in governing mitochondrial dynamics and cellular senescence.

SINGLE-CELL TRANSCRIPTOMIC INSIGHTS INTO AGING-RELATED CHANGES IN ADIPOSE TISSUE

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Aging is a complex biological process involving gradual molecular and cellular changes that lead to a decline in overall function. Among various tissues, adipose tissue is known to undergo age-related changes more rapidly, making it a useful model for studying the biological mechanisms of aging. In this study, we aimed to investigate aging-related changes in adipose tissue by analyzing publicly available single-cell RNA sequencing (scRNA-seq) data (GSE137869). This dataset comprises scRNA-seq from adipose tissues of young (5 months old) and old (27 months old) rats, including both males and females. We first analyzed gene expression changes between age groups, followed by cell clustering using the Seurat package and cell-type identification with the Azimuth tool. UMAP visualization revealed that the Macrophage 2 (Mac2) cluster and the Adipose Stem & Progenitor Cells 2 (ASPC2) cluster showed the most significant differences with aging. To further investigate how gene expression patterns changed over time, we applied pseudotime trajectory analysis using the Monocle3 package, setting the young group as the starting point. This approach allowed us to classify genes into two main groups: those that increased with aging and those that decreased. The results showed a noticeable increase in immune-related pathways, particularly those linked to inflammatory signaling and immune response regulation in older adipose tissue. This suggested immune activity was accompanied by an increase in macrophages and persistent low-level inflammation, often referred to as inflammaging. In contrast, genes involved in maintaining the collagen-containing extracellular matrix (ECM) were significantly reduced with aging, suggesting that the structural integrity of adipose tissue declines over time. To validate these findings, we performed cell-cell communication analysis using the CellChat package, which confirmed an increase in inflammatory signaling between macrophages and other adipose cell types, supporting the idea that immune cell interactions become more active with aging. Additionally, signaling pathways related to ECM maintenance were found to be weakened, reinforcing the observation that the extracellular matrix deteriorates with age. These results highlight the close relationship between inflammation and ECM breakdown as key processes in adipose tissue aging. Our findings suggest that increased immune activity and loss of ECM structure are major characteristics of adipose aging and may contribute to broader metabolic and inflammatory conditions in older individuals. Although further functional validation is needed, the results from trajectory modeling and intercellular communication analysis may help identify potential strategies to mitigate aging-related tissue dysfunction and promote healthier aging.

AQUAPORIN-4 DEPENDENT MECHANOTRANSDUCTION REGULATES ASTROCYTE AGING AND COGNITIVE FUNCTION

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Aquaporin-4 (AQP4), an astroglial water channel, plays a crucial role in regulating brain water balance and facilitating perivascular cerebrospinal fluid (CSF) influx and interstitial solute clearance. However, the impact of biomechanical stimuli associated with the aged brain microenvironment—such as fluid shear stress induced by interstitial fluid flow—on AQP4 function remains largely unexplored.

In this study, we investigated the effects of enforced aging on primary astrocytes using D-galactose treatment, which resulted in hallmark aging phenotypes, including reduced lamin B1 expression and decreased nuclear circularity. Interestingly, when these aged astrocytes were exposed to a microfluidic platform mimicking the fluid dynamics of a youthful glymphatic system, their aging-associated characteristics were significantly alleviated. Notably, aged astrocytes exhibited impairments in amyloid-beta uptake and glutamate homeostasis, but these functional deficits were restored upon exposure to fluid shear stress.

Mechanistically, the shear stress-induced recovery of astrocytic function was abrogated when AQP4 expression was silenced via siRNA, indicating that AQP4 is essential for this rejuvenating effect. However, overexpression of AQP4 did not further enhance the recovery, suggesting a threshold effect in AQP4-mediated functional restoration. Importantly, while fluid shear stress did not increase AQP4 expression levels, it promoted AQP4 polarization at the astrocytic membrane, which was closely associated with the observed reversal of aging-related impairments.

Our findings suggest that biomechanical forces, particularly fluid shear stress, play a pivotal role in mitigating aging-induced astrocytic dysfunction by modulating AQP4 polarization rather than its overall expression. These results highlight the potential of targeting astrocytic mechanotransduction as a therapeutic strategy for aging-related neurodegenerative conditions.

LUNG-VASCULAR SENESCENCE: ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR

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Chronic obstructive pulmonary disease (COPD) is characterized by alveolar destruction and airflow obstruction, with aging and cigarette smoke (CS) exposure as major risk factors. Macrophage migration inhibitory factor (MIF) regulates innate immunity, cellular homeostasis, and survival. We previously reported lower plasma MIF levels in COPD patients. Here, we investigate MIF's role in CS-induced senescence and test a novel MIF agonist in a COPD model. HUVECs were treated with CS extract (CSE) in combination with recombinant MIF, MIF agonist (MIF20), MIF siRNA, anti-MIF-mAb, or MIF antagonist (MIF98). ERK inhibitor U0126 was used to assess MIF's protective mechanisms. MIF KO mice received a lentiviral vector to restore MIF expression. Mice were exposed to 4 months of CS and treated orally with MIF20 (3 mg/mouse) in the last 2 months. Cell type composition and MIF levels in human lung tissues were analyzed using Xenium, with age groups categorized as young/intermediate-old (<40y) and old (>60y). CSE-induced replicative senescence transcripts (p16, p21) and a generally accepted biomarker SA-β-gal activity in HUVECs, which were attenuated by MIF/MIF20 but enhanced by MIF siRNA, anti-MIF-mAb, or MIF98. This anti-senescent effect was abolished by inhibiting ERK signaling, essential for cell proliferation and survival. CS-exposed MIF KO mice showed accelerated COPD, as measured by lung compliance. CS-induced Ec-p21 expression was mitigated by MIF restoration via lentiviral MIF transduction. MIF20 attenuated the CS-induced upshift in the pressure-volume curve, indicating increased lung compliance and reduced p16 & p21 mRNA expression. Spatial data revealed an age-related increase in CD4 T cells and macrophages, while ECs and fibroblast decreased, alongside downregulated MIF in aged human lung tissues. These findings suggest MIF augmentation may be a novel strategy to prevent and potentially reverse CS-related COPD. In future studies, we will explore the relationship between MIF and age-related changes in cell-type proportions, as well as its co-expression with senescence marker genes.

SENESCENCE CONFERS THERAPEUTICALLY EXPLOITABLE APL-LIKE PLASTICITY TO NEWLY DIAGNOSED NON-M3 ACUTE MYELOID LEUKEMIA

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Senescence-related aberrant cellular plasticity is an emerging research topic and may have important ramifications for conceptually novel treatment strategies. Therapy-induced senescence (TIS) is considered an 'apoptosis back-up' response of tumor cells to various types of cancer therapeutics. Recent evidence increasingly focuses on the 'dark side' of senescence, tumor-promoting effects exerted by senescent cancer cells through cell-autonomous (such as senescence-associated stemness, our own finding) or non-cell-autonomous mechanisms (such as pro-inflammatory and mitogenic effects caused by the senescence-associated secretory phenotype). Notably, and similar to apoptosis, there is virtually no robust data on the potentially tumor-suppressive function of TIS when it is enforced in newly diagnosed (nd) malignancies.

Here, we investigated the impact of TIS on the long-term outcome of nd acute myeloid leukemia (AML), which is a (cyto-)molecularly extremely heterogeneous and therapeutically challenging blood cancer, excluding the acute promyelocytic leukemia (APL/M3) subtype. We applied bulk and single-cell analysis of primary AML patient samples in a flow-based ex vivo senescence assay after exposure to cytarabine or daunorubicin, two standard chemotherapy agents used in intensive induction therapy for most patients. We made the following key observations: (I) TIS capacity, determined in primary leukemia blasts ex vivo, predicted superior long-term outcomes in three independent cohorts comprising > 90 patients with nd AML. (II) Unexpectedly, a 13-gene classifier derived from non-M3 AML samples in TIS identified M3 AML cases at their basal transcriptome level with high sensitivity and specificity across several large AML cohorts (nearly 1,000 patients). (II) Entering TIS biologically unified the highly heterogeneous spectrum of non-M3 AML cases by conferring M3/APL-like plasticity, with the polycomb-repressive complex 2 (PRC2) component Suz12 operating as the underlying epigenetic mechanism. (IV) TIS uniquely rendered non-M3 AML cells susceptible to otherwise M3-reminiscent ATRA-mediated 'seno-differentiation', suggesting a conceptually novel state switch-dependent therapeutic approach in cancer.

These findings mark TIS as a beneficial treatment response to initial drug encounter, translating into superior prognosis, and as a mechanism of profound epigenetic remodeling that may present novel therapeutically exploitable vulnerabilities.

HSP90β/α-CRYSTALLIN CHAPERONE SYSTEM PROTECTS NORMAL LENS DEVELOPMENT AND PREVENTS CATARACTOGENESIS

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Purpose: Cataract is derived from protein aggregation due to genetic mutations, stress and aging. The damaged protein aggregates are bound by α-crystallins, the small heat shock proteins which were shown to possess chaperone activities initially demonstrated by Dr. Horwitz's laboratory in 1992 and subsequently by many others. Lack of ATP binding and hydrolysis ability limit their functions as chaperones, and could not recycle the bound proteins into cytoplasm. Thus, maintaining lens transparency requires functions of true chaperone proteins. Here, I will discuss systematically the new chaperone system in the ocular lens.

Methods: Human lens capsular epithelia, lens epithelial cell lines, zebrafish and mice were used as testing systems. QRT-PCR and Wes were used to analyze mRNA and protein expression. Morppholino Oligos and CRISPR/Cas9 technology were used to silence gene expression in ex vivo and in vivo.

Results: HSP90, HSP70, HSP60 and HSP40 in normal and cataract human lenses, and in young and aged mouse lenses were analyzed, and it is found that HSP90β is a dominantly expressed heat shock protein in lens. From transparent human lens to cataract patients, also from young to aged mouse lens, HSP90β is significantly downregulated. Silence of HSP90β expression in lens epithelial cells of zebrafish caused significant in vivo apoptosis followed by cataractogenesis.

Conclusions: The HSP90β/α-crystallis chaperone system protects normal lens development and prevent cataractogenesis. (Supported by NSFC grants #82271071, #81970787, #81770910, and NSFG joing grant #2019B1515120014), and the Fundamental Funds, 3030901010111 of the State Key Laboratory of Ophthalmology of Zhongshan Ophthalmic Center).

DOES THE MEDITERRANEAN DIET SUPPRESS PREDICTED PATHOGENIC FUNCTIONS IN THE GUT MICROBIOTA IN AD?

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The gut microbiota is altered in Alzheimer's disease (AD) patients and may contribute to AD by secreting microbial metabolic product that affect immunologic and/or neuronal function. The gut microbiota metabolic output is largely dependent on dietary composition as shown in clinical trials and animal models. Epidemiological studies have shown that consuming a Mediterranean diet is associated with improved cognitive function and reduced risk of AD. A clinical trial further found that the Mediterranean diet improved AD biomarkers in CSF and was linked to changes in the gut microbiome and microbially-produced short-chain fatty acids. On the contrary, the Western diet is linked to increased AD risk and has differential effects on the gut microbiota. Whether the Mediterranean or Western diet affects AD pathogenesis via modulating microbiota metabolic function has not been extensively investigated in mechanistic animal models. We designed three rodent diets based on human dietary patterns including a control diet, a Mediterranean diet, and a Western diet. We hypothesize that the Mediterranean diet ameliorates Alzheimer's disease by suppressing pathogenic gut microbiota and microbial metabolites, and conversely, a Western high fat high sugar diet worsens AD by increasing microbiota pathogenic functions. Mouse weight and fecal samples were collected on day 0 (mouse age of 8-week), day 7 and monthly until mice were 8-month of age. Mice gained more weight on both the Mediterranean diet and the Western diet compared to the control diet regardless of genotype and sex. Male mice gained more weight on the Mediterranean diet compared to Western diet regardless of genotype. Furthermore, APP/PS1 male mice on Mediterranean and Western diet gained more weight than WT male mice. Analysis of the gut microbiota showed that diet was the major variable that affected microbiota composition, and genotype and sex also contributed to microbiota compositional differences to a less extent. Furthermore, we assessed amyloid plaque burden in the hippocampal and parietal cortical regions and investigated the effect of diet on microglial transcriptional signatures. Preliminary data of Aβ immunohistopathology of female mice (n=4) showed the Mediterranean diet reduced Aβ plaque burden compared to the control diet by a 5-fold change. Consistent with previous studies, these data suggest that the Mediterranean diet has a protective effect on the AD pathogenesis and provides an opportunity to link this effect to changes in the gut microbiota and inflammatory responses in the brain which we are identifying in on-going analyses.

BONE-SPECIFIC ENDOTHELIAL CELLS MEDIATE BONE REGENERATION VIA DISTINCT EXPRESSION OF KIT LIGAND

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Bone quality progressively deteriorates in the aging process, increasing susceptibility to fractures, reducing the regenerative capacity, and contributing to diseases such as osteoporosis. These age-related bone defects underscore the urgent need for effective bone regeneration strategies, which remain a significant challenge in clinical practice, particularly due to the limitations associated with autologous bone grafting, such as donor site morbidity and limited bone availability, as well as a lack of comprehensive understanding of the mechanisms regulating the osteogenic activity *in vivo*. This study investigated the potential of human bone-derived endothelial cells (b-ECs) in promoting bone regeneration, especially in conjunction with bone marrow-derived mesenchymal stem cells (bm-MSCs). Our results demonstrated that b-ECs retain unique osteoinductive properties post-isolation, crucial for promoting bone formation *in vivo*. Utilizing ectopic and orthotopic xenograft models in immunodeficient mice, our findings revealed that the synergistic interaction of b-ECs and bm-MSCs induced rapid and substantial bone formation, highlighting the therapeutic potential of b-ECs in bone repair strategies. Notably, the distinct expression of KIT ligand (KITLG) in b-ECs was identified as a key factor in these processes. KITLG expression by b-ECs facilitated the recruitment of c-Kit+/CD34+ hematopoietic progenitor cells to the osteovascular niche, leading to robust osteogenic differentiation of bm-MSCs, a process regulated by Notch signaling. Moreover, inducing KITLG expression in non-bone-derived endothelial cells conferred similar osteoinductive capabilities. These findings not only enhance our understanding of the role of bone-specific endothelial cells in osteogeneis, but also open avenues for developing innovative cell-based therapies for bone regeneration and aging-related bone diseases.

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EPIGENETIC REPROGRAMMING VIA OVEREXPRESSION OF *OCT4*, *SOX2*, *KLF4* (OSK) IS A MECHANISM TO SAFELY REJUVENATE AGED AND DISEASED MOTOR UNIT CELLS.

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Motor system degeneration is a feature of both natural aging and severe neurodegenerative diseases. The motor unit (MU) consists of a motor neuron in the spinal cord and the myofibers it innervates at the neuromuscular junction (NMJ). Accelerated epigenetic age co-occurs with diseases of MU decline, which range from age-related sarcopenia to more severe conditions such as Amyotrophic Lateral Sclerosis (ALS). Epigenetic reprogramming via overexpression of Yamanaka factors *Oct4*, *Sox2*, *Klf4* (OSK) can safely restore the epigenetic landscape and rejuvenate neuronal tissues. Here, we applied OSK reprogramming to improve MU maintenance and resilience in both chronological aging and genetic disease. We first studied the MUs of mice which were aged via epigenetic damage (Inducible Changes to the Epigenome ["ICE"] mice). At 18 months (18m), ICE mice did not show any differences to chronologically-aged 24m wildtype (WT) mice in multiple measures of MU physiology, including compound muscle action potential, motor unit number estimation, and single motor unit potential. However, 18m ICE mice showed significant decline in MU physiology compared to age-matched controls. Next, we applied OSK reprogramming to determine the impact on the terminal feature of the MU, the NMJ. Neuronal OSK expression in 24m mice restores several metrics of age-related NMJ decline, including both presynaptic (nerve terminal area, axon diameter, length of presynaptic branches) and postsynaptic measures (acetylcholine receptor area, endplate area). We next applied OSK reprogramming to a disease of the MU, ALS ($SOD1^{A5V}$), using human iPSC-derived motor neurons. $SOD1^{A5V}$ neurons are known to demonstrate hyperactivity in vitro. We transduced $SOD1^{A5V}$ and WT iPSCs with a doxycycline-inducible OSK cassette. After OSK induction, $SOD1^{A5V}$ neurons show increased neuron skeleton length and branching. Using multielectrode array, we replicated the physiological hyperactivity in $SOD1^{A5V}$ neurons and demonstrate that this hyperactivity resolves to WT levels after induction of OSK without any differences in survival. Co-incubation of neurons with vincristine mimics ALS-related axonopathy. When treated with vincristine, the skeleton length of $SOD1^{A5V}$ neurons decreases, and this length is restored upon induction of OSK. Taken together, these results indicate that MU decline is a feature of epigenetic aging and that applying epigenetic reprogramming is able to improve MU morphology and function in aged and diseased motor unit cells.

REGRESSION OF AGE-ASSOCIATED AND HIGH FAT INDUCED HEPATIC FIBROSIS BY VITAMIN D SUPPLEMENTATION

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Metabolic dysfunction-associated fatty liver disease (MAFLD) is the most common cause of chronic liver diseases worldwide and can further progress into metabolic dysfunction-associated steatohepatitis (MASH) and cirrhosis. Hepatic fibrosis is a critical step in the progression of the disease and is often difficult to reverse. For the first time, we demonstrate that vitamin D3 supplementation both in 22-month-old C57BL6 mice and high-fat-fed mice effectively reverses not only hepatic lipid accumulation but also hepatic fibrosis by enhancing mitochondrial functions.
We observed that consumption of 60% high-fat diet (HFD) for 4 months promoted metabolic impairments such as oral glucose tolerence test (OGTT) and an increased ALT, cholesterol, HDL, and LDL levels. However, when HFD is consumed with vitamin D3 (20,000 IU/kg), these effects are alleviated. Moreover, the increased hepatic steatosis, inflammation, and fibrosis caused by HFD were prevented by vitamin D3 accompanied with the elevation of mitochondrial functions. Also, metabolic abnormalities and hepatic fibrosis due to aging were alleviated by supplementaion of vitamin D3. Interestingly, the degrees of reduction in inflammation and fibrosis in vitamin D3.-supplemented mice were similar to that of mice performed endured exercise 3 times per week for 4 months. Our results suggest that activation of vitamin D metabolism could be a potential therapeutic strategy for preventing metabolic dysfunction-associated steatohepatitis due to obese or aging.

SIRT6 AS A CENTRAL PLAYER IN AGING AND GLAUCOMATOUS NEURODEGENERATION IN THE RETINA

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Glaucoma is a leading age-related neurodegenerative disease characterized by the progressive loss of retinal ganglion cells (RGCs) and optic nerve degeneration. Although elevated intraocular pressure (IOP) is a major risk factor, many patients, particularly those with normal tension glaucoma (NTG), continue to lose vision despite normal IOP. Here, we identify Sirtuin 6 (Sirt6), a nuclear NAD^+-dependent deacetylase with known anti-aging properties, as a key regulator of RGC survival. Sirt6 is highly expressed in RGCs, and its deletion, either globally or specifically in RGCs, causes age-related RGC loss and optic nerve degeneration without IOP elevation, which mimics NTG pathology. Mechanistically, Sirt6 deficiency accelerates cellular senescence through Caveolin-1 upregulation and induces mitochondrial dysfunction. In models of high-tension glaucoma, Sirt6 levels were reduced following IOP elevation, while genetic overexpression of Sirt6, both globally and in RGCs, protected against IOP-induced neurodegeneration. Furthermore, pharmacological activation of Sirt6 or AAV2-mediated gene therapy delivering Sirt6 preserved RGC structure and function in both acute and chronic glaucoma models. These findings position Sirt6 as a critical molecular link connecting aging, senescence, and glaucomatous neurodegeneration, and highlight its potential as both a therapeutic target and a biomarker of neuronal resilience. Targeting Sirt6 may offer a novel strategy to prevent vision loss in glaucoma that does not rely on lowering IOP.

MITOCHONDRIA-LYSOSOME COUPLING CONTRIBUTES TO LYSOSOME ACIDIFICATION AND AGING

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Nearly all cellular processes are pH dependent. The acidic pH inside the lysosome (vacuole in yeast) is essential for cellular content degradation, signaling, and autophagy. Defect in lysosome/vacuole acidification is a conserved hallmark of aging and age-related diseases. Traditionally, lysosome/vacuole is thought to import free protons (H^+) from the surrounding neutral cytosol. In this study, we uncovered a previously unrecognized, conserved lysosome/vacuole acidification mechanism, involving lysosomal/vacuolar uptake of H^+ pumped out by mitochondrial electron transport chain through membrane contacts between mitochondria and lysosomes/vacuoles. Aging/senescence-associated disruption of mitochondria-lysosome/vacuole contacts causes lysosomal/vacuolar de-acidification, which can be reversed by expressing a linker to connect these organelles and through an asymmetry-dependent rejuvenation process in daughter cells. Preserving lysosomal acidification in senescent human cells prevents the induction of major senescence-associated secretory phenotype factors and enhances autophagic flux. These findings reshape our current understanding of the mechanisms underlying lysosomal/vacuolar (de-)acidification in both young and aged/senescent cells.

DETRIMENTAL INTERPLAY BETWEEN HIV-1 AND AMYLOID FIBRILS ASSOCIATED WITH NEURODEGENERATIVE DISEASES

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HIV-1 accelerates aging-associated diseases, and infected individuals may develop HIV-associated neurocognitive disorders (HAND) even under effective antiretroviral therapy (ART). In this study, we analyzed the interplay between HIV-1 and brain amyloids, which are known to be associated with neurological disorders such as Parkinson's and Alzheimer's disease. We found that α-synuclein and (to a lesser extent) Aβ fibrils, significantly enhance HIV-1 entry and replication in human macrophages and microglia, the major viral target cells in the brain (Olari, Liu et al., *Nat. Commun.* 2025). Mechanistically, these amyloids facilitate viral attachment and fusion, likely by bridging viral and cellular membranes despite their overall negative charge. Furthermore, we demonstrate that amyloidogenic fragments of the HIV-1 Envelope protein can cross-seed and accelerate the formation of α-synuclein and Aβ amyloids. Extracts from human brains enhanced HIV-1 infection, and notably, the magnitude of the effect correlated with the levels of binding Thioflavin T, a dye commonly used to stain amyloids. Our results suggest that HIV-1 infection may exacerbate age-related microglial senescence through amyloid-driven enhancement of viral replication. Currently, we are investigating potential synergies between HIV-1 and brain amyloids in inducing senescence pathways and inflammatory responses. Our preliminary data show that HIV-1 replicates in cerebral organoids containing innately developed microglia and triggers an immune response that may drive accelerated aging. Moreover, exogenously introduced α-synuclein and Aβ amyloids seem to enhance HIV-1 infection in this organoid model. Understanding the interplay between HIV-1 and brain amyloids may open new avenues for anti-amyloid or senolytic therapies as adjuvants to ART for treating HIV-associated neurodegenerative disorders.

LIFESPAN EXTENSION BY CHEMICAL ENHANCEMENT OF DNA REPAIR

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DNA damage is a central driver of the aging process. We previously found that KIF2C, known to play a role in DNA repair, is repressed in aged cells. Here, we investigated if increased KIF2C activity counteracts DNA damage and its effects on aging phenotypes. We show that a small-molecule agonist of KIF2C enhances DNA repair in two distinct genetic disorders exhibiting DNA damage and accelerated aging, the Hutchinson-Gilford progeria (HGPS) and Down (DS) syndromes. Mechanistically, the KIF2C agonist improves the repair of DNA double-strand breaks by inducing nuclear envelope invaginations poked by cytoplasmic microtubules, which translated into amended epigenetic and transcriptional signatures of HGPS and DS. Moreover, subcutaneous administration of the KIF2C agonist in progeria mice mitigated aging phenotypes, extending their healthspan. Our study discloses a unique geroprotective pharmacological approach targeting DNA damage.

CHARACTERIZING SENESCENT CELLS AND THEIR TISSUE MICROENVIRONMENTS WITH SPATIAL TRANSCRIPTOMICS

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Background and objectives: Senescent cells play diverse roles in health and aging; however, their heterogeneity across these contexts remains poorly understood. While their beneficial roles include the facilitation of wound healing, and blood-tissue barrier maintenance, they have also been shown to actively drive diseases such as atherosclerosis, Alzheimer's disease, and scleroderma. Senescent cells are believed to exert both their beneficial and detrimental effects through secreted factors that alter their local microenvironment. Although it is known that the secretory profiles of senescent cells can vary, this variation has primarily been studied in cell culture, rather than in a natural tissue context. Thus, despite strong interest in targeting senescent cells as a disease intervention, safely developing such strategies requires a deeper understanding of their heterogeneity. Newly developed spatially resolved transcriptomics (ST) technologies present an unparalleled opportunity to deeply characterize senescent cells in situ without compromising their microenvironment. Methods and results: Here we present preliminary results using 1) a sequencing-based ST technology with whole transcriptome detection, (Visium HD) and 2) an imaging-based ST platform with high detection efficiency (Xenium) to identify senescent cells in lymph nodes from adults aged 55+. Both platforms yielded high-quality data, enabling us to identify cells expressing senescent-associated genes and perform in-depth analysis of these cells and their associated neighborhoods/niches. Discussion and impact: With this approach, we can distinguish senescent cells in various tissues, differentiating between normal aging and disease contexts, and gain insights into their impact on neighboring cells. Understanding such features of cellular senescence across different indications is critical to developing therapeutics that specifically target detrimental senescent cells while preserving the benign/beneficial ones.

ALTERED DEVELOPMENTAL SENESCENCE AS THE BASIS OF CHARGE SYNDROME INNER EAR DEFECTS

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CHARGE syndrome is a rare disease that presents different symptoms among which are coloboma, heart defects, choanal atresia, delay in growth and development, genitourinary disorders and abnormalities in the ear that include malformations of the semicircular canals. Most people with CHARGE syndrome have mutations in the CHD7 gene that encodes a DNA helicase. However, the cell mechanisms that link CHD7 with the pathophysiology of CHARGE syndrome are not known.
Cellular senescence is a state of permanent cell cycle arrest in which cells remain metabolically active secreting a wide variety of factors such as proinflammatory cytokines that can induce local inflammation. Although traditionally linked to cancer and aging, we now know that senescent cells are present in a tissue-specific manner during vertebrate development. This recent discovery has provided a new framework to understand how morphogenesis and remodeling take place during embryogenesis, also making senescence a key program to consider in the pathophysiology of developmental diseases. Considering that: 1) CHARGE defects are mostly associated to regions where developmental senescence takes place; 2) Many CHARGE defects are related to lack or excess of cell elimination; 3) CHD7 loss of function modifies the expression of the main senescence mediators p21, p16, p27 and p53, we propose that CHARGE defects due to CHD7 deficits are due to misregulation of developmentally-programmed senescence. Our study focusses on the development of the inner ear in vertebrate models as the most reliable criteria associated with CHD7 loss for diagnosing CHARGE syndrome. Our results suggest a possible mechanistic link between cell senescence and the known role of CHD7 in cancer progression.

ARTIFICIAL INTELLIGENCE DRIVEN CO-PROFILING OF TRANSCRIPTIONAL AND EPIGENETIC LANDSCAPES IN HUMAN HEMATOPOIETIC STEM CELLS ACROSS THE LIFESPAN

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Aging in hematopoietic stem cells (HSCs) is characterized by functional decline, clonal dominance, and an increased risk of hematologic disorders. While murine studies have shown that HSC rejuvenation (e.g., intermittent fasting, senolytics) improves blood regeneration and longevity, the molecular drivers of human HSC aging remain poorly defined, limiting translational progress. To address this gap, we present the first single-cell multiome atlas of human HSC/progenitor cells (HSPCs) across the lifespan (newborn to 80 years), integrating transcriptional and chromatin accessibility landscapes to dissect age-related mechanisms.

To construct this 'Human HSC Atlas' across transcriptional and epigenetic levels, we performed single-cell multiome sequencing (scMultiome-seq, simultaneously examining gene expression and chromatin accessibility in the same cell) on FACS-sorted CD34+ HSPCs from human umbilical cord blood and bone marrow of healthy donors across different age groups (newborn–18, 20–50, and 60–80 years). Unbiased clustering analysis identified 22 distinct cell populations, including an HSC/MPP subset marked by canonical stemness genes (e.g., MLLT3, HLF). Differential gene expression analysis in HSCs/MPPs across ages, combined with Gene Ontology and KEGG pathway analysis, revealed age-dependent biological processes and signaling pathways. Aged HSCs/MPPs exhibited increased metabolism, DNA damage repair, and heightened cell cycling. HSC transcriptional signatures, characterized from independent scRNA-seq datasets, were progressively downregulated with age, reflecting compromised self-renewal. In parallel, we analyzed dynamic changes in chromatin accessibility within HSCs/MPPs during aging, including epigenetic HSC signatures and lineage-specific motif activities.

To translate these findings, we are leveraging scGPT, a generative AI framework, to develop a reinforcement learning model and map the molecular states of HSCs to their 'biological clock'. The model will also integrate multiomic aging signatures with targeted interventions, including signaling pathways (e.g., mTOR), cellular processes (e.g., autophagy, NAD+ metabolism), and genetic factors (e.g., Yamanaka factors). Our goal is to use this well-trained model to predict combinatorial intervention strategies with the potential to rejuvenate aged human HSCs.

This work provides a unique database that deciphers the comprehensive transcriptional and epigenetic landscapes of human HSCs/MPPs from birth to aging, establishing a quantitative foundation for targeting HSC aging and proposing AI-driven rejuvenation strategies with therapeutic potential.

ATM IN THE SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE: INHIBITION ≠ KNOCKDOWN

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Ataxia telangiectasia mutated (ATM) kinase, a master regulator of the DNA damage response, plays a critical role in cellular senescence by orchestrating cell cycle arrest and influencing the composition of the senescence-associated secretory phenotype (SASP). While ATM activation is known to promote senescence following genotoxic stress, its dynamic regulation of the SASP remains incompletely characterized. Our study reveals a key distinction between pharmacological inhibition and genetic knockdown of ATM in modulating the SASP profile. Using primary human fibroblasts and U2OS osteosarcoma cells induced to senescence via doxorubicin treatment, we demonstrate that ATM inhibition with AZD-1390 produces a markedly different secretome compared to shRNA-mediated ATM knockdown. Specifically, shRNA knockdown of ATM reduced canonical pro-inflammatory SASP factors (IL-6, IL-8) five days after senescence induction, whereas ATM catalytic inhibition enhanced these pro-inflammatory secretions. U2OS cells expressing catalytically dead ATM similarly secreted higher levels of IL-6 and IL-8 compared to wild-type ATM-expressing U2OS. Furthermore, AZD-1390 increased SASP secretions in p53-null U2OS cells, indicating that AZD-1390's effect on the SASP operates independently of p53. Mouse models in previous literature have shown that catalytic inhibition of other ATM-related PIKK enzymes such as ATR and DNA-PKcs results in more genotoxic stress in cells than the removal of these same enzymes. Our findings contribute to this growing body of literature concerning intervention methodology when targeting PIKKs and provide novel insights into the complex regulatory networks ATM governs in the SASP.

AGE- AND SEX-DEPENDENT DIFFERENCES IN NEUROIMMUNE AND METABOLIC RECOVERY FOLLOWING SEPSIS

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Sepsis is a leading cause of long-term cognitive and behavioral impairments, with older individuals at a higher risk of developing dementia. However, the molecular mechanisms driving these age-related differences remain unclear, limiting the development of targeted treatments. Additionally, studies have largely focused on the acute effects of sepsis, and most do not account for sex differences. Here, we investigate how aging and sex influence the response to a septic insult using a clinical isolate of E. coli, a common cause of sepsis. We performed bulk RNA sequencing of brain tissue from young and aged mice at two key time points: the acute phase (2 days post-infection) and the recovery phase (21 days post-infection). At baseline, compared to young mice, aged mice exhibited heightened neuroinflammation, characterized by complement system activation and microglial priming, alongside reduced neuronal markers, suggesting pre-existing vulnerability. At two days post-infection, both age groups showed innate immune activation; however, older mice exhibited a response dominated by complement-driven innate immunity, with minimal adaptive immune involvement. Aged mice also displayed signs of blood-brain barrier dysfunction and greater metabolic suppression, particularly in lipid metabolism and mitochondrial pathways. By 21 days post-infection, young mice exhibited partial recovery, with sustained upregulation of pathways related to neuronal plasticity and metabolic regulation, including insulin signaling, though synaptic transmission pathways remained impaired. In contrast, aged mice showed limited gene expression changes, suggesting stalled recovery. The absence of further neuronal pathway downregulation likely reflects pre-existing decline rather than new sepsis-induced damage. Whether the partial recovery in young mice indicates ongoing repair or permanent neuronal loss remains unclear. Our findings highlight complement activation as a potential driver of age-related impairments in immune regulation and neuronal resilience. Interestingly, females exhibited a stronger adaptive immune and metabolic response than males in both age groups, which warrants further investigation . These results emphasize the need to account for age and sex differences in developing targeted therapies for sepsis-induced cognitive and behavioral decline. Ongoing single-cell RNAseq and histological analyses will further clarify the cellular mechanisms driving long-term deficits and identify potential targets for intervention.

EXPLORING GLIAL SENESCENCE IN CHRONIC CUPRIZONE-INDUCED DEMYELINATION: IMPLICATIONS FOR CELLULAR AGING IN MULTIPLE SCLEROSIS

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Aging is the most common risk factor for Multiple Sclerosis (MS) disease progression. Cellular senescence (CS), the irreversible state of cell cycle arrest, is a main driver of aging and has been found to accumulate prematurely in neurodegenerative diseases, including Alzheimer's and Parkinson's disease. Recently, several studies have shown that MS patients also exhibit higher levels of CS in glial cells of the brain. How demyelination induces CS, and the impact of CS on myelin repair, or remyelination, remains unclear. To explore this, we use the chronic cuprizone toxicity model of demyelination, which recapitulates the remyelination failure and reactive gliosis observed in progressive MS patients. Focusing on the subcortical white matter, we first confirmed that the chronic cuprizone diet significantly decreased mature oligodendrocytes and increased the number of reactive astrocytes and microglia. We also observed a significant increase in senescent cells, as indicated by SA-β-gal and p16INK4A expression. Further inspection of these senescent cells led to the discovery that approximately 50% of the senescent cells were microglia. Senescent astrocytes and oligodendrocyte lineage cells did not constitute a significant proportion of the senescent cell population in chronically demyelinated white matter. Interestingly, removal from the cuprizone diet for four weeks failed to restore mature oligodendrocyte numbers and significantly reduce p16INK4A expressing senescent cells in the white matter. These lingering senescent cells appeared to be primarily microglia. Together, these results suggest that chronic demyelination induces CS within microglia, which are not efficiently cleared, even in young adult mice. Whether removal of senescent cells enhances remyelination will be examined in our future studies.

DISSECTING THE COMPLEXITY OF NUTRIENT IMPACT ON CELLULAR AGING: THE IMPACT OF AMINO ACIDS ON YEAST REPLICATIVE LIFESPAN

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Cellular metabolism is central to aging; amino acids regulate a range of metabolic pathways associated with aging, and amino acid interventions have been shown to influence lifespan (Dato, S. *et al.*, *Biogerontology*, 2019). We are interested in understanding the underlying mechanistic details by which amino acids impact the cellular aging process and identifying ways to extend lifespan. We have chosen the model system, *Saccharomyces cerevisiae*, to address these problems, because its core metabolic functions and pathways are conserved within the eukaryotic kingdom. As a prototrophic organism, it is also capable of growing with or without amino acids, enabling us to systematically study the effect of each individual amino acid on lifespan in a way that is not possible in other organisms.

We have developed a microfluidic system, the Yeast Lifespan Machine (YLM), that measures yeast replicative lifespan in a high-throughput manner, by capturing individual cells within microfluidic traps, imaging them throughout their lifespans, then calculating the number of cell divisions and total survival time for each cell using machine learning (Thayer, N. *et al., bioRxiv*, 2022). Using the YLM, we have directly measured the lifespans of thousands of cells in each media condition, enabling us to screen the effects of all 20 amino acids on lifespan in different genetic backgrounds.

Compared with minimal media (containing no amino acids), we identified amino acids that decreased median lifespan by 50%, and increased median lifespan up to 70% in a wild type strain. We then applied a series of mutations that were previously known to impact yeast lifespan (Defossez P. *et al., Mol. Cell.*, 1999). From this screen, we discovered:

1. Activation of a key nutrient sensing pathway, the SPS amino acid sensing system (Forsberg, H. *et al., Mol. Microbiol.*, 2001), can have both a positive and negative effect on lifespan.
2. We identified those amino acids that shorten lifespan by increasing the genomic instability pathway of Extrachromosomal rDNA Circles (ERCs) accumulation.
3. By combining mutations in the SPS pathway and ERC accumulation pathway, we identified amino acids that impact ERC accumulation through amino acid sensing.
4. Lastly, we identified amino acids which impact lifespan through pathways other than nutrient sensing or ERC accumulation.

This approach has allowed us to dissect some of the complexity of interactions between Genetics and Environment that need to be ascertained in order to develop a clearer understanding of how nutrients such as amino acids have such a dramatic effect on cellular lifespan.

p53 IS AN AGE-DEPENDENT REGULATOR OF THE SENESCENCE PHENOTYPE

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p53 is a potent tumor suppressor known to control multiple phenotypes of cellular senescence in cell culture models, but its role in physiological aging is unclear. Here we show that p53 activity restrains senescence-associated inflammation in vivo. MERSCOPE spatial profiling of aged mouse liver showed altered p53 activity, as marked by expression of its target genes, in a subset of senescent-like cells. In vivo AAV-mediated CRISPR knockout of p53 in aged, but not young, hepatocytes increased the age-associated expression of senescence-associated inflammatory genes. Conversely, pharmacological activation of p53 with small molecule MDM2 inhibitors suppressed age-associated inflammation and reversed age-associated repression of genes associated with lipid metabolism. These data suggest that p53 is a master regulator of senescent cell phenotype, or “senotype”, and that this function is targetable, potentially for the benefit of healthy aging.

GLOBAL EPIGENOMIC SCREENINGS RELATED TO PRO-AND ANTI-VASCULAR AGING USING A NONLINEAR DOWN SYNDROME TRISOMY MODEL.

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Down syndrome (DS) is the most frequent chromosomal disorder in human genetics caused by dysregulation of the transcription factor, nuclear factor for activated T cells (NFAT). Currently, epidemiological studies have shown that despite the high risk of neurological diseases-including early Alzheimer's disease, muscle weakness, leukemia, and male infertility, the incidence of solid cancers and progressive atherosclerosis is remarkably low in individuals with DS. In other words, DS is associated with premature aging but exhibits a nonlinear phenotype of vascular metabolism that is intensely anti-aging. Such a nonlinear phenotype sharply contrasts with Werner and Hutchinson-Gilford syndrome, known as early aging models.

Our genome-wide expression analysis revealed that *DSCR-1*, a factor linked to DS located on human chromosome 21 (and murine chromosome 16), is strongly induced in endothelial cells (ECs) in an NFAT-dependent manner during angiogenesis and chronic inflammation. The stable expression of DSCR-1 can appropriately modulate NFAT activation from VEGF signaling, contributing to the attenuation of pathological vascular neogenesis and inflammation. This mechanism is believed to be a leading cause of the anti-cancer activity observed in DS patients.

In our research, we conducted a comparative analysis of the epigenomics and proteomics from aged DS patient-derived trisomy-induced pluripotent stem (iPS) cells and differentiated these cells into ECs. We also examined DS model mice and their controls. High-throughput chromatin conformation capture (Hi-C) analysis revealed some unique topologically associated domains (TAD) in DS-derived iPS cells. Additionally, DS-iPS-derived ECs showed significant increases in anti-inflammatory gene sets, including *DSCR-1*. Moreover, using an aged mice model, we found that lung ECs downregulated closed chromatin territory through ATAC-seq, accompanied by the induction of immune-related gene sets via RNA-seq as the mice aged. In contrast to the lung microenvironment, oxidative stress-related proteins were significantly elevated in ECs from the brain. These global multi-omics screenings are expected to lead to novel therapeutic and preventive approaches not only for DS-related nonlinear early aging but also for lifestyle-related severe vascular diseases.

GEMclear: ELEVATING ACCURACY OF SPATIAL PROTEOMICS BY REMOVING BACKGROUND WITH GEL ELECTROPHORESIS

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Spatial proteomics transforms research on disease mechanisms and drug target exploration by detecting which and where proteins interact. Proximity Labeling Mass Spectrometry (PL-MS) is one of the most widely used spatial proteomics approaches that identifies proteins near a target protein or subcellular structure. By fusing a labeling enzyme (e.g., HRP, TurboID) to the target, nearby proteins are biotinylated, enriched with streptavidin beads, and characterized by mass spectrometry. This reveals local interactomes, providing high-resolution insights into cellular organization and function.

Despite its strengths, PL-MS can generate numerous false positives in MS readouts, lowering its accuracy of spatial proteome detection. A primary cause of these false positives is the nonspecific pulldown of non-biotinylated background proteins by streptavidin beads. Because background proteins are often orders of magnitude more abundant than the target proteins, the specific signals can easily be obscured by noise during MS analysis. Currently, prior knowledge and intensive control experiments are used to reject false positives. Here, we aim to physically remove the background proteins for high-fidelity PL-MS.

This method is named Gel Electrophoresis Mass-spectrometry background clearing (GEMclear). It integrates proximity labeling, hydrogel embedding, and electrophoresis to remove non-biotinylated proteins before MS detection. Our imaging results demonstrated that GEMclear effectively depleted non-biotinylated proteins while preserving the biotinylated proteins. To finally validate the accuracy of MS measurement, we performed GEMclear to proximity labeled mitochondria and nuclear lamina, which have well-characterized proteome. Successfully, the GEMclear MS method accurately generated subcellular organelle proteome libraries, capturing known marker proteins while effectively removing background signals from non-specific contaminants.

Furthermore, we showcased the versatility of GEMclear with different PL enzymes in live cells and fixed tissues. GEMclear worked with HRP in fixed cells and TurboID in live cells. It also accurately profiled astrocytes in fixed mouse brain tissues. Given its high-accuracy spatial proteome profiling and its versatility, GEMclear holds promise for studying organellar proteomics and protein-protein interactions. It can also be a powerful tool for in vivo single-cell type profiling, such as cell types associated with Alzheimer's disease.

THYROID AND KLOTHO: A NOVEL REGULATORY AXIS IN KIDNEY AGING-RELATED PATHOLOGY

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Klotho derived from the kidney has anti-aging properties where it maintains renal homeostasis and protects against aging-related kidney dysfunction. Klotho diminishes with age, which heightens the risk of chronic kidney disease (CKD) and other renal pathologies. The Wnt/β-Catenin signaling is a key regulatory target of Klotho, where renal overactivation promotes fibrosis and progression to CKD. Aging is also associated with a marked decline in triiodothyronine (T3), a thyroid hormone essential for various physiological functions. Subclinical hypothyroidism is a common feature of people after the age of 60. Given the concurrent decline in Klotho and T3, we investigated whether these hormones interact to influence kidney health in the context of aging BALB/c mice.

We found that treatment with T3 significantly enhanced renal Klotho expression by activating the ATF-3/p-c-Jun transcription factor pathway. This impact of T3 was particularly strong in aged mice, which otherwise have low baseline Klotho. The T3-induced Klotho subsequently inhibits the Wnt/β-Catenin pathway by binding to Wnt ligands, thereby preventing aberrant GSK-3β expression. This ability of T3 to indirectly inhibit Wnt/β-Catenin activity highlights the therapeutic potential of Klotho to target aging-associated kidney diseases.

TARGETING AUTOPHAGY AND SENESCENCE IN COLORECTAL CANCER DRUG-TOLERANT PERSISTERS

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Colorectal cancer (CRC) is the second most fatal cancer worldwide. Emerging evidence has demonstrated that cancer cells can enter a reversible drug-tolerant persister (DTP) state to escape death from chemotherapy, and it cannot be explained on the basis of genetic mutations. After chemotherapy ceases, DTPs regrow and remain sensitive to chemotherapy. Thus, targeting DTPs before the development of irreversible drug resistance elicited by long-term chemotherapy represents a potential therapeutic opportunity. We previously reported that key macroautophagy genes and autophagic flux were upregulated in irinotecan (CPT-11)-induced CRC DTPs. More recently, we obtained preliminary data showing phenotypes of senescence in CRC DTPs, including increased senescence-associated-β-galactosidase (SA-β-gal) activities, and upregulated expression of the lysosome marker LAMP1 and anti-apoptotic Bcl-2 proteins. Here, we hypothesize that the survival of CRC DTPs is dependent on both autophagy and senescence. Using patient-derived CRC cell lines, the dynamics of autophagy and senescence are examined before, during and after the DTP state induced by camptothecin (CPT), a DNA topoisomerase I inhibitor. Co-staining of two patient-derived CRC cell lines using LC3B puncta (autophagy) and SA-β-gal (senescence) demonstrated that both processes are enriched in the DTP state as compared to control treated cells. Whether autophagy and senescence are being activated in two different subpopulations or simultaneously in all DTP cells requires further investigation and additional colorectal cancer cell lines. Our preliminary data also demonstrates that simultaneous inhibition of autophagy and senescence by an ULK1/2 inhibitor (SBI-0206965) and senolytic (BH3 mimetic ABT-263), respectively, prevents DTP regrowth *in vitro*. To determine the effects on DTPs, and elucidate how these pathways are interacting to maintain DTP growth requires further mechanistic investigations. Taken together, our study will unravel the implications of autophagy and senescence in CRC DTPs. It may also reveal a novel therapeutic strategy in targeting DTPs.

CANCER TREATMENT RESISTANCE TO PLATINUM-BASED CHEMOTHERAPY IS DRIVEN BY A TARGETABLE TGF-β SENESCENT SECRETOME

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Platinum-based chemotherapy is commonly used for non-small cell lung cancer (NSCLC) and high grade serous ovarian cancer (HGSOC) treatments, yet clinical outcomes remain poor. Cellular senescence and its associated secretory phenotype (SASP) can have multiple tumour-promoting activities, although are largely unexplored in these cancers. Here we show that cisplatin-derived SASP enhances the malignant phenotype of lung cancer cells. Using xenograft, orthotopic and KrasG12V-driven murine NSCLC models, we demonstrate that cisplatin-induced senescent cells strongly promote tumour progression. Of note, infliction of platinum-induced chemotherapeutic damage in lungs of naturally-aged mice exacerbates tumour development when compared to young mice. Mechanistically, we find that a TGF-β ligands-enriched SASP drives stimulatory proliferative effects in nearby lung cancer cells through TGFβR1 and Akt/mTOR pathway activation, and propose it as a novel cancer therapy resistance mechanism. Importantly, TGFβR1 inhibition with galunisertib or senolytic treatment significantly reduces tumour progression driven by cisplatin-induced senescence. We demonstrate, using distinct murine NSCLC models, that concomitant use of TGFBR1 inhibitors with platinum-based chemotherapy significantly reduces tumour burden and improves survival. Finally, we validate the translational relevance of tumour-promoting TGF-β-enriched SASP and its correlation with residual disease and relapse by using clinical NSCLC and HGSOC samples from patients who received neoadjuvant platinum-based chemotherapy. Altogether, our results provide pre-clinical proof-of-concept for future trial designs in cancers of unmet need.

INTRANASAL INSULIN MITIGATES SURGERY-INDUCED MEMORY IMPAIRMENT IN A MOUSE MODEL OF HIPPOCAMPAL AGING

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Perioperative neurocognitive disorder (PND), i.e., long-lasting postoperative cognitive decline is a significant concern in elderly patients. Effective treatments remain limited. In the hippocampus, a region critical for learning and memory, aging is associated with a decline in somatostatin-positive (Sst+) GABAergic interneurons in the dentate hilar region, contributing to memory impairment. We previously demonstrated that selective ablation of Sst+ interneurons induce cognitive dysfunction, generating a pseudo-aged mouse model of hippocampal aging. Additionally, brain insulin and insulin receptors decline with age. Intranasal insulin (INS) has shown promise in improving cognitive function in Alzheimer's disease, but its role in surgery-induced memory impairment remains unclear. In this study, we examined the effects of INS on surgery-induced memory deficits in pseudo-aged mice with genetically ablated dentate hilar Sst+ interneurons. Pseudo-aged mice were generated via bilateral injection of AAV5-EF1α-mCherry-flex-dtA into the dentate hilus of Sst-IRES-Cre mice (3-4 months old), while young control mice (also 3–4 months old) received AAV5-EF1α-mCherry virus. Following a three-week recovery period, mice underwent exploratory laparotomy under isoflurane anesthesia. INS was administered daily for nine days -six days pre-surgery and three days post-surgery. Locomotor function was assessed using the open field test, while cognitive performance was evaluated using the Y-maze test, the trace fear conditioning test, and the Morris water maze (MWM). Surgery impaired working memory in the Y-maze and caused deficits in non-declarative associative memory in trace fear conditioning and MWM tests in pseudo-aged mice, whereas no impairments were observed in control mice injected with the control vector (AAV5-EF1α-mCherry). Notably, INS treatment significantly improved memory performance in pseudo-aged mice across all behavioral tests. These findings suggest that INS may hold therapeutic potential for alleviating surgery-induced cognitive decline in aged individuals. Further research is warranted to elucidate the underlying mechanisms and to explore its translational potential in clinical settings.

LINEAGE-SPECIFIC RESPONSES OF SKIN STEM CELLS TO ENDOGENOUS RETROVIRUS REACTIVATION DURING MURINE SKIN REGENERATION

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Transposable elements (TEs), often regarded as molecular fossils or genomic parasites, are interspersed genomic repeats constituting ~40% of the human genome. Primarily residing in the heterochromatin, TEs are dynamically expressed and essential for early development but are mostly silenced in adult tissues via histone and DNA methylation. Aberrant TE expression is observed in neurodegeneration, cancer, and aging, whose function remains poorly understood.

Previously, we reported closely coupled expression of histone methyltransferase SETDB1 in activated hair follicle stem cells (HFSCs) during hair follicle regeneration in the murine skin. Conditional ablation of Setdb1 leads to reactivation of endogenous retroviruses (ERVs, a class of TEs), which generate retroviral peptides and viral-like particles, causing hair loss and stem cell exhaustion. Furthermore, we showed phenotype rescue upon antiviral treatment, indicating a causal role of ERVs.

Here, we seek mechanistic insights into how ERV reactivation impacts different skin stem cells. Using single-cell multi-omics analysis, we showed cell-type-specific responses to ERV reactivation, revealing two spatially distinct pathways. On the one hand, epidermal and upper hair follicle cells strongly induce a host-antiviral reaction in the mutant, leading to their exhaustion. Conversely, transient amplifying cells (TAC) do not mount antiviral response despite diminished proliferation. Instead, they are lost due to replication stress. We propose that this distinct response to ERV reactivation is due to the inherent nature of these stem cells and their signaling niches- epidermal and hair follicle stem cells are essential for maintaining barrier function, enabling them to mount an effective immune response. In contrast, TACs signaling hubs for regeneration prioritize tissue renewal over antiviral defense.

Interestingly, we also observed that antiviral genes exhibit distinct regulatory patterns, with some showing coaccessibility with nearby ERVs upon H3K9me3 loss. In contrast, others do not exhibit co-regulated ERVs or H3K9me3, indicating that part of the inflammatory responses is decoupled from ERV reactivation, alluding to indirect mechanisms such as DNA damage-induced inflammation.

In summary, we provide novel mechanistic insights into stem cell activity and TE suppression during adult tissue regeneration.

THE cGAS/STING PATHWAY IMPACTS THE TUMOR-SUPPRESSIVE FUNCTION OF p53

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Tumor suppressor wild-type p53 can orchestrate immune responses through the degradation of TREX1, a DNA exonuclease, leading to cytosolic DNA accumulation. cyclic GMP–AMP synthase (cGAS) can sense cytosolic DNA, initiating the STING-mediated secretion of type I interferons, thereby activating the immune system to execute p53's tumor suppressive function. We found p53's tumor suppressive function can be compromised by disruptions in the cGAS/STING signaling axis. Limited research has explored the correlation between p53 and the cGAS/STING pathway, leaving the mechanism by how cGAS/STING influences p53 function elusive. Herein, we aim to elucidate how cGAS and STING affect p53's tumor-suppressive function.

Firstly, we aim to investigate whether cGAS or STING directly impact p53's regulatory mechanisms, thereby affecting its function. Our findings reveal that loss of STING alters its stability resulting in reduced p53 protein levels. Elevated STING levels can inhibit MDM2-mediated degradation of p53 through decreasing MDM2 expression. Additionally, the cGAS/STING downstream effector kinase, TBK1 can also reduce MDM2 expression. This reduction in MDM2, caused by higher TBK1 levels can be prevented by a phosphorylation site mutation in MDM2. As TBK1 can function as a kinase, this suggests that TBK1 may phosphorylate MDM2, thereby regulating MDM2's activity and expression, which in turn modulates p53's expression and function.

Secondly, we seek to identify which specific cellular processes regulated by cGAS or STING are responsible for mediating p53's tumor suppression. P53 exerts multiple functions to impede tumor growth, particularly through senescence, apoptosis, and autophagy. Using a lung carcinoma H1299 cell line with inducible p53 expression, we determined that cGAS/STING loss could abolish p53-mediated senescence and cell cycle arrest. Understanding the reciprocal regulation between p53 and cGAS/STING sheds light on potential combination therapies utilizing p53 activators with cGAS or STING agonists. Such strategies hold promises in enhancing the immunogenicity of tumor cells and facilitating their subsequent elimination.

SINGLE-CELL MULTI-OMICS ANALYSIS OF AGE-ASSOCIATED EPIGENOMIC CHANGES BY INTEGRATING TRANSCRIPTOMIC AND CHROMATIN PROFILING

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Epigenomic state is one of the key indicators for tracking age-related changes. However, it is essential to detect changes in cell types and states before analyzing the epigenomic landscape to study aging at the organismal level. Therefore, we have developed a novel single-cell multi-omics approach that integrates transcriptomic profiling to classify cell types and states, followed by epigenomic analysis.
Here, we present single-cell combinatorial indexing multi-target chromatin integration labeling followed by sequencing (sci-mtChIL-seq). This allows simultaneous single-cell analysis of RNA polymerase II binding to chromatin and epigenomic factors such as transcription factors and histones. As a proof of concept, we applied sci-mtChIL-seq to analyze the binding dynamics of the skeletal muscle-specific transcription factor MyoD during mouse embryonic myogenesis. This model system validated our approach by demonstrating how MyoD binding transitions from genome-wide occupancy in muscle progenitors to enrichment at muscle-specific genes on active chromatin in differentiated myocytes.
By applying sci-mtChIL-seq to aging mouse cells, we comprehensively integrated transcriptomic and epigenomic analyses to elucidate age-related cellular changes. This method provides a powerful tool to capture the molecular dynamics of cellular aging and enables a deeper understanding of the mechanisms underlying age-associated epigenomic transitions.

THERAPY-INDUCED SENESCENCE AS A TRANSITIONAL STATE LEADING TO TRASTUZUMAB RESISTANCE IN HER2+ BREAST CANCER CELLS

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Human epidermal growth factor receptor-positive (HER2+) breast cancer constitutes approximately 20% of all cases. It is primarily managed with Trastuzumab (TZB), a monoclonal antibody that targets the HER2 receptor to inhibit its downstream signaling. Although trastuzumab is initially effective, acquired resistance limits its long-term therapeutic success. Importantly, whether TZB exerts its therapeutic effects through the induction of therapy-induced senescence (TIS) and the potential role of TIS as a cellular intermediate leading to TZB resistance are unknown. Here, we performed multiomic profiling and characterized the dynamics of TIS in response to TZB treatment in two cell culture models of HER2+ breast cancer.

Resistance was established during a 4-month time course of chronic trastuzumab treatment (10 μg/mL) in HER2+ breast cancer cell lines (SKBR3 and BT474) in vitro. At the initial stages, BT474 cells underwent a pronounced growth arrest, characterized by decreased HER2 expression, elevated SA-β-gal activity, and increased expression of cyclin-dependent kinase inhibitors (p16, p21, and p27) which correlated with a reduced colony formation capacity. SKBR3 cells also exhibited reduced proliferation compared to control cells, though without the pronounced growth arrest observed in BT474. Senescence markers and HER2 expression were also increased in the SKBR3-treated cells while colony formation ability was reduced relative to the control. The differences in population doubling between the two cell lines may reflect intrinsic cellular responses as well as donor-specific factors, as the SKBR3 cell line was derived from a patient previously treated with radiation, steroids, cyclophosphamide, and 5-fluorouracil, unlike the BT474 donor. Over time, HER2 expression was restored in both cell lines, accompanied by a gradual and persistent increase in population doubling rates. After approximately 3 months, trastuzumab-treated cells regained colony-forming ability, concurrent with a decline in SA-β-gal staining, indicating the acquisition of resistance. Multiomics analyses for bulk ATAC and RNA sequencing suggest that these phenotypic changes are linked to the reprogramming of gene regulatory networks during TIS, highlighting TIS as a potentially critical cellular intermediate in the development of resistance to TZB.

TARGETING THERAPY-INDUCED SENESCENCE: REPURPOSING APPROVED DRUGS TO SUPPRESS SASP IN CANCER CELLS

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Hayflick first characterized cellular senescence as the state of irreversible cell cycle arrest. It is known that chemotherapeutic agents used in cancer treatment can trigger senescence, called therapy-induced senescence, and influence tumor progression through the secretion of bioactive molecules, collectively termed senescence-associated secretory phenotype (SASP). In order to prevent these detrimental effects, the development of senomorphic compounds that inhibit SASP secretion in senescent cells has emerged as a critical treatment strategy. In our previous study, the Rho kinase (ROCK) inhibitor Y27632 was demonstrated to suppress SASP in senescent HeLa cells, suggesting that compounds with ROCK inhibitory activity may possess senomorphic properties.

In the present study, the approved drugs parecoxib and moxifloxacin, which were identified through in silico analyses as potential alternatives to the specific ROCK inhibitor Y27632, were evaluated for their senomorphic effects in A549 and PC3 cancer cell lines.

Senescence was induced in A549 and PC3 cells by treatment with 300 nM doxorubicin for 4 days. Subsequently, the culture medium was replaced with serum- and phenol red-free DMEM, followed by treatment with parecoxib (100 μM) and moxifloxacin (100 μM) for 48 hours. Then, the cell secretomes were collected, and the IL-6 and total protein amounts were measured in the secretome.

Senescence induction with doxorubicin resulted in a significant increase in IL-6 secretion in both A549 and PC3 cells compared to the control group. While moxifloxacin and parecoxib reduced IL-6 secretion in senescent A549 cells, only moxifloxacin reduced IL-6 secretion in senescent PC3 cells, but parecoxib did not. Thesefindings suggest that moxifloksasin and parecoxib may have different effects depending on cell type. Evaluation of total protein secretion in the conditioned medium revealed an increase in both A549 and PC3 cell lines following doxorubicin-induced senescence, which was subsequently reduced by treatment with moxifloxacin and parecoxib. Additionally, the intracellular total protein amount per cell was elevated in senescent cells, and the treatment of moxifloxacin and parecoxib reduced this increase.

Senomorphic drugs offer an important strategy for cancer treatment to both control the tumor microenvironment and reduce treatment-related side effects. Our findings suggest that parecoxib and moxifloxacin may exert senomorphic effects by modulating
the secretion of SASP-related proteins, suggesting their potential therapeutic utility in cancer treatment. However, their potential therapeutic applications need to be further investigated.

TARGETING STRESS RESPONSE TRANSCRIPTIONAL NETWORKS TO IMPROVE MUSCLE HEALTH AND AGING

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Skeletal muscle plays a pivotal role for healthy aging by regulating whole-body metabolism and maintaining mobility. Muscle mass declines with age (sarcopenia), as do its stem cells and repair capacity, increasing our susceptibility to chronic disease. Cellular stress responses are activated when damage is sensed to temporarily alter physiology until homeostasis can be returned. Chronic transcriptional activation of stress responses precludes proper return to homeostatic physiology and has been shown to contribute to age-associated phenotypes and disease. How stress responses reprogram physiology and metabolism of damaged organs lacking regenerative capacity, such as aging muscle, is understudied. Using systems biology approaches in Drosophila, we found that endoplasmic reticulum unfolded protein response (UPR) or nuclear factor-kappa B (NF-κB)-induced inflammation decrease fly healthspan and lifespan when ectopically activated in adult muscle and are endogenously activated in naturally aged fly muscle. Combining classical fly genetics with multi-omics (whole-body single-nuclei RNA-sequencing (snRNA-seq) and tissue-dissected mass spec metabolomics/lipidomics), we found that chronic activation of these stress response pathways in muscle decreases carbohydrate metabolism, increases aberrant lipid biosynthesis, and dysregulates systemic insulin signaling. Moreover, our transcription factor (TF) analyses of published snRNA-seq from aging flies (available from the Aging Fly Cell Atlas) shows significant overlap in stress response networks and naturally aged transcriptional networks in muscle, suggesting endogenous stress response TF activity may drive age-associated muscle decline and metabolic dysfunction.

MODULATING SENESCENCE IN PANCREATIC ADENOCARCINOMA WITH TARGETED NANOTHERAPIES

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers, due to its resistance to chemo- and immunotherapies. Inducing senescence can expose therapeutic vulnerabilities, but lingering senescent cells pose risks both for relapse and aging-related toxicities. We developed a Galectin-3-targeted lipid nanoparticle system to selectively deliver a senolytic BRD4 degrader after senescence induction. This targeted approach significantly reduced tumor growth and improved survival in preclinical PDAC models. Single-cell transcriptomics revealed remodeling of the tumor microenvironment, with suppression of pro-tumor and pro-inflammatory pathways in both cancer cells and fibroblasts. These findings point to a promising strategy for overcoming PDAC resistance and targeting pathological senescence in cancer and potentially other age-related diseases.

TRANSCRIPTIONAL ELONGATION/3'-END PROCESSING CONTROL BY NELF AND ELOA IN REVERSIBLE GROWTH ARREST

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Negative elongation factor (NELF) is a transcriptional regulator that is primarily known for its role in the promoter-proximal pausing of RNA polymerase II (RNAPII). Here, we show that acute depletion of NELF subunits results in a reversible growth arrest phenotype driven by upregulated expression of senescence-associated genes, within a small subset of genes at which NELF depletion induces RNAPII release. Our genetic suppressor screen and long read analyses identify the transcription elongation factor Elongin A (ELOA) mediating the NELF depletion-induced growth arrest and demonstrate that mechanistically, this phenotype is suppressed by ELOA loss due to specific changes in the 3'-end processing of ELOA target genes, which function in cellular senescence. Accordingly, we also demonstrate that ELOA loss suppresses cellular senescence in aging human fibroblasts. The findings presented here establish the existence of a crosstalk between splicing factors, 3'-end processing machinery and the elongation factors ELOA/NELF in the regulation of gene expression relevant to cellular senescence and aging.

uPAR-TARGETED NANOPARTICLES ENHANCE THE EFFICACY OF DASATINIB AND QUERCETIN IN SUPPRESSING ADIPOSE TISSUE SENESCENCE AND IMPROVING INSULIN RESISTANCE IN OBESE MICE

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Obesity induces adipose tissue (AT) inflammation and senescence, results in the development of insulin resistance. Urokinase-type plasminogen activator receptor (uPAR) expression is upregulated in senescent cells including AT of obese mice.The combination of dasatinib and quercetin (DQ) is a senolytic agent and improves insulin resistance by suppressing AT senescence. We developed DQ complex nanoparticles targeting uPAR and investigated whether it improves AT senescence and insulin resistance more efficiently than the existing drug combination. A polymer-lipid hybrid nanoplatform utilizing a uPAR antibody was developed for the systemic delivery of the DQ combination targeting uPAR (DQ-LPHN@uPAR). Male C57BL/6 mice were fed a high-fat diet for eight weeks, followed by weekly administration of DQ-LPHN@uPAR via tail vein injection for seven weeks (uTNP), while maintaining the high-fat diet. The mice in the control group (Con) and free drug group (FD) were injected with a vehicle and DQ, respectively, via the tail vein. Nanoparticle containing DQ without uPAR antibody was also injected into tail vein (NTNP). Glucose metabolism was assessed using a glucose tolerance test, and insulin sensitivity was evaluated using an insulin tolerance test. AT aging was analyzed through SA-β-galactosidase staining and the levels of aging biomarker using Western blotting. Fasting glucose levels did not differ significantly among the groups, whereas fasting insulin levels were significantly lower in the NTNP and uNTP groups compared to the Con. Following glucose injection, blood glucose levels and the glucose area under the curve (AUC) were significantly lower in uTNP mice compared to the Con. Similarly, after insulin injection, blood glucose levels and AUC were significantly lower in the uTNP group but not in the NTNP or FD groups. Accordingly, the levels of phosphorylated Akt were significantly higher in uTNP. SA-β-galactosidase staining was significantly reduced in uTNP mice compared to the Con and FD groups. Additionally, the expression of aging biomarkers and the percentage of crown-like structure-positive cells were lower in uTNP mice. These results suggest that DQ-LPHN@uPAR is more effective in suppressing adipose tissue senescence and improving insulin resistance in obese mice than the DQ alone. Therefore, the development of targeted drug against uPAR holds promise as a senotherapeutic strategy for addressing adipose tissue senescence and insulin resistance.

CELLULAR SENESCENCE AS A PROVIRAL HOST STRESS RESPONSE FOR VIRAL PERSISTENCE

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Cellular senescence is a state of stable cell cycle arrest thought to function as an anti-tumorigenic cellular stress response. Due to the reduced proliferative capability of senescent cells, senescence can also function as an antiviral response. However, it has been recently reported that numerous viruses can exploit senescence to promote a proviral microenvironment. Merkel cell polyomavirus (MCPyV) is a human tumor virus that causes a highly aggressive and metastatic skin cancer called Merkel cell carcinoma (MCC) that predominantly affects elderly and/or immunocompromised patients. Though MCC has a high mortality rate (~46%), little is known about the exact mechanism MCPyV utilizes to induce the onset of MCC. Therefore, more research is required to understand how MCPyV can persist and survive within its host cell. Previous studies have shown that an MCPyV oncoprotein, the large tumor antigen (LT), can promote cell growth inhibition. We found that LT could induce cellular senescence through observation of senescence-associated-β-galactosidase (SA-β-gal) staining, senescence-associated secretory phenotype (SASP) expression, and activation of the p53-p21 pathway. Notably, induction of senescence was dependent on the Merkel cell polyomavirus unique region (MUR), a domain that is not conserved in any other human polyomavirus LT. Activation of p53 was caused by nucleolar stress (NSR), a host stress response induced through inhibition of ribosomal biogenesis. LT expression resulted in the nuclear sequestration of RPL5, downregulation of phospho-RPS6, and reduced global protein translation. Knockdown of p21 could reverse numerous senescent phenotypes demonstrating that LT-induced senescence was regulated by p21. Importantly, inhibition of senescence through p21 knockdown or treatment of the senolytic, navitoclax, could decrease MCPyV DNA replication over time, indicating that cellular senescence could regulate MCPyV genome persistence. In conclusion, these data demonstrate the ability of a human tumor virus to manipulate various host stress responses for its own benefit and implicates the potential for cellular senescence to contribute to MCPyV pathogenesis.

BEHAVIORAL ANALYSIS OF HAAO -/- AND KMO -/- MICE AND RNA SEQUENCING OF BCG-TREATED HAAO -/- MICE TO IDENTIFY NEUROINFLAMMATORY GENE TARGETS

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The kynurenine pathway (KP), a catabolic route of tryptophan metabolism, produces neuroactive metabolites, some of which become neurotoxic under inflammatory conditions, particularly with aging. Identifying key modulators of the KP may help mitigate oxidative damage from age-related inflammation. To explore the impact of KP dysregulation on cognition and mood, we generated transgenic mice with conditional knockouts of key enzymes: Kynurenine 3-Monooxygenase (KMO -/-) and 3-Hydroxyanthranilate 3,4-Dioxygenase (HAAO -/-). Mice underwent behavioral assessments, including the open field, olfactory habituation/dishabituation, Y-maze, and Barnes maze tasks. Preliminary Y-maze results showed KMO -/- mice had significantly higher spontaneous alternations than wild-type (WT) ($p \leq 0.05$) and HAAO -/- ($p \leq 0.01$) mice, suggesting KMO deletion may protect against age-related working memory decline. In the Barnes maze, aged KMO -/- mice had significantly reduced latency compared to HAAO -/- ($p \leq 0.001$), further supporting a cognitive benefit. To examine gene expression changes under inflammatory conditions, RNA sequencing was performed on brain tissue from HAAO -/- mice treated with Bacillus Calmette-Guérin. These findings may provide insight into how KP dysregulation influences cognition, mood, and neuroinflammatory responses.

EVALUATING THE EFFECTS OF AGE, E2/P4 AND NRF2 ON FEMALE NEURAL STEM PROGENITOR CELL QUIESCENCE AND SENESCENCE

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Cellular senescence is one of the hallmarks of aging and adult neural stem progenitor cells (NSPCs) are not immune. With aging, adult quiescent NSPCs manifest in cellular states like senescence and deeper quiescence contributing to reduced neurogenesis. However, the dynamics of these cellular states with advancing age is less understood. Previous studies from our lab have established a direct correlation between adult neurogenesis and the expression of the redox-sensitive transcription factor nuclear factor (erythroid-derived 2)-like 2 or Nrf2 during a critical middle age in male rodents. Interestingly, while Nrf2 also plays a crucial role in maintaining cellular quiescence and delaying senescence in other tissues, its effects on these processes in NSPCs, particularly in the female brain, is unknown. To address this gap, we are investigating NSPC aging and the influence of the sex hormones, 17β Estradiol (E2) & Progesterone (P4), in female wild-type (WT) and Nrf2 knock-out (KO) F344 rats. Our data indicates an age-related decline in neurogenesis in both the subventricular zone (SVZ) and subgranular zone (SGZ) of the female rats (aged 2, 6, 9 and 14 months), as well as changes in NSPC-associated behaviors of fine olfactory discrimination, pattern separation and reversal in the Morris Water Maze. In addition, a vulnerable period at ~9-10 months of age was seen in relation to the SVZ, when the loss of E2 and P4 significantly affects NSPC activity and function. Moreover, preliminary results suggest an even more reduced fine olfactory discrimination and pattern separation abilities in the NRF2 KO animals compared to WT rats. Markers of quiescence (BrdU/Ki67 -ve and Id4 +ve) and senescence (p16 and p21) in the NSPC niches are being evaluated. Broadly, these studies will provide insights into the role of E2/P4, Nrf2 and aging on NSPC quiescence and senescence in females.

LOCAL UNIQUENESS OF MICROCIRCULATION AND ANGIOGENESIS WITHIN THE INFUNDIBULUM OF THE OVIDUCT OFFERS INSIGHT INTO ITS VULNERABILITY TO CELLULAR STRESS AND SUSCEPTIBILITY TOWARDS HGSOC.

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High-Grade Serous Ovarian Carcinoma (HGSOC), the most common ovarian cancer, originates from epithelial cells within the infundibulum fimbriae, the distal tip of the fallopian tube. Traditionally, it is thought that the proximity to the ovary causes regional vulnerability to DNA damage because of ovarian ROS chemicals emanating during ovulation. On the other hand, our recent studies have revealed the presence of distinct cell populations at the infundibulum and a unique aging tissue microenvironment, suggesting that the unique vulnerability stems from the infundibulum's distinct developmental, cellular, and physiological properties.

Microcirculation is the flow and communication of blood within microvasculature, such as capillaries, and the surrounding interstitial fluid, which directly support the cells' metabolic activities through delivery of oxygen and nutrients within each tissue. The precise regulation of this process is essential for maintaining tissue homeostasis yet has remained understudied. We have found that the mouse oviduct, a highly translucent and small-sized organ, is a highly suitable model system to study wholemount microcirculation with experimental ease.

The oviduct is known to receive blood supply from the ovarian and uterine arteries, yet how they arborize into microvasculature that subsequently penetrate the different regions of the tube remains undescribed. Using different high-resolution imaging techniques to visualize endothelial cells and circulation, we have identified the detailed network of penetrating arterioles and capillaries within each oviductal region.

Interestingly, the microcirculation pattern within the oviduct changed alongside the estrus cycle, pregnancy, and aging. Particularly, the infundibulum region had no microcirculation during estrus (ovulation), wherein persistent angiogenic sprouting of endothelial cells and no pericyte coverage was observed. Recently, we reported the presence of vacuolating multiciliate cells within the aged infundibulum. However, we have found that oxidative stress is sufficient to induce vacuolation within ex vivo young infundibulum. We are currently testing whether the mitochondrion within the different epithelial populations of the infundibulum exhibit any observable signs of oxidative stress across estrus cyclicity and aging, and if pregnancy may be protective against acquiring such phenotypes.

Thus far, our data provides novel insight into how the distinct tissue microenvironment within the infundibulum may uniquely predispose the region to increased cellular stress and susceptibility towards transformation.

LEVERAGING NONINVASIVE IMMUNOPET IN PANCREATIC CANCER TO QUANTIFY IL-6 AND uPAR AS THERANOSTIC TARGETS DURING CHEMOTHERAPY INDUCED SENESCENCE

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Pancreatic cancer is a stromal rich immunologically suppressed cancer microenvironment, where immunoPET imaging can quantify tumor heterogeneity. Previous reports have shown the tumor environment with stroma secrete interleukin-6 (IL-6) which can be further increased with chemotherapy as part of the senescence associated secretory phenotype. Here we examined murine IL-6 (mIL-6) in a KPC pancreatic cancer model, using a [^{89}Zr]Zr-DFO-mIL-6 antibody showing accumulation in the "tumor" by 144 hours post injection whether the KPC tumor was implanted 1, 2, 3 or 4 weeks prior. The addition of chemotherapy treatment with gemcitabine and nab paclitaxel at 30 and 100 mg/kg respectively, significantly increased [^{89}Zr]Zr-DFO-mIL-6 targeting in the tumor from 8.3 to 20.7 %ID/g. Lastly we switched mIL-6 from immunoPET imaging to a radiotherapy, creating [^{225}Ac]Ac-macropa-PEG4-mIL-6. Administration of 500 nCi [^{225}Ac]Ac-macropa-PEG4-mIL-6 saw a decrease in tumor growth in combination with gemcitabine + Nab paclitaxel, while single arm therapy failed to decrease KPC tumor progression.

Separately, in a cell binding assay, KPCs identified minimal uptake with [^{89}Zr]Zr-DFO-mIL-6, and a robust uptake with [^{89}Zr]Zr-DFO-muPAR. We have explored previously uPAR as a marker of chemotherapy induced senescence as well as quantified basal expression and distribution in male and female c57bl6j mice up to one year old. As a radiotherapy, [^{225}Ac]Ac-macropa-PEG7-muPAR was shown to have a synergistic effect with the combination of trametinib and palbociclib, leading to a static tumor size after a single dose administration through 3 weeks, where single arm TP or radiotherapy failed to prevent tumor growth.

To address both stroma and pancreatic cancer cells, we then combined radiotherapies targeting IL-6 and uPAR as a two hit punch in a KPC flank study. By targeting simultaneously both populations, we saw a near complete reduction in tumor size for 2-3 weeks after a single dose administration (300nCi). Complete blood chemistry will be described alongside other welfare metrics of body weight and mouse condition scores. Current ongoing work combines IL-6 and uPAR radiotherapy with gemcitabine and nab paclitaxel in weekly fractionated doses to eliminate KPC tumors. Future work will define immune populations present in the microenvironment for each therapy arm and additional targeted therapeutic avenues.

SENESCENCE MEDIATES AGE-RELATED TUMORIGENESIS AND METASTASIS

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Aging profoundly affects both the immune system and the tissue microenvironment, predisposing older adults to chronic diseases such as cancer. While most prominent first-line treatments are effective in younger populations, many older patients exhibit reduced tolerance, often compromising their prognosis. Here, I propose a strategy to mitigate tumor burden and limit tumor spread without adverse effects by targeting senescent populations that arise with aging. This approach aims to sustain antitumor immunity and preserve optimal organ function. By establishing lung adenocarcinoma tumors in young, middle-aged, and old mice, we found that the aged lung microenvironment accelerates tumor burden and significantly promotes the dissemination of tumor cells to lymph nodes and distant organs. To determine if age-associated senescent cell accumulation contributes to creating a conducive environment for tumor growth and pre-metastatic niches, we employed the *INK-ATTAC* system, which selectively eliminates p16-positive cells upon drug treatment, in an orthotopic model of primary lung adenocarcinoma. Preliminary findings indicate that lifelong removal of senescent cells reduces tumor burden, limits tumor spread, and extends the survival of aged mice with cancer. We anticipate that our data will serve as a foundation for understanding the systemic effects of senescent cell accumulation on lung cancer progression and metastasis.

DEVELOPING A MOLECULAR MAP OF HEMATOPOIETIC AGING

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The hematopoietic system is a regenerative tissue that becomes dysregulated during aging, leading to systemic pathologies such as anemia and impaired infection response. These age-related changes, including regenerative decline and reduced lymphoid cell production, are initiated in hematopoietic stem cells (HSCs), which sit atop a complex hierarchy of progenitors that produce all mature blood and immune cells. Aging research so far has largely focused on dichotomous comparisons of young vs. old hematopoietic cells, especially HSCs, primarily using mouse models and often mixing male and female animals. As a result, our understanding of the temporal progression of hematopoietic aging is currently limited. Defining the succession of age-related events is critical to elucidate causative relationships and identify targets for preventing or reversing hematopoietic decline. To this end, we profiled the hematopoietic system of large cohorts of male and female mice every 3 months (mo) from young adulthood (~3 mo) to geriatric ages (~30 mo). We performed multimodal analyses on bone marrow (BM), spleen, and blood populations to assess hematopoietic output (flow cytometry) and conducted detailed investigations on isolated HSCs to establish their transcriptomic (RNA-seq) and epigenomic (ATAC-seq and Methyl-seq) state, as well as regenerative potential (transplantation) across the age-range, building a roadmap of molecular and functional changes associated with HSC aging. Interestingly, HSC regenerative decline was aligned between both sexes and significantly decreased by midlife (~12 mo), while epigenetic and transcriptomic alterations were observed before midlife (~9 mo). This identified a set of molecular changes that precede functional alterations, which we are currently investigating. In contrast, in their native BM niche environment, male and female hematopoietic systems aged differently, with accelerated B cell attrition and a compensatory expansion of lymphoid-biased multipotent progenitors (MPP4s) in females, as opposed to delayed B cell attrition and contraction of MPP4s in males. Molecularly, HSCs from female mice prominently displayed an inflammatory response gene signature at an earlier timepoint than HSCs from male mice, which might be associated with precocious inflammaging of the BM niche in females, which we are currently testing. To probe the importance of inflammation in HSC aging, we also aged cohorts of hematopoietic-specific Socs3-deficient mice. Without the protective effects of SOCS3 dampening the JAK-STAT pathway, we observe premature aging features in the blood and BM of both sexes, loss of HSC maintenance in the BM, and premature death (~12 mo). We are now profiling the molecular state of prematurely aged Socs3-deficient HSCs to assess the consequences of unmitigated inflammation response. Our intention with this approach is to identify key inflection points of aging to target for interventions to delay hematopoietic aging.

ATLAS OF LYSOSOMAL AGING REVEALS A MOLECULAR CLOCK OF STORAGE-ASSOCIATED METABOLITES

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The idea that lysosomal dysfunction contributes to aging has been proposed since the organelle's discovery, yet the molecular mechanisms underlying this process have remained elusive due to a lack of appropriate tools. Here, we leveraged a recently developed transgenic mouse line expressing a lysosomal epitope tag for rapid organelle purification to systematically map aging-associated deficiencies. In aged mice, we observed lysosomal accumulation of glycerophosphodiesters (GPDs) and cystine – metabolites known to accumulate in juvenile lysosomal storage disorders – supporting a long-standing hypothesis linking these diseases to the lysosomal dysfunction observed in old age. Using a novel "tag-free" lysosomal isolation approach, we confirmed that this metabolic signature is present in aged wild-type mice and found that lysosomal GPD and cystine levels progressively increase throughout the lifespan, correlating with chronological age. Notably, caloric restriction, a lifespan-extending intervention, mitigated these changes in the heart but not in the brain. Further, we demonstrated that this lysosomal signature is conserved in aged rats, suggesting that it may represent a broader feature of mammalian aging. Finally, we found that GPD and cystine accumulation is associated with a compositional shift in bis(monoacylglycero)phosphates (BMPs), lysosome-specific lipids essential for maintaining lysosomal function. Together, our findings provide new insights into lysosomal metabolism in aging and may inform our understanding of age-related diseases.

SENOLYTIC TREATMENT CAUSES REGION-SPECIFIC TRANSCRIPTIONAL CHANGES IN THE AGED MOUSE BRAIN

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There is a well-established association between senescence and aging, however, the precise impact of senescence across an aging brain with region specificity remains unclear. This work applies spatial transcriptomics to uncover the region-specific impact of senolytic therapeutics - drugs that induce apoptosis of senescent cells. Previous work has shown that senolytics delay aging-associated phenotypes in mouse models and demonstrated promise in clinical trials of senescence-associated conditions such as fibrotic diseases. One such senolytic, navitoclax (ABT-263), is a Bcl-2 inhibitor known to decrease tau aggregation, improve cognitive function, and promote a pro-regenerative state. Spatial transcriptomic techniques are uniquely suited for revealing region-specific gene expression changes in response to senolytic treatment. In this study, aged (24-month-old) male mice were treated daily with navitoclax (50 mg/kg in DMSO, n=2) or DMSO alone (n=2) via oral gavage for a total of 10 days. On day 11, brains were harvested and processed using IRISeq (Imaging Reconstruction using Indexed Sequencing), our optics-free spatial transcriptomics approach utilizing polyA and polyT bead connections. This enables region-specific analysis of gene expression changes across coronal brain sections. Clustering analysis revealed 11 distinct brain regions, including the hippocampus, cortex, thalamus, and amygdala. Across brain regions, we identified 165,847 differentially expressed gene (DEG) transcripts and classified 356 of these as senescence-associated genes. Notably, Cdkn1a expression was significantly lower in the amygdala, hippocampus, hypothalamus, thalamus, and white matter of navitoclax-treated mice ($p < 0.05$). Expression of senescence markers, Igfbp3 and Igfbp7, also decreased in the ventricles of the treatment group. Gene ontology term enrichment of significant DEGs revealed upregulation of genes involved in “synaptic transmission,” “neuron projection development,” and “neurotransmitter receptor activity” in treated animals. These striking transcriptional changes, observed after only short-term senolytic treatment, highlight the efficacy of navitoclax as a senolytic therapy in aging brains. Importantly, this study demonstrates the strength of our spatial transcriptomic approach in identifying senescence signatures and elucidating region-specific changes following senescent cell clearance in aged mice.

TARGETING THE P21-CYCLIND1-CDK6 AXIS TO MITIGATE SENESCENCE-DRIVEN INFLAMMATION AND AGE ASSOCIATED FUNCTIONAL DECLINE

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Aging, a multifaceted process linked to increased risk of numerous diseases, is marked by the accumulation of pro-inflammatory, proliferation-arrested senescent cells. Cyclin-D1 (CCND1), a key stimulator of proliferation, paradoxically shows upregulation in these senescent cells. We investigated this by knocking down CCND1 in senescent IMR90 cells, which significantly decreased Senescence Associated Secretory Phenotype (SASP) and Interferon Stimulated Genes (ISGs). Knocking down CCND1's kinase partner, CDK6, but not CDK4, and treating senescent cells with Palbociclib, a CDK4/6 inhibitor, similarly downregulated SASP and ISGs, suggesting a non-canonical CCND1/CDK6 role in SASP and ISG activation. Through RNA sequencing, IP-mass spectrometry, and immunofluorescence, we show that the CCND1-CDK6 complex promotes DNA damage, leading to cytoplasmic chromatin fragments (CCFs), which activate CGAS-STING signaling and drive SASP and ISG expression. Intriguingly, these effects are further regulated by the P53-CDKN2A (P21) axis. P21 knockdown impaired DNA repair, increased CCFs, and elevated SASP and ISGs, effects fully rescued by Palbociclib. Profiling Ccnd1 in hepatocytes from young and old mouse livers revealed increased Ccnd1 and inflammation in aged livers despite no observable proliferation. To test Ccnd1's role in vivo, we used AAV2/8 to deliver hepatocyte-specific CRISPR-saCas9-mediated Ccnd1 knockout and administered Palbociclib by oral gavage in old wild-type mice. Both these interventions significantly reduced DNA damage, SASP, and ISGs in the liver, extending our in vitro findings to an in vivo context. Moreover, Palbociclib-treated old mice showed a prevention of the age-related increase in frailty and significantly longer endurance on the rotarod test, underscoring the functional benefits of targeting the CCND1/CDK6 pathway. In summary, our data reveals a novel role for CCND1/Ccnd1 in non-proliferating senescent cells and aged hepatocytes, extending beyond cell cycle regulation to include modulation of DNA damage, inflammation, and functional aging phenotypes. These findings highlight the potential of therapeutically targeting CCND1/CDK6, via Palbociclib or other strategies to mitigate age-related inflammation, tissue dysfunction, and decline in physical performance.

SEX-SPECIFIC METABOLIC ADAPTATIONS IN HYPOTHALAMIC NEURONS EXPOSED TO AMYLOID-β: IMPLICATIONS FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by impaired brain glucose metabolism. Despite relatively well-documented sex differences in metabolic shifts in the hippocampus and cortex in AD, the role of the hypothalamus remains underexplored. The permeable BBB within the medio-basal hypothalamus increases its susceptibility to peripheral metabolic disruptions but also allows for early therapeutic interventions. This study investigates sex-specific glucose metabolic responses to amyloid-beta (Aβ) toxicity in male and female mouse hypothalamic neuronal cell lines exposed to 5 μM oligomerized Aβ. Summary of our findings:

Male Aβ-treated neurons exhibited reduced hexokinase activity and increased lactate dehydrogenase B expression, suggesting a buildup of pyruvate and a bottleneck in glycolysis. Concomitantly, pyruvate dehydrogenase expression was suppressed, indicating reduced conversion of pyruvate to acetyl-CoA. Aβ-treated cells also showed a trend of reduced glycolytic reserve in the seahorse glycolysis test, reinforcing the notion of compromised glycolytic function. Despite these impairments, ETC components Cyt C and SCO1 were upregulated, suggesting a compensatory shift toward OXPHOS. Inhibiting mitochondrial pyruvate entry with UK5099 reduced mitochondrial maximal respiration as expected. However, Aβ-treated males showed slightly lower maximal respiration in response to the inhibitor than controls, suggesting a subtle decrease in mitochondrial flexibility by Aβ.

Female Aβ-treated neurons exhibited reduced GLUT3 and PFK1 protein levels and unchanged hexokinase activity. Interestingly, they showed a trend toward increased basal glycolysis and a significantly higher glycolytic reserve than their male counterparts, indicating a metabolic shift toward glycolysis despite reduced glucose transport capacity. Citrate synthase and isocitrate dehydrogenase gene expression were downregulated, indicating a suppressed TCA cycle flux and a possible citrate accumulation. Unlike males, ETC genes were largely unchanged, but maximal and ATP-linked mitochondrial respiration was reduced, indicating a metabolic shift away from mitochondrial respiration. Aβ-treated females exhibited slightly higher maximal respiration in the absence of pyruvate than controls, suggesting enhanced metabolic flexibility and possibly a greater reliance on alternative fuel sources in response to Aβ.

Overall, male hypothalamic neurons rely on oxidative glucose catabolism in response to Aβ, whereas female neurons demonstrate a metabolic reprogramming reminiscent of the Warburg effect, prioritizing glycolysis and alternative fuel utilization.

THE SOURCE OF CHRONIC STRESS IN AGED NEURONS

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Aging is one of the most prominent risk factors for neurodegeneration, yet the molecular mechanisms underlying the deterioration of old neurons are mostly unknown. To efficiently study neurodegeneration in the context of aging, we transdifferentiated primary human fibroblasts from aged healthy donors directly into neurons, which retained their aging markers. Here we show that aged neurons are chronically stressed, leading to the formation of sub-micron stress granule-like inclusions and basal phosphorylation of eIF2α. We demonstrate that stress granules fail to resolve due to the loss of G3BP1 ubiquitylation and segregation from chaperones like HSP90α. Importantly, chronic stress dampens neuronal resiliency to future stress events, and transdifferentiated neurons are unable to activate transcription of HSP chaperones in response to new stressors. We further show that the source of chronic stress in aged neurons is mitochondrial-derived double-stranded RNA, which binds to PKR, promotes phosphorylation of eIF2α, and causes assembly of chronic stress granules. Importantly, key facets of the chronic stress response are also present in aged human brain tissue. Together our data demonstrates that aging-linked chronic activation of the stress response is a key driver of poor resiliency in aged neurons and is likely a major risk factor for neurodegeneration.

MODELING AGING IN hESC-DERIVED RETINAL PIGMENT EPITHELIAL CELLS

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Retinal degeneration is a leading cause of vision impairment and complete blindness. Some pathologies involve the depletion of retinal pigment epithelium (RPE) during aging. Contrary to fetal RPE cells, which possess proliferative capacities, adult RPE cells that are lost due to injury or disease fail to regenerate and replenish the damaged structures leading to photoreceptor degeneration and vision loss. While current therapies slow disease progression, they do not restore vision, highlighting the need for strategies to counteract RPE and photoreceptor loss.
To address this, we use a well-establised in-lab protocol to differentiate human embryonic stem cells (hESC) into RPE cells rapidly and reproducibly. These cells express mature RPE cell markers, show increased barrier function and provide an accessible in vitro model for human RPE research. Interestingly, we observed that RPE cell numbers decline over time, accompanied by hypertrophy, reduced phagocytic activity, and impaired wound healing – features also observed in vivo during aging.
To further investigate age-related RPE dysfunction, we developed a novel in vitro injury model, that facilitates controlled RPE cell removal at specific time points. Young RPE cultures retained proliferative capacity upon cell loss, whereas older cultures exhibited decreased proliferation, leading to hypertrophy and dysfunction, mirroring aging-related RPE degeneration in vivo. Transcriptomic comparisons revealed an upregulation of senescence-associated pathways. Notably, treatment of old RPE cells with a senolytic drug improved their recovery in the injury model. Further studies will explore whether senolytic treatment enhances RPE cell transplantation outcomes in mammalian models of RPE damage in vivo.

INNOVATIVE ANTI-AGING APPROACHES: MARINE BIOACTIVES AND DRUG REPURPOSING TO REPAIR DNA DAMAGE AND MODULATE CELLULAR SENESCENCE

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Cellular senescence and genomic instability are critical hallmarks of aging, contributing to the progressive decline in cellular function. The accumulation of DNA damage, exacerbated by senescence-associated inflammatory and metabolic alterations, impairs repair pathways and reinforces senescence-associated phenotypes. Developing strategies to mitigate DNA damage and modulate senescence pathways represents a promising avenue for anti-aging therapeutics.

Marine ecosystems are a rich source of unique bioactives with antioxidant, anti-inflammatory, and cytoprotective properties. In this study, we evaluate the potential of bioactive compounds derived from marine resources to alleviate DNA damage and senescence. In parallel, we also test known pharmacological compounds (for drug repurposing) in the same conditions. Cells were exposed to oxidative stress to induce DNA lesions, mimicking aging-associated genotoxic conditions and subsequently treated with libraries of either marine-derived bioactive compounds or drugs. DNA damage was assessed using single-cell gel electrophoresis (comet assay) and cell senescence with the senescence-associated beta-galactosidase staining.

Our results identified 25 compounds capable of significantly reducing DNA damage compared to controls. Among these, a subset of compounds further demonstrated an impact on senescence-associated pathways, suggesting that these compounds not only mitigate genotoxic stress but also address cell senescence, a key driver of aging processes.

This research shows the complementary potential of natural marine-derived bioactives and pharmacological agents for anti-aging applications. By targeting DNA integrity and cell senescence, these compounds offer dual benefits for cellular longevity and resilience against senescence while presenting as promising avenues for innovative anti-aging therapeutic strategies. Ongoing studies aim to elucidate their mechanisms of action and explore their translational potential in anti-aging formulations.

Work developed in the scope of "AgeTech - Investigate New Compounds for the Development of Innovative Anti-Aging Cosmetic Products" project (COMPETE2030-FEDER-01193900), co-financed by COMPETE 2030 and Algarve2030.

EXTRACELLULAR VESICLES FROM SENESCENT RETINAL PIGMENT EPITHELIAL CELL MONOLAYERS CONTRIBUTE TO MITOCHONDRIAL DYSFUNCTION IN NAIVE RECIPIENT CELLS.

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Cells communicate through multiple mechanisms, such as direct contact or the secretion of signaling molecules. Increasing evidence highlights the role of extracellular vesicles (EVs) as key mediators in intercellular communication. EVs are lipid-bound vesicles secreted by nearly all cell types, containing bioactive molecules such as proteins, lipids, and RNAs. These vesicles influence recipient cell behavior by transferring their cargo, which may reflect the physiological or pathological state of the donor cell. Previously we have shown that EVs derived from acutely stressed cells retinal pigment epithelial cells (ARPE-19) induce cellular damage in naïve recipient cells upon their uptake (PMID: 29684588). However, acute oxidative stress may not reflect chronic levels of oxidative stress occurring in age-related diseases such as age-related macular degeneration (AMD). Here we utilized a chronic oxidative stress model, exposing polarized, fully differentiated ARPE-19 monolayers to long-term H_2O_2. Senescence was confirmed, testing for senescence markers β-galactosidase and p53. Furthermore, treatment with H_2O_2 led a reduction in monolayer integrity, increased ROS production and mitochondrial elongation. Proteomics analysis of senescence-associated EVs revealed an enrichment of mitochondrial proteins, suggesting that these EVs may serve to eliminate dysfunctional mitochondrial components or actively transmit mitochondrial signals to affect neighboring cells. In transfer assays, apical EVs released from H_2O_2-treated donor cells were found to quickly induce alterations in the barrier function of naive recipient cells. Additionally, recipient cells treated with senescent EVs exhibited a downregulation of fission (DRP1 and MFF) and mitophagy proteins (PINK1), along with an upregulation of fusion proteins (OPA1 and MFN2) as assessed by Western blotting. Confocal microscopy imaging of mitochondrial networks using MitoTrackerDeepRed staining and the MiNA (Mitochondrial Network Analysis) plugin in Image J confirmed an increase in mean mitochondrial length. These findings suggest that senescent EVs can communicate stress messages to healthy RPE cells, potentially contributing to RPE dysfunction by modifying the mitochondrial machinery of recipient cells.

REGULATORY NETWORK INFERENCE OF INDUCED SENESCENT MIDBRAIN CELL TYPES REVEALS CELL TYPE-SPECIFIC SENESCENCE-ASSOCIATED TRANSCRIPTIONAL REGULATORS

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Cellular senescence of brain cell types has become an increasingly important perspective for both aging and neurodegeneration, specifically in the context of Parkinson's Disease (PD). Recent advancements have been made to provide a 'minimum criteria' to define a senescent cell *in vivo* based on observed phenotypes, however senescent cells *in vivo* are very low in number and transient in their presence as they can be cleared within days by immune cells. In contrast, *in vitro* models of senescence are advantageous where an unlimited number of cells can be generated and molecular investigations into the nuanced differences in the expression of senescent phenotypes across different cell types can be made more accessible. Here, we describe a cell type-specific profile of senescence through the investigation of various canonical senescence markers in five human midbrain cell lines (astrocytes, endothelial cells, microglia, oligodendrocytes, and dopamine-like neurons) using chronic 5-bromodeoxyuridine treatment as a model of DNA damage-induced senescence. Importantly, our findings highlight cell type-specific profiles of key hallmarks displayed in cellular senescence. We used principal component analysis and subsequent regulatory transcriptional network inference to define both unique and common senescence profiles in the cell types investigated, as well as revealed senescence-associated transcriptional regulators (SATRs). Functional characterization of one of the identified regulators, transcription factor AP-4, further highlights the cell type-specificity of the expression of the various senescence hallmarks. Taken together, our data indicates that SATRs modulate cell type-specific profiles of induced senescence in key midbrain cell types that play an important role in the context of aging and PD. Our characterization of cell type-specific senescence can inform more precise, effective, and safe therapeutic interventions, particularly in complex diseases like PD where evidence suggests that multiple cell types contribute to pathology.

BREAKING THE BALANCE: HOW OXIDATIVE STRESS DISRUPTS DEUBIQUITYLASES ACTIVITY IN VERTEBRATES AGING BRAINS

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Protein ubiquitylation regulates a broad spectrum of cellular processes, primarily tagging proteins for degradation via the proteasome. Altered proteasomal activity occurs during aging and in age-related diseases (Davidson & Pickering, 2023). We previously observed significant changes in protein ubiquitylation in the brains of old mice and killifish compared to young ones, with reduced proteasome activity accounting for at least 35% of these changes (Marino et al., 2023).

Deubiquitylases (DUBs) act as molecular erasers for the ubiquitylated proteome, and pharmacological inhibition of DUBs has been shown to influence lifespan in nematodes (Koyuncu et al., 2021), prompting us to investigate their role in aging vertebrate brains. In this study, we applied chemoproteomics to examine the **activity of cysteine DUBs** in young and old mouse and killifish brains. We found a global decline in DUB activity in aged brains, which could be rescued by a reducing agent, suggesting the role of oxidative stress as a key factor in this decline. Further, enrichment of ubiquitylated proteome upon DUB inhibition in iPSC-derived iNeurons could modestly (compared to proteasome inhibition) but significantly recapitulate age-induced ubiquitylation changes seen in mice brains (Marino et al., 2023). Additionally, a temporal study in mouse brains revealed a **sequential relationship between oxidative stress, DUB inhibition, and proteasome impairment during aging**, suggesting a cascade of events culminating in proteostasis collapse.

Taken together, our findings emphasize the underappreciated role of DUBs in brain aging and suggest that their dysfunction - probably triggered by oxidative stress - may contribute to the age-related proteostasis decline.

Keywords: Aging, oxidative stress, ubiquitylation, deubiquitylases, proteasome impairment, chemoproteomics

A NOVEL ROLE FOR TRANSCRIPTION FACTOR CEH-62/GBX1 IN RESTORATION AND RECOVERY FROM ADULT REPRODUCTIVE DIAPAUSE

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Fasting and refeeding is a form of dietary intervention that has benefits on healthspan across species. Upon starvation at mid-L3 larval stage, *C. elegans* enters a fasting induced sleep-like quiescence known as Adult Reproductive Diapause (ARD), forming mini-adults that harbor quiescent germline stem cells and survive up to 80 days without food. Upon refeeding, such worms undergo restoration, reactivating the germline and growing to reproductive adults. Our previous work has shown that HLH-30/TFEB loss leads to a metabolic collapse during ARD leading to a novel senescent phenotype which thwarts regrowth and recovery of germline upon refeeding. An unbiased screen revealed that downregulation of DAF-1/TGFß signaling rescued *hlh-30*-induced metabolic collapse, senescence and reproductive ability. Comparison of differentially downregulated genes in the poorly recovering *hlh-30* mutants with those upregulated in better recovering *hlh-30;daf-1* mutants revealed a strong enrichment of certain metabolic pathways that may be important for recovery.
To identify differentially regulated genes involved in mediating recovery, we performed RNAi mediated gene knockdown in wild type worms recovering from long term starvation in ARD. As a molecular marker for metabolic changes, we examined *acdh-1* expression, which responds to perturbations in many of the enriched metabolic pathways in our transcriptomics. Our screen revealed 84 candidates that regulated *acdh-1* activity, which we screened further to identify genes that also impact body size upon refeeding as a marker for restoration. A promising candidate whose knockdown increased body size was *ceh-62/GBX1*, a neuronal transcription factor. To elucidate the mechanism through which it may be impacting body size, we performed proteomic analysis of recovering worms during *ceh-62* knockdown, which revealed upregulation of pathways such as fatty acid and propanoate metabolism amongst others. Furthermore, neuronal specific knockdown of *ceh-62* was sufficient to reproduce the body size phenotype, strengthening the hypothesis that neuronal to soma signaling may be involved. We now aim to investigate how *ceh-62*, a gene linked with neuronal differentiation, regulates these metabolic pathways, and posit that understanding its role will help reveal novel pathways of restoration and repair potentially involving cross-tissue signaling. As restoration from stress response is often impaired with aging, our studies will help uncover novel mechanisms of enhancing this process to improve healthspan with age.

SEX-DEPENDENT CHANGES IN AMPAR EXPRESSION AND NA, K-ATPASE ACTIVITY IN THE CEREBELLUM AND HIPPOCAMPUS OF α-KLOTHO-HYPOMORPHIC MICE

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Aging is characterized by a functional decline in several physiological systems. Klotho protein is involved in aging and in the regulation of synaptic plasticity in the hippocampus. Klotho-hypomorphic mice (Kl-/-) exhibit accelerated aging and cognitive decline. Glutamatergic signaling is important for synaptic plasticity, since N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors participate in long-term potentiation (LTP) and long-term depression (LTD). The Na^+, K^+-ATPase (NaK) pump regulates cell membrane potential and participates in neurotransmitter signaling. Therefore, the aim was to investigate the expression of glutamatergic receptors and NaK isoforms activity in the cerebellum and hippocampus of male and female Kl-/- mice. Cerebellum and hippocampus of Kl-/- and Klotho-wild-type (Kl+/+) male and female mice (n = 7; 8 week-old) were collected, after genotyping the animals through conventional PCR. Western Blotting assays were performed to investigate the protein expression of the subunits GluN1 (NMDA) and GluA1 (AMPA), using β-actin as an internal control. For NaK activity assay, each sample was used in three distinct tubes containing histidine buffer, adenosine triphosphate (ATP), and differents concentrations of ouabain (3 μM or 3 mM), a NaK inhibitor, to determine α1 and α2/3 isoforms and total ATPase activities (NaK-ATPase and Mg^{2+}-ATPase). To determine the absorbance of the samples, the quenching solution (0.5% ammonium molybdate) and the colorimetric reagent Fiske-Subarrow were added. Data was statistically analyzed by unpaired Student's t-test, Two-way ANOVA, and Tukey's multiple comparisons. Differences were considered significant for $p < 0.05$. The project is approved by the Ethics in the Use of Animals Committee (CEUA 8613021222). In the cerebellum, Kl-/- male mice show reduced expression of GluA1 (AMPA) compared to Kl+/+ male (51% of reduction; ($F(1,22 = 4.862)$; $p = 0.0082$) and Kl-/- female (51% of reduction; ($F(1,22 = 9.391$ for interaction); $p = 0.0079$)). No differences were observed in the expression of GluA1 in the hippocampus or GluN1 in the both regions. Also, Kl-/- male mice show reduced α2/3-NaK activity compared to Kl+/+ male in the cerebellum (t, $df = 3.136, 8$; $p = 0.0139$), but not in the hippocampus, while Kl-/- female mice show reduced Mg^{2+}-ATPase activity in the cerebellum (t, $df = 3.340, 12$); $p = 0.0059$). The findings suggest that Klotho could influence the expression of AMPA receptor and the activity of NaK-ATPase in the cerebellum in a sex-dependent manner. Therefore, Klotho deficiency may result in sex-specific alterations in AMPAR expression and NaK-ATPase activity, which may contribute to the cognitive decline found in these animals.

A NOVEL MULTI-MODAL PLATFORM FOR THE ACCURATE IDENTIFICATION AND ISOLATION OF SENESCENT CELLS

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Cellular senescence is a hallmark of aging associated with age-related diseases. However, deciphering its specific role in disease is challenging, given high heterogeneity. Identification of senescent cells relies on the combination of different markers because no single marker is specific and expressed over a cells' lifecycle. Bulk approaches or methods bypassing this problem with orthogonal validation across multi-modal measurements (e.g., proteins, genes, morphology) are sub-optimal, in part, because no single technology longitudinally collects these metrics from the same, single cell. Thus, populations identified as senescent are likely impure, making it challenging to employ fundamental cell biology approaches (e.g., cell quantification, co-cultures). Hence, there is an urgent need to decipher senescent cells at single cell resolution to enable the convergence of these multi-modal measurements.

To address these challenges, we developed a method for identifying and quantifying senescent cells by leveraging a novel cell biology platform. This platform compartmentalizes tens of thousands of single cells within permeable hydrogel enclosures, integrating imaging and nucleic acid extraction to enable linked, multi-modal analysis of the same cell across functional, phenotypic and genomic data.

We validated this method to detect multi-modal hallmarks of senescence from the same, single-cells (morphology, SASP, SA-β-gal, transcriptome) and leveraged it to study primary human preadipocytes. Preadipocytes were selected as our first case study, due to their vulnerability to senescence and the significant role of adipose tissue in metabolic age-related diseases. Etoposide-treated senescent preadipocytes displayed high levels of heterogeneity across multi-modal measurements encompassing morphology, SA-β-gal, SASP and gene expression compared to non-treated cells. Multi-modal data analysis uncovered and quantified populations expressing different combinations of senescence attributes, otherwise obscured in bulk or single-modality approaches. Because this method can unambiguously link converged markers to the transcriptome, we were further able to identify shared and distinct gene signatures across these populations. These results demonstrate the potential of using this technology to generate multi-modal data across cell types and tissues to establish comprehensive benchmarks for discovering novel, specific senescence markers. We believe this method can play a meaningful role in developing consensus on the identity and utility of senescent cells which could accelerate Atlassing initiatives, efforts to discover specific markers across tissues, and / or clarify the specificity of senolytic drugs.

TISSUE AGING: EFFECTS OF THE APOLIPOPROTEIN E GENOTYPE ON ADIPOSE-TISSUE STEM CELLS (ASC) PHYSIOLOGY AND DIFFERENTIATION

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Apolipoprotein (Apo) E is vital for transporting lipids, especially cholesterol, inter- and intracellularly. It is involved in lipid metabolism and cell communication. In humans, an APOE4/4 genotype associates with less functional ApoE and links to a higher risk of metabolic and neurodegenerative diseases. In order to investigate ApoE function in stem cell and tissue aging, the ApoE-knockout rabbit model was used. Primary adipose-tissue stem cells (ASC) were isolated from subcutaneous adipose tissue of female New Zealand White (ZIKA hybrids) wild-type (WT) and ApoE-knockout (KO) rabbits and cultured in vitro. ASC lines from different donors (n=22) at low passages (P4-7) and high passages (P 19-25) were characterised based on their morphology, proliferation kinetics and immunological phenotype. Both primary ASCs from WT and ApoE-KO rabbits exhibited the mesenchymal stem cell morphology and expression of stem cell markers. While the multipotency markers were expressed, the pluripotency markers could not be detected in both groups. The ASC lines were positive for cluster of differentiation (CD)14, CD34, CD105 and negative for CD45, CD73 and CD90. CD44 expression was significantly higher in ApoE-KO ASCs compared to WT controls at low passages. The metabolic phenotype of the ASC lines was analysed using the Seahorse Cell Mito Stress Test (Agilent). ApoE-KO rabbit ASCs showed significant differences in maximal respiration, reserve respiratory capacity, proton leak and coupling efficiency, demonstrating that mitochondrial efficiency of energy conversion is affected by APOE mutations in ASCs. The metabolic differences between ApoE-KO and WT ASCs emphasise a functional role for ApoE in stem cell metabolism and differentiation.
Funded by DFG GZ JU 31463-1 and DFG GRK 2155 ProMoAge

ROLE OF CHAPERONE-MEDIATED AUTOPHAGY IN CELLULAR SENESCENCE IN AGING

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Autophagy malfunctioning and senescence are drivers of aging; however, little is known about their joint role in aging. Although senescent cells are of transient nature under physiological conditions, abnormal accumulation of senescent cells occurs with age and has been shown to contribute to functional loss and the increased vulnerability to disease of old organisms. Using functional fluorescent reporters to track chaperone-mediated autophagy (CMA), a selective type of lysosomal degradation that declines with age, we have found that CMA is upregulated during senescence in multiple cell types and in response to different pro-senescence stimuli. Cells with genetic downregulation of CMA, to mimic the changes of this pathway in aging, still engage into senescence but show quantitative and qualitative differences in their ability to attenuate protein translation during senescence and in the composition of their senescence-associated secretory phenotype. We have found a higher abundance of senescent cells in tissues from a mouse model with systemic blockage of CMA. Interestingly, blockage of CMA only in macrophages in vivo is sufficient to recapitulate the accumulation of senescent cells observed upon systemic CMA blockage, thus highlighting an important role of macrophage CMA in senescence cell recognition/clearance. We found that both genetic prevention of CMA decline with age in mice or pharmacological activation of CMA in old mice, lead to significantly lower levels of senescent cells in most organs providing proof-of-concept that preservation and activation of CMA activity could be effective in reducing senescence cell load. Furthermore, we demonstrate that activation of CMA in a mouse model of lung fibrosis, an age-related disease closely linked to senescence, reduces pathology severity and progression. Our work provides new insights on the functional interplay of two aging drivers, reduced CMA and persistence of senescence and highlights CMA upregulation as a possible target to promote senescence resolution in old organisms.

CELL-SURFACE LAMP1 IS A SENESCENCE MARKER IN AGING AND IDIOPATHIC PULMONARY FIBROSIS

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The accumulation of senescent (SEN) cells with age promotes chronic inflammation and drives the progression of age-related diseases. Therapeutically eliminating SEN has been shown to extend healthspan and lifespan in preclinical models, underscoring the urgent need for strategies that selectively target these cells. We identify Lysosomal-Associated Membrane Protein 1 (LAMP1) as a robust, surface-expressed biomarker of cellular senescence. LAMP1 is consistently upregulated in SEN across human and murine cells in cell culture. Analysis using flow cytometry shows an increase in the expression of cells with surface-LAMP1$^+$ in various tissues (liver, lung, and kidney) of aged mice compared to young mice cells, and real-time PCR confirms the co-expression of canonical senescence markers in surface-LAMP1$^+$ cells compared to unsorted and cells with surface-LAMP1-. In another in vivo model, we observe a marked increase in LAMP1$^+$ cells in fibrotic lungs of bleomycin-treated mice, and the RNAseq analysis of the LAMP1$^+$ cells from the control (sham) and bleomycin-treated mice reveals an enrichment of several senescence-related genes, further confirming LAMP1 as a reliable surface biomarker of senescence. Finally, we demonstrate that a dual antibody-drug conjugate (ADC) approach targeting LAMP1$^+$ cells eliminated SEN cell culture. These findings highlight LAMP1 as a tractable senescence biomarker and provide proof-of-concept for a novel ADC-based senolytic therapy.

AGE-RELATED DYSREGULATION OF INNATE IMMUNITY IN HUMAN PLATELETS

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Platelets are anucleate cells that aside from mitochondrial DNA, are transcriptionally silent, inheriting RNAs from megakaryocyte precursors. Platelets mediate coagulation, but also secrete inflammatory mediators and express a full complement of innate immune pattern recognition receptors such as Toll-like Receptors (TLRs). Activated platelets express CD40-ligand (CD40L) and CD62p, the ligand of PSGL-1, thus promoting leukocyte interactions; platelets are present in enormous numbers (~150,000-450,000/µl blood) and are likely, yet incompletely understood, contributors to age-associated innate immune dysregulation. We carried out platelet RNA-seq at baseline and following influenza vaccination in young (age 21-35) and older (≥65) adults (subdivided into largely non-frail community-dwelling (Comm) and nursing home (NH)-resident adults who nearly all met criteria for frailty). We found a pre-vaccine, age-related increase in expression of signal transduction and mitochondrial RNAs and decrease in translation-related RNAs (e.g. eIFs and ribosomal RNAs). Tensor decomposition analysis revealed distinct trajectories of RNA expression of genes in platelet activation and translation pathways in young, Comm and NH adults at days 2, 7 and 28 post-vaccine. Elevated platelet expression of CD40L, CD62p and CD63 protein was found in Comm vs. young adults both prior to and post-vaccination; NH adults showed lower expression of activation proteins compared to Comm adults that could result from decreased expression of translation-related RNAs (which was lowest in NH adults). We also demonstrate age- and frailty-related alterations in TLR-induced platelet activation, with decreased activation in Comm vs. young adults, but increased activation in NH adults comparable to that in young individuals. These marked alterations in the platelet transcriptome and activation response despite the absence of genomic transcription implicate platelets in age-associated chronic inflammation and the increased incidence of thrombotic and pro-inflammatory diseases in older adults.

INHIBITION OF DNA POLYMERASE EPSILON LEADS TO ROBUST TUMOR REGRESSION IN MODELS OF TRIPLE-NEGATIVE BREAST CANCER (TNBC)

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Breast cancer remains the second leading cause of cancer-related mortality in women. Triple-Negative breast cancer (TNBC) accounts for 15-25% of all breast cancer cases, exhibiting a relatively poor five-year prognosis. As no targeted therapies exist in TNBC, identifying novel treatment regimens is critical for improving patient outcomes. We discovered that TNBC is especially sensitive to partial suppression of leading strand replicative polymerase, DNA polymerase epsilon (POLE). POLE suppression in TNBC cells leads to replication fork stalling, DNA damage, and a senescence-like state or cell death, phenotypes not observed in other breast cancer subtypes or non-transformed cells. Transcriptomic analysis revealed that suppression of POLE in TNBC cells, but not LUBC cells, led to an increase in inflammatory cytokine transcripts known to be targeted by the NF-κB transcription factor. Suppressing POLE in a mouse 4T1 model of TNBC results in a dramatic reduction in tumor burden and increased immune infiltration in vivo. Together, these findings suggest an interesting hypothesis: that replication stress caused by POLE inhibition leads to robust tumor regression and an alteration in the immune microenvironment of TNBC in a way that can impact cancer therapy. These findings are impactful as they may illuminate an oncology-based strategy for clinically targeting POLE and, in turn, other pathways sensing genotoxic stress.

METABOLIC NANOSCOPY FOR STUDYING AGING AND DISEASES

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Understanding metabolism in living organisms is crucial for uncovering the fundamental mechanisms underlying various biological processes. We developed an optical multimodal metabolic nanoscopy, which integrates DO-SRS, MPF, FLIM, and SHG into a unified molecular imaging platform for studying metabolic dynamics in living organisms at sub-cellular resolutoin. This approach utilizes various deuterated molecules—including glucose, amino acids, fatty acids, and water etc. as bioorthogonal metabolic probes. The enzymatic incorporation of deuterium results in carbon-deuterium (C-D) bonds in newly synthesized molecules, which can be detected by DO-SRS in the Raman spectrum's spectral cell-silent region, distinguishing them from older molecules. This nanoscopy provides novel insights into metabolic heterogeneity across different cell types and organ tissues under both physiological and pathological conditions. For example, it revealed that overexpressed tau proteins significantly disrupt lipid metabolism in aged and Alzheimer's-affected brains, leading to an excessive accumulation of newly formed lipid droplets in glial cells—an effect that can be mitigated by AMPK activation. This advanced nanoscopy imaging platform holds significant potential for disease detection, diagnosis, drug discovery, and evaluating drug efficacy or resistance. It can serve as a tool for understanding the fundamental mechanisms of aging and disease progression.

HISTONE ACETYLTRANSFERASES IN LONGEVITY

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Aging is characterized by a continuous decline in normal physiological functions. Over the past decades, various epigenetic studies have supported the fact that the accumulation of epigenetic alterations accompanies the aging process, which significantly contributes to age-related diseases, such as cancer, metabolic disorders, and Alzheimer's disease. Therefore, understanding the epigenetic mechanisms in aging will provide new avenues to develop strategies to improve health. We have recently reported for the first time that activation of the histone acetyltransferase PCAF-1, the sole homolog of mammalian KAT2A and KAT2B in *C. elegans*, extends the nematode lifespan. Moreover, our findings indicate a tissue-specific role of PCAF-1 in mediating longevity, where neuronal and intestinal activation of this acetyltransferase is sufficient to promote longevity, suggesting a cross-tissue and cell nonautonomous regulation of lifespan. We are currently investigating the downstream mechanisms of PCAF-1 longevity, including epigenetic modifications, transcriptional regulation, and gene expression changes. Overall, our research highlights the importance of histone acetylation in promoting longevity. It also emphasizes the need to study evolutionarily conserved histone acetyltransferases to uncover epigenetic mechanisms that enhance health and longevity across multiple organisms.

THE DISCOVERY OF NUCLEAR LC3 SEQUESTRASOME AND ITS FUNCTION

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The clearance of up-taken exogenous or endogenous material depends critically on LC3, which governs endosome and autophagosome intracellular dynamics. We discovered a novel mechanism that determines the levels of cytoplasmic LC3 through nuclear sequestration. We identified a nuclear multi-protein complex, hereafter referred to as the LC3 sequestrasome. At the core of this complex is the bromodomain-containing protein BRD2, which binds to LC3 and controls, in a signal-dependent fashion, the release of LC3 from the nucleus to the cytosol. BRD2 plays an essential role in LC3 sequestrasome function, and deficiency in BRD2 leads to LC3 compartmentalization exclusively in the cytosol. The greatly elevated level of cytoplasmic LC3 in the absence of BRD2 is accompanied by an increase in the formation of LC3-decorated endosomes and promotes rapid cleavage of the up-taken exogenous material, including β-amyloid. We will discuss the mechanism of sequestrasome formation and the processes responsible for the nuclear retention and release of LC3, as well as the physiological significance of the LC3 sequestrasome in β-amyloid clearance during Alzheimer's disease in mice.

THE ROLE OF THE CG SITE IN DYNAMIC DNA SEQUENCE MUTAGENESIS AND MAMMALIAN LIFESPAN

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Many sequences in the genomes of all mammals are dynamic. That is to say, under certain conditions they can adopt several unusual structures like the G-quadruplex, i-Motif or Triplex. When these structures form in a controlled manner, they may influence gene expression at the level of transcription and translation. These positive functions are balanced by their uncontrolled tendency to form during transcription, where the G-quadruplex often stabilizes R-loops, and during replication where they can hinder replication fork movement often generating point mutations, expansions and deletions. Previous work [2,3,4] showed that the genomic frequencies of representative dynamic sequences scale negatively with lifespan, thus diminishing the potential for mutagenesis in long-lived mammals. In this study, the genomic frequencies of these same representative dynamic sequences were found to scale with published lifespan data on species-specific mutation rates. Moreover, the genomic frequencies of these sequences were also found to scale with published lifespan data on DNA methylation rates. These results are consistent with data showing that DNA methyltransferase specificity and DNA methylation patterns are disrupted by mutation and repair processes. Importantly, mutagenic dynamic sequences containing CG sites do not scale negatively with lifespan. This suggests that DNA methylation and methylated DNA binding proteins function in the suppression of structure formation by mutagenic dynamic sequences containing CG sites. Thus, two endogenous pools of mutagenic dynamic sequences are present in the 126 mammalian species studied here. One comes to a model in which species-specific mutation rates will be enhanced, beyond traditional sources of endogenous mutagenesis, not only by the inborn frequency of dynamic sequences lacking CG sites, but also by the age dependent release of normally latent mutagenic dynamic sequences containing CG sites caused by the mutation linked decay of methylation patterns. These results suggest a key role for dynamic sequences in genomic evolution and provide a link between the observed linear increase in mutations with age and the observed net linear decrease in CG methylation with age in humans. Moreover, they offer an explanation for several conundrums in the field of DNA methylation. Among these are: the absence of DNA methylation in many eukaryotic organisms and the shortened lifespan and elevated mutation rates seen in methylated DNA binding protein deficient mice.

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REORGANIZATION OF THE F-ACTIN CYTOSKELETON IN AGING *DROSOPHILA* NEURONS

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Aging disrupts biomolecule relative concentrations, altering subcellular homeostasis in ways that are still poorly understood. In neurons, an age-dependent increase in filamentous actin (F-actin) levels and aggregation has been reported, yet its underlying mechanisms and consequences for neuronal fitness remain to be elucidated. We uncovered a dramatic age-related rearrangement of the actin cytoskeleton in *Drosophila* neurons, marked by the progressive formation of F-actin dense structures. Correlative light-electron microscopy (CLEM) on aged brains revealed that these structures are bundles of tightly packed, parallel actin filaments localized near the plasma membrane. To uncover key regulators involved in this remodeling, we performed a targeted RNAi screen, thus identifying conserved actin cross-linkers and non-conventional myosins, some of which show increased expression with age. Among them, Myosin XV localizes at the tips of these bundles and drives their elongation when overexpressed. To assess the functional consequences of age-dependent F-actin bundling, we performed co-localization analyses and found a close association between F-actin bundle and endolysosomal markers. Furthermore, downregulating F-actin bundling suppressed the accumulation of lysosomes we normally observed in aged brains, indicating that F-actin reorganization drives age-related changes in intracellular trafficking. Remarkably, this phenomenon is not restricted to *Drosophila*, as we observed a significant increase in F-actin bundling in aged mouse DRG neurons, pointing to an evolutionarily conserved mechanism. Together, our findings showcase how age-related changes in the stoichiometry of actin regulators can alter the neuronal actin cytoskeleton, ultimately affecting cell compartmentalization and homeostasis. This work sheds new light on the subcellular mechanisms of aging that contribute to neuronal dysfunction and may participate in age-associated neurodegeneration.

EVALUATION OF THE HALLMARKS OF AGING USING SENESCENT PRIMARY HUMAN ASTROCYTES

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Introduction: Aging is considered the primary risk factor for neurodegenerative diseases. Astrocytes are the most abundant glial cells in the brain that play a crucial role in central nervous system homeostasis and repair. Importantly, impairment of astrocyte function is linked with cognitive decline and neurodegeneration. Here we seek to understand cellular health across the hallmarks of aging in astrocytes as cells approach senescence.

Methods: To induce senescence, primary human astrocytes were serially passaged until loss of proliferation (a total of 20 passages). We then compared early passage cells (passage 4) versus late passage cells (passage 18) for senescence using Western blots for P16 and P21, astrocyte transition to a reactive phenotype using immunofluorescence for GFAP, and Nanopore sequencing for message RNA profiling. Additionally, we examined mitochondrial health with Seahorse XF assays for metabolic function and live cell staining using flow cytometry with JC-10, Mitotracker, and Mitosox dyes for membrane potential, abundance, and efficiency, respectively.

Results: Late passage cells exhibited increases in P16 and P21 expression compared to early passage cells. We also identified an increase in GFAP expression in late passage cells. Mitochondrial health was also impacted, with the JC-10 assay revealing reduced mitochondrial membrane potential in late passage cells. Interestingly, basal mitochondrial respiration was significantly higher in late versus early passage, yet early passage cells exhibited greater spare respiratory capacity. Ongoing work will examine mitochondrial abundance and efficiency as well as mRNA profiles between early and late passages.

Discussion: Our findings indicate that primary human astrocytes undergo significant phenotypic and functional changes with serial passaging, indicative of cellular aging and senescence. The upregulation of GFAP in late passage astrocytes suggests a reactive state, which is often linked to aging and neuroinflammation. Additionally, the loss of mitochondrial membrane potential may explain the increase in basal respiration and loss of spare respiratory capacity in late passage astrocytes that could result in loss of ATP production and increased oxidative stress. Our future work will explore the capacity of senescent astrocytes to respond to induced stressors (i.e., resilience), the relation of these phenomena to other hallmarks of aging, and examine the molecular mechanisms of astrocyte aging and the connection to neurodegenerative diseases.

Funding: NIH NIA K07AG060266, VA BLRD BX004369, University of Kansas Medical Center.

EXPLORING NOVEL FACTORS THAT IMPACT THE DEVELOPMENT AND HIJACK OF CELLULAR SENESCENCE IN CANCER

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Senescence is a cellular state prevalent at all stages of tumorigenesis, acting as an intrinsic barrier against full-blown malignancy. Multiple cancer associated stressors, like oncogene activation and chemotherapy, can induce senescence and growth arrest. While senescence was initially considered irreversible, we and others have recently uncovered that this arrest can be breached, giving rise to highly invasive, chemo-resistant cancer cells with unstable genomes. However, the molecular mechanisms that govern senescence escape and its contribution to disease progression remain poorly understood. To uncover novel drivers of senescence escape and assess their therapeutic relevance, we conducted a genome-wide CRISPR/Cas9 knockout screen in physiologically relevant senescent cell models. Notably, this screen revealed metabolic pathways as critical players, highlighting a potential link between cellular metabolism and therapy resistance. Our preliminary findings further support this notion, showing elevated DNA damage, mitochondrial dysfunction and altered metabolic phenotypes in cells that have bypassed p21-mediated senescence. High-content imaging, electron microscopy, and Seahorse metabolic profiling revealed progressive mitochondrial cristae deterioration and a switch from oxidative phosphorylation to glycolysis in both senescent and senescence-escaped cells. These shifts suggest that senescent cells undergo metabolic reprogramming that may influence their ability to escape, a process with profound implications for tumor evolution and therapy resistance.

VACCINATION-INDUCED TH17 T CELLS OFFSET THE AGE-RELATED DECLINE IN DURABLE T CELL IMMUNITY

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Older adults are susceptible to infections, in part due to waning of immune memory. To determine mechanisms that fail to sustain long-lasting immunity, we examined varicella zoster virus (VZV) vaccination as a model system. We compared VZV-specific T cells several years after vaccination in adults who had been vaccinated at young (<20 years) or older age (>50 years) with a live-attenuated vaccine that confers durable protection only when given at young age. VZV-specific CD4+ T cells were deficient in an interferon signature at older age but maintained diversity and lacked features of cellular senescence or exhaustion. CD8+ T cells were more susceptible to age and lost diversity and stem-like features while gaining end-differentiated, NK-like signatures. No difference was seen for cellular senescence or exhaustion. Immunization with an adjuvanted VZV component vaccine that elicits durable immunity in older adults did not reverse age-associated defects in CD8+ T cells. Instead, it selectively improved functionality of VZV-specific Th17 CD4+ T cells and prevented their acquisition of Treg features, likely as consequence of lipid metabolic pathways. Our data indicate that effective vaccination in older adults can compensate CD8+ T cell defects by inducing a durable CD4+ Th17 population that resists mis-differentiation into Tregs.

IDENTIFICATION OF SENESCENT NEURONS IN ALZHEIMER'S DISEASE BRAINS WITH HIGH-PLEX, SINGLE-CELL SPATIAL PROFILING PLATFORMS

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Cellular senescence is a change of cell fate triggered by programmed physiological processes or stress responses. These cells are resistant to apoptosis and elicit a harmful secretome, contributing to many age-associated diseases, including Alzheimer's disease (AD) and related dementia. However, identifying senescence cells *in situ* remains challenging due to loss of cell identity and heterogeneous cellular and molecular features. In this study, we employed three spatial proteogenomics platforms to explore postmortem human AD brains and identified a unique type of neuron arrested in a senescent-like cell state we refer to as "G_X".

Using the GeoMx Digital Spatial Profiler, which supports high plex, multi-omic molecular profiling while preserving spatial context, we analyzed discrete regions of interest (ROIs) containing groups of cells in FFPE tissue sections. We selected the stackable multi-omics assay, incorporating the Human Neural Proteome Atlas (500+ targets), Human Immuno-Oncology Proteome Atlas (570+ targets), and the Whole Transcriptome RNA panel. This approach allowed us to examine cellular and molecular changes in relation to AD-associated amyloid and tau pathologies, as well as the proteogenomic features of senescent cells. Our analysis included postmortem hippocampal tissues from individuals with AD, age-matched controls, and young healthy controls. To complement this assay, we utilized the CosMx Spatial Molecular Imager, which enables ultra-high-plex detection of spatially resolved RNA and protein at subcellular resolution. The targeted protein panel focuses on neural cell typing and AD pathology, while the RNA assay provides an unbiased view of 18,000+ protein-coding genes. Finally, we analyzed serial tissue sections with CellScape, which detects low-abundance markers of senescence that were previously difficult to quantify.

Overall, our data provide evidence for a novel neuronal cell state and type, which we refer to as "G_X" arrested neurescent cells, and their spatial proximity to AD neuropathology. Serotherapies clearing senescent cells in AD are currently in Phase 2 clinical testing. The newly identified molecular features of neurescence will aid in the development of neurescent biomarkers and refine target engagement evaluation.

MOLECULAR MECHANISM OF FERROPTOSIS RESISTANCE IN SENESCENT AND CANCER CELLS

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Cellular senescence is a state of irreversible cell cycle arrest induced by many stressors. In the cancer microenvironment, senescent stromal cells induced by various stresses secrete many inflammatory and pro-proliferative factors via the senescence-associated secretory phenotype (SASP), promoting the development and progression of cancer. Recently, multiple therapeutic approaches have been developed to eliminate senescent cells by targeting their cell death resistance pathways. Senescent cells exhibit resistance to ferroptosis, a form of iron-dependent cell death; however, the underlying mechanisms remain unclear. Here, we discovered that lysosomal function was crucial for lipid peroxidation and ferroptosis induction by cystine deprivation. In senescent cells, the expression of component of V-ATPase was downregulated, leading to lysosomal dysfunction and resistance to lipid peroxidation and ferroptosis. V-ATPase inactivation increased inhibited lipid peroxidation and ferroptosis in proliferating cells. Conversely, V-ATPase activation restored ferroptosis sensitivity in senescent cells and therapy-induced senescent cancer cells. Furthermore, the induction of ferroptosis sensitivity prevented pancreatic cancer development in xenograft and Kras mutant mouse models. Our findings reveal a link between lipid peroxidation and the regulation of ferroptosis during cellular senescence, suggesting that inducing ferroptosis in senescent cells could be a therapeutic strategy for the treatment of age-related diseases.

LIFE-EXTENDING INTERVENTIONS AND COMPRESSION OF MORBIDITY: A CASE EXAMPLE DEMONSTRATING A RIGOROUS ANALYTICAL APPROACH

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Despite extensive research on life-extending interventions, rigorous statistical techniques to determine whether these interventions also impact compression of morbidity (CoM) are rarely used. In this paper, we present an illustrative case example of an analytical method to examine the effect of caloric restriction and intermittent fasting on CoM by comparing the rates of vitality decline and survival decline. We hypothesized that the difference between the rates of vitality decline and survival decline would be smaller in the intervention group than in the control group, indicating that life-extending interventions not only extend lifespan but also compress morbidity. Utilizing available data from a previous experimental study in mice, we calculated the average rate of vitality decline by fitting exponential decay models to individual vitality trajectories and compared it with the rate of survival decline estimated from the Cox proportional hazards model. Contrary to our expectations, the results showed that life-extending interventions did not lead to CoM. Instead, these interventions, particularly caloric restriction, suggested a potential expansion of morbidity, as evidenced by a greater difference between the rates of vitality decline and survival decline in the intervention group compared to the control group. These findings challenge the assumption that lifespan extension necessarily compresses morbidity, highlighting the need to consider lifespan, healthspan, and CoM as endpoints when evaluating anti-aging interventions. While we do not claim that life-extending interventions categorically fail to achieve CoM, we present a case example demonstrating how a rigorous analytical approach can be applied to test the CoM hypothesis. Our framework offers a valuable tool for future studies, and further refining this method will be crucial to determine under which circumstances lifespan extension leads to CoM.

Funding: Supported in part by NIH grants P30AG050886, U24AG056053, and the Gordon and Betty Moore Foundation. The opinions expressed are those of the authors and do not necessarily represent those of the NIH or any other organization.

SPATIOTEMPORAL PROTEOSTASIS REWIRING DURING SENESCENCE PROGRESSION REVEALED BY COMPREHENSIVE TRANSLATOME AND MITOCHONDRIAL IMPORTOME ANALYSES

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Cellular proteostasis declines with age, and mitochondria are essential for maintaining proteostasis. Mitochondrial dysfunction drives the formation of senescent cells, marked by the accumulation of mitochondria. Despite the critical role of mitochondrial protein import (MPI) in maintaining proteostasis, the regulation of MPI and global cellular translation during senescence progression remains elusive. Leveraging our newly developed multiplexed enhanced protein dynamics (mePROD) proteomics approach, we quantified time-resolved global changes in translation and mitochondrial protein uptake in response to replicative, DNA damage, and mitochondrial stress-induced senescence.

Our study identified a reduction in the global translation profile during senescence progression, with mitochondrial protein import decreasing as a result of reduced mitochondrial translation. However, essential mitochondrial processes, such as anti-apoptotic pathways, fatty acid beta-oxidation, glutathione metabolism, and branched-chain amino acid processing, continue to be imported to support the senescent state. Senescence-associated secretory processes are enriched in the translatome, in contrast to the diminished cellular proteostasis processes. Despite the overall reduction in translation, we observed the accumulation of specific organelle proteomes, particularly in mitochondria, lysosomes, and peroxisomes, suggesting impairments in both proteome and organelle degradation pathways during senescence. Additionally, we noted a decline in chaperone translation, along with alterations in macro-autophagy, selective mitophagy, pexophagy, and an elevated immune proteasome. Our findings enhance the understanding of proteostasis dysfunction during senescence progression and open new avenues for therapeutic interventions to attenuate aging-related phenotypes.

ENHANCED SURVIVAL AND MUSCLE FUNCTION IN NATURALLY AGED MICE: A LINK TO INFLAMMAGING?

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Aging is the primary risk factor for many neuromusculoskeletal diseases, therefore, systemic therapies which impact multiple tissues simultaneously can be more impactful than tissue-specific approaches. Our previous findings show that systemic transplantation of our unique adult multipotent muscle-derived stem/progenitor cells (MDSPCs) from young mice—but not old—delays the onset of aging-related diseases and doubles the lifespan in mouse models of progeria. In addition, induced neovascularization in muscle and brain—where no transplanted cells were detected—strongly suggests a therapeutic secretome-related paracrine/endocrine mechanism. This hypothesis was further supported in systemically transplanted 22mo-old naturally aged mice, which exhibited increased ambulation speed, voluntary activity, muscle vascularization, and reduction of articular cartilage inflammation 2mo post-injection. Our current findings, from a cohort of 23mo-old naturally aged mice systemically transplanted with young MDSPCs (n=16, NA-IP), exhibited significantly increased survival (100%) compared to a cohort of PBS-injected mice (n=18, NA-PBS; 66.7%) 1mo post-transplantation. Longitudinal functional performance was tested by the four-limb hang test, a clinically relevant measure of muscle fatigability. Starting at 1wk post-injection, NA-IP mice show significantly less muscle fatigability as measured by holding impulse (hang time x body weight) than NA-PBS. Similarly, post-treatment scores normalized to pre-treatment averages demonstrated consistent improvement each week for NA-IP mice. The NA-PBS cohort, however, never achieved its pre-injection score again. Normalized holding Impulse scores were significantly greater in NA-IP mice, compared to NA-PBS mice, at 3wk and 4wk post-transplantation, indicating less fatigability in cell treated mice. Gastrocnemius and quadriceps weights, normalized to body weight, were significantly greater in NA-IP mice compared to NA-PBS and show significant positive correlation with function. Interestingly, we observed significant weight variance of the spleen, a major immune organ, in NA-PBS mice, due to drastic spleen weight increases in mice with poor function. By plotting normalized spleen weight against muscle function, we discovered a significant inverse correlation, indicating a possible connection between the spleen, chronic inflammation, and muscle function. Ongoing serum and spleen analysis should provide a better understanding of the connection between inflammaging, systemic immune signaling through the aging spleen, and their impact on function. Together, these novel findings provide further evidence of systemically delivered young MDSPCs functionally rejuvenating aged tissues and their potential impact on inflammaging. The concurrent nature of this systemic pro-angiogenesis and immune-modulatory therapy may serve as a novel intervention strategy to promote healthy neuromusculoskeletal aging.

AGE-RELATED TRAJECTORIES OF MITOCHONDRIAL FUNCTION IN LONG-LIVED AND SHORT-LIVED AD-BXD MOUSE GENOMETYPES

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The link between mitochondrial dysfunction and age-related diseases, especially Alzheimer's (AD), is well known, but its mechanism remains unclear. A major gap is the unknown interaction between mitochondrial function, genetics, and health. Many animal models use a one genome in one environment approach, limiting the scope and span of the studied phenotypes such as longevity or environmental robustness failing to reflect human genetic and environmental diversity. This issue is compounded by the many factors affecting mitochondrial function.

Our study explores how genotype, environment, and mitochondrial function interact. We present data on the age-related mitochondrial function changes in crosses between long- and short-lived BXD recombinant inbred strains and 5XFAD mice, creating isogenic, replicable crosses (AD-BXD), displaying the age-related Aβ toxicity effect. We use two separate long-lived AD-BXD crosses (different genetic backgrounds) to search for traits reliably associated with longevity. We provide detailed, tissue-specific and electron transport chain complex-specific data on mitochondrial function characterized by the combination of age, sex, genetic background, and 5XFAD transgene status. Our analysis takes two approaches: on the group level, to assess sex, age, or transgene effects; and on the strain level, to look for associations with other phenotypes collected in the population, including behavior and neuroanatomy.

Our data show how respiratory profiles shift in AD-BXD mice from 6 to 14 months. We used high-resolution respirometry (HRR), a method with low throughput which limits studies to a small number of groups, precluding the production of large multi-factor datasets. However, by using an incomplete nested design and linear mixed models we constructed bioenergetic profiles that show the contribution of each factor. This approach overcomes key throughput challenges in bioenergetics while leveraging the AD-BXD's well-defined genetics.

Our findings clarify how genetics, environment, and mitochondrial function interact in aging and AD, advancing our understanding of the factors behind longevity and laying the groundwork for future translatable interventions.

Supported by NIH/NIA R01AG075813-01, AG070913-01, UTHSC CITG.

HIGHLY MULTIPLEXED IMAGING TO ANALYZE SPATIOTEMPORAL CELLULAR SENESCENCE

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Cell states are regulated by responsive signal pathways upon ligand binding to the receptor and inter-cellular interactions. Thus, high-resolution imaging has been attempted to explore the dynamics of signaling. Recently, multiplexed imaging has been introduced to profile cell state by acquisition of comprehensive spatial protein information in the cells. However, it is still challenging to extract the dynamics of multiple signal activations due to compromised resolution for visualizing the molecules.

In this study, we developed a fluo-erasable antibody 'Precise Emission Canceling Antibody (PECAb)' for multiplexed imaging. Antibodies were reacted with DBCO-SS-NHS ester and then with azide-modified fluorescent dyes to label the antibodies through the SS linker. Immuno-staining with the PECAb indicated the detection of the antigen-specific signal and erasing the signals by using reducing reagent TCEP. After erasing, other antigen-specific signals were detected by using other PECAbs. These results indicated that sequential staining with PECAb is feasible.

To analyze cell state dynamics, we used IMR90 oncogene-induced senescence (OIS) model. The PECAbs allowed high-resolution sequential imaging using 206 antibodies and it allowed reconstruction of the spatiotemporal dynamics of signaling pathways during OIS. Additionally, combining this approach with a spatial transcriptome method, seq-smFISH, can effectively classify cells and identify their signal activation states in human tissue. Overall, the PECAb system serves as a comprehensive platform for analyzing complex cell processes.

CELLULAR SENESCENCE IN THE REGENERATION OF *SYLLIS MALAQUINI (SYLLIDAE, ANNELIDA)*

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Syllis malaquini is a marine annelid with remarkable regenerative abilities, capable of regenerating both anterior and posterior structures, including complex tissues such as the nervous system. Cellular senescence promotes tissue development during embryogenesis and participates in tissue regeneration from fish to mammals. In this work we have explored the role of cellular senescence after *S. malaquini* amputation. Our results show positive SA-β-gal areas associated to negative proliferation along with the presence of MMP1 during the regeneration process, particularly in the early stages of blastema development. The impact of senolytic drugs on regeneration reveals that modulating these pathways affects both SA-β-gal staining and regenerative outcomes. Our findings emphasize the utility of *S. malaquini* as a powerful invertebrate model for studying cell senescence during regeneration in systems with advanced tissue complexity, expanding the understanding of regeneration mechanisms beyond traditional vertebrate models.

DIETARY RESTRICTION AMELIORATES METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE VIA ACTIVATING CISD2 PROLONGEVITY GENE

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CISD2 is one of the eight pro-longevity genes identified in mammals, functioning on modulating intracellular Ca2+ homeostasis and mitochondrial functions. Notably, a persistently high level of CISD2 extends a healthy lifespan in natural aging mice. However, the molecular mechanism underlying the regulation of CISD2 expression remains elusive. Intriguingly, using CISD2 reporter mice, we found 24-hour fasting significantly enhances CISD2 expression at the transcriptional level. In addition, both dietary restriction (DR) and CISD2 transgenic mice exhibit similar health benefits, including increase of lifespan and improvement of metabolic dysfunction-associated fatty liver disease (MAFLD). In this study, we investigate the role of CISD2 in DR using a mouse model of Western-diet (WD)-induced MAFLD in wild-type and hepatocyte-specific Cisd2 knockout mice. Three findings are pinpointed. Firstly, DR enhances hepatic CISD2 expression, ameliorates WD-induced insulin resistance and glucose intolerance, and improves MAFLD in a CISD2-dependent manner. Secondly, liver transcriptomic study revealed that in the mice treated with DR for 6 months, the CISD2-dependent differential expression genes (DEGs) are mainly involved in peroxisomal lipid metabolism, glucose metabolism, and glutathione-mediated detoxification. Thirdly, analysis of transcriptome profiles identified the Aryl hydrocarbon receptor (AhR), which is involved in the maintenance of hepatic mitochondrial homeostasis, as a potential upstream regulator for CISD2. Interestingly, AhR knockdown in AML12, a hepatocyte cell line, attenuates the induction of CISD2 under fasting condition, suggesting that AhR is a transcriptional activator upstream to CISD2 gene expression. In summary, our findings demonstrate that CISD2 plays a crucial role in mediating the beneficial effects of DR on glucose homeostasis and MAFLD, and highlights CISD2 activator as an alternative therapeutic approach to treat WD-induced metabolic disorders.

DISSECTING THE CELLULAR MECHANISMS OF LUNG PARENCHYMA AGING

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Aging affects lung function, predisposing older adults to respiratory diseases; however, the cellular and molecular mechanisms of lung aging are not fully understood. Leveraging scRNA-seq data from 184 lung parenchyma samples, we built an analytical platform to dissect the cell composition, gene expression modules, and regulatory changes linked to multiple hallmarks of lung aging. Our findings show cell type-specific age-association of senescence markers and a decline in alveolar cell proliferation, autocrine WNT signaling, and stemness indicators with advancing age. Analysis of myeloid cells reveals a global reduction in macrophage subsets and a surge in mitochondrial dysfunction and inflammatory signaling. In contrast, T cells expanded with age and showed heightened interferon gamma expression, cytotoxic activity, and exhaustion in older lung and blood samples, indicative of age-related immune dysfunction. Cell-cell communication analysis revealed aberrant myeloid-T cell cross-talk, leading to increased T cell chemotaxis and activation. Lastly, we built a machine learning-based predictor of lung biological age that identified new biomarkers of disease risk.

MULTIOMIC PROFILING OF HER2-MEDIATED ONCOGENE INDUCED SENESCENCE IN HUMAN MAMMARY EPITHELIAL CELLS

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Oncogene-induced senescence (OIS) is a critical tumor-suppressive mechanism that prevents malignant transformation in response to aberrant oncogenic signaling. However, recent evidence suggests that OIS is not always a permanent barrier to tumorigenesis, as some cells can evade the proliferative arrest and acquire enhanced growth advantages. This is particularly relevant in early-stage breast cancer, where studies on ductal carcinoma in situ (DCIS) have shown that the co-expression of senescence and proliferation markers, such as p16 and Ki67, is associated with subsequent tumor progression and poor prognosis. Despite its importance, the gene regulatory mechanisms that regulate entry into and escape from OIS in the early stages of breast cancer remain unclear. This can be mainly attributed to the absence of an *in vitro* model that can accurately reproduce OIS in human mammary epithelial cells (HMECs) directly relevant to human breast cancer (BC). Here, we generated a highly robust and flexible doxycycline (DOX)-inducible system of human epidermal growth factor receptor 2 (HER2/ERBB2) overexpression in primary non-immortalized HMECs (HMEC-iERBB2). Using this system, we successfully overexpressed HER2 in a doxycycline-dependent manner in normal HMECs to induce proliferative arrest accompanied with phenotypic and molecular changes characteristic of an OIS response. By employing a time-resolved multiomic analysis that our lab has previously established, we uncovered the transcriptional and epigenetic changes that define the evolution of the OIS phenotype over time both in bulk and at single-cell resolution. Analysis of the transcriptome over the first two weeks of HER2-induction reveals a highly dynamic OIS response with a shared core gene signature in common with the well characterized model of WI38 fibroblasts undergoing $HRAS^{G12V}$-mediated OIS. Intriguingly, HER2-mediated OIS upregulates substantially anti-viral interferon (IFN) responses along with pathways associated with cell-plasticity, which is also corroborated by the expression of several TFs involved in inflammation and stemness, including POU5F1/OCT4, KLF3/4 and RELB. Furthermore, our preliminary data of time resolved single-cell profiling demonstrate increased heterogeneity of the HER2-mediated OIS responses and reveal cell populations with shared features of premalignant senescent cells.

REGULATION OF BECLIN1/BCL-2 COMPLEX IN DRUG-TOLERANT PERSISTER (DTP) COLORECTAL CANCER CELLS

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Colorectal cancer (CRC) represents the second most lethal cancer worldwide. Standard-of-care treatment typically includes surgery and chemotherapy. While many patients initially respond positively to chemotherapy, drug resistance often develops over time. Before the emergence of genetic mutations, cells are described to enter a drug-tolerant persister (DTP) state. Upon cessation of treatment, these cells can regenerate parental tumors. Targeting DTP cells before the development of genetic mutations is considered a potential therapeutic strategy against CRC. Our research group has identified crucial roles for the autophagy and anti-apoptotic pathways in CRC DTP cell survival. It is well established that the Beclin1/Bcl-2 complex is a key regulator of both pathways. In response to cellular stressors, Beclin1 and Bcl-2 family members dissociate, triggering Beclin1-dependent autophagy and apoptosis inhibition via Bcl-2 proteins. The Beclin1/Bcl-2 complex interaction has been associated with aging. Enhanced autophagy mediated by impaired interaction between Beclin1 and Bcl-2 family members improved health and extended lifespan. In cancer research specifically, much remains to be understood regarding the complex interaction. We hypothesize that the DTP state is dependent on the separation of Beclin1 and Bcl-2 family members, which increases both autophagy and anti-apoptotic signaling. To better comprehend the molecular mechanisms that drive and maintain the DTP state, we aim to investigate the Beclin1/Bcl-2 complex expression, its potential regulators and the effects of modulating this interaction. Preliminary data indicate that Beclin1 and Bcl-2 family members are upregulated in CRC DTP cells at both mRNA and protein levels while Proximity Ligation Assays (PLA) further demonstrate that interactions between both Beclin1/Bcl-2 and Beclin1/Bcl-xL are disrupted in the DTP state. Moreover, key regulators of the complex, including Klotho, MST1, and JNK, exhibit altered expression in DTP cells. Identifying the mechanisms that keep Beclin1 and Bcl-2 family members bound together and inducing complex interaction might prevent CRC cells from entering the DTP state and therefore prevent tumor relapse.

INVESTIGATING ENDOSOME-mRNA ASSOCIATION IN AGING NEURONS.

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The endocytic pathway is a highly dynamic and interconnected system that carries out the transport of specific cargos throughout the cytoplasm between distinct membrane-bound cellular compartments. In neurons, defects in endosomal trafficking have been reported to occur during aging and age-related disorders, and genetically mimicking these alterations was found to cause a neurodegenerative cascade. It appears therefore essential to understand what causes the observed age-related decline in endosomal trafficking and the related functional consequences. Recently, mRNAs and ribosomes have been found on endosomes suggesting a direct role for these organelles in controlling protein synthesis in neurons. However, the cause-effect relation between dysfunctional endosomal trafficking and dysregulation of mRNA distribution and translation in aged neuronal cells is still unknown.

Here, we report that early endosomes harbor a complex and rich transcriptome in mature neurons. Isolation of Rab5-associated endosomes coupled with NGS sequencing revealed the presence of hundreds of transcripts associated with multiple functions and organelles. These findings were validated by single molecule in situ hybridization, further showing specific endosome-mRNA associations in different neuronal compartments. We next established in vitro and in vivo models of neuronal aging, confirming age-related defects in early endosomal trafficking. We now aim at identifying changes in the endosomal-associated transcriptome in aged neurons and determine the related functional consequences.

A GOLGI-STRESS PATHWAY RESPONSIVE TO MEMBRANE LIPIDS IS ACTIVATED IN A LONG-LIVED *C. ELEGANS* MODEL

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Methylation is a central regulatory mechanism that links metabolism of methionine and folate to control of gene expression through histone methylation and affects membrane properties through phosphatidylcholine (PC) production. In *C. elegans*, the methyl donor S-adenosylmethione (SAM) can be produced by one of four synthases *sams-1, sams-3, sams-4* and *sams-5*. One physiological pathway that is particularly sensitive to SAM levels is the cellular stress response. Each external stress response elicits a specific gene expression program and we have found that loss or *sams-1* or *sams-4* have distinct effects on survival during heat stress, gene expression and histone modification profiles, demonstrating that the enzymatic source of SAM can influence how it is used. Stress responses can also be generated from internal sources, such as membrane stress responses that occur when phospholipids levels become unbalanced, or acyl chain desaturation is altered. SAM derived specifically from *sams-1* can induce membrane stress, as production of the methylated phospholipid PC is compromised. This stress response appears distinct from ER derived lipid bilayer stress and blocks cycling of the GTPase ARF-1, disrupts its localization on the Golgi ministacks. Using TEM, we also find that Golgi ministack structure is lost. We previously showed oss of Golgi integrity promoted proteolytic maturation of the lipogenic transcription factor SBP-1/SREBP1. Recently, we found that maturation of another ER-resident transcription factor, LET-607/CREBH, is similarly stimulated by low PC and drive the activation of *warf-1/arf-1.1*. WARF-1 accumulates on Golgi puncta in low PC, replacing ARF-1. Interestingly, low PC-induced *let-607* maturation occurs in the intestine, but not the hypodermis, where ER stress regulators appear to have a greater impact on its regulation. Our RNA seq experiments also suggest distinctions between Golgi and ER stress gene expression programs after loss of PC. Taken together, our work highlights the distinct ways metabolism and stress responses can interact to regulate transcriptional programs. Understanding how and where SAM acts and how that influences cellular methylation pathways is made will allow integration of nutritional and metabolic processes with molecular pathways regulating stress, aging and lipogenesis.

S-ADENOSYLMETHIONINE SYNTHASE SPECIFIC METABOLIC CHANGES ARE LINKED TO INCREASED MITOPHAGY IN LONG-LIVED SAMS-1 ANIMALS

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The 1-carbon cycle and one of it's central metabolites, S-adenosylmethione (SAM), is linked to lifespan extension. SAM can be produced by one of two synthases in mammals (MAT1A or MAT2A). MAT1A is thought to be specific to the liver and MAT2A is required for viability, making it difficult to dissect pathway-specific functions. However, in C. elegans, this family of enzymes has expanded to 4 enzymes, *sams-1, sams-3, sams-4* and *sams-5*. While knockdown of each synthase decreases SAM similar amounts, loss of sams-1 is associated with increased lifespan and survival in heat stress whereas *C. elegans* lacking *sams-4* die rapidly under heat stress. This suggests that SAM from SAMS-1 or SAMS-4 each has unique roles in aging and stress responses. In order to determine how SAM from these enzymes might be used differently, we compared multiple -omics techniques and identified changes in gene expression, H3K4me3 patterns, lipids, polar metabolites, proximity proteomes and lysine methyl proteomes that are specific to each synthase. Strikingly, while *sams-1(RNAi)* animals show many changes in levels of steady state metabolites, *sams-4* animals exhibit few changes in comparison to wild type, in basal or heat shocked conditions. This suggests metabolic changes are not linked to the limited survival of *sams-4(RNAi)* animals after heat shock. Changes in several key metabolites were specific to *sams-1*, including increases in lipid classes of phosphatidylcholine (PC) and triglycerides, changes in polyamines and a general decrease in mitochondrial metabolites. Published studies have linked changes in the 1-carbon cycle and *sams-1(RNAi)* to increase mitochondrial fission (Wei and Ruvkun, PNAS 2020) which were hypothesized to stem from epigenetic changes. We also find increased mitochondrial fission after *sams-1(RNAi)*, but this is not found in *sams-3, -4 or -5 RNAi* animals, suggesting that lowering SAM is not sufficient to induces mitochondrial phenotypes. We found that mitochondrial fission defects were rescued by dietary choline, which restores PC levels and phenocopied by RNAi of PC biosynthetic enzymes. This suggests that changes in PC downstream of SAMS-1 are important for mitochondrial changes. Supporting importance of these phenotypes to SAMS-1 biology, we found a dramatic downregulation of genes acting in mitochondria as *sams-1* animals age, decreases in mitochondrial proteins in proximity proteomics and reduction in methylation of mitochondrial proteins. Autophagy has been shown to increase in hypodermal tissues after loss of *sams-1*, based on this data, we next asked if increases in mitophagy might explain the loss of mitochondrial proteins and metabolites. Indeed, strains harboring intestine-specific LGG-1::DFP along with mKate::TOMM-20 showed increases in mitochondria within autophagosomes. As increases in mitophagy have been linked with heat stress survival and aging, we hypothesize that loss of SAM from *sams-1*, but not *sams-4*, has specific effects on mitophagy, driving changes in lifespan and stress resistance.

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INVESTIGATING METHIONINE RESTRICTION AND ITS LINK TO SAM SYNTHASE-SPECIFIC PHENOTYPES IN *CAENORHABDITIS ELEGANS*

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Methionine restriction (MetR) has been shown to extend lifespan across various species, but isolating the underlying mechanisms is challenging due to methionine's involvement in numerous pathways. Central to methionine metabolism is the Met/SAM cycle, where S-adenosylmethionine (SAM), a key methyl donor, is synthesized from methionine and ATP by S-adenosylmethionine synthases (SAMS). In *Caenorhabditis elegans*, loss of different SAM synthases produces distinct phenotypic outcomes. After heat shock, *C. elegans* lacking *sams-1* live longer, whereas *C. elegans* lacking *sams-4* die more rapidly. Additionally, *sams-1* loss causes fertility defects, which *sams-4* loss does not. These contrasting phenotypes provide an opportunity to investigate how MetR affects downstream metabolism. We have developed models for restricting methionine in the *C. elegans* diet, and our findings could reveal new targets for interventions aimed at promoting longevity through metabolic manipulation.

FOLATE STRESS ELEVATES THE LEVEL OF ENDOGENOUS GENOTOXIC FORMALDEHYDE

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Formaldehyde is a reactive chemical that causes toxicities in humans following elevated exposure. Whilst formaldehyde is typically associated with environmental chemicals, recent evidence reveals that high levels of formaldehyde is produced by mammalian metabolism to cause DNA damage, which can lead to accelerated aging and cancer. This is devastating for children with genetic deficiencies in formaldehyde detoxification enzymes (ALDH2 and ADH5) and the Fanconi anemia (FA) DNA repair pathway. Afflicted children accumulate spontaneous formaldehyde-DNA damage leading to accelerated aging, loss of blood stem cells, and cancer. There is a current knowledge gap on the *in vivo* physio-pathological factors that impact the level of endogenous formaldehyde. Without this knowledge, we will not understand how endogenous formaldehyde impacts genome stability and aging in the broader general population. We hypothesize that folate metabolism plays an important role in maintaining formaldehyde homeostasis in mammalian cells. Previous studies have shown that folate metabolites can biochemically release formaldehyde *in vitro*, but the *in vivo* contribution of folate metabolism to endogenous formaldehyde has not been studied. To address this, we have optimized sensitive mass spectrometry methods to quantify endogenous formaldehyde in mammalian tissues. Our preliminary results reveal that, in contrast to the *in vitro* release of formaldehyde by folates, excessive supplementation of folic acid in mice do not result in accumulation of endogenous formaldehyde. We proceeded to test the effect of folate deficiency on endogenous formaldehyde using a transgenic mouse strain with deletion of the gut folate receptor PCFT. These mice develop progressive folate deficiency following birth, leading to megaloblastic anemia and failure to thrive. Strikingly, we observe elevated formaldehyde in the liver, bone marrow and spleen. To further dissect this elevated formaldehyde triggered by folate-depletion, we generated mice with deletions in both formaldehyde detoxification enzyme ADH5 and PCFT. These double knockout mice show synergistic increase in endogenous formaldehyde, associated with worsening red cell macrocytosis and micronuclei, a marker of hematopoietic DNA damage. Overall, our work identifies a novel mechanism of formaldehyde-DNA damage arising from folate deficiency. Furthermore, we highlight folate metabolism as an important safeguard against the toxic accumulation of endogenous formaldehyde.

PD-LINKED LRRK2 G2019S MUTATION IMPAIRS ASTROCYTE MORPHOLOGY VIA ERM HYPERPHOSPHORYLATION

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Astrocytes are highly complex cells that mediate critical roles in synapse formation and maintenance by establishing thousands of direct contacts with synapses through their perisynaptic processes. Here, we found that the most common Parkinsonism gene mutation, LRRK2 G2019S, enhances the phosphorylation of the ERM proteins (Ezrin, Radixin, and Moesin), components of the perisynaptic astrocyte processes in a subset of cortical astrocytes. The ERM hyperphosphorylation was accompanied by decreased astrocyte morphological complexity. Dampening ERM phosphorylation levels in LRRK2 G2019S mouse astrocytes restored their morphology in the anterior cingulate cortex. To determine how LRRK2 mutation impacts Ezrin interactome, we used an in vivo BioID proteomic approach, and we found that astrocytic Ezrin interacts with Atg7, a master regulator of autophagy. The Ezrin/Atg7 interaction is inhibited by Ezrin phosphorylation, thus diminished in LRRK2 G2019S astrocytes. Importantly, the Atg7 function is required to maintain proper astrocyte morphology. Our data provide a molecular pathway through which the LRRK2 G2019S mutation alters astrocyte morphology in a brain-region-specific manner.

PROTEIN AGGREGATE DISASSEMBLY INTO FUNCTIONAL PROTEIN MONOMERS TO RE-INSTALL PROTEIN HOMEOSTASIS AND TO TREAT NEURODEGENERATIVE DISEASES.

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Age dependent impairment of protein homeostasis is discussed as one cause of increased accumulation of protein aggregation. In turn, age-dependent protein misfolding diseases interfere with efficient protein homeostasis – leading to a viscous cycle. Neurodegenerative protein-misfolding diseases, like Parkinson's and Alzheimer's disease (AD), are driven by prion-like self-replicating and propagating protein assemblies of Aβ, α-synuclein, and many more. The conformation of these proteins in the aggregated state is thermodynamically more stable than their physiological monomer conformation. Therefore, we have developed all-D-enantiomeric peptide ligands that are specific for the respective target protein and bind the monomeric protein of interest with high affinity, thereby stabilizing them in their native conformation. This leads to the disassembly of oligomers and fibrils into monomers, and allows re-installation of homeostasis.
The ligand for α-synuclein, SVD-1a, disassembled preformed α-synuclein fibrils (PFF) as shown by AFM and DLS. SPR and NMR demonstrated picomolar affinity of SVD-1a to α-synuclein monomers, while keeping them in their physiological IDP conformation.
The ligand for Aβ, RD2, demonstrated ex vivo target engagement and disassembled Aβ oligomers obtained from brain tissue of former AD patients (Kass et al., Cell Rep. Med. 3, 100630, 2022). A clinical phase Ib, double-bind, placebo-controlled study with 20 mild cognitively impaired (MCI) and mild AD patients treated once daily orally with RD2 for four weeks with an additional four weeks follow-up period yielded good safety and tolerability. Also, as demonstrated and published before with four different animal models in four different laboratories, patients treated with RD2 improved their short term memory abilities significantly, as shown with the Word List assay of the CERAD battery of neurocognitive testing. A placebo controlled double-blind proof-of-concept phase II study with 300 patients treated orally over 12 to 24 months with RD2 at two different doses or placebo has finished patient recruitment.
I will also acknowledge the many contributors of both developments that are too many to be included here in the abstract.

OXR1 REGULATES CELLULAR SENESCENCE AND NEURONAL AGING THROUGH RETROMER-MEDIATED ACTIN BRANCHING

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The accumulation of senescent cells in the aging brain is associated with numerous age-related disorders but its link to the retromer complex and endolysosomal trafficking are unknown. The neuronal retromer plays an essential role in trafficking endocytosed lipids and proteins, and loss of retromer function contributes to neuron decline and neurodegenerative disease. We previously reported that the neuronal stress response protein Oxidation Resistance 1 (OXR1) maintains retromer function. We now find that fibroblasts from patients with bi-allelic loss of function *OXR1* mutations become senescent at an increased rate, marked by senescence-associated markers such as p16 and p21, cytoplasmic DNA, and increased release of the senescence-associated secretory phenotype (SASP). Similarly, knockdown of *OXR1* in iPSC-derived neurons recapitulates these senescence-associated markers and inhibits neuronal network formation. We show that loss of OXR1 disrupts retromer interaction with the WASH complex, thereby inhibiting F-actin branching. This leads to destabilization of the nuclear membrane and activation of the cGAS-STING inflammation pathway. Pharmacological stabilization of the retromer with the compound R55 rescues these phenotypes. Further, we find that neuronal overexpression of OXR1 improves spatial learning and memory in mice. In summary, the endolysosomal function through OXR1 and retromer function is essential to prevent cellular senescence and serves as a valuable target to promote healthy brain aging.

ACTIVATION OF THE LACTATE RECEPTOR RESTORES LIPID METABOLISM AND REVERSES VASCULAR SENESCENCE IN VITRO AND IN VIVO

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Vascular senescence is associated with systemic metabolic dysfunction, including impaired lipid metabolism and increased lipid accumulation. Excessive lipid accumulation is well-documented to contribute to lipotoxicity, atherosclerosis and impaired cardiovascular function.

A previous study in our lab reported that loss of the lactate receptor, GPR81, is associated with impaired fatty acid metabolism and skeletal muscle aging using a mouse model of premature aging (progeria). Building on this, we investigated the role of GPR81 activation in alleviating vascular senescence. Specifically, we treated senescent vascular cells, human umbilical vein endothelial cells (HUVECs) and vascular smooth muscle cells (VSMCs), with GPR81 agonist, 3-chloro-5-hydroxy BA (CHBA), for 7 days and examine lipid content and hallmarks of cellular senescence. Interestingly, CHBA treatment resulted in significant reduction in lipid accumulation, as well as increased cell proliferation and marked decrease in key senescence markers including inflammatory cytokines, DNA damage, reactive oxygen species (ROS) and expression of p21 as compared to untreated cells. Seahorse analysis further revealed that GPR81 activation restored lipid oxidation, fueling mitochondrial oxidative phosphorylation that was impaired by aging. Notably, CHBA decreased lipid peroxidation and the intracellular ferrous iron pool, reducing the levels of ferroptosis, a widely recognized programmed cell death pathway that is active in aged tissues and cells.

In addition, we employed a mouse model of premature aging to evaluate the role of GPR81 agonist in aged arteries. We discovered that treatment of progeria mice with CHBA for one month reduced lipid accumulation and reversed many of the aging hallmarks in aged arteries, including the levels of SA-β-gal, p21 and the high mobility group box 1 (HMGB1). Notably, activating GPR81 preserved elastin fibers and collagen content that were both compromised by aging.

Overall, our studies demonstrate the potential of lactate receptor GPR81 activation to restore lipid metabolism and reverse vascular senescence, suggesting that GPR81 agonist, CHBA, may be a promising therapeutic candidate in reversing or slowing cardiovascular aging and improving health span.

TISSUE REMODELING AND SENESCENCE UNDERLY AGE-RELATED ADIPOSE TISSUE INFLAMMATION

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Adipose tissue dysfunction is a hallmark of aging and is associated with numerous age-related diseases, including metabolic, cardiovascular and neurodegenerative diseases (1-2). Characterizing age-associated adipose tissue changes is crucial for elucidating how adipose tissue contributes to overall health.

We performed a comprehensive analysis of adipose tissues throughout the murine lifespan and uncovered significant age-related remodeling. Adiposity, characterized by increased adipose tissue weight and lipid deposition in the liver, increased through midlife before declining in later life. Interestingly, while adipocyte size coincided with peak adiposity, the number of adipocytes continued to increase with age. Using single-nucleus transcriptomics, we identified two waves of increased inflammation in the adipose tissue: one associated with the peak of adiposity and one with advanced age. Furthermore, senescent-cell burden increased with age and positively correlated with adipose tissue inflammation. Given these results, we hypothesized that senescent cells play a causal role in adipose tissue aging.

To investigate the contribution of senescent cells to aging, we transplanted senescent or control preadipocytes into young mice. These transplanted cells accumulated in visceral adipose tissue. Consistent with prior studies (3), senescent cell exposure induced physical dysfunction in young mice. We observed several age-related transcriptome changes in the adipose tissue of senescent cell recipients. Furthermore, our data suggest a novel mechanism by which senescent cells remodel the vasculature to enhance CD4+ T cell recruitment into aged adipose tissue.

Together, these findings suggest a critical role of tissue remodeling and senescent cells in driving age-related adipose tissue inflammation.

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SPATIAL TRANSCRIPTOMICS REVEALS SENESCENCE-ASSOCIATED TISSUE REMODELING IN AGING MOUSE LIVER

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Senescent cells accumulate in aging tissues and contribute to the deterioration of tissue function and increased susceptibility to aging-related diseases. Despite their importance, the mechanisms underlying the emergence of senescent cells within native tissues during aging, their spatial and transcriptional organization, and their interactions with the surrounding microenvironment remain poorly understood. Here we applied image-based spatial transcriptomics to young and aged mouse livers and generated a comprehensive liver-senescence-focused spatial transcriptomic atlas at true cellular resolution. This spatial atlas, comprising 526,318 cells, revealed a marked periportalization associated with liver aging, sex- and lobe-dependent patterns in senescent cell distribution, spatial clustering of senescent cells, and cell-type-specific influences of senescence cells on their local microenvironment. These findings provide new insights into the aging-related, cell-type-specific senescent phenotypes (senotypes), highlighting opportunities for developing targeted senotherapeutic and immunotherapeutic interventions. Additionally, we identified contrasting immune microenvironments and signaling dynamics in aged versus senescent cells, underscoring the importance of further exploring the interplay between aging and cellular senescence.

HYPOAD: VOLUMETRIC AND SINGLE-CELL ANALYSIS OF THE HUMAN HYPOTHALAMUS IN AGING AND ALZHEIMER'S DISEASE

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The hypothalamus integrates peripheral signals to regulate fundamental homeostatic and survival-related functions, including metabolism, sleep, and stress responses. Although Alzheimer's disease (AD) is primarily characterized by progressive memory decline, hypothalamic dysfunction - commonly characterized as abnormal eating behaviors, weight loss, sleep disturbances, and neuroendocrine imbalances - occurs early and persists throughout the progression of the disease. Despite these well-documented symptoms, the impact of AD on distinct hypothalamic subregions and cell types remains poorly understood. Here, we present *hypoAD*, a comprehensive analysis of hypothalamic subregion-specific volumetric and molecular changes during aging and early-stage AD. By integrating high-resolution functional MRI from 202 individuals with single-cell transcriptomic profiling of hypothalamic nuclei from AD and age-matched non-dementia controls (7 individuals per group), we identified significant volumetric changes in nuclei governing metabolic homeostasis, stress regulation, sleep, and circadian rhythms. Single-cell sequencing of 614,403 nuclei reveals 10 major cell types and 254 neuronal subtypes, highlighting a transition in microglial states from aging to disease trajectories. Notably, neurons within key nuclei exhibit the most pronounced alterations, with inhibitory neurons showing significant disruptions in ligand-receptor interactions and GPCR signaling. These findings provide a high-resolution volumetric map and a comprehensive cell type-specific transcriptomic atlas of the human hypothalamus in AD, offering mechanistic insights into disease progression and establishing a foundation for targeted interventions to mitigate hypothalamic dysfunction and slow early AD progression.

MOAP-1 IS A NEGATIVE REGULATOR OF STEATOSIS, INFLAMMATION AND CELLULAR SENESCENCE IN LIVER DURING AGING

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Dysregulation of hepatic lipid homeostasis promotes development of metabolic dysfunction-associated steatotic liver disease (MASLD), which affects 32.4% of the global population and is now the fastest-growing cause of hepatocellular carcinoma (HCC). MASLD can progress from benign steatosis to metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, HCC, while increasing risks of extrahepatic cancer. This disease poses a significant health threat worldwide, especially in times of the rapidly rising global aging population.

MOAP-1 is a Bax-binding protein that facilitates mitochondrial apoptosis signalling. Our laboratory previously demonstrated that MOAP-1 deficient liver and hepatocytes exhibited hyperactive Nrf2 signalling. MOAP1 negatively regulates the p62-Keap1-Nrf2 signalling by binding to the PB1-ZZ domain of p62 to disassemble the p62 bodies, thereby freeing up Keap-1 for suppressing nuclear translocation of Nrf2 (Tan et al., EMBO Reports, 22: e50854, 2021). Interestingly, in an observational study using 18-24 months old mice, which mimics the human aging population, approximately half of the aged MOAP-1 knockout (KO) cohort exhibited macroscopic abnormalities in liver, while none of the corresponding wild-type (WT) mouse showed any notable defect. Histopathological analyses of livers showed that many of the MOAP-1 KO mice developed tumours and lesions with elevated levels of inflammation and/or steatosis, resembling pathological features commonly observed in livers of patients with MASLD-associated diseases.

To understand the role of MOAP-1 in MASLD, we subjected WT and MOAP-1 KO mice to the STAM paradigm, which recapitulates the development and progression of MASLD-associated disease axis in a timely manner, from steatosis to MASH and eventually HCC (Fujii M et al., Med Mol Morphol, 46:141-152, 2013). Comparing to the WT counterpart, the MOAP-1 KO mice exhibited elevated levels of hepatic steatosis, inflammation, cellular senescence, p62 bodies and tumour burden. In vitro studies using WT and MOAP-1 KO LO2 human hepatocytes showed that palmitic acid (PA) treatment selectively induced lipid accumulation in the MOAP-1 KO, but not the WT LO2 cells. Moreover, PA treatment promoted cellular senescence in the MOAP-1 KO LO2 cells, exacerbating mitochondrial ROS production and DNA damage. Preliminary data showed that MOAP-1 deficient cells expressed low levels of p21, a critical DNA repair protein, suggesting that MOAP-1 may also play a role in DNA damage repair response. Further work is ongoing to elucidate the molecular mechanisms underlying the putative protective role of MOAP1 in mitigating the development and progression of pathologies linked to MASLD-associated disease axis during aging.

LOSS OF NUCLEAR LAMINA INTEGRITY INDUCES A SENESCENCE-LIKE SLOW-CYCLING PHENOTYPE

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The nuclear lamina consists of an intricate network of nuclear lamins and nuclear envelope transmembrane proteins that tightly regulate nuclear mechanotransduction and perinuclear heterochromatin organization to determine cellular identity and function. Lamin B1 is downregulated during cellular senescence, a stable form of cell cycle arrest, in which perinuclear heterochromatin becomes internalised and can be condensed into distinct senescence-associated heterochromatic foci (SAHFs). While lamin B1 downregulation is an established senescence marker, less is known about how other nuclear lamina components change in this context. Here, we show that lamin A/C depletion in non-transformed human diploid fibroblasts (HDFs) elicits a senescence-like slow-cycling phenotype: they show, like in senescent cells, upregulation of some secretory factors but no classic senescence markers, such as DNA damage foci and increased lysosomal activity. Instead, they are characterised by a sharp division or "de-capping" of the remaining nuclear lamina that is now lamin B1-dominant. In contrast, lamin A/C depletion has little effect on cell cycle and nuclear integrity across multiple cancer cell lines. Out of the common nuclear envelope proteins tested, LBR was identified to be lowly expressed in HDFs but highly expressed in cancer cell lines. We, therefore, hypothesized that LBR may compensate for lamin A/C to maintain an even distribution of lamin B1 around the nucleus. To test this hypothesis, we knocked down both LBR and lamin A/C in A549 lung cancer cells and created the de-capping phenotype, while rescued the de-capping phenotype in TIG3 HDFs by overexpressing LBR. Interestingly, our preliminary data suggest that, while lamin A/C depletion in HDFs leads to upregulation of IL1β and TGFβ2, ectopic LBR only rescues the former, suggesting that lamin A/C loss contributes to gene expression through distinct mechanisms. Our data may explain why lamin B-predominant cell types, such as embryonic stem cells and certain cancer cells, are able to maintain nuclear integrity.

HUMANIZED *MTERT* CONFERS HUMAN-LENGTH TELOMERES AND HAYFLICK LIMIT IN MICE

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Telomere shortening is a key driver of replicative senescence, a hallmark of human aging. However, mice possess excessively long telomeres and widespread telomerase activity, diverging from human telomere biology and complicating their use in aging and disease research. To bridge this gap, we humanized the mouse telomerase reverse transcriptase gene (*mTert*) by replacing its regulatory regions (5' intergenic region, introns 2 and 6) with human counterparts. The resulting *hmTert* allele preserved the wildtype mTert protein but recapitulated human *TERT* expression—restricted to gonads and a subset of immune cells, and silenced in somatic tissues. Sequential breeding of $Tert^{h/-}$ mice progressively shortened telomeres, stabilizing at human-like lengths (7–9 kb in $Tert^{h/h}$ "HuT" mice, compared to ≥50 kb in C57BL/6J mice).

Despite somatic telomerase silencing, HuT mice maintained normal body weight and tissue homeostasis in highly proliferative organs (testes, intestine, bone marrow). However, under stress (e.g., ulcerative colitis-like pathology), HuT colonocytes showed reduced proliferative capacity compared to wildtype mice, highlighting telomere-dependent limitations on cellular renewal. Notably, embryonic fibroblasts (MEFs) from HuT mice displayed a human-like Hayflick limit. While both HuT and wildtype MEFs senesced within 10 population doublings (PDs) under atmospheric oxygen (20%), only HuT MEFs underwent senescence after approximately 40 PDs under physiological oxygen (3%), accumulating senescence-associated β-galactosidase and other markers. Wildtype MEFs, by contrast, proliferated indefinitely. This senescence was correlated with telomere length and genotype and was rescued by ectopic mTert expression, directly linking the Hayflick limit to telomere reserves in HuT mice.

Our findings establish that *mTert* humanization resets mouse telomeres to human lengths and imposes telomere-dependent replicative senescence, recapitulating defining features of human somatic cell aging. The HuT mouse model bridges a critical gap in translational research, offering a robust platform to study human aging, cancer, and telomere-associated diseases.

IMMUNOTHERAPY APPROACHES FOR THE ELIMINATION OF SENESCENT CELLS IN FIBROTIC DISORDERS AND AGING

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The process of cellular senescence has recently been identified as a major culprit in the initiation and progression of fibrosis. Senescent cells that accumulate in tissues have acquired the ability to bypass the surveillance by the host immune system. Nonetheless, the mechanisms that underlie immune evasion remain largely undefined. Thus, we aim to identify the immunological signals (Senescence Associated Immune Checkpoints, SAICs) that enable senescent cells to avoid immune clearance. To this purpose, we performed a proteomic screen of surface proteins and bulk RNA-seq in senescent cells and identified Galectin-9 (Gal-9) as a candidate SAIC. Further, in vitro validation confirmed the overexpression of Gal-9 upon senescence induction in various cell lines and primary cells. Interestingly, Gal-9 receptor TIM-3, which is canonically expressed in immune cells, was also found to be overexpressed in senescent cells, together with its other putative ligands – phosphatidylserine and CEACAM1. Using mouse models of pulmonary and kidney fibrosis, we detected higher expression of Gal-9 and TIM-3 at the mRNA level in those tissues. In a pulmonary fibrosis model, we found that targeting Gal-9 and TIM-3 with neutralizing antibodies reduced the expression of collagen genes and attenuated the development of fibrosis. Overall, our results highlight Galectin-9, as well as its receptor TIM-3, as putative SAICs that could be harnessed for developing novel anti-senescence immunotherapy approaches.

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CODE OF CONDUCT FOR ALL PARTICIPANTS IN CSHL MEETINGS

Cold Spring Harbor Laboratory (CSHL or the Laboratory) is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom. To this end, the Laboratory aims to maintain a safe and respectful environment that is free from harassment and discrimination for all attendees of our meetings and courses as well as associated support staff, in accordance with federal, state and local laws.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth expectations for the professional conduct of all individuals participating in the Laboratory's meetings program, both in person and virtually, including organizers, session chairs, invited speakers, presenters, attendees and sponsors. This Code's prohibition against discrimination and harassment is consistent with the Laboratory's internal policies governing conduct by its own faculty, trainees, students and employees.

By registering for and attending a CSHL meeting, either in person or virtually, participants agree to:

1. Treat fellow meeting participants and CSHL staff with respect, civility and fairness, without bias based on sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all CSHL facilities, equipment, computers, supplies and resources responsibly and appropriately if attending in person, as you would at your home institution.

3. Abide by the CSHL Meeting Alcohol Policy (*see below)*.

Similarly, meeting participants agree to refrain from:

1. Harassment and discrimination, either in person or online, in violation of Laboratory policy based on actual or perceived sex, pregnancy status, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, creed, nationality or national origin, immigration or citizenship status, mental or physical disability status, veteran status, military status, marital or partnership status, marital or partnership status, familial status, caregiver status, age, genetic information, status as a victim of domestic violence, sexual violence, or stalking, sexual reproductive health decisions, or any other criteria prohibited under applicable federal, state or local law.

2. Sexual harassment or misconduct.

3. Disrespectful, uncivil and/or unprofessional interpersonal behavior, either in person or online, that interferes with the working and learning environment.

4. Misappropriation of Laboratory property or excessive personal use of resources, if attending in person.

BREACHES OR VIOLATIONS OF THE CODE OF CONDUCT

Cold Spring Harbor Laboratory aims to maintain in-person and virtual conference environments that accord with the principles and expectations outlined in this Code of Conduct. Meeting organizers are tasked with providing leadership during each meeting, and may be approached informally about any breach or violation. Breaches or violations should also be reported to program leadership in person or by email:

- Dr. David Stewart, Grace Auditorium Room 204, 516-367-8801 or x8801 from a campus phone, stewart@cshl.edu
- Dr. Charla Lambert, Hershey Laboratory Room 214, 516-367-5058 or x5058 from a campus phone, clambert@cshl.edu

Reports may be submitted by those who experience harassment or discrimination as well as by those who witness violations of the behavior laid out in this Code.

The Laboratory will act as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

If you have questions or concerns, you can contact the meeting organizers, CSHL staff.

For meetings and courses funded by NIH awards:

Participants may contact the Health & Human Services Office for Civil Rights (OCR). See this page for information on filing a civil rights complaint with the OCR; filing a complaint with CSHL is not required before filing a complaint with OCR, and seeking assistance from CSHL in no way prohibits filing complaints with OCR. You may also notify NIH directly about sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported events.

For meetings and courses funded by NSF awards:

Participants may file a complaint with the NSF. See this page for information on how to file a complaint with the NSF.

Law Enforcement Reporting:

- For on-campus incidents, reports to law enforcement can be made to the Security Department at 516-367-5555 or x5555 from a campus phone.
- For off-campus incidents, report to the local department where the incident occurred.

In an emergency, dial 911.

DEFINITIONS AND EXAMPLES

Uncivil/disrespectful behavior is not limited to but may take the following forms:

- Shouting, personal attacks or insults, throwing objects, and/or sustained disruption of talks or other meeting-related events

Harassment is any unwelcome verbal, visual, written, or physical conduct that occurs with the purpose or effect of creating an intimidating, hostile, degrading, humiliating, or offensive environment or unreasonably interferes with an individual's work performance. Harassment is not limited to but may take the following forms:

- Threatening, stalking, bullying, demeaning, coercive, or hostile acts that may have real or implied threats of physical, professional, or financial harm
- Signs, graphics, photographs, videos, gestures, jokes, pranks, epithets, slurs, or stereotypes that comment on a person's sex, gender, gender identity or expression, sexual orientation, race, ethnicity, color, religion, nationality or national origin, citizenship status, disability status, veteran status, marital or partnership status, age, genetic information, or physical appearance

Sexual Harassment includes harassment on the basis of sex, sexual orientation, self-identified or perceived sex, gender expression, gender identity, and the status of being transgender. Sexual harassment is not limited to sexual contact, touching, or expressions of a sexually suggestive nature. Sexual harassment includes all forms of gender discrimination including gender role stereotyping and treating employees differently because of their gender. *Sexual misconduct* is not limited to but may take the following forms:

- Unwelcome and uninvited attention, physical contact, or inappropriate touching
- Groping or sexual assault
- Use of sexual imagery, objects, gestures, or jokes in public spaces or presentations
- Any other verbal or physical contact of a sexual nature when such conduct creates a hostile environment, prevents an individual from fulfilling their professional responsibilities at the meeting, or is made a condition of employment or compensation either implicitly or explicitly

MEETING ALCOHOL POLICY

Consumption of alcoholic beverages is not permitted in CSHL's public areas other than at designated social events (wine and cheese reception, picnic, banquet, etc.), in the Blackford Bar, or under the supervision of a licensed CSHL bartender.

No provision of alcohol by meeting sponsors is permitted unless arranged through CSHL.

Meeting participants consuming alcohol are expected to drink only in moderation at all times during the meeting.

Excessive promotion of a drinking culture at any meeting is not acceptable or tolerated by the Laboratory. No meeting participant should feel pressured or obliged to consume alcohol at any meeting-related event or activity.

VISITOR INFORMATION

EMERGENCY (to dial outside line, press 3+1+number)	
CSHL Security	**516-367-8870 (x8870 from house phone)**
CSHL Emergency	**516-367-5555 (x5555 from house phone)**
Local Police / Fire	**911**
Poison Control	**(3) 911**

CSHL SightMD Center for Health and Wellness ***(call for appointment)*** Dolan Hall, East Wing, Room 111 **cshlwellness@northwell.edu**	**516-422-4422** **x4422 from house phone**
Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2000**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400**

GENERAL INFORMATION

Meetings & Courses Main Office

Hours during meetings: M-F 9am – 9pm, Sat 8:30am – 1pm
After hours – See information on front desk counter
For assistance, call Security at 516-367-8870
(x8870 from house phone)

Dining, Bar

Blackford Dining Hall (main level):
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Blackford Bar (lower level): 5:00 p.m. until late

House Phones

Grace Auditorium, upper / lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

Books, Gifts, Snacks, Clothing

CSHL Bookstore and Gift Shop
516-367-8837 (hours posted on door)
Grace Auditorium, lower level.

Computers, E-mail, Internet access

Grace Auditorium
Upper level: E-mail and printing in the business center area
WiFi Access: GUEST (no password)

Announcements, Message Board Mail, ATM, Travel info

Grace Auditorium, lower level

Russell Fitness Center
Dolan Hall, east wing, lower level
PIN#: **(On your registration envelope)**

Laundry Machines
Dolan Hall, lower level

Photocopiers, Journals, Periodicals, Books
CSHL Main Library
Open 24 hours (with PIN# or CSHL ID)
Staff Hours: 9:00 am – 9:00 pm
Use PIN# (On your registration envelope) to enter Library
See Library staff for photocopier code.
Library room reservations (hourly) available on request between 9:00 am – 9:00 pm

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Local Interest

Fish Hatchery	631-692-6758
Sagamore Hill	516-922-4788
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City
Helpful tip -
Take CSHL Shuttle OR Uber/Lyft/Taxi to Syosset Train Station
Long Island Railroad to Penn Station
Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681
Executive Limo Service	516-826-8172
Limos Long Island	516-400-3364
Syosset Taxi	516-921-2141
Orange & White Taxi	631-271-3600
Uber / Lyft	

Trains

Long Island Rail Road	718-217-LIRR (5477)
Amtrak	800-872-7245
MetroNorth	877-690-5114
New Jersey Transit	973-275-5555

CSHL Campus Map

CSHL Map