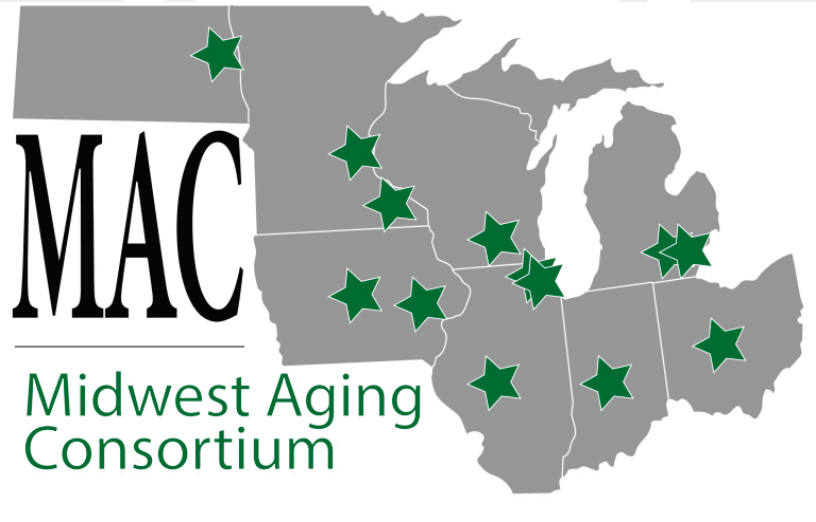
Program Book

5th Annual MAC Aging Research Symposium

April 28-30, 2024

Blackwell Inn and Pfahl Conference Center,

Ohio State University, Columbus, OH



THE OHIO STATE UNIVERSITY

WEXNER MEDICAL CENTER

Welcome and Overview

We are honored to welcome everyone to Columbus and **The Ohio State University** for our **5th Midwest Aging Consortium (MAC)** meeting. Your presence here is a testament to the importance and impact of our collective efforts in aging research.

Since its inception, the MAC has been a unique platform for collaboration, uniting institutions in the upper Midwest to propel aging research. The MAC leverages the distinct strengths of each member institution, including the **Mayo Clinic and the Universities of Wisconsin, Minnesota, Michigan, Iowa, Indiana, Northwestern, North Dakota, Ohio State, Iowa State, Illinois-Chicago, and Southern Illinois,** to foster a culture of collective advancement. Our community is expanding, as evidenced by the participation of twenty-two institutions in the 2024 MAC Conference. We express our deep gratitude to the exceptional faculty and their trainees who are instrumental in pushing the boundaries of aging research.

This year, we are privileged to host two distinguished Keynote Speakers, **Dr. Ana Maria Cuervo**, The Robert and Renee Belfer Chair, Co-director of the Institute for Aging Research at the Albert Einstein College of Medicine, and **Dr. Ananda Roy**, the Assistant Director in the Transformational Science & Discovery Office of Strategic Coordination – Common Fund, Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director, NIH. Both are esteemed leaders in the field of aging, and each, from their unique vantage point, champions the advancement of basic and translational research on aging.

Finally, we want to thank the sponsors, including **NIH, Glenn Foundation, Hevolution/AFAR, Boehringer Ingelheim, and from OSU the Division of Pulmonary, Critical Care and Sleep Medicine, the Davis Heart Lung Research Institute, and the Infectious Disease Institute**. In addition, special thanks to the support and tireless help of our administrative team, Tyler Fox, Jyotsna Nateri, and Jennifer Martin; without them, this event would not have been possible.

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

2024 MAC Planning Committee
Ana L. Mora, MD
Mauricio Rojas, MD
Maria Mihalova, PhD

Sponsors and Partners

The Midwest Aging Consortium wants to thank all of the donors, sponsors, and partners that have provided financial support to make this event possible.

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10X

Agenda

Sunday, April 28

6 - 8 p.m. **Welcome Reception** Room – Stadium View Dining

Monday, April 29

Breakfast and Registration

7 - 8 a.m.

Welcome Remarks

Room - Ballroom

8:15 a.m. **Carol R. Bradford, MD, MS, FACS**, Dean, College of Medicine, Vice President for Health Sciences, Wexner Medical Center, Leslie H. and Abigail S. Wexner Dean's Chair in Medicine

Loren Wold, PhD, Associate Dean for Research Operations and Compliance in the College of Medicine, and a Professor in the Department of Surgery, The Ohio State University

Keynote Presentation 1

8:25 a.m. "*Selective autophagy, fighting aging one protein at a time"*
Ana Maria Cuervo, MD, PhD The Robert and Renee Belfer Chair, Co-director Institute for Aging Research, Albert Einstein College of Medicine

Session Chair: ***Laura Niedernhofer, MD, PhD*** *Director of Institute on the Biology of Aging and Metabolism and Medical Discovery Team on the Biology of Aging at Univ. of Minnesota*

Cellular Senescence and Aging

9:00 *"Cellular Senescence in the Aging Brain"*
Marissa Schafer, PhD, Department of Physiology and Biomedical Engineering Department of Neurology, Mayo Clinic

9:25 - 9:55 Abstract presentations: Englund Davis, Zhang Lei, Noah Lepola

Session Chairs: **Hua Bai, PhD**, Genetics, Development and Cell Biology, Iowa University

Nathan LeBrasseur, PT, PhD, Professor, Dept. of Physical Medicine & Rehabilitation; Director, Robert & Arlene Kogod Center on Aging; Co-Director, Paul F. Glenn Center for Biology of Aging Research, Mayo Clinic

Break

10 a.m.

Tribute to Judith Campisi

10:25 a.m. Laura Niedernhofer, University of Minnesota; Paul Robbins, University of Minnesota

Metabolic Interventions and Aging

10:35 *"A role for brown adipose tissue to protect against cardiovascular aging"*
Kristin Stanford, **PhD**
Associate Director, Diabetes Metabolism Research Center, The Ohio State University

11- 11:30 Abstract presentations: Anna Carey, Demarco Rafael, Yang Yeh

Session Chairs: **Christin Burd, PhD**, Departments of Molecular Genetics, Cancer Biology and Genetics, The Ohio State University

Paul Robbins, PhD, Co-Director, Institute of Aging and Metabolism, Medical Discovery Team Biology of aging, University of Minnesota

Poster teasers – Young Investigator Forum

11:30 a.m. Maria Ford, Yinan Zhang, Natalia Vanegas, Masashi Tabuchi, Lorena Rosas, Magdalena Blaszkiewicz, Reji Babygrija, Maria Festing, Gonzalo Garcia, Yang Liu, Jinoh Kim, Eric MacGregor.

Facilitators: **Mariana Sadagurski, PhD,** Institute of Environmental Health Sciences, I Bio Center, Wayne State University

Shijiao Huang PhD, Department of Biochemistry and Molecular Biophysics, Kansas State University

Scott Leiser, PhD, Internal Medicine, Geriatric and Palliative Medicine,

University of Michigan Medical School

Lunch

Room – Ballroom

Noon *Trainee Lunch Session: Development Awards from National Institute of Aging*

Registration required Room - Pfahl 340

Christy Carter, **PhD,** Health Scientist Administrator, Division of Aging Biology, National Institute on Aging

Metabolism and Aging

1:00 *"Single-cell whole-genome sequencing discovers somatic mutations in aging"*
Lei Zhang, PhD, Institute on the Biology of Aging and Metabolism, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota.

1:25- 1:55 Abstract presentations: Ping Kang, Wei Wei, Neelanjana Roy

Session Chairs: **Dudley Lamming, PhD,** Director, UW-Madison Comprehensive Diabetes Center Mouse Phenotyping and Surgery Core, University of Wisconsin-Madison

Scott Budinger, MD Chief of Pulmonary and Critical Care in the Department of Medicine, Ernest S Bazley Professor of Airway Diseases, Northwestern University

Break

2 p.m.

Nutrition and Exercise in Aging

2:35 *"Are rapamycin and exercise compatible for healthy longevity?"*
Adam Kanopka, PhD, Geriatrics, Department of Medicine, University of Wisconsin

3:00 - 3:30 Abstract presentations: Joan Serrano, Christian Elliehausen, Matthew Johnston

Session Chairs: **Rozalyn Anderson, PhD,** Director, Pilot and Feasibility Program; UW Comprehensive Diabetes Center, University of Wisconsin-Madison

Rochelle Buffenstein, PhD, Professor, University of Illinois Chicago

Poster Session

Room - Dale Family Pavilion and Terrace

3:30 - 5:30 p.m.

Facilitators: **Christy Carter**, **PhD,** Health Scientist Administrator, Division of Aging Biology, National Institute on Aging

Kristy Townsend, PhD, Department of Neurological Surgery, Director, Women in Medicine, and Science (WIMS), The Ohio State University, College of Medicine

Daniella Chusyd, PhD, Indiana University Bloomington, Department of Environmental and Occupational Health

Holly Brown Borg, PhD, UND Chester Fritz Distinguished Professor, Assistant Dean for Gender Equity, Department of Biomedical Sciences, University of North Dakota School of Medicine & Health Sciences.

Dinner

Room – Ballroom

6 - 8 p.m.

Tuesday, April 30

Breakfast Room – Ballroom

7:30 - 830 a.m.

Keynote Presentation 2

8:30 a.m. "*SenNet--The NIH Common Fund Cellular Senescence Network"*
Ananda Roy, PhD, Assistant Director, Transformational Science & Discovery Office of Strategic Coordination – Common Fund, Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director, NIH, Adjunct Investigator, NIA

Session Chair: **Mauricio Rojas, MD**, Scientific Director Comprehensive Transplant Center Biorepository, Vice Chair of Research, The Ohio State University

Redox Mechanisms and Biomarkers of Aging

9:00 *Prothrombotic markers of aging*
Sanjana Dayal, PhD, FAHA, Internal Medicine-Hematology, Oncology, and Blood and Marrow Transplantation, University of Iowa

9:25 *Resiliency Among Older Adults Receiving Lung Cancer Treatment*
Carolyn Presley, MD, MHS, Co-Director of the Cancer and Aging Resiliency Clinic, Associate Medical Director of the Oncogeriatrics Program, The Ohio State University Comprehensive Cancer Center

9:50-10:10 Abstract presentations: Haylee Hamilton, Min-Ae Song

Session Chairs: **David Allison, PhD,** Dean Distinguished Professor Provost Professor School of Public Health Indiana University Bloomington

Anne Bronikowski, Professor, Integrative Biology Professor, Kellogg Biological Station Professor, Ecology, Evolution & Behavior Program, Michigan State University

Break

10:10 a.m.

Stress Response and Aging

10:25 *Promoting lung recovery: epigenetic and metabolic control of Treg cell function during aging*

Benjamin Singer, MD Lawrence Hicks Professor of Pulmonary Medicine, Biochemistry and Molecular Genetics, Northwestern University

10:45 – 11:15 Abstract presentations: Ajay Bhat, Correa Alzate

Session Chairs: **Kristin Stanford**, **PhD**
Associate Director, Diabetes Metabolism Research Center, The Ohio State University

Wilber Escoria, PhD Department of Biology, Xavier University

Poster prize and Travel awards

11:15 a.m.

Business meeting

11:35a.m. - Noon Organizing Committees 2024 and 2025

Adjournment

Noon

Keynote Speakers

Ananda Roy

Dr. Ananda L. Roy joined the Office of Strategic Coordination, NIH in 2015. Dr. Roy earned his Ph.D. from the University of Nebraska, studying mechanisms of mammalian peptide chain initiation, and did his postdoctoral training at the Rockefeller University where he studied mechanisms of transcription initiation and gene regulation. He biochemically discovered and molecularly cloned TFII-I transcription factor. He went on to join Tufts University School of Medicine in 1993 and stayed there till he joined the Office of Strategic Coordination. At Tufts, he further developed the biochemistry and biology of the TFII-I family of proteins in health and disease. He also studied genome-wide transcriptional and epigenetic regulation of gene expression in the immune system. Dr. Roy has trained many graduate students and post-doctoral fellows and directed the Graduate Biomedical Program in Genetics at Tufts. Dr. Roy has been awarded several NIH and Foundation grants, chaired external grant review panels and currently serves on several journal editorial boards. Dr. Roy maintains a research program at the Laboratory of Molecular Biology and Immunology, National Institute on Aging (NIA), focusing on transcriptional signatures associated with immune-cell activation.[1]

[1] Biography from https://loop.frontiersin.org/people/86815/bio

Ana Maria Cuervo

Dr. Cuervo is co-director of the Einstein Institute for Aging Research, and a member of the Einstein Liver Research Center and Cancer Center. In October 2001 she started her laboratory at Einstein, where she studies the role of protein-degradation in aging and age-related disorders, with emphasis in neurodegeneration and metabolic disorders.

Dr. Cuervo's group is interested in understanding how altered proteins can be eliminated from the cells and their components recycled. Her group has linked alterations in lysosomal protein degradation (autophagy) with different neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's disease. They have also proven that restoration of normal lysosomal function prevents accumulation of damaged proteins with age, demonstrating this way that removal of these toxic products is possible. Her lab has also pioneered studies demonstrating a tight link between autophagy and cellular metabolism. They described how autophagy coordinates glucose and lipid metabolism and how failure of different autophagic pathways with age contribute to important metabolic disorders such as diabetes or obesity.

Dr. Cuervo is considered a leader in the field of protein degradation in relation to biology of aging and has been invited to present her work in numerous national and international institutions, including name lectures as the Robert R. Konh Memorial Lecture, the NIH Director's, the Roy Walford, the Feodor Lynen, the Margaret Pittman, the IUBMB Award, the David H. Murdoxk, the Gerry Aurbach, the SEBBM L'Oreal-UNESCO for Women in Science, the C. Ronald Kahn Distinguished Lecture and the Harvey Society Lecture. She has organized and chaired international conferences on protein degradation and on aging, belongs to the editorial board of scientific journals in this topic, and is currently co-editor-in-chief of Aging Cell.

Dr. Cuervo has served in NIH advisory panels, special emphasis panels, and study sections, the NIA Scientific Council and the NIH Council of Councils and has been recently elected member of the NIA Board of Scientific Counselors and member of the of the Advisory Committee to the NIH Deputy Director. She has received numerous awards for the pioneerign work of her team such as the 2005 P. Benson Award in Cell Biology, the 2005/8 Keith Porter Fellow in Cell Biology, the 2006 Nathan Shock Memorial Lecture Award, the 2008 Vincent Cristofalo Rising Start in Aging Award, the 2010 Bennett J. Cohen Award in Aging Biology, the 2012 Marshall S. Horwitz, MD Faculty Prize for Research Excellence and the 2015 Saul Korey Prize in Translational Medicine Science. She has also received twice the LaDonne Schulman Teaching Award. In 2015 she was elected International Academic of the Royal Academy of Medicine of the Valencia Community and in 2017, she was elected member of the Real Academia de Ciencias Exactas, Fisicas y Naturales. She was elected member of the American Academy of Arts and Sciences in 2018 and member of the National Academy of Science in 2019.[2]

[2] Biography from https://www.einsteinmed.edu/faculty/8784/ana-maria-cuervo

NIH Safety Document

Dear Participant:

This conference is supported in part by the National Institutes of Health (NIH) under Award No 1R13AG087673-01.

Ohio State University is committed to working in an environment that is free from harassment and discrimination and is committed to fostering a safe and just environment for its students, faculty, staff, and visitors. All the members of our community had training on how to identify any type of discriminatory behavior.

OSU does not tolerate discrimination in any form holding Respect and Integrity, Well-Being and Sustainability, Equity and Justice, and Innovation and Discovery as our shared core values. Harassment, Discrimination, Retaliation, and Sexual Misconduct are destructive and contradict our core values. Participants in OSU programs and activities (including this event) must comply with OSU's Policies on Non-Discrimination and Sexual Misconduct.

Please review the following information regarding OSU's policies, as well as how to report any violations of these policies, how to make a complaint, and how any complaints will be resolved.

The policies and procedures outlined below have been developed to ensure the safety and well-being of all members of the OSU community are of easy access at the:

https://equity.osu.edu

The OSU is Committed to creating an environment that is equitable, fair, and just.

The Office of Institutional Equity (OIE) coordinates Ohio State's response to complaints of all protected class harassment, discrimination and sexual misconduct affecting students, employees, graduate associates, appointees, volunteers, visitors, suppliers and contractors, and provides information on rights and options for formal investigation.

In addition to responding to complaints of harassment, discrimination, and sexual misconduct (Title IX), OIE oversees the handling of issues involving the Americans with Disabilities Act (ADA), Affirmative Action and Equal Employment Opportunity (AA/EEO), and Youth Activities and Programs.

Protected class harassment, discrimination, sex and gender-based misconduct (Title IX)

- Responds to all incidents of protected class harassment and discrimination including sex- and gender-based misconduct, sexual assault, sexual harassment, relationship violence, stalking, sexual exploitation and sex and gender-based discrimination.

- Provides coordinated intake and outreach support to members of the university community. This includes access to on- and off-campus resources, including confidential resources and options for investigations.

- Provides supportive measures to assist students and employees with academic or workplace needs. Among the measures available are no-contact directives, changes in class or

work schedule, emergency housing, counseling, academic support, and pregnancy related accommodations.

- Oversees prevention education and training around the non-discrimination policy and sexual misconduct for the university community.

Youth Protection

- Promotes the safety and welfare of youth who participate in activities and programs with minors entrusted to the university's care.

- Outlines what is required of individuals interacting with youth with the goal of safeguarding their wellbeing.

- Informs individuals of reporting obligations in instances of known or suspected child abuse or neglect.

Affirmative action and equal employment opportunity (AA/EEO)

- Ensures equity in all employment processes from time of hire to separation through the university's Affirmative Action and Equal Employment Opportunity (EEO) programs.

- Consults with the university community to educate on the key principles of Affirmative Action and EEO and works to strategically align internal operations with applicable regulations.

- Coordinates the collection, analysis, and reporting of key demographic and operational data to assess program impact.

Americans with Disabilities (ADA)

- Guides the university's efforts to provide seamless access to all of its programs, the physical and digital environments.

- Focused on disability-based discrimination such as exclusion, refusal to accommodate, harassment and retaliation.

- Provides oversight for the reasonable accommodation of students, employees, visitors and other program participants.

- Provides training, information and resources on access and accommodations.

All OSU Non-Confidential Employees, including students employed at OSU, who learn of or observe conduct that they believe may be a violation of these policies are required to report it to the Title IX Coordinator or Official with Authority. Failure of a Non-Confidential Employee to report an incident or incidents of Sexual Misconduct of which they become aware is a violation of Policy and the violator could be subject to disciplinary action. No employee is authorized to investigate or resolve reports of prohibited conduct without the involvement of the Title IX Coordinator. All OSU members are encouraged to report actual or suspected violations of these policies. Third Parties (e.g., contractors, visitors, etc.) are encouraged to report actual or suspected violations of these policies.

Some examples of harassing behaviors may be:

- Unwelcome sexual advances or requests for sexual favors.

- Unwelcome comments or behavior, including but not limited to drawings, written statements, social media posts, or verbal statements, related to an individual's race, ethnicity, color, national origin, sex, disability, religion, age, gender, gender identity, disability, marital status, sexual orientation, veteran status, or other protected class.
- Unwelcome teasing, joking, or flirting based on actual or perceived gender identity, gender expression, or sexual identity/orientation.
- Retaliation.

OSU is committed to conducting prompt and equitable investigations and will appropriately address violations of OSU policy. Upon completion of an investigation, students and employees found to have engaged in acts of harassment, discrimination or retaliation will be promptly disciplined. If circumstances warrant, discipline for OSU students or employees may include suspension, expulsion, or termination and corrective action may be pursued related to individuals who are not OSU students or employees.

Any person may report discrimination, harassment, or retaliation in person, by mail, by telephone, or by electronic mail, using the links and contact information listed below. Reports may be made at any time (including during non-business hours) by using the telephone number, email, online reporting form, or by mail to the OAC address, listed below. Reports may be submitted in person at the OAC Office during business hours or by appointment if after business hours.

Office of Institutional Equity

1501 Neil Ave. | Columbus, OH 43210

Phone: 614-247-5838

Email: equity@osu.edu

Individuals who have questions, concerns, or complaints related to -discrimination and harassment are encouraged to call 614-247-5838 or TTY 614-688-8605, or email equity@osu.edu.

Individuals may also notify NIH (https://public.era.nih.gov/shape/public/notificationForm.era or 301-480-6701) or file a complaint with HHS OCR at the address below about concerns of harassment, including sexual harassment, discrimination, and other forms of inappropriate conduct at NIH-supported conferences.

U.S. Department of Health and Human Services

200 Independence Avenue, SW

Room 509F, HHH Building

Washington, D.C. 20201

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https://www.hhs.gov/ocr/complaints/index.html

Save The Date

Be sure to join us next Spring at The Mayo Clinic for the 2025 Midwest Aging Consortium Conference.

Abstracts

Table Of Contents

1. Oxidative stress-evolved bacteria enhance stress resistance and lifespan in C. elegans via the p38-MAPK Pathway

Ajay Bhat [1], Rebecca L. Cox [1], Brice Graham Hendrickson [1], Nupur K. Das[1], Megan L. Schaller[1], Angela M. Tuckowski[3], Emily Wang[1], Yatrik M. Shah[1,2] and Scott F. Leiser[1,2]

1. Molecular & Integrative Physiology Department, University of Michigan, Ann Arbor, MI 48109
2. Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, USA.
3. Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI, 48109, USA

Organisms are frequently exposed to a variety of environmental and systemic stressors, such as xenobiotics, fluctuating temperatures, oxidative stress, protein misfolding, and nutritional imbalances. In response, they have evolved signaling pathways to sense and effectively respond to stress, enabling them to survive and restore homeostasis. However, aging leads to a decline in stress response efficacy, contributing to the accumulation of damage and age-related disorders. Compounding this complexity, organisms do not live in isolation but are surrounded by and in many cases host millions or trillions of microorganisms. The short generation time and large population of microbes enable them to evolve and resist stress much faster than their hosts. This raises the question of whether microbial evolution during stress could play a role in modulating the stress tolerance and lifespan of their hosts.

In this study, we employ the nematode *Caenorhabditis elegans* and its bacterial diet as a model to explore how bacterial adaptation to oxidative stress influences the host's lifespan and stress response. Through adaptive laboratory evolution, we genetically modify OP50, a strain of *E. coli* commonly used as *C. elegans'* food source, to enhance resistance to oxidative stress. We find that worms fed these bacteria also develop resistance to oxidative stress and exhibit increased lifespan. Furthermore, through whole genome sequencing, we identify a mutation in the iron sulfur cluster regulator (iscR) gene in our lab-evolved bacteria. This mutation contributes to heightened host stress resistance and underscores the significance of the bacterial Fe-S pathway in regulating worm lifespan and stress tolerance. Additionally, we observe elevated iron levels in stress-evolved bacteria, which colonize the gut of worms and trigger an innate immune response through the mitogen-activated protein kinase (MAPK) pathway. In conclusion, we find evidence that microbial adaptation to stress could be leveraged to improve health and slow aging.

2. A potential harmful effect of acrylonitrile, a chemical compound in cigarette smoke, on epigenetic aging of lungs of smokers

Ajmal Khan[1], Fatima Jahan[1], Amarnath Singh[2], Joseph P. McElroy[2], Daniel Y. Weng[2], Sarah A. Reisinger[2], Mark D. Wewers[3], Peter G. Shields[2], Min-Ae Song[1]

[1]Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH; [2]Comprehensive Cancer Center, The Ohio State University and James Cancer Hospital, Columbus, OH; [3] Pulmonary and Critical Care Medicine, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA

Cigarette smoke contains large amounts of volatile organic compounds (VOCs) that are attributed to age-related respiratory diseases. We and others reported a significant association of smoking with accelerated biological age determined by DNA methylation. However, little is known about the relationship between specific VOCs, lung aging, and inflammation among smokers. This study assessed the well-studied DNA methylation-age estimates (mAge) that are linked to mortality (Grim-mAge and Pheno-mAge).

Epigenetic age estimates were assessed in the lung epithelium of 31 never-smokers (NS) and 13 smokers (SM) with no pulmonary diseases. We examined the differences in mAge between SM and NS using regression models after adjusting for potential confounders, including chronological age, sex, and race. In smokers, we investigated the relationship between ten smoking-related VOCs (cotinine-adjusted), the mAge-acceleration (-mAA), and lung inflammatory cytokines after adjusting for the covariates.

While there was no statistical difference in the chronological age between SM and NS (25.3 and 26.2, p=0.4), SM had significantly older GrimAge (p=0.0002) and Pheno-mAge (p=0.03) compared to NS. SM had up to 30-fold higher levels of VOCs compared to NS. In SM, of metabolites measured, a higher level of 2-cyanoethyl mercapturic acid (CEMA, a metabolite of acrylonitrile) was associated with faster Pheno-mAA (r=0.85, p=0.002), while a borderline significance was found for Grim-mAA (r=0.63, p=0.05). A borderline significant positive association was found between Pheno-mAA and IL-1β (p=0.05).

Our data suggest a potential contribution of acrylonitrile to premature lung aging among smokers, which supports a future larger study of its contribution to pulmonary diseases.

Contact information:
Ajmal Khan (khan.1266@osu.edu)
Min-Ae Song (Song.991@osu.edu)

3. Intestinal Gasdermin C Expression is Dependent on Nutrient Status and Immune Environment

Andrea Keller[1,2], Kubra Akkaya[1,2], Maria Festing[1,2], Amanda Cao[3], Vincenzo Coppola[2,4], Maria M. Mihaylova[1,2]
[1]Department of Biological Chemistry and Pharmacology, Ohio State University, Columbus, OH; [2]Comprehensive Cancer Center, Ohio State University, Columbus, OH; [3]Harvard Medical School, Boston, MA; [4]Department of Cancer Biology and Genetics, Ohio State University, Columbus, OH

Intestinal homeostasis depends on a balance between epithelial cells of the crypts and villi, the microbiota, and resident immune cells. With advancing age, intestinal metabolism, stem cell function, and barrier integrity all decline, as well as the ability to mount a robust immune response. Previous studies have investigated dietary interventions such as caloric restriction and fasting that improve intestinal function and delay aging-associated pathologies, but their influence on intestinal immunity is less clear. In the body's periphery, pathogen-infected immune cells can undergo pyroptosis, a process mediated by the Gasdermin (GSDM) family of proteins that culminates in lytic death of the infected cell and release of pro-inflammatory cytokines. GSDMC and D are also found in the intestine, but little is known about their regulation under changing intestinal conditions. We have found by bulk RNA-sequencing that *Gsdmc2-4* are among the most down-regulated genes following short-term (24hr) fasting in mouse small intestinal crypts. We have also observed downregulation of *Gsdmc2/4* in crypts isolated from aged (>24mo) mice, severe combined immunodeficient mice, and germ-free mice, indicating a dependence on microbial sensing and immune crosstalk with epithelial cells. Conversely, *Gsdmd* is unaffected by nutrient deprivation or aging. Using *ex vivo* intestinal organoid models, we have shown that *Gsdmc2/4* are induced by IL-4 and IL-13, which are produced by Type 2 innate lymphoid cells (ILC) in response to parasitic infection. Ongoing work is focused on delineating changes in ILC populations and cytokine production occurring with aging and dietary interventions. These results suggest that *Gsdmc2/4* are uniquely able to integrate signals from resident immune cells and the microbiome to provide innate protection against infection, a balance which is critical to maintain when intestinal homeostasis is disrupted with aging.

4. The role of *fmo-4* in longevity and stress response

Angela Tuckowski[1], Marshall Howington[1], Hillary Miller[1], and Scott F. Leiser[1,2,3]

1. Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI 48109.
2. Molecular & Integrative Physiology Department, University of Michigan, Ann Arbor, MI 48109.
3. Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109.

The link between dietary intake, metabolism, and long-term health is well-established in biomedical research. Despite this, our understanding of the mechanisms that connect metabolism to resilience and longevity remains limited. Many longevity pathways, like dietary restriction (DR), involve active modification of metabolism to potently and reproducibly improve health and longevity across species. Our lab identified the *flavin containing monooxygenase* family of enzymes as key regulators of longevity in *C. elegans.* Behind initial work focusing on *fmo-2,* we and others discovered that the widely conserved FMO family of xenobiotic enzymes are upregulated in multiple models of longevity, including nematode and mouse models. Furthermore, *C. elegans fmo-2* is both necessary and sufficient to extend lifespan under DR and hypoxic conditions, while also improving healthspan and stress resistance. In focusing on the conserved nature of the FMO gene family, this project studies the role of *C. elegans* FMO-4, a structurally similar gene to FMO-2 that is expressed in a different tissue. Our data show that *fmo-4* confers stress resistance, is required for DR-mediated longevity, but is not required for the hypoxic response. We also find that FMO-4 regulates calcium signaling and stress response to promote healthy aging. Together, the data produced by this project provide evidence as to 1) the metabolic, stress, and aging processes in which FMO-4 activity plays a role, and 2) the likely mechanisms for these roles. My current and future work will focus on *fmo-4* and its interactions with established longevity pathways, the distinct mechanisms of *fmo-4,* and the downstream processes modulated by *fmo-4* to regulate longevity. The resulting data will provide a model for the metabolic impact of FMO-4 and FMO enzymes in general that can be further interrogated in mammalian systems. Since the pathways and roles of FMO enzymes are important for age-associated diseases, this knowledge may allow us to identify potential therapeutic targets to improve human health.

5. Age-associated accumulation of B cells promotes macrophage inflammation and inhibits lipolysis in adipose tissue during sepsis

[1-3]Anna Carey, [2,3]Katie Nguyen, [2,3]Pranathi Kandikonda, [2,3]Victor Kruglov, [2,3]Claire Bradley, [2-3]Korbyn J. V. Dahlquist, [2,3]Stephanie Cholensky, [3,4]Whitney Swanson, [5]Vladimir P. Badovinac, [3,4,6]Thomas S. Griffith, [1-3]Christina D. Camell

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[2]Institute on the Biology of Aging and Metabolism, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, 55455, USA
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[6]Minneapolis VA Health Care System, Minneapolis, Minnesota 55417, USA

Non-canonical lipolysis induced by inflammatory cytokines or Toll-like receptor ligands is required for the regulation of inflammation during endotoxemia and sepsis. Canonical lipolysis induced by catecholamines declines during aging due to factors including an expansion of lymphocytes, pro-inflammatory macrophage polarization, and an increase in chronic low-grade inflammation; however, the extent to which the non-canonical pathway of lipolysis is active and impacted by immune cells during aging remains unclear. Therefore, we aimed to define the extent to which immune cells from old mice influence non-canonical lipolysis during sepsis. We identified age-associated impairments of non-canonical lipolysis and an accumulation of dysfunctional B1 B cells in the vWAT of old mice. Life-long deficiency of B cells resulted in restored non-canonical lipolysis and reductions in pro-inflammatory macrophage populations. Our study suggests that targeting the B cell-macrophage signaling axis may resolve metabolic dysfunction in aged vWAT and attenuate septic severity in older individuals.

6. Caloric restriction and RNA processing: RNA helicases, mito-nuclear crosstalk, and metabolic flexibility

Anshit Singh[1,2], Spencer A. Tye[1,2], Andrew J. Engeler[2], Josef P. Clark[3], John M. Denu[1,4,5], Ricki J. Colman[1,6], Rozalyn M. Anderson[1,3,7], Timothy W. Rhoads[1,2]

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RNA processing has emerged as a key mechanism engaged in the response to caloric restriction (CR), a dietary intervention known to extend healthspan and lifespan in numerous model organisms. Extensive data has established the impact of CR on metabolism, but the mechanisms regulating this and how it might intersect with transcriptional remodeling are not known. Prior work in non-human primates that identified RNA processing as part of the CR response also uncovered lysine acetylation sites on RNA helicase enzymes that strongly responded to CR. To examine the potential mechanistic implications, we generated lysine to glutamine (KQ) and lysine to arginine (KR) acetylation-mimic mutants of lysine 162 (K162) on the endogenous helicase DDX39B in HepG2 cells. Downstream analysis of metabolism revealed significant changes in mitochondrial respiration and cell growth. KQ mutant cells showed a strong and statistically significant increase in maximal respiration, while KR mutants showed a dampening of both basal and maximal respiration. We also observed significant alterations in the mitochondrial membrane potential as well as overall ATP levels in the cells. Furthermore, we saw changes in the stoichiometry of mitochondrial respiration complexes, indicating alterations in mito-nuclear crosstalk. These observations implicate acetylation of DDX39B and the associated RNA processing machinery the TRanscription-EXport (TREX) complex in the downstream regulation of metabolism; the potential impact on metabolic flexibility will be the target of follow-up investigations.

7. Proteostasis collapse and disruption of Integrated Stress Response pathway in aging and neurodegenerative disorders.

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Proteasome function decreases with age as measured by proteasome activity and accumulation of ubiquitinated proteins in human tissues and many model organisms. Proteasome dysfunction is associated with ubiquitin-containing inclusion bodies in neurodegenerative diseases. This project aims to understand the molecular mechanisms of proteostasis collapse, a hallmark of aging and neurodegeneration. Proteostasis is the coordinated synthesis, processing, and degradation of proteins. The Ubiquitin Proteasome System (UPS) is the primary protein degradation pathway. Proteasome collapse is associated with activating the Integrated Stress Response (ISR) signaling pathway. During the ISR, exposure to stressful conditions leads to mRNA translation inhibition, and coalescing of mRNAs and RNA-binding proteins into biomolecular condensates called Stress Granules (SGs). Defects in the ISR pathway and persistent SGs are also associated with aging and neurodegenerative diseases. The correlation between ISR and UPS is apparent, but the process of SG accumulation during proteasome inhibition remains unclear. We are applying biochemical assays, fluorescent microscopy, and live cell imaging to determine the effects of proteasome inhibition on translation activity and SG accumulation upon stress. Strikingly, by using fluorescent microscopy and western blot we found that chronic, low-level proteasome inhibition activates the ISR, but does not form SGs. This observation suggests translation initiation and elongation may be impaired when the proteasome is suppressed. We observed that stress granule formation was inhibited, and cell death was increased in acutely stressed cells with low proteasome activity. These results suggest two possible implications of an aberrant ISR upon catastrophic proteasome suppression associated with aging: 1) proteostasis collapse may impair stress granule formation and post-transcriptional gene regulation, and 2) failure of stress granule assembly could impair cell adaptation to and survival of stress. Understanding how proteasome overload is connected to ISR dysfunction at the molecular level will help us identify potential targets for aging and neurodegeneration.

8. Loss of Endogenous Nox2-NADPH Oxidase does not Prevent Age-induced Platelet Activation and Arterial Thrombosis in Mice

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Background: Reactive oxygen species (ROS) are known to contribute to platelet hyperactivation and thrombosis during aging, however, mechanistic contribution of the specific oxidative pathways remains elusive. We hypothesized that during aging, endogenous Nox2-NADPH oxidase contributes to platelet ROS accumulation and that loss of Nox2 will protect from platelet activation and thrombosis. **Methods**: We studied littermates of Nox2-knockout (Nox2-KO) and wild type (WT) mice at young (3-4 months) or old (18-20 months) age. We examined agonist-induced platelet activation, oxidant generation, aggregation, secretion, and susceptibility to *in vivo* thrombosis in mice. **Results**: Compared to young WT mice, aged WT mice showed a significant increase in platelet αIIbβ3 activation, granule release, oxidant generation, aggregation and secretion and enhanced susceptibility to platelet-induced pulmonary thrombosis. Aged Nox2-KO mice showed similar enhancement in platelet activation and susceptibility to thrombosis as in aged-WT mice. Adoptive transfer of platelets from aged WT or aged Nox2-KO mice to the aged host mice resulted in similar time to develop occlusive thrombus in the carotid artery after photochemical injury. **Conclusions**: We conclude that the loss of endogenous Nox2 does not protect against age-related platelet activation and arterial thrombosis in mice.

9. The sweet taste receptor TAS1R2 regulates myocyte differentiation in C2C12 muscle cells

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Sweet Taste Receptors (STRs) are G protein coupled receptors formed by the obligate heterodimerization of Tas1r2 and Tas1r3. Historically, STRs have been associated with nutrient sensing and metabolic regulation in the tongue, gut, and pancreas. Our current research has focused on the recent discovery that STRs are widely expressed in skeletal muscle. We have observed that Tas1r2 deletion increases muscle mass and fitness in young and aged mice. These findings are clinically relevant because of the age-associated decline in muscle mass and function. To further investigate the effects of STRs in skeletal muscle, we used Crispr-cas9 gene editing to delete Tas1r2 in C2C12 muscle cell line. Following a standard differentiation protocol (2% HS), Tas1r2-deficient C2C12 cells (cKO) displayed partial differentiation but were unable to fuse and form myotubes. Through q-PCR analysis, we identified consistent upregulation of myogenic progenitors (pax7, myoD) in cKO cells, alongside significant downregulation of myogenic transcription factors (klf2, klf4) crucial for mediating fusion. Additionally, markers of myotube formation (tnnt3, mck) were upregulated during differentiation, albeit to a lesser extent than observed in wild-type C2C12 cells (cWT). Supplementation of the differentiation protocol with 90 nM insulin facilitated fusion of cKO myocytes, resulting in longer and thicker myotubes compared to cWT cells. Interestingly, despite the enhanced expression of myogenic progenitors, fusion and myotube markers (pax7, myoD, myoG, myomaker, myomerger, myh4, tnnt3, mck), the expression of transcription factors klf2 and klf4 remained low in cKO cells, suggesting a potential regulation of the ERK5-klf2/4 signaling axis by Tas1r2. Insulin signaling appeared capable of bypassing these deficits. These results corroborate our previous findings in a mouse model, indicating that deletion of STR signaling enhances myofiber size and muscle mass in a physiological context.

10. Improving mitochondrial function via drug treatments can reduce cellular senescence.

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Aging is a complex process culminating in loss of organ function and organism resilience. A great challenge in aging research is determining cause and effect: which insult(s) instigate aging and which are a consequence of it? For example, mitochondria dysfunction can be both upstream and downstream of cellular senescence. Our approach to addressing this challenge is to use a well-defined system in which, by definition, the primary insult is increased genotoxic stress in the nuclei of cells. We use *Ercc1*$^{-/-}$ mice and mouse embryonic fibroblasts (MEFs), in which *Ercc1* is deleted and this destabilizes a critical DNA repair endonuclease ERCC1-XPF. As a consequence, the cells are missing several key DNA repair mechanisms required to protect the nuclear genome. Cells and tissue from mutant mice accumulate oxidative DNA damage faster than wild-type (WT) cells and mice and display accelerated onset of senescence. *Ercc1*-deficient cells and mice also display mitochondrial dysfunction. Here, we seek to understand the mechanism by which mitochondrial dysfunction arises. We treated *Ercc1*$^{-/-}$ MEFs with mitochondrial-targeted drugs and synthetic molecules to inhibit signaling pathways, then measure the effect on mitochondrial ROS production, mitochondrial membrane potential, mitochondrial mass, mitochondrial bioenergetics, and markers of senescence and mitochondrial function. Preliminary results suggest that improving mitochondrial function reduces cellular senescence. Furthermore, targeting upstream signaling has more impact on mitochondrial health than trying to target mitochondrial *per se*. Future work is aimed at discovering better mitochondrial targeted therapeutics that can improve mitochondrial homeostasis while reducing cellular senescence.

11. Can rapamycin and exercise improve health in older female mice?

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Since rapamycin (Rapa) and exercise independently improve healthy longevity, it is tantalizing to suspect the combination of rapa and exercise would further extend healthy longevity than either treatment alone. However, the status quo indicates that Rapa prevents several health benefits of exercise by abrogating the acute increase in mTOR signaling after exercise. These studies have only been performed in young rodents using involuntary models of resistance exercise or focused on acute responses. No studies have examined the impact of Rapa on the metabolic, physical, or skeletal muscle adaptations to voluntary exercise in older mice. We tested the hypothesis that frequent Rapa (2 mg/kg, 5d/week) would inhibit several health benefits of Progressive Weighted Wheel Running (PoWeR) in older mice. Older female C57BL6/J mice (22 months old, N=10-14/group) were acclimated to 1 week of unweighted wheel running before being allocated to 8 weeks of sedentary control or PoWeR with either vehicle or Rapa. PoWeR distance averaged ~3km/day and was not different between groups. PoWeR improved body composition through decreased fat mass and this was not influenced by frequent Rapa. Frequent Rapa prevented the increase in insulin sensitivity and glucose tolerance after PoWeR. The increase in maximal exercise performance after PoWeR was attenuated by frequent Rapa. Intrinsic to exercise capacity and insulin sensitivity, skeletal muscle mitochondrial respiratory capacity increased with PoWeR but was not affected by frequent Rapa. Frequent Rapa treatment partially or completely inhibited the greater soleus and plantaris muscle mass in PoWeR versus Sed but did not influence the increased FDL muscle mass. These preliminary data suggest that frequent Rapa (2g/kg, 5d/wk) treatment may prevent or attenuate several metabolic, physical, and skeletal muscle adaptations to voluntary exercise in older female mice. These data highlight the need to find alternative Rapa dosing regimens to cooperate with exercise for the purpose of further extending healthy longevity.

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12. PD1 blockade improves survival and CD8+ cytotoxic capacity, without increasing inflammation, during normal microbial experience in old mice

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Abstract: The elderly and those with chronic diseases have a 2 to 10-fold increased risk of hospitalization and mortality following infection as compared with healthy adults. A significant contributor to disease severity is the aging of the immune system, including an expansion of exhausted T cells. These cells simultaneously promote excessive levels of inflammation and display impaired T cell activation and function. How exhausted T cells from aged individuals respond to infection and whether acute restoration of T cell activation further exacerbates the inflammatory environment or generates functional T cells is unclear. Previously, we used a mouse model of normal microbial experience (NME), where old specific pathogen-free (SPF) mice show 100% mortality following exposure to multiple microbes ordinarily found in pet store mice. In the present study, we show that when exposed to NME, old SPF mice have increased expression of multiple inflammatory pathways and exhibit elevated frequencies of a heterogenous exhausted $CD8^+$ T cell pool that expresses differing combinations of the inhibitory receptor programmed cell death protein 1 (PD1), TOX, and CXCR5. Pre-treatment or intervention with an anti-PD1 monoclonal blocking antibody during the exposure of old SPF mice to NME significantly improves both antibody production and survival, without altering the acute inflammatory response. Anti-PD1 checkpoint blockade-mediated survival is dependent on $CD8^+$, but not $CD4^+$ T cells. Correspondingly, $CD8^+$ $PD1^+$ T cells from old mice given anti-PD1 monoclonal antibody have increased granzyme B production. These data reveal a new approach for reducing vulnerability to infections in the elderly by targeting $CD8^+$ T cell exhaustion through PD1 checkpoint blockade immunotherapy.

13. Fisetin reverses T cell aging and immunosenescence

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Age-related immune dysfunction, or immunosenescence, poses a significant health risk to cancer survivors. We and others have shown that common cancer treatments including chemotherapy, radiation, and hematopoietic stem cell transplantation accelerate immunosenescence. As a result, cancer survivors face compromised immune function and an increased risk of cancer recurrence. Senolytic compounds may mitigate the age-accelerating consequences of cancer therapy by eliminating aged, senescent cells throughout the body. Prior studies show that a short-term treatment with the senolytic, fisetin, reduces the expression of senescence markers ($p16^{INK4a}$ and $p21^{CIP1}$) in $CD3^+$ T cells. However, the efficacy of senolytic compounds is often cell-type specific, and $CD3^+$ T cells are extremely diverse, participating in various suppressive, cytotoxic, and inflammatory immune responses. An in-depth profiling of immune cell sensitivity to fisetin has not yet been conducted. Here, we tested the hypothesis that fisetin modulates T cell survival signals in a subset-dependent manner, leading to the elimination of senescent cells. In preliminary studies, the short-term treatment of aged mice with fisetin mitigated immunosenescent phenotypes in $CD3^+$ T cells. Specifically, fisetin administration increased the CD4:CD8 T cell ratio, reduced the skewing of $CD8^+$ T cells toward effector memory phenotypes, restored costimulatory molecule (CD27, CD28) expression and decreased production of IL-6, a cytokine associated with cellular senescence. Using single-cell RNA sequencing, I have characterized the T cell subset changes and transcriptional pathways associated with fisetin sensitivity in mice. I also report the effect of fisetin on T cell survival and PI3K-Akt signaling in vitro. Understanding how fisetin impacts T cell aging could lead to novel strategies to combat immunosenescence.

14. Skeletal muscle-specific p21 overexpression induces metabolic dysfunction

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We have shown that senescent cells accumulate in skeletal muscle during aging. In particular, we discovered a distinct population of senescent muscle fibers with high levels of the cyclin-dependent kinase inhibitor p21. The direct impact of these cells on skeletal muscle aging and systemic health remains to be established. To address this gap, we generated a mouse model that allows for inducible and muscle fiber-specific overexpression of p21 (**SKM-p21**). We found that elevation of p21 in adult mice induces manifestations of skeletal muscle aging and metabolic dysfunction. To interrogate the relationship between p21 and metabolic health, we assigned adult SKM-p21 mice and age-matched controls to either a conventional diet or a fast-food diet (**FFD**) of high-fat chow plus high fructose water for ten weeks. SKM-p21 and control mice assigned to the FFD experienced ~20% weight gain over the intervention. Interestingly, when examining body composition, SKM-p21 mice had greater increases in fat mass and reductions in lean mass than controls on the FFD. Moreover, SKM-p21 mice also exhibited exacerbated FFD-induced impairments in clearing circulating glucose, as assessed by a glucose tolerance test (**GTT**). To gain molecular insight into p21-mediated metabolic dysfunction, we performed RNA-seq on skeletal muscle and epididymal fat. Enrichment analysis revealed that p21 amplifies the transcriptional activity of several pathways associated with the FFD, including cellular senescence and inflammation, in muscle and fat. Further, insulin-stimulated glucose uptake pathways were reduced in the muscle of SKM-p21 mice on the FFD, consistent with results from the GTT. We are now examining insulin-driven signaling events at the protein level, which may reveal the mechanisms by which p21 compromises glucose uptake. Future studies will utilize loss-of-function models to determine if reducing levels of p21 in muscle fibers is protective against skeletal muscle aging and metabolic stress. Taken together, this work will evaluate p21 as a mediator of skeletal muscle health, which has the potential to reveal new druggable targets to counteract age-related metabolic dysfunction.

15. mTOR-inhibition prevents post-traumatic osteoarthritis pain behavior in female mice.

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Age and injury are the greatest risk factors for osteoarthritis (OA), a degenerative joint disease which can cause pain and disability. Mechanistic target of rapamycin (mTOR) signaling is increased in joint tissues and in the pain-sensing dorsal root ganglia (DRG) during OA and in other pain models. mTOR-inhibition has decreased post-traumatic OA (PTOA) severity in some, but not all, studies using young male rodents. However, it remains unknown if mTOR-inhibition can offer similar effects in female mice or mitigate PTOA pain. Therefore, we performed a non-invasive ACL rupture (ACLR) on the left hind limb of 24 female C57Bl/6J mice at 5-months of age to induce PTOA followed by treatment with vehicle (n=12) or the mTOR-inhibitor rapamycin (rapa; n=12; 2mg/kg, 3x/wk) for 8-weeks. Bodyweight was collected weekly, and blood glucose was collected at 2-, 4-, and 8-weeks post-ACLR. Knee hyperalgesia (pain sensitization) and mechanical allodynia (mechanical sensitization) were measured before and every 2-weeks following ACLR via pressure application measurement and Von Frey filament testing, respectively. At sacrifice, both hind limbs were fixed in formalin for microCT and histopathology. Bodyweight increased over the course of the study and was similar between groups. Expectedly, blood glucose was elevated in rapa-treated mice at all timepoints measured. ACLR-induced knee hyperalgesia was delayed in rapa-treated mice up to 6-weeks post-ACLR, though both groups showed similar withdrawal thresholds by 8-weeks. Mechanical allodynia was evident after ACLR but was persistently lower in rapa-treated mice versus vehicle throughout the study. Interestingly, microCT revealed increased calcification of the medial meniscus, fabellae, and regions of the quadriceps tendon. Together, these data show that, despite increasing intra-articular calcification, mTOR inhibition may delay or lessen aspects of PTOA pain. Further work will evaluate histological OA severity and the mechanistic basis for decreased pain behavior during mTOR inhibition.

16. Reprogramming-based vasculogenic and neurogenic cell therapies drive improved memory and reduced neuropathological burden in a mouse model of Alzheimer's Disease

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Numerous studies indicate a strong correlation between cerebrovascular impairment and the development of Alzheimer's Disease (AD). These cerebrovascular alterations precede the formation of amyloid plaques, tangles, and the onset of cognitive decline, suggesting that impaired cerebrovascular function plays a key role in the onset and/or progression of AD neuropathology. In consequence, therapeutic interventions aiming to restore cerebrovascular function constitute crucial strategies to attenuate the progression of the disease. Cell-based therapies constitute promising strategies to address vascular and neurogenic deficiencies in the brain. To implement safe and efficient cell therapies in AD, we used electroporation, to deliver 3 pro-vasculogenic and 3 pro-neurogenic transcription factors to reprogram mouse primary embryonic fibroblasts (pMEFs) into induced endothelial cells (iECs) and induced neurons and (iN) respectively. To evaluate their therapeutic potential, pMEFs pre-labeled with 5-bromo-2'-deoxyuridine (BrdU) and transfected with *Etv2, Foxc2, Fli1 (EFF)* or a control empty plasmid were delivered with 3 intracranial injections into the lateral ventricles (LV) of females from the triple transgenic murine model of AD (3xTg-AD) or Wild-Type Controls. Within each cage mice with the same genotype were randomly assigned to either vasculogenic, or control cell injections. Two weeks after the last injection spatial memory was analyzed with the Barnes Maze. Subsequently, brain tissue was processed for immunostaining and biochemical analysis. Similarly, pMEFs pre-labeled with BrdU and transfected with *Ascl1, Brn2, Myt1l (ABM)* or an empty plasmid were injected into the hippocampus of 3xTg-AD or Wild-Type Controls females. 4 weeks after the injection (51 weeks old mice) spatial and recognition memory was analyzed. One week later, brain tissue was processed for immunostaining and biochemical analysis. Our results indicate that pMEFs pre-programmed into vasculogenc iECs and delivered to the LVs induce an increase in global cerebral blood flow (CBF) as early as 7 days post-injection. Vasculogenic cells also lead to a reduction of the spatial memory deficits in the 3xTg-AD mice. Histological analysis show that the injected cells were able to migrate to multiple brain regions and survive for at least 4 weeks in close contact with brain blood vessels. Notably, animals injected with vasculogenic cells show an increase in the total

vascular area in the cortex and show a reduced amyloid-beta load. On the other hand, pMEFs pre-programmed into iNs can survive for at least 6 weeks and express markers of mature neurons inside the brain parenchyma and induce a reduction of amyloid-beta levels in the hippocampus of 3xTg-AD mice. Together our results indicate that the development of electroporation-based cell therapies by direct reprogramming constitute a promising approach to treat AD and AD-related dementias.

17. Hypoxia extends lifespan through neuronal HIF-1 stabilization, bioamine and neuropeptide signaling, and cell-nonautonomous induction of FMO-2.

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Exposure to a hypoxic environment extends lifespan in *C. elegans* but is not a practical solution to improve lifespan and healthspan in mammals. The mechanism of hypoxia-mediated longevity requires neuronal stabilization of HIF-1, which in turn leads to cell nonautonomous induction of the pro-longevity effector, FMO-2. However, HIF-1 and FMO-2 have broad effects on transcription and metabolism, respectively. In this work, we seek to understand the detailed neuronal circuit through which hypoxia extends lifespan in order to identify highly specific neuronal longevity targets. We have identified a role for both high- and low-oxygen sensing neurons in hypoxia-mediated longevity, suggesting that despite HIF-1 activation, these the interplay between these neurons is still crucial. We have also established that stabilization of HIF-1 in a single serotonergic neuron pair is sufficient to extend lifespan by more than 25%. Downstream of this serotonergic signal, we examine key serotonin receptor-expressing interneurons, and find the SER-7-expressing RIS neuron is required for hypoxia-mediated longevity. We also find that the neurotransmitters octopamine and GABA as well as the neuropeptide NLP-17 signal to communicate information about oxygen availability to peripheral longevity effectors. Together, this project identifies key players in the cell non-autonomous hypoxic circuit, which are distinct but comparable from other, similar longevity circuits.

18. Multi-omics Approach to Characterize Adipose Senescence in Subcutaneous and Visceral Depots

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Abstract

Background: Cellular senescence is implicated in the progression of adipose dysfunction and inflammation with aging and obesity. The distribution of subcutaneous and visceral adipose depots changes with age and contributes differently to aging phenotypes.

Objectives: To characterize senescence in adipose and investigate how senescent cells contribute to aging in subcutaneous versus visceral depots.

Methods: Subcutaneous and visceral adipose tissue from bariatric surgery patients ages 25-64 were evaluated. Senescence associated beta-galactosidase (SAβ-Gal) assay and qPCR for common molecular markers of senescence were used to identify overall level of senescence burden for the tissue. Single nuclei RNAseq (snRNAseq) was used for phenotyping and cell typing senescent cells. Finally, 10X Genomics Visium and was used to spatially characterize senescence and the surrounding area. A senescence associated gene list, SenMayo was utilized to identify senescence hotspots within the tissue.

Results: The subcutaneous adipose has higher SAβ-Gal staining and increased *CDKN1A* expression than visceral adipose from the same subjects. The snRNAseq results identified adipocytes and adipocyte progenitors as driving much of this increased *CDKN1A* expression in subcutaneous adipose. T-cells were identified to have increased levels of *CDKN2A* expression in both subcutaneous and visceral depots from older subjects. Visium analysis identified senescence hotspots primarily adjacent to vasculature and within fibrotic areas of adipose tissue.

Conclusion: Subcutaneous adipose has increased p21 expression and SAβ-Gal staining than visceral adipose from the same subjects. Interestingly, p21 expression in adipocytes decreased with age. In addition, most spatial senescence hotspots are adjacent to vasculature and within fibrotic areas of the tissue which have few adipocytes. Therefore, increased senescence in subcutaneous adipose could be due to the increased fibrosis and inflammatory cells found in this depot.

19. Reversal of neuronal tau pathology, metabolic dysfunction, and electrophysiological defects via adiponectin pathway activation

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The specific mechanisms of cognitive decline and dementia associated with Alzheimer's disease (AD) remain unclear; however, changes in brain mitochondrial metabolism are coincident with functional decline associated with AD. Direct links between pathology and cellular energetics have not been established, nor have therapeutic strategies explored this connection. Here, we interrogate the therapeutic potential of mitochondrial targeting via the adiponectin receptor activator AdipoRon (AR). Adiponectin is an adipose-tissue-derived hormone previously linked to metabolic syndrome and aging, and adiponectin receptors are expressed in all cell types of the brain. Here, we show that AR clears neurofibrillary tangles (NFTs), a hallmark of AD, and rescues diverse tauopathy-associated defects in primary neurons. Specifically, AR reduced levels of phospho-tau and lowered NFT burden by a mechanism requiring AMPK, an energy-sensing kinase linked to adiponectin signaling. The transcriptional response to AR extended beyond the expected reprogramming of mitochondrial metabolism, influencing post-synaptic receptors, cellular maintenance, and homeostatic pathways. At the organelle level, activation of lysosomal pathways involved increased protein levels of LC3 and p62 and was dependent on AMPK. Regarding metabolism, the negative consequences of NFTs on mitochondrial activity, ATP production, and lipid stores were corrected. Furthermore, AR restored decreases in dendritic complexity caused by tauopathy, and this effect was dependent on AMPK and the stress-responsive kinase JNK. Whole-cell patch-clamp experiments identified NFT-associated defects in electrophysiological passive parameters (e.g., resting potential, resistance, spiking profiles) and active parameters (e.g., action potential firing), both of which were corrected by AR. Together, these data reveal a neuronal intracellular network linking mitochondrial function to cellular maintenance processes and electrical aspects of neuronal function that can be targeted via adiponectin receptor activation.

20. Changes in Fat Metabolic Enzymes During Aging are Regulated by the ERK Signaling Pathway via Activation of Chaperone-Mediated Autophagy.

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Proteomic analysis of anti-aging drugs, diets, and mutations (including acarbose, caloric restriction, canagliflozin, and the Snell dwarf and GHRKO stocks) have suggested a reduction in many fat metabolic enzymes in the liver. These results suggest common mechanism(s) and signaling pathway(s) between multiple mice models regulating aging and tissue health. The specific signaling pathways involved are, however, unknown. Data from our lab has shown these interventions lead to a reduction in pathways regulated by two sets of protein kinases, i.e. mTORC1 and ERK1/2. Here we report that fat metabolism in these models is under the control of ERK signaling rather than mTOR. In vitro and vivo treatments with Trametinib, a drug that inhibits ERK signaling, can mimic the changes seen in fat metabolism, and others, without significant effects on the mTOR pathway. Our data suggest that reduction in ERK activity is a fundamental pathway regulating aging that may be independent of mTOR signaling. Further, we show that alteration in enzymes involved in fat metabolism in these slow-aging mice largely involve post-transcriptional controls, including effects of chaperone-mediate autophagy, rather than alterations in the corresponding mRNAs. We draw two conclusions: a) Transcriptome analysis may miss important changes in metabolic pathways related to slowed aging, including those that modulate fat metabolism; and b) Posttranscriptional mechanism in the degradation/translation pathway, such are CMA or cap-independent translation (CIT), could be fundamental in modulating lifespan extension.

21. The TAS1R2 sweet taste receptor regulates skeletal muscle mass recovery following hindlimb immobilization

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The sweet taste receptor TAS1R2 is a G protein-coupled receptor (GPCR) that is traditionally associated with sugar detection and taste perception on the tongue and insulin and incretin secretion in the intestine and pancreas. Our lab has recently demonstrated that TAS1R2 is expressed in the skeletal muscle of mice and has roles in regulating skeletal muscle fitness. Intriguingly, mice with muscle-specific deletion of *Tas1r2* (mKO) have increased muscle mass, strength, and mitochondrial capacity. Additionally, untreated mKO muscles showed moderate reduction of protein degradation (i.e 20S proteasome activity) and enhanced protein synthesis (i.e. SunSeT). These data indicate a role of TAS1R2 in the regulation of muscle mass turnover. To further test this hypothesis, we subjected young (13-16 weeks) control (mWT) and mKO male mice to 7 days of unilateral hindlimb immobilization (UHI) to induce muscle atrophy and then monitored their recovery for 15 days. The uncasted hindlimb was used as an internal control. We observed a significant increase in the cross-sectional area (CSA) and greater relative recovery of casted muscles of mKO mice compared to mWT mice. The casted gastrocnemius muscles of the mKO mice exhibited higher overall mass gain following recovery compared to mWT mice. Additionally, we observed increased overall physical activity of mKO mice compared to mWT immediately following cast removal. To understand the molecular mechanisms mediating these effects, we assessed mRNA and protein expression of genes relevant to muscle synthesis and degradation. In mKO muscles, we observed a decreased baseline expression of genes and proteins related to mitophagy (i.e Mfn2, Fis1) and autophagy (i.e Murf1, MAFbx, Foxo3, LC3B) and an increased baseline expression and phosphorylation of proteins related to muscle synthesis (i.e Akt, p-p70S6K). Following UHI, total ubiquitination and autophagy markers increased, but in the mKO the effect was ameliorated. In contrast, there were no significant differences in expression or phosphorylation of proteins related to synthesis. Our results indicate that deletion of *Tas1r2* in skeletal muscle improves muscle mass recovery through the regulation of protein turnover and autophagy. This suggests a potential role of TAS1R2 in attenuating muscle mass loss and dysfunction associated with conditions such as obesity, cancer, or aging.

22. Sex-specific neuroprotective mechanisms of Canagliflozin in the aged hippocampus and Alzheimer's Disease

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Aging is the primary risk factor for numerous chronic diseases, including neurodegenerative conditions like Alzheimer's disease (AD). Emerging evidence suggests that pharmacological interventions targeting various cellular and molecular pathways implicated in aging can delay its progression and mitigate late-life illnesses. We have recently demonstrated that an FDA-approved anti-diabetes drug, Canagliflozin (Cana), a sodium-glucose transporter 2 (SGLT2) inhibitor, extended the lifespan in the genetically diverse UM-HET3 male mice by 14%, without an effect on females. Moreover, Cana exhibited neuroprotective properties, such as improved central insulin responsiveness and reduced region-specific neuroinflammation. Furthermore, Cana treatment improved locomotor activity and exploratory behavior in aged male mice. Here, we investigated whether Cana has beneficial effects on the amelioration of AD pathologies. Transcriptomic analysis revealed significant changes in the hippocampus of aged 25-month old male but not female mice treated with Cana, as characterized by negative regulation of genes related to Toll-like receptor, Il-6, and p53 signaling pathways. Further assessment suggested that Cana treatment in the hippocampus delayed cellular senescence and neuroinflammatory signaling, in aged Cana-treated male mice. Moreover, microglial transcriptome, identified sex-specific genes related to microglial activation in Cana-treated aged mice. Accordingly, we detected increased levels of extracellular ASC, which is released upon NLRP3 inflammasome activation, in the hippocampus from aged, control males compared with Cana-treated males. Moreover, exposure of primary adult microglia cells to LPS induced the upregulation of NLRP3, IL-1β, and TNF-α gene expression, while treatment with the specific NLRP3 inhibitor, MCC95052 resulted in significant inhibition of LPS-induced gene expression. Similarly, Cana treatment reduced LPS-induced NLRP3, TNF-α, and IL-1β gene expression in primary microglia. Evaluation in a Cana treated 5xFAD mouse model of AD showed promising results in cognitive performance across various behavioral tests, including rotarod, grip strength, open field test and Y-maze. Our study highlights Cana's potential as a therapeutic agent for AD and provides insights into novel cellular and molecular mechanisms underlying its pharmacological effects.

23. FABP7: A link between neuroinflammation, aging and Alzheimer's disease

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Aging is one of the most important risk factors for the development of Alzheimer's Disease (AD), pointing towards a likely complex and intertwined relationship between the two. The aged central nervous system (CNS) is characterized by impaired immunoregulation, displaying an exacerbated and prolonged innate immune response. Many studies implicate neuroinflammation as a key process in the onset and progression of multiple neurological disorders, including AD. Astrocytes are critical players in the regulation of the CNS' innate immune response. Fatty acid binding proteins (FABPs) participate in fatty acid metabolism by regulating uptake and transport, but can also deliver and/or sequester ligands to regulate signaling processes. We previously showed that the upregulation of FABP7 induces a proinflammatory phenotype in astrocytes. Herein, we investigated a potential role for FABP7 in the regulation of the inflammatory response during aging and AD. We observed that FABP7 is upregulated in primary hippocampal astrocyte cultures after exposure to amyloid β peptide fragment 25-35 ($A\beta_{25-35}$). We confirmed that in the brain of AD patients and APP/PS1 mice, a widely used AD mouse model, FABP7 expression is upregulated in astrocytes and particularly evident in those surrounding amyloid plaques. Remarkably, astrocytes displaying high FABP7 levels showed higher expression of inflammatory markers. Conversely, silencing FABP7 in astrocyte cultures decreased the upregulation of inflammatory markers induced by different mediators. Strikingly, an upregulation in FABP7 expression in GFAP+ astrocytes was also observed in the cerebral cortex and hippocampus of 2-year-old mice, suggesting a potential role for FABP7 in the regulation of neuroinflammation during aging. Collectively, our results suggest the potential of FABP7 as a therapeutic target to prevent the establishment of an exacerbated inflammatory response in aging and AD.

24. Overcoming Barriers in Recruiting Vulnerable Patients with Lung Cancer in South Central Ohio: The Nutricare Study, a "Food is Medicine" Intervention

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Introduction: Despite recent efforts to expand clinical trial eligibility criteria to involve traditionally underserved patient populations, less than 5% of patients diagnosed with cancer choose to participate in clinical trials (lifestyle, behavioral or pharmaceutical), of which only a small fraction represents underserved or vulnerable populations. The barriers that contribute to these trends are undoubtedly multifactorial. Examining sociodemographic and clinical characteristics may provide insight into successful participant recruitment and retention of currently, underrepresented groups in clinical trials.

Methods: The NutriCare study (NCT04986670) is a national randomized controlled trial evaluating the impact of medically tailored meals plus weekly nutritional counseling on the outcomes of vulnerable patients with lung cancer. Eligibility criteria included: ≥65 years of age; underrepresented minority; rural resident; no health insurance; or income ≤130% of federal poverty line, diagnosis of non-small cell or small cell lung cancer starting cancer treatment. To remain on study, participants had to meet the eligibility criteria and complete 10 baseline surveys within 8 weeks of consenting to the study. A comprehensive list of screened patients was maintained throughout the course of the study, including approach attempts and patient reasons for declining participation. Strategies to improve recruitment included obtaining referrals from the treating physician, and inclusion of the study dietitian (or another trusted member of the oncology care team) during the informed consent process. Descriptive statistics were used to summarize the recruitment strategy required to meet enrollment goals.

Results: Overall, 261 participants were screened in Phase I of this study at The Ohio State University (Nov, 2020 – Aug, 2022), of which 69 (26%) participants consented to the study. Of these 69 participants, 40 (58%) were 65 years of age or older, 34 (49%) lived in a rural area, 7 (10%) identified as a racial or ethnic minority, and 22 (32%) met federal poverty levels. While 58

(84%) participants subsequently enrolled in the study, the remaining 16% did not complete the baseline assessments and were withdrawn from the study due to the following reasons: 1) unsatisfied with the arm they were randomized to, 2) feeling overwhelmed with their diagnosis/treatment, or 3) felt baseline survey completion was too burdensome.

Conclusion: Barriers to recruitment and willingness to participate in this clinical trial, especially for vulnerable cohorts, included effort and time burden required, feeling overwhelmed with recent diagnosis, and unwillingness to be in the control arm. Future randomized trials should consider crossover study designs to prevent dropout after randomization and measure time toxicity as an outcome to improve clinical trial participation and better meet the supportive care needs of vulnerable patients with lung cancer.

25. MAPLE

Hyeongseon Jeon

While various statistical and computational tools have been developed for high-throughput spatial transcriptomics (HST) data analysis, most of the currently existing methods focus on the analysis of a single sample. Hence, they cannot be utilized for analyzing HST data collected longitudinally and/or in multiple conditions, e.g., those from aging-related studies or multi-sample experiments to compare changes in tissue architecture between diseases vs. controls. To address this limitation, we developed MAPLE, which enables the joint analysis of multiple HST samples, allows differential abundance analysis (DAA), and provides uncertainty quantification. MAPLE combines the advantages of both deep learning and Bayesian modeling through a two-stage approach that derives spatially aware gene expression features using a graph neural network and fits a Bayesian spatial multivariate finite mixture model to identify cell sub-populations and implement DAA. It has been demonstrated that (i) MAPLE improves the accuracy and stability of the tissue architecture identification through effective information sharing; (ii) its embedded multinomial regression framework allows quantification of statistical significance for longitudinal changes or differences between conditions; and (iii) its Bayesian framework provides uncertainty quantification for tissue architecture identification and DAA, while also allowing the incorporation of various biological knowledge. To showcase the power of MAPLE in analyzing HST data related to lung tissues, we analyzed slides from healthy lungs and samples with fibrosis. MAPLE was able to identify the fibrotic zones with high resolution according to the anatomical region, as well as find spatial clusters between both groups.

26. Aging Glial Cells and Inflammaging exacerbated CNS Autoimmune Demyelinating Disease

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Age is a risk factor for the development of progressive multiple sclerosis (pMS). Accumulation of disability during pMS, as opposed to relapsing-remitting MS, correlates weakly with the frequency of actively inflamed white matter lesions, and is relatively unresponsive to therapies that target peripheral immune cells. Conversely, slowly expanding lesions with a rim of activated microglia, as well as widespread microglial activation, are more characteristic of pMS. Collectively, these observations have led to the hypothesis that CNS injury in pMS is mediated to a greater extent by CNS-resident cells than peripheral leukocytes. To assess the impact of aging on autoimmune neuroinflammation and glial activation, we compared clinical disease and pathology of Th17 adoptive transfer experimental autoimmune encephalomyelitis (EAE) in young adult versus middle-aged (M.A.) mice. M.A. mice exhibited a severe clinical course with high peak disability scores and mortality rates, and non-remitting course. In contrast, young mice displayed a milder course with no mortalities, and the majority underwent clinical remission. While the number of infiltrating immune cells in the CNS did not differ between groups, M.A. mice had a relative preponderance of donor $CD4^+$ T cells and neutrophils, and a dearth of B cells. Experiments with reciprocal bone marrow chimeras demonstrated that the age of non-hematopoietic compartment, as opposed to peripheral immune cells, determined the clinical phenotype. Microglia in the inflamed aged CNS exhibited a distinctive pro-inflammatory phenotype and transcriptome. A systemic chronic inflammatory state normally arises with aging, commonly referred to as "inflammaging", that is characterized by elevated pro-inflammatory plasma factors. We questioned whether age-related changes in the levels of circulating factors could contribute to the distinctive properties of microglia and the exacerbated course of EAE that we observed in M.A. mice. We found that microglia displayed a more reactive phenotype following culture with plasma from aged donors, compared to young adult donors. Serial transfers of heterochronic plasma were capable of modulating EAE clinical disease severity *in vivo*. Young mice receiving aged plasma displayed an exacerbated chronic clinical course, compared to PBS-treated controls. In contrast, aged mice receiving young plasma exhibited an ameliorated late phase of EAE compared to PBS-treated controls. Moreover, microglia from young mice receiving aged plasma displayed a more reactive phenotype compared to controls, while the inverse was observed for middle-aged mice receiving young plasma. Collectively, these results suggest that the aged CNS microenvironment, which includes highly activated glia, supports a robust encephalitogenic T cell response and drives a non-remitting clinical course. Furthermore, age-associated plasma components can modulate microglial activation, neuroinflammation, and clinical phenotype during EAE.

27. Single Cell RNA Sequencing Technologies: Evaluation of Cell Type Capture Efficiency in Human Lung Tissue and The Superiority of FRP in Conserving High Cellular Diversity and Cellular Senescence.

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Rationale: Modern genomics research has undergone a revolution thanks to technologies that enable transcriptomic analyses at both single-cell and nucleus levels, revealing unprecedented cellular heterogeneity in human lung tissue. Among these technologies, single nucleus RNA sequencing (snRNA-seq) and single-cell fixed RNA profiling (scFRP-seq) have provided foundational insights. Challenges related to time, depletion of specific cell populations, and sample viability can impact data interpretation. To address this, we evaluate the efficiency of cell type capture and its implications for detecting potential cellular senescence. Methods: Human donor lung samples were obtained from OSU (for scFRP-seq) and were compared with another, different, independent cohort from GSE171524 (PMID:33915568) performed by snRNA-seq. The samples were processed using bioinformatics analysis to process and integrate the data. We used the SCTransform method to normalize data from each sample. Finally, we evaluated canonical senescence markers like CDKN1A and CDKN2A and all the SenMayo database markers. Results: After integrating all samples, we noted variations in cell proportions. The scFRP-seq technology showed a more balanced representation of epithelial, immune, endothelial, and mesenchymal lineages in the lung samples than the snRNA-seq method. scFRP-seq technology demonstrated better capturing of potential cellular senescence cells compared to single nucleus analysis, identifying more senescence in mesenchymal, immune, and endothelial cells, especially in subpleural fibroblasts, smooth muscle cells, EC general capillary, EC arterial, and dendritic cells. Conclusions: Our results reveal that the use of the single cell fixed technique allows for a more efficient and detailed detection of senescence markers compared to the analysis of individual nuclei. The scFRP-seq technology not only offers a more balanced representation of lung cell composition compared to other methods but also surpasses them by allowing the assessment of not only nuclear changes but also cytoplasmic and cell surface alterations, which are fundamental for a comprehensive characterization of senescence.

28. Peroxisomal Dysfunction and Aging: Exploring the Role in Cellular Senescence and Mitochondrial Homeostasis

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Abstract

Peroxisomes recently emerged as an important regulator of aging. Recent studies from our group and others have revealed that the function of peroxisomal protein import is impaired in aged animals. This impairment leads to dysregulated lipid metabolism, elevated oxidative stress, increased inflammation, and altered mitochondrial morphology and function. However, how peroxisomal dysfunction contributes to aging remains largely unknown. To further understand how peroxisomal dysfunction contributes to aging, we performed a pooled genome wide CRISPR screen to interrogate the cellular responses to defective peroxisome. Our CRISPR screen identified SCAF1 as a novel regulator for cellular fitness and mitochondrial homeostasis in response to defective peroxisomal import. SCAF1 is a member of the serine/arginine (SR)-rich splicing factor family and is normally localized to nuclei. Intriguingly, SCAF1 is localized to mitochondria under peroxisomal import stress as well as mitochondrial stress. TurboID proximity labeling and proteomics analysis shows that SCAF1 interacts with many mitochondrial matrix proteins involved in mitoribosomal biogenesis, highlighting the critical role of SCAF1 in mitochondrial translation. Moreover, cells with mitochondrial-localized SCAF1 exhibit enlarged and flattened cell morphology and are protected from cell death, implying a connection between SCAF1 and cellular senescence. This is consistent with our transcriptomic analysis that shows peroxisomal dysfunction induces the expression of senescence-associated secretory phenotype (SASP) factors as well as anti-apoptotic BCl-2 members. Taken together, our study identified a novel SR-splicing factor in regulated mitochondrial homeostasis and potentially cellular senescence upon peroxisomal dysfunction.

29. A partial loss-of-function variant (Ile191Val) of the TAS1R2 glucose sensor is associated with improved exercise responses in older adults: a retrospective analysis

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Sweet taste receptors (STR), composed of the TAS1R2/TAS1R3 heterodimer, play crucial roles in sugar detection on the tongue and regulate incretin and insulin secretion in the intestine and pancreas. Recently, our lab identified TAS1R2 as a nutrient sensor in mouse skeletal muscles, activating poly(ADP-ribose) polymerase-1 (PARP1) and reducing nicotinamide adenine dinucleotide (NAD) levels upon activation. Consequently, skeletal muscle TAS1R2 deficiency in mice led to enhanced mitochondrial capacity, increased running endurance, and improved responses to exercise training compared to wild-type counterparts. Given the association between aging and muscle loss/dysfunction, we assessed the impact of TAS1R2 in 27-month-old mice. Aged TAS1R2-knockout mice exhibited increased muscle size/mass, improved strength, higher mitochondrial density, and enhanced exercise endurance compared to aged wild-type controls.

These preliminary findings prompted exploration of TAS1R2's role in older human adults. For this, we evaluated the TAS1R2-Ile191Val partial loss-of-function variant in older obese individuals undergoing a 6-month trial of diet-induced weight loss with exercise training (WLEX), diet-induced weight loss alone (WL), or an education control (CON). Participants were retrospectively genotyped as Ile/Ile (conventional TAS1R2 function) or Val/_ (partial loss-of-function) within each group. We examined parameters that are directly comparable to those assessed in the mouse models of Tas1r2 loss-of-function. The impact of WL or WLEX interventions appeared minimal without considering the TAS1R2 genotype. WL reduced body mass, with WLEX yielding modest improvements in body composition, and select indices of aerobic performance and *ex vivo* mitochondrial capacity. However, considering the TAS1R2 genotype revealed differential responses.

In the WLEX group, Val/_ carriers showed enhanced responses to exercise training compared to Ile/Ile counterparts (skeletal muscle index, RER peak, fasting glucose, and Hba1c). Additionally, TAS1R2 partial loss-of-function unveiled significant effects otherwise masked in the WLEX group compared to CON, including increased skeletal muscle mass, improved *in vivo* mitochondrial capacity and aerobic performance, and reduced fasting glucose, HbA1c, and HOMA-IR. These effects were absent in Ile/Ile counterparts. Val/_ carriers ranked above Ile/Ile participants in an overall composite index (sum of all measured outcomes), indicating a substantial effect size difference (Cohen's d=1.79).

These findings demonstrate genotype-dependent adaptations to exercise training, suggesting that TAS1R2 partial loss-of-function in humans yields beneficial effects on muscle mass and function, similar to observations in mice. Ongoing efforts aim to validate these findings across broader human populations (UK Biobank and the Study of Muscle, Mobility and Aging-SOMMA).

30. Spatial Transcriptomic Analyses of the Aging and IPF lungs Reveals Age and Disease Senescence Niches

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3. Department of Biomedical Informatics, Ohio State University, Columbus, OH, United States Rationale.

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Rationale. Aging has intricate effects on lung function and disease susceptibility. In idiopathic pulmonary fibrosis (IPF), senescent cell accumulation is a hallmark, but the shift from senescence to lung pathology remains unclear. Omics technologies, particularly spatial transcriptomics (ST), shed light on these changes by revealing distinct lung regions with unique ST signatures. This study delves into ST patterns in healthy (young and old) and fibrotic lungs to uncover senescent niches. **Methods.** We conducted a Visium ST experiment involving nine lung samples: two young, three old, and four IPF cases. Our focus was on healthy lung regions and the upper and lower parenchymal areas in IPF samples. We introduced the MAPLE bioinformatics framework, combining deep learning and Bayesian modeling to integrate multiple ST samples. We annotated ST clusters using LungMAP-LGEA markers and assessed the expression of IPF-associated genes. Additionally, we performed deconvolution analysis, pairing single-cell RNA sequencing profiles with each ST sample and computing senescence acores using the SenMayo database. **Results.** Our approach recovered eight novel ST clusters emerged, with five associated with alveolar regions, two with bronchial regions, and one with smooth muscle cells. The alveolar region clusters exhibited unique biological signatures compared to typical alveoli (Alveoli I). Notably, SenMayo+ spots were predominantly concentrated in the parenchymal region of older donors, with the highest density in the peribronchial area. Furthermore, the CDKN1A+ cluster in SenMayo+ spots from older lungs were linked to endothelial cells in Alveoli III and IV, connected to lipid metabolism, extracellular matrix production, and ER activity. In addition, we localized the aberrant alveolar region (KRT5-/KRT17+) with a shared expression of CTHRC1+ in IPF samples, which have previously demonstrated in single cell RNA-seq studies a senescent phenotype, offering spatial insight and for potential use of novel senescent specific regions. **Conclusions.** In summary, our study uncovered eight novel ST clusters, five of which are associated with unique alveolar regions in the lung. These alveolar clusters provided valuable insights into lung processes, emphasizing their relevance to senescent niches. Additionally, we identified the aberrant alveoli subcluster in IPF samples, shedding light on the transition from senescence to lung pathology.

31. Nutrient receptor Tas1r2 loss-of-function variant preserves muscle and metabolic function in aging mice

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Understanding the mechanisms underlying muscle and metabolic health in aging populations is crucial for developing interventions to maintain these functions. The sweet taste receptor Tas1r2, traditionally associated with taste perception, has been identified in non-taste tissues, including skeletal muscle, suggesting a broader physiological role. Our data indicate that body (bKO) or muscle-specific (mKO) deletion of Tas1r2 increases muscle mass and strength and improves mitochondrial function and endurance. Notably, 27-month-old bKO mice preserve these phenotypic attributes compared to wild-type (bWT) counterparts. To better understand the molecular mechanisms that mediate these effects, we performed transcriptomic analyses of the soleus muscle of young (y; 4 months) and old (o; 27-months) bWT and bKO mice and. We also independently explored Tas1r2 expression variation and regulation in muscles of genetically diverse BXD mice using transcriptomics data sourced from GeneNetwork.org. Gene Set Enrichment Analysis (GSEA) findings reveal that young bKO mice have enriched pathways related to muscle biogenesis, protein turnover, and mitochondrial function – all related to the maintenance of muscle health, enhanced energy metabolism, and the mitigation of age-related muscular degeneration and metabolic decline. Remarkably, old bKO mice had identical GSEA pathway signatures as young bKO, suggesting a protective role of Tas1r2 loss-of-function against age-related decline in muscle and metabolic health. In BXD mice, Tas1r2 expression was correlated with genes that regulate GPCR binding and processing. Significantly, BXD mice with the lowest and highest Tas1r2 expression levels (Tas1r2-low vs. Tas1r2-high) exhibited transcriptional signatures mirroring those observed in the comparison between bKO and bWT muscle tissue. This pattern was consistently validated through GSEA, where pathway signatures in young bKO versus bWT mice aligned with those in BXD mice across the spectrum of Tas1r2 expression. This validation reinforces our confidence in the robustness of the signatures observed in knockout mice, closely emulating the effects of loss-of-function Tas1r2 variants across diverse age groups. Such insights pave the way for future studies to explore these mechanisms in a genetically diverse population, reflecting a more realistic application and potential therapeutic avenues for muscle and metabolic health in aging.

32. Impact of CBX5 Mutations on Telomere and Centromere Stability in Cancer Development

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Abstract

Chromobox 5 (CBX5), a critical component in gene regulation, plays a significant role in cellular growth and development by binding to and silencing specific DNA regions, particularly in centromeres and telomeres. This process helps prevent aberrant gene expression and suppresses tumor metastasis. However, mutations in CBX5 can disrupt this regulatory mechanism, leading to untimely gene activation and contributing to carcinogenesis. In our study, we utilized data from the Catalogue of Somatic Mutations in Cancer (COSMIC) to investigate the patterns and impacts of CBX5 mutations. By employing the Cancer-Related Analysis of Variants Toolkit (CRAVAT), we analyzed mutations within both coding and noncoding sequences of CBX5 across various cancers and tissues. Our findings highlight a predominance of mutations in noncoding regions, suggesting that CBX5's regulatory control is mainly compromised at the gene expression level rather than through structural protein alterations. Notably, missense substitutions were identified as the most common mutation type, with a significant occurrence in carcinomas, particularly affecting the large intestine, liver, and breast in older adults (61-80 years). Despite the presence of mutations across different domains of the protein, our statistical analysis revealed no significant correlation between mutation location and functional disruption, indicating a widespread impact on CBX5's regulatory functions. Our research underlines the importance of CBX5 in maintaining cellular homeostasis and its potential role in the onset of various cancers. By connecting the frequency and nature of these mutations with patient age, we provide new insights into the age-related risks of developing CBX5-associated cancers. This study contributes to a better understanding of the genetic alterations leading to cancer and highlights the need for targeted research into CBX5's role in gene silencing and tumor suppression.

33. Ablation of Discoidin Domain Receptor 1 (DDR1) in Murine Model alters Bone Aging.

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I would like to be considered for an oral presentation

Bone is a composite tissue primarily comprised of collagen type I and hydroxyapatite mineral. Peak bone mass is achieved in young adulthood and bone mass begins to decrease around middle age. Loss of bone is exacerbated due to menopause and can lead to osteoporosis type I. Dysregulation of bone remodeling by osteoclasts and osteoblasts is the primary cause of bone loss. The impact of cell surface collagen receptors on bone loss is an area of increasing interest, as they may lead to therapeutic applications.

In this study we investigate the role of Discoidin Domain Receptor 1 (DDR1), a receptor tyrosine kinase present in both osteoclasts and osteoblasts. Studies were conducted on DDR1 knockout mice and their wild type littermates between 2 to 12 months of age. Our results show a compromised bone quality (reduced stiffness) in 12-month-old DDR1 knockout mice. This was accompanied by decreased osteoclast activity in the cortical bone of 12-month DDR1 KO mice femurs. In vitro osteoclastogenesis of bone marrow derived cells was also reduced in DDR1 knockout (KO) mice. Transmission electron microscopy analysis revealed an increase in collagen fibril diameter while, picrosirius red staining showed increased immature collagen in the DDR1 KO mice. These results signify that attenuation of DDR1 can lead to impaired bone loss due to decreased osteoclast activity, which may be an attractive therapeutic target for osteoporosis a disease prevalent in aging populations.

34. Characterizing metabolic and age-related changes through complementary mass spectrometry imaging and histological methods

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There is a real need to better understand how different cell types within an organ respond to environmental changes and determine if they display similar age-dependent decline. Previous work from our lab and others has shown that there is an age-dependent decline in intestinal tissues that is accompanied by metabolic changes. To capture this better, we have developed several approaches, along with collaborators, to better map metabolites spatially at near single-cell resolution using Mass spectrometry imaging (MSI). This powerful approach allows researchers to identify many single molecules simultaneously by preserving their spatiotemporal distribution on a single tissue cross-section. Our lab has used intestinal organoids to study stem and progenitor cell turnover in relation to diet and aging which have been a powerful tool for modeling hereditary and age-related diseases, cancer, toxicology, and regenerative medicine. Researchers have reported protocols to analyze various tissues, spheroids, and single-cell layers of organoids by MSI. However, further optimization was necessary to preserve the fragile 3D budding organoid structures often comprised of a heterogeneous population of epithelial cells or for combined complementary immunostaining techniques. Therefore, we have tested different polymer solutions and conditions to design the most suitable matrices for studying budding intestinal organoids by MSI analysis. Mouse small intestinal organoids were generated from wild-type C57BL/6 mice and embedded in different hydrogels for MALDI-MSI. Single-layer budding organoid sections were then analyzed by a timsTOF fleX MALDI-2 mass spectrometer in the negative ion mode and data was analyzed by using Scils Lab. In addition, we were able to couple MSI with immunofluorescent staining from consecutive sections of single-cell layer budding organoids. We found out that HPMC-PVP hydrogels provide the best physical conditions to preserve organoid morphology and analysis by both MALDI-MSI and immunohistology. In parallel, we further optimized tissue preparation of small intestinal and colon tissues for MALDI-MSI analysis and discovered putative lipid species that are differentially abundant in young versus aged tissues. We believe that the combination of these two important approaches will help develop a comprehensive spatial analysis and maps of aging tissues and may lead to novel biomarkers that can be used to improve the treatment of age-related diseases.

35. Influence of Excess Nicotine Exposure on Dopamine Dysregulation in Caenorhabditis elegans During Development

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Abstract

Our study explores the effects of nicotine on biological aging processes, employing *Caenorhabditis elegans* as experimental model. We specifically examine how varying concentrations of nicotine (0 mM, 0.1 mM, 1 mM, and 10 mM) influence physiological health during the developmental, sexual maturity, and senescence stages of *C. elegans*. Initial findings during development reveal significant alterations in neuron and neuronal cluster fluorescence intensities in early L4 stage worms, reflecting potential changes in neural wiring integrity. Nicotine, known to act as an agonist for nicotinic acetylcholine receptors (nAChR), plays a crucial role in neuromuscular synaptic transmission. This interaction is critical in both human physiology and in our *C. elegans* model, specifically the BZ555 strain, which provides visual markers for dopamine (DA) expression. Given the role of DA and acetylcholine (ACh) in modulating locomotion and behavior, our observations offer insights into nicotine's impact on neuron functionality and integrity, which could be reflective of early life stage exposure impacts in humans. Preliminary results demonstrate a dose-dependent relationship between nicotine exposure and changes in neuromuscular function, as evidenced by variations in pharyngeal pumping rates. Notably, the highest nicotine concentration (10 mM) showed a significant decrease in neuronal fluorescence density, suggesting a toxic effect that may impair neuronal function. These findings raise concerns regarding the potential early onset of dopamine insufficiency diseases, such as Parkinson's Disease, and highlight the necessity for further investigation into dose-dependent neuronal impacts. Future research will focus on comparing these effects with those induced by the neurotoxin hydroxydopamine, and examining the combined effects of nicotine vape fluid components, such as metals and microplastics. This comprehensive approach aims to uncover the broader implications of nicotine and vaping product exposure on aging and neuron function, contributing valuable insights to the field of aging research.

36. Novel lipid senolytics targeting senescent cell vulnerability *via* ferroptosis

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Abstract:
Cellular senescence is a key mechanism and driver of the aging process. Senescence is conventionally characterized by halted cell proliferation, resistance to apoptosis, and a complex senescence-associated secretory phenotype. With age, senescent cells accumulate in various organs and contribute to tissue dysfunction and various age-related conditions. Senolytics, drugs that selectively eliminate senescent cells, have been proven as an effective strategy to extend healthspan and treat many age-related diseases. Despite the promise, only a small number of senolytics have been reported to date and many exhibit undesirable side effects or pharmacokinetic profiles that limit their clinical translation. Thus, the discovery of new senolytics is needed. Through phenotypic cell-based drug screening, we have recently identified a class of polyunsaturated fatty acids as novel senolytics. These lipid senolytics demonstrated potent activity in various human and mouse senescent cell models. Their senolytic efficacy was further validated in progeria and naturally aged mice models, where they not only reduced senescent cell burden in multiple tissues but also improved healthspan of mice. Intriguingly, our preliminary studies found that these novel lipid senolytics induced senescent cell death through ferroptosis, unlike the apoptosis pathway targeted by traditional senolytics. Ferroptosis is a form of cell death mediated by iron-dependent lipid peroxidation. The senolytic activity of these lipids can be suppressed by ferroptosis inhibitors and iron chelators, but not by apoptosis or necrosis inhibitors. We are currently conducting lipidomics and RNAseq analyses aiming to further elucidate the mechanisms underlying this novel class of ferroptosis-inducing lipid senolytics. Collectively, our research has discovered a new class of lipid senolytics that exploit ferroptosis to eliminate senescent cells. These findings not only expand our understanding of senolytic mechanisms, but also offer a novel senotherapeutic approach by targeting a distinct vulnerability of senescent cells *via* ferroptosis.

37. Pro-fibrotic fibroblast MOXD1 expression is associated with impaired copper transport and cellular senescence in Idiopathic Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an age-related disease of unknown etiology, characterized by the accumulation of senescent cells. It has been reported that intracellular accumulation of copper (Cu^{2+}) due to defective homeostasis can promote senescence in fibroblasts through impaired autophagy, increased ROS, and an increase in expression of pro-fibrotic proteins such as collagen and TGF-β, thus playing an important role in the pathogenesis of IPF. Previously we discovered, using single-cell RNA sequencing (scRNA-seq), that IPF fibroblasts showed a significant increase in expression of Cu^{2+}-binding proteins, particularly MOXD1, a Cu^{2+}-binding enzyme. However, the mechanisms behind the increased MOXD1 expression and its functional role in IPF and senescence remain unknown. Therefore, we aimed to assess whether changes in the expression of two major regulatory intracellular transporters of Cu^{2+}, ATPase Cu^{2+} transporting alpha (ATP7a) and Cu^{2+} transporter 1 (Ctr1), are associated with an upregulation of MOXD1 in fibroblasts as a consequence of an intracellular Cu^{2+} imbalance. Due to the recognized heterogeneity in fibroblasts in fibrotic lungs, we measured the expression of MOXD1, ATP7a, and Ctr1 in whole lung tissue from healthy donors and IPF patients by scRNA-seq analysis. Subsequently, we validated our data in whole lung tissue and human lung fibroblast (hLF) by Western blot and immunofluorescence assays. We also performed the same analysis in human precision cut-lung slices (hPCLS) treated with bleomycin (BLM), a chemotherapeutic agent known to induce cellular senescence and IPF-like characteristics in human lung tissue. Our data show that MOXD1 expression increased in pro-fibrotic fibroblast population in whole lung and hLF from IPF lung. Interestingly, MOXD1-positive fibroblasts exhibited ATP7a (Cu^{2+}-exporter) downregulation while Ctr1 (Cu^{2+}-importer) is upregulated in IPF. Comparable to IPF fibroblasts, scRNA-seq analysis in BLM-treated hPCLS showed a decreased expression of ATP7a with increased levels of MOXD1. In addition, we observed that labile Cu^{2+} accumulated in BLM-treated hPCLS. Our findings suggest that increased expression of MOXD1 is associated with rising Cu^{2+} levels and may indicate a compensatory mechanism in response to the downregulation of ATP7a. These results indicate an important role of MOXD1 in the pathogenesis of IPF.

38. 17α-Estradiol longevity treatment improves metabolic and peripheral nerve parameters in aged male and female mice

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Age-related peripheral neuropathy (PN) is a top cause of small fiber PN in humans, and we previously demonstrated that PN also impacts subcutaneous adipose tissue of aged mice and humans. We and others reported less severe diabetic PN in female mice, with protection from age-related PN until reproductive senescence, but we found that PN in skin, muscle and adipose was not mitigated with rapamycin longevity treatment in aged HET3 mice of either sex. Genetically diverse HET3 mice are utilized by the NIA's intervention testing program (ITP) to systematically test longevity treatments. Given our observed sex differences in age-related PN, we tested another validated longevity treatment,17α-Estradiol (17α-E2). 17α-E2 is a non-feminizing sex hormone that extends lifespan in male but not female mice. However, its effects on health measures in either sex have been little examined. Middle aged (~51 wks) and older (~84 wks) male HET3 mice received 17α-E2 in diet for 17 weeks. We observed increased grip and plantarflexion muscle contractility and improved NMJ function in middle aged 17α-E2 treated mice. 17α-E2 treatment improved intraepidermal innervation (a histological measure of small fiber PN) only in the older mice. Reduced fat mass was seen in all 17α-E2-treated mice regardless of age, but glucose sensitivity was improved only in treated older males. Increased energy expenditure was seen in 17α-E2 treated middle aged mice. These metabolic and neural health tests also varied by genetic strain contribution. For example, grip strength improvement was most evident when C57BL/6 or DBA/2 founder contribution to the HET3 strain was low, as measured by SNP%. Studies in female HET3 mice given 17α-E2 across the lifespan are underway; thus far reduced fat mass was seen in 17α-E2-treated mice of both sexes. Overall, we found that 17α-E2 treatment is a translationally relevant way to maintain metabolic and nerve health across aging.

39. Repairing the Skin Barrier: Utilizing Topical Senolytics for Diabetic Wound Healing

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Abstract

More than 25% of the United States population aged 65 and above are affected by diabetes mellitus, with the aging demographic playing a substantial role in fueling this epidemic. Diabetes mellitus, a metabolic disorder characterized by elevated blood sugar levels, initiates chronic, low-grade inflammation, rendering patients more susceptible to infections and increasing the likelihood of developing diabetic foot ulcers (DFUs). Studies reveal that senescent cells can both promote and inhibit cutaneous wound healing. Cellular senescence, defined as an irreversible cell cycle arrest, can be pharmacologically targeted using senolytics, portending an effective strategy to expedite chronic wound closure. The aim of this study was to examine the effect of topical fisetin, a senolytic inhibitor of PI3K/AKT/mTOR. To evaluate if senescent cell clearance via topical fisetin can promote wound closure, full-thickness excisional wound was created on the dorsum of db/db$^{-/-}$ type-2 diabetic model; these animals were treated with topical vehicle or topical fisetin for three consecutive days per week for three consecutive weeks (n=9 total topical applications). Skin re-epithelialization and epidermal thickness was measured by histological analysis. Effect of topical fisetin on p16^{INK4a} and p21$^{Waf1/Cip11}$ senescent cells was analyzed by RNA- in situ hybridization (ISH) and SA-β-gal. RNA expression of senescence-associated secretory phenotype (SASP) was evaluated by qPCR. Topical fisetin did not significantly mitigate wound closure but increased of epithelial thickness and reduced p21$^{Waf1/Cip1}$- accumulation and SA-β-gal+ senescent cells. It further reduced SASP gene expression in wound healing in type-2 diabetic mice. This study shows that pharmacological targeting of survival pathways in senescent cells can be translated into interventions that enhance chronic wound healing, and may yield new strategies to treat chronic skin conditions.

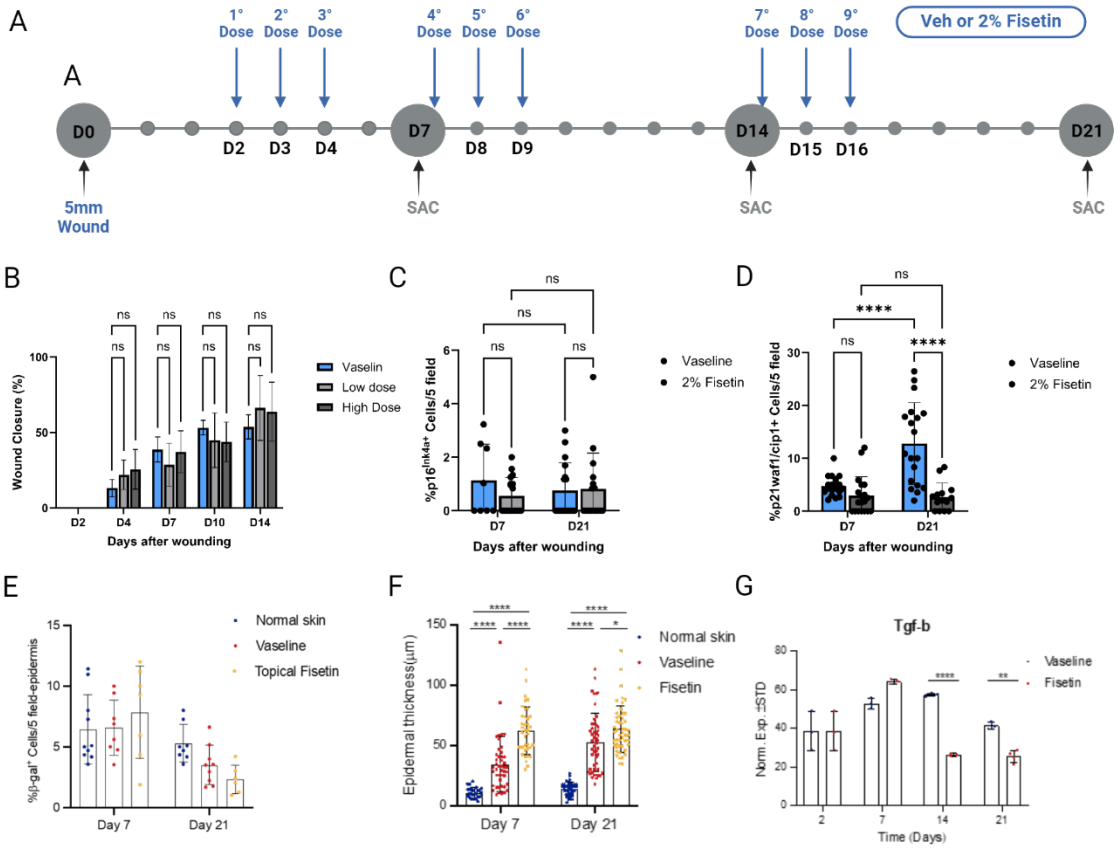

Figure 1. Fisetin treatment clearance senescence cells and increased the epithelial thickness in Type-2 diabetic mouse wound. A. Schematic diagram of the procedure, full thickness excisional skin wound was created on the backs of db/db$^{-/-}$ type-2 diabetic model (17-weeks-old) and was administrated topical treated with vehicle or Fisetin for three consecutive days per week on three consecutive weeks. B. Wound contracture assessment as a function of % wound closure. Quantification of p16^{Ink4a} (C), p21$^{Waf1/Cip1}$ (D) and SA-β-gal (E) positive cells in dermal tissue. F. Epithelial thickness graphic representation. G. Relative expression of senescence and SASP markers in Vaseline- and Fisetin-treated wound skin after 2, 7,14 and 21-days post-wounding. Measurements are expressed as mean ± SEM. Statistical analysis was performed using Student's t-test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

40. Determining regional age and diet dependent adaptations in the gut using single-cell analysis

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The mammalian gastrointestinal tract is one of the largest surface areas in the human body and is constantly exposed to dietary nutrients and microbially derived metabolites. Its rapid regeneration relies on a defined pool of stem cells, which are influenced by environmental factors and nutrient availability. We and others have shown that diet composition and nutrient availability regulate key nutrient-sensing pathways in intestinal stem and progenitor cells, affecting stem cell fate decisions. Acute nutrient deprivation in the form of fasting primes intestinal stem cells to uptake and utilize fatty acids, influencing intestinal stem cell clonogenicity, proliferation, and gut regeneration. Additionally, we have shown that aged intestinal stem cells have altered metabolic state and differential response to nutrient availability. In particular, intestinal stem and progenitor cells of aged animals have reduced utilization of fatty acids and reduced proliferation compared to young animals. Employing a multi-omics scRNAseq approach and analysis, we have further investigated age dependent and region dependent changes in the mammalian small intestine and colon, confirming some of our previous findings and discovering new metabolic and immune pathways deregulated in aged intestinal stem cells. These observations provide a potential dietary and metabolic strategy for improving intestinal regeneration and host defense in old age.

41. Chronic Allergen Challenge Induces a Distinct Lung Inflammatory Milieu in Aged Mice

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RATIONALE: Individuals 65+ years of age with asthma have been found to have greater inflammation and decreased sensitivity to corticosteroid treatment, which leads to more difficulty managing their asthma symptoms. Neutrophil infiltration and IL-17A, an effector cytokine in type 17 inflammation, levels are more commonly found increased in aged individuals with asthma. This unique inflammatory environment involves pathways related to inflamm-aging and may contribute to diminished sensitivity to corticosteroids. Potential differences in inflammatory responses in the young vs. aged asthmatic lung has yet to be fully characterized. In the present study, we aimed to understand the differential immune responses to chronic mixed allergen exposure in young and aged mice.

METHODS: Six-week-old (young) and 13- and 18-month-old (aged) wild type C57BL/6 mice were exposed intranasally to either PBS or *Alternaria alternata*, *Aspergillus fumigatus*, house dust mite, ovalbumin, and cyclic-dimeric guanosine monophosphate (MA+GMP) three times a week for four weeks. Additionally, young and aged mice were administered 4 mg/kg IgG or IL-17A monoclonal antibody (mAb) intraperitoneally twice a week for the last three weeks of the allergen protocol. Bronchoalveolar lavage (BAL) fluid was collected 24 hours after the last exposure. BAL immune cell levels were evaluated by differential analysis.

RESULTS: MA+GMP exposure significantly increased total BAL cell counts in young and aged mice, with increases in eosinophils, neutrophils, macrophages, and lymphocytes. Compared to young mice, MA+GMP challenged aged mice had significantly greater BAL lymphocyte levels, while macrophage, neutrophil, and eosinophil counts were comparable. Upon administration of IL-17A mAb, young mice challenged with MA+GMP had lower neutrophils and eosinophils than young mice given MA+GMP and IgG. Conversely, aged mice given MA+GMP and IL-17 mAb had no significant decrease in immune cell populations compared to aged mice administered MA+GMP and IgG. There was also no significant difference in the immune cell populations in response to MA+GMP and IL-17A mAb between male and female mice for both age groups.

CONCLUSIONS: Our data suggests that there are differences between young and aged mice in response to chronic mixed allergen exposure. Neutralizing IL-17A was shown to only be effective in reducing airway immune cell populations, specifically neutrophils and eosinophils, in young mice and not aged mice. Our model can be used to further explore distinct mechanisms in allergic airway inflammation and the aging asthmatic lung.

42. Lifelong restriction of dietary valine has sex-specific benefits for health span and lifespan in mice

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Abstract

Despite prevailing dietary advice, higher protein consumption is associated with increased rates of cancer, diabetes, and diabetes mortality. In contrast, protein restriction (PR) promotes metabolic health in humans, and in rodents, improves glycemic control and promotes leanness. The Lamming lab has previously shown that many of the benefits of PR are the result of a reduced consumption of dietary branched-chain amino acids (BCAAs; leucine, valine and isoleucine). The restriction of BCAAs extends health span and lifespan in mice. The metabolic benefits of BCAA restriction are mediated by isoleucine and valine, with isoleucine restriction being sufficient to extend lifespan in mice. However, it remains unknown whether valine restriction improves lifelong health and extends lifespan. In this study, we utilized male and female mice (n=25 mice per diet per sex) and placed them on a control (CTL; 21% protein, 100% valine) or valine restricted (Val-R; 21% protein, 33% valine) diet starting at 4 weeks of age and followed them longitudinally. Every 6 months, we performed physical fitness and metabolic phenotyping assessments until 24 months of age as well as assessed frailty and body composition longitudinally.

Val-R-fed male and female mice displayed significantly improved glucose regulation and increased energy expenditure, coinciding with decreased body weight and adiposity. These results are independent of pro-longevity hormone, FGF21. We found that the Val-R animals are less frail and have maintained physical fitness, despite lower valine-related muscle growth. Strikingly, Val-R extends median lifespan in male mice by 24%, but not female mice. In conclusion, Val-R improves multiple aspects of health in mice of both sexes as well as extends lifespan in male mice. This suggests translational potential of valine restriction on protection from aging and age-related diseases in humans.

43. Age-Related Changes in R5 Structure and Neural Network that Induce Sleep Instability in *Drosophila*

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As sleep homeostatic regulatory mechanisms deteriorate with aging, sleep becomes more irregular and fragmented. Deleterious effects of disrupted sleep underlie neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases. While the circadian clock has been well-studied at the molecular level, the sleep homeostasis model is less understood. We focused on the R5 ring neurons within the ellipsoid body in *Drosophila* to assess sleep drive influence on sleep quality across the lifespan. Sleep homeostasis reflects the pressure for sleep that builds up with longer periods of wakefulness. Sleep pressure is altered by membrane potential fluctuations. Brain activity measured by local field potential revealed that network coordination is disturbed in aged flies compared to young, which may lead to an age-dependent reduction in sleep drives. RNAi screening was performed to locate age-inducing molecules affecting the structure of R5 neurons. Several young RNAi samples were clustered with aged control samples, indicating that the knockdown of these molecules modified young neurons to have similar morphology as aged neurons. Ly6, a protein that participates in sleep modulation, is one of the molecules that display this change.

An RNA sequencing analysis of young and aged flies characterized various Ly6 genes as downregulated and upregulated. In congruence with the downregulation of genes involved in metabolism and protein synthesis during aging, we postulate that the loss of some Ly6 genes may also promote aging characteristics, and therefore the restoration of these genes will intervene with aging effects. On the other hand, the upregulation of Ly6 genes may be a compensatory response, revitalizing lost sleep regulation functions by increasing the expression of related genes. Overall, the dysregulation of Ly6 gene expression contributes to sleep instability. By identifying how Ly6 gene expression alters R5 neuronal structure and function, we can determine the consequences of structural deterioration on sleep quality and quantity and understand how sleep disruption aggravates neurodegenerative disorders. This insight may contribute to the development of a remedy to prevent or mitigate disease progression during aging.

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44. Skeletal Muscle Fitness and Maintenance of Long-Lived Dward Mice

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Ames dwarf mice live 50% longer than control littermates due to a genetic defect in growth hormone (GH) signaling, displaying delayed ageing processes in multiple body systems. A relatively unexplored organ system of this model is skeletal muscle (SM), which dictates energy consumption and serves as a good determinant of overall health. Here we compare the function, morphology, and maintenance of Ames dwarf mice SM against chronologically age-matched wild-type controls. SM function was evaluated across a six-month period by having mice perform grip strength, rotarod, and endurance running experiments. Cohorts were divided into young, middle, and aged groups with regular plasma collections for future cytokine analysis. Dwarf mice show improved scores in relative grip strength, walking gait, and endurance running times compared to age-matched wild type mice. Proposed mechanisms behind increased function include improved oxidative and regenerative capacity in dwarf mice, paired with a higher percentage of type I fibers owing to muscular endurance. Histological analysis of muscle fiber quality and size was conducted via H&E and laminin staining of sectioned tibialis anterior muscle taken from both groups. Subsequent analysis using ImageJ software was conducted to quantify cross-sectional area (CSA) of individual muscle fibers. Dwarf mice show healthy nucleation yet considerably smaller (CSA) of than wildtypes - unsurprising due to their dwarfism. Future analysis of nucleation, stem cell population, and fibrotic environment of muscle taken from aged individuals should especially elucidate any meaningful differences. Upon completion of fitness testing, transcript analysis from harvested muscle should identify differentially expressed genes between genotypes and the cellular pathways they influence. Utilizing a longevity model to study SM function and maintenance is a novel approach to gain insight into the seemingly inverse relationship between GH signaling and mammalian longevity.

45. Mutations in the Growth Hormone Receptor Gene and Their Effect on Human Cancers

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Abstract

The Growth Hormone Receptor (GHR) gene plays a crucial role in regulating the growth hormone pathway and various growth-associated processes. Mutations in this gene can disrupt normal cell growth and replication, which are processes intricately linked to aging. As the risk of mutations increases with age, we investigated the relationship between GHR mutations and cancer development. Our study utilized data from the Catalogue of Somatic Mutations in Cancer (COSMIC) and employed the Cancer-Related Analysis of Variants Toolkit (CRAVAT) to analyze mutations based on patient age, cancer type, and mutation characteristics. Our findings indicate that mutations in the GHR gene, particularly missense substitutions, are predominantly observed in skin, hematopoietic, lymphoid tissues, and prostate cancers. These mutations are most prevalent among individuals aged 51-80, suggesting a significant age-related component. Notably, mutations in the conserved domains of the GHR gene were found to be more pathogenic and strongly associated with cancer, highlighting the importance of these regions in maintaining normal GHR function. Through our analysis, we identified several mutations that disrupt GHR functionality, acting as potential cancer drivers. This correlation between pathogenic mutations in the GHR gene and cancer suggests a crucial link between disrupted growth hormone signaling and carcinogenesis. Our study contributes to the understanding of how age-related changes in the GHR gene can influence cancer risk and underscores the importance of monitoring these mutations as potential biomarkers for cancer development.

46. Lung Microbiome Associated with Accelerating Epigenetic Aging Reveals Smoking Effects: A Pilot Study

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Lungs harbor a variety of microbiomes that can affect pulmonary health. Aging is linked to declined respiratory function, and premature lung aging may lead to an increased risk of pulmonary diseases. A recent study shows airway microbiomes to be linked to lung function in an age-dependent manner. However, no study has been reported on a relationship between microbiomes and biological aging of the lungs. As an exploratory study, we identified bacteria species associated with accelerated lung aging in conjunction with a host factor, such as smoking.

We obtained lung epithelium for biological aging measured by DNA methylation profiles (mAge) using the IlluminaEPIC chip and bronchial alveolar lavages for microbiomes using RNA sequencing from healthy young individuals (21-30, n=26). We analyzed GrimAge acceleration, referring to faster or slower mAge than predicted based on chronological age, which is known to predict lifespan and mortality independently of chronological age and genetic influences. We used the k-means clustering to classify samples into either a faster mAge or slower mAge group. Using the XGBoost algorithm, we identified bacteria species that predict a faster or slower mAge group. We then validated these bacteria species using the Leave-One-Out Cross-Validation method and performed a logistic regression model to associate bacteria species and Grim-Age. We also studied the association between identified bacteria species and smoking status (cigarette smoking, electronic cigarette use, and neither [never-smokers]) using a regression model after adjusting for chronological age. For smokers, we associated bacteria species with cotinine-adjusted volatile organic compounds (VOCs, n=10) and polycyclic aromatic hydrocarbons (PAHs, n=8), measured in urine using a regression model after adjusting for chronological age.

Our study found that in the lungs of healthy individuals, two bacteria species, *Alistipes finegoldii* and *Arachidicoccus sp. BS20*, can predict a faster or slower mAge group with 81% accuracy, 82 % sensitivity, and 80% specificity. The fitted model accurately predicted a faster or slower mAge group using *Alistipes finegoldii* (*P*=0.03) and *Arichidicoccus sp. BS20* (*P*=0.04). The area under the curve (AUC) index was 0.95. Smokers (n=7) had significantly lower *Alistipes finegoldii* (P=0.002) and *Arachidicoccus sp. BS20* level (P=0.001), compared to never-smokers (n=10). Neither bacteria species were associated with electronic cigarette use. Interestingly, in smokers, a

higher level of *Alistipes finegoldii* was significantly associated with higher levels of 2-Hydroxypropyl Methacrylate (a metabolite of propylene oxide, *P*=0.02), Acrylamide Mercapturic Acid (a metabolite of acrylamide, *P*=0.01), and 1-hydroxyphenanthrene (a PAH metabolite, *P*=0.004). Also, a higher level of *Arachidicoccus sp. BS20* was associated with monohydroxybutenyl-mercapturic acids (a metabolite of 1,3-butadiene, *P*=0.03).

We identified *Alistipes finegoldii* and *Arachidicoccus sp. BS20* as bacteria species that predict lung epigenetic aging which provides insight into the relationship between microbiomes and lung health. Also, we found that smoking has an impact on the levels of these bacteria species, and they are affected differently by different smoking-related toxicants. These findings highlight the need for further research on the potential role of lung microbiota composition in accelerating lung biological aging and their roles in pulmonary health.

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47. Identification of Aging and Senescent Cells in Different Anatomical Regions of Healthy Lungs Using Fixed RNA Profiling

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Rationale. The study of lung cell populations in response to aging has been challenged by the aging process and the complex lung cellular composition. Therefore, we aimed to identify at the single-cell level aging and senescence transcriptional signatures specific to cell types and anatomical regions within healthy lungs.

Methods. We used single cell Fixed RNA Profiling by 10x genomics on young and aged lungs from three different anatomical regions parenchymal, subpleural and peribronchial. The single cell data analysis was performed using the workflow of Seurat v4.1 and R v4.2.1. Similarly, the bioinformatics integration of the different areas was applied using the Reciprocal PCA (RPCA) method. Cell subpopulation identities were defined using the Human Lung Cell Atlas annotation. Finally, the scores were calculate using the module score method by Seurat, for aging score we used a list of genes related to lung parenchymal aging generated by our group (LungAge Score) and for senescence we considered the SenMayo database.

Results. The results showed a higher LungAge Score in immune cells, such as alveolar macrophages and monocytes in the aged subpleural samples, followed by alveolar and adventitial fibroblasts, which had a higher score in the peribronchial samples. We did not observe significant changes in the endothelial and epithelial lineage. On the other hand, samples from the subpleural region exhibited a high senescence score in adventitial fibroblasts, while in the parenchymal and peribronchial were the alveolar ones. We also obtained high senescence scores but now of alveolar macrophages in the parenchymal samples, likewise epithelial cells such as basal, club, ciliated, AT2 (alveolar type 2 cells) and transitional state exhibited a high senescence score in the subpleural and parenchymal region.

Conclusion. We identified transcriptional signatures related to different anatomical regions of the aged lung with a specific pattern of aging and senescence for different lung cell types such as alveolar and peribronchial fibroblasts, as well as alveolar macrophages, which may be related to dysregulated processes in these senescent cells and showing a heterogeneous effect of aging on lung cells.

48. Regulation of lifespan and metabolism by the transcription factor Jim

Authors:

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Aging is characterized by complex remodeling of the epigenome, including loss of repressive heterochromatin that may subsequently lead to aberrant gene expression and de-repression of retrotransposons (RTE). However, how specific perturbations to heterochromatin affect the aging process are not fully understood. Using the model system *Drosophila melanogaster* (fruit flies), we identified the transcription factor "*Jim*" in a genetic screen for genes that are necessary for RTE silencing. We found that reducing *Jim* expression enhances RTE mobilization in an RTE reporter line. *Jim* overexpression increases silencing in a heterochromatin reporter line and increases H3K9me2 levels, a histone modification associated with heterochromatin. Whole-body *Jim* overexpression dramatically shortens lifespan while increasing starvation resistance in male flies. Oenocyte (analogous to liver)-specific *Jim* overexpression also shortens lifespan and produces major deficits in desiccation resistance and cuticle waterproofing. *Jim* overexpression led to differential expression of several thousand target genes, which were enriched for genes involved in lipid and carbohydrate metabolism. We found that *Jim* knockout or knockdown during development is lethal and adult-specific knockdown radically shortens lifespan in both sexes. We conclude that *Jim* is a major regulator of heterochromatic silencing, metabolism, and lifespan.

49. T cell receptor signaling induces p16^{INK4a} expression

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T cell senescence in peripheral blood, measured by p16^{INK4a} expression, is a frequently used marker of biological aging in human populations. However, it remains unclear what triggers increased p16^{INK4a} expression in these cells or how this important cell cycle regulator becomes elevated with age. We monitored *p16^{INK4a}* mRNA levels during repeated rounds of T cell receptor (TCR) engagement in mouse cells and found increased levels of *p16^{INK4a}* during stimulation. Furthermore, *p16^{INK4a}* levels remained higher than baseline during the rest period between stimulations. To understand how TCR signaling increases p16^{INK4a} expression, we constructed a luciferase reporter driven by 1.5 kilobases of the human *p16^{INK4a}* reporter. In T cells derived from a human lymphoma (Jurkat), we found that stimulation with anti-CD3 antibody was sufficient for maximal reporter activation. However, in primary human T cells, both CD3 and IL-2 signaling was needed to achieve peak p16^{INK4a} expression. Further testing in primary T cells with anti-CD3 in combination with TGF-B, IL-6, or TNF-a revealed that none of these cytokines known to influence T cell fate, increased elevated *p16^{INK4a}* levels as strongly as CD3 and IL-2. To narrow down where transcription factors could be binding to the promoter, we created several variants of the reporter with different promoter lengths, from approximately one kilobase to 130 nucleotides. Using these constructs, we narrowed the region of transactivation to between -520 and -260. Using PROMO we identified predicted binding sites for NF-AT1, AP-1, Sp1, and E2F-1 within the transactivation area. These data identify a 260 bp region of the *p16^{INK4a}* promoter transactivated by factors downstream of CD3 and IL-2 signaling. Further, we establish a cyclical pattern of *p16^{INK4a}* expression during T cell stimulation and rest, which does not restrain proliferation.

50. Impaired Fatty Acid Oxidation Influences Nuclear Dynamics in Alveolar Type 2 Epithelial Cells.

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RATIONALE: Fatty acid oxidation (FAO) is the process by which fatty acids are transported into the mitochondria and broken down into acetyl-CoA units that feed the TCA cycle for energy production or serve as substrate for protein acetylation. Histones are highly acetylated proteins, their acetylation regulates transcriptional processes, sustains nuclear lamina stability, and is affected during senescence. Our lab has shown that aged and IPF AT2 cells exhibit mitochondrial metabolic defects associated with the development of fibrosis. How these mitochondrial defects influence the epigenome affecting AT2 homeostasis remains unexplored. We have aimed to elucidate the mechanisms by which metabolic reprogramming regulates nuclear transactions in AT2 cells.

METHODS: Cpt1a is the FAO rate limiting enzyme responsible for transporting fatty acids into the mitochondria. We employed control and Cpt1aKO MLE12 cells and assessed global histone changes by bottom-up mass spectrometry. Mass spec was validated using confocal microscopy. Lamin B1 levels were assessed via western blot and immunofluorescence before and after acetate treatment. Nuclear size was assessed using DAPI staining, qPCR was used to assess gene expression and B-gal staining to measure levels of senescence.

RESULTS: Cpt1aKO cells exhibited decreased acetyl-CoA production, increased expression of profibrotic and senescence genes, and a higher percentage of B-gal positive cells. Mass spectrometry revealed that histone acetylation is significantly reduced in Cpt1aKOs, with concomitant increased H3K9me2 in KO cells. H3K9me2 regulates chromatin-nuclear lamina interactions. Lamin-B1 is a structural component of the nuclear lamina which has been shown to interact with H3K9me2 regulating gene expression. Additionally, Lamin-B1 loss is a hallmark of senescence, that has been associated with mitochondrial dysfunction. Here we found that in contrast to increased H3K9me2, Lamin-B1 total protein levels were decreased in KO cells. We also found that Cpt1a loss induced Lamin-B1 gaps, these gaps were associated with nuclear blebbing and increased nuclear size, also hallmarks of senescence. This phenotype was rescued by restoring acetyl-CoA pools via acetate treatment.

CONCLUSIONS: Our studies indicate that FAO serves as an important regulator of histone post-translational modifications, nuclear lamina homeostasis, and senescence through acetyl-CoA production. Our current studies focus on the implications of these chromatin changes in gene expression by mapping the genomic location of the affected marks. Investigating how reduced FAO shapes the epigenome of lung alveolar epithelial cells will shed light on the mechanisms by which metabolic defects influence nuclear transactions; and how these defects contribute to the development of diseases such as IPF.

51. Ptth regulates lifespan through temporal and spatial activation of STING/NF-κB signaling during *Drosophila* metamorphosis

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Abstract

The prothoracicotropic hormone (Ptth) is well-known for its role in controlling insect developmental timing and body size by promoting the biosynthesis and release of ecdysone. However, the role of Ptth in adult physiology is largely unexplored. Here, we show that Ptth null mutants (both males and females) are long-lived and increase resistance to oxidative stress. Transcriptomic analysis reveals that age-dependent upregulation of the innate immunity pathway is attenuated by Ptth mutants. Intriguingly, we find that Ptth regulates the innate immunity pathway specifically in fly oenocytes, the homology of mammalian hepatocytes. We further show that oenocyte-specific knockdown of NF-κB/Relish extends lifespan, while oenocyte-specific overexpression of NF-κB/Relish attenuates the lifespan extension of Ptth mutant. In addition to the oenocyte-specific regulation of the innate immunity pathway, we find that Ptth mutants exhibit reduced STING and NF-κB/Relish signaling during pupal development. Surprisingly, knockdown of either STING or NF-κB/Relish in pupal oenocytes significantly prolongs lifespan of adult flies. Thus, our findings reveal an unexpected longevity regulation mediated by Ptth hormonal factor and STING/NF-κB signaling in developing hepatocytes.

52. Role of HAT1in Mammalian Development, Aging and Cancer

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The acetylation of newly synthesized histones is catalyzed by enzymes known as type B histone acetyltransferases. Histone acetyltransferase (Hat1) is a type B HAT that belongs to the Gcn5-related N-acetyltransferase (GNAT) family. Hat1 is responsible for the acetylation of newly synthesized histone H4 on lysines 5 and 12 during the process of chromatin assembly. Although it was the first histone acetyltransferase discovered, its biological function(s) remain poorly understood. To understand the biological role of Hat1 in mammals, we have generated a conditional Hat1 mouse model using loxP/Cre technology. Global deletion of Hat1 leads to neonatal lethality with lack of movement, respiratory distress, minor growth retardation and craniofacial skeletal defects. Pathological examination reveals lung hyperproliferation leads to neonatal lethality due to reduced aeration and death via respiratory failure at the time of birth. Haploinsufficiency of Hat1 results in defects consistent with an early aging associated phenotype and these defects include shortened life span, lordokyphosis (hunchback), muscle atrophy, minor growth retardation, reduced skin fat deposition, cancer, paralysis and loss of vitality. At the cellular level, Hat1 mutant mouse embryonic fibroblasts (MEFs) show growth defects, delays in cell cycle, increased in genome stability, accumulation of γ-H2AX and sensitivity to DNA damaging agents. Haploinsufficient Hat1 MEFs undergo early senescence and accumulate high levels of p21. In addition, liver tissue specific Hat1 knockouts exhibit a variety of liver disease phenotypes, including non alcoholic fatty liver diseases (NAFLD), lipidosis, hepatocellular carcinoma (HCC), hepatoblastoma (HB) and cholangiocarcinoma (CC) in older age in Hat1 mutant animals. Overall, our results show that loss of Hat1 induces mammalian developmental defects, multiple hallmarks of early-onset aging and cancer.

53. Spatially-Resolved Lipidomic Analysis of Normal and Idiopathic Pulmonary Fibrosis (IPF) Human Lungs Using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI)

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Idiopathic pulmonary fibrosis (IPF) is an age-associated progressive lung disease with meagre treatment options. Previous analyses of surfactant lipid composition in bronchoalveolar lavage and whole lung tissue lipidomics revealed profound disturbances accompanying the progression of IPF. However, spatially-resolved lipidomic data is lacking, considering patchy involvement of the parenchyma in IPF with areas of fibrosis interspersed with uninvolved alveolar regions. We sought to evaluate the lipidomic profile of tissues from IPF and healthy donor lungs with an accurate anatomical localization. Human lung samples from IPF patients and age-matched donors were embedded in HPMC-PVP medium and fast-frozen. Samples were sectioned and thaw-mounted onto ITO slides and regular glass slides. ITO slides were sprayed with DAN matrix and analyzed on Bruker tims TOF flex at 5 and 20 μm spatial resolution. Serial sections were stained with H&E. We detected > 900 individual mass species in negative and > 1100 in positive ion mode. We identified m/z 687.5 peak, putatively corresponding to sphingomyelin (SM (d16:1/17:0)) as the best for visualizing lung architecture when overlaid with the H&E staining. We hypothesize that SM gives a better localization specificity compared to phosphatidylcholine due to its abundance in outer cell membranes compared to organelles' membranes. Analysis of the surfactant composition, based on the species co-localizing with surfactant components DPPC in positive mode and POPG in negative mode, revealed overall reduction of monounsaturated glycerophospholipids such as POPC and DG (P-16:0/16:1) in IPF samples, while polyunsaturated isoforms were increased. We then focused on the differential abundance of lipids between the samples and found a marked reduction of free fatty acids (stearic, oleic, and arachidonic acid) in IPF, with a major shift towards long chain fatty acid-CoA species seen only in the IPF lung samples, including visibly intact parenchyma.. We confirmed altered surfactant composition in situ and identified differential abundance of several lipid species in IPF tissues. High resolution spatial lipidomics of the lung is a promising tool that can help decipher metabolic processes in specific areas of the lung, and the pathological implications.

54. Age-related lipid accumulation promotes loss of homeostasis in the male germline stem cell niche through niche-to-stem cell conversion

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The capacity of stem cells to self-renew or differentiate has been attributed to distinct metabolic states. In the Drosophila testis, we have shown that both germline stem cells (GSCs) and somatic cyst stem cells (CySCs) employ strategies to control lipid levels to promote stem cell maintenance. When lipid catabolism is impaired, neutral lipids accumulate in lipid droplets (LDs) and GSCs and CySCs exit the niche to differentiate, suggesting a role for lipid metabolism in the control of stem cell maintenance versus differentiation. However, the precise mechanisms involved in the loss of stem cell homeostasis triggered by lipid accumulation remains unknown.

In the testis, both niche and stem cell number decreases with age. Recent data from our lab shows that LDs accumulated with age in “hub” cells, a main component of the stem cell niche in the testis. Downregulation of the lipogenesis factor sterol regulatory element binding protein (SREBP) prevented LD accumulation in hub cells from older individuals, while ectopic activation of SREBP caused hub cell loss through their conversion into CySCs. Furthermore, SREBP activation downregulated the levels of Escargot, a member of the Snail family of transcription factors involved in hub maintenance. While some mechanisms have been described to contribute to the conversion of hub cells into stem cells, this is the first time that a lipid metabolism gene has been implicated in such phenomenon. Our findings highlight a critical role for lipid homeostasis in stem cell maintenance, providing a framework for investigating the impact of aging and metabolic diseases on stem cell function and tissue homeostasis.

55. Sex-dependent responses to calories and fasting uncouple cognition from neuropathology in a mouse model of Alzheimer's Disease.

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Caloric restriction (CR), involving reduced food intake without malnutrition or starvation, has been shown to slow the onset of Alzheimer's disease (AD) in various animal models. However, in mice CR regimens not only restrict calories but also induce prolonged fasting between meals. While CR has been shown to improve metabolic and cognitive functions and suppress pathological markers in AD mouse models, the specific contributions of fasting versus calorie reduction remains unclear. Here, we investigated the contribution of fasting to the beneficial effects of CR on AD progression. Male and female 3xTg mice, starting at 6 months, were assigned to (1) AL, ad libitum diet; (2) DL, Diluted ad libitum diet—animals had ad libitum access to a low-energy diet, reducing caloric intake by ~30% (Non-Fasting); and (3) CR—calories restricted by 30%, with once-daily feeding (Fasting). We assessed the effect of these diets on metabolic health, AD neuropathology, cognition, and survival of 3xTg mice. Additionally, targeted metabolomics in the brain identified metabolite abundance and dysregulated pathways. We observed substantial metabolic benefits of both prolonged fasting and reduced calories in both sexes, promoting leanness and reducing adiposity, although CR-fed male 3xTg mice regained much of the lost weight by the end of the study. Fasting was crucial for CR-induced improvements in insulin sensitivity in both sexes. Furthermore, CR-fed 3xTg male mice exhibited enhanced long-term memory compared to DL-fed animals. However, both fasting and energy restriction reduced plaque deposition in females, but not in males and fasting was necessary for improvements in tau phosphorylation only in females. Fasting was required for the inhibition of mTORC1 and activation of autophagy in both sexes. Metabolic signatures of CR-fed and DL-fed mice shared similarities and overlapping pathways in both sexes and were distinct from AL-fed mice. Finally, CR-fed 3xTg males showed improved survival compared to the DL group. Our data suggests that beneficial effects of prolonged fasting in a CR regimen on AD symptom progression vary by sex and uncouples pathology from cognitive improvements providing crucial mechanistic understanding in translating CR to human interventions.

56. Aging Rate Indicators: Shared Molecular Traits in 10 Kinds of Slow-Aging Mice

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A "biomarker of aging" is conceptualized as an index of how far an individual has moved along the path from youth to old age. In contrast, an Aging Rate Indicator ("ARI") represents a measure of speed, rather than distance, i.e. a measure of how rapidly the individual is moving towards the phenotypic changes typical of old age. Here we present and review recent data documenting common, shared characteristics of slow-aging mice, whether the slowed aging is caused by a mutant allele, the calorie restriction diet, or drugs that slow aging and extend mean and maximal lifespan. Some of the candidate ARIs, shared by 10 varieties of slow-aging mice, are physiological changes seen in fat, fat-associated macrophages, muscle, liver, brain, and plasma. Others are molecular measurements, reflecting activity of mTORC1, selective mRNA translation, or each of 6 MAP kinases in two distinct MAPK cascades in liver, muscle, or kidney. Changes in ARIs are notable in young adult mice after 8 months of drug or diet exposure, are detectable in mutant mice at least as early as 4 – 6 months of age, and persist until at least 18 – 22 months. Many of the candidate ARIs are thought to play an influential role in cognition, inflammation, exercise responses, and control of metabolic rate, and are thus plausible as modulators of age-related physiological and neurological illnesses. In principle, screening for drugs that induce alterations in ARIs in normal young adult mice might facilitate the search for preventive medicines that can retard aging and late-life illnesses in mice or in human populations.

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57. Is Cancer a Death Sentence? The impact of Cancer Community Events, Age, Cancer knowledge, Screening, and Current health on Cancer Fatalism

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Background

Nigeria has the highest cancer burden, with 124,815 incidences and 78,899 deaths in sub-Saharan Africa. Popular fatalistic beliefs such as "*cancer* is *a death sentence", and "cancer is not my portion"* exist in this population, which potentially affects cancer prevention. Yet, factors responsible for these cancer fatalistic beliefs have remained unknown. This study examined the association between age, cancer knowledge, screening, and current health on cancer fatalism in Nigeria.

Method

The analytical sample was 1457 participants, 18-68 years old, from those who attended cancer community events (CCEs) in Abuja and those who have never attended any CCE. The participants completed the measures of cancer fatalism and knowledge. Multiple linear regression was used to analyze the data.

Results

Our findings showed that participants who have never attended any CCE had higher cancer fatalism (β=1.21, $p<0.001$) than those who have attended CCEs. Cancer knowledge (r (1455) = -0.22 $p<0.001$), screened for cancer (t (378) = 6.97 $p<0.001$), knowing someone diagnosed with cancer (t (1156) = 6.41 $p<0.001$), and having chronic health conditions (t (1455) = 2.10 $p=0.036$) were statistically associated with cancer fatalism. Hence, increasing the cancer knowledge by one item decreased the cancer fatalism by 0.21 units (β=-0.21, $p=0.002$) when controlling for other variables. Additionally, participants aged 30 to 49 had higher cancer fatalism (β=0.46, $p=0.049$), being married (β=0.51, $p=0.029$), living in rural areas (β=0.62, $p=0.006$), and lower income (β=1.05, $p=0.002$) were associated with higher cancer fatalism.

Conclusion

To reduce the burden of cancer in this population, there is an urgent need to address cancer fatalism (i.e., the fatalistic belief that cancer is a death sentence) through CCEs across rural communities, among lower-income earners, older adults, and married people in Nigeria. Nigeria's National Institute on Cancer Research and Treatment needs to encourage more CCE, leveraging global health days for awareness and democratizing cancer awareness at state and local levels.

58. "I am Still Alive": An Interpretative Phenomenological Analysis of Older Women Living with Metastatic Breast Cancer

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Abstract

Background

Metastatic breast cancer (mBC) is cancer that has spread from the primary organ (breast) to other distant organs such as bone, spine, lungs, brain, and liver. It is a treatable but incurable cancer. While the burden of mBC is devastating to all patients, older mBC patients have poorer prognoses, poor access to care and experience social isolation. This current study is aimed to explore the experiences of older women living with mBC and the impact of belonging to a peer support group (PSG) in Nigeria.

Method

Using interpretative phenomenological analysis (IPA), eight women living with mBC aged 50 and above participated in the semi-structured interview.

Results

Our findings showed that participants encountered psychological, physiological, and social challenges that are interrelated across four central themes: "Tormented by Pain," "I am the One that is Going to Die," "I am Not alone," and "Winning the 'war' against mBC." While the experiences of older women living with mBC were dominated by different levels of pain, death anxiety, stigma, and financial burden; belonging to a PSG brought participants hope, information, self-worth, and courage.

Conclusion

This study has shown PSG should be an important component of cancer control. Hence, healthcare leaders should support and fund the establishment of PSG across Nigeria. Whether mBC is curable or not, the last days of older women living with mBC should be filled with dignity, peer support, and the absence of pain.

59. Implications of CBX1 mutations that dysregulate centromeric stability in cancers

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Abstract

The Chromobox 1 (CBX1) gene encodes a highly conserved non-histone protein enriched in heterochromatin and centromeres. It essential for embryonic development, stem cell maintenance, and the regulation of cell proliferation and apoptosis. However, mutations in this gene can lead to transcriptional dysregulation, giving DNA polymerase inappropriate access to DNA and potentially causing cancer. Our study utilized data from the Catalogue of Somatic Mutations in Cancer (COSMIC), focusing on patient age, mutation type, cancer type, primary histology, and whether mutations occurred in coding or noncoding DNA sequences. Using the Cancer-Related Analysis of Variants Toolkit (CRAVAT) and Clustal Omega for sequence alignments, we aimed to understand CBX1 mutations' significance. We found a higher frequency of mutations in noncoding regions than in coding regions, with missense substitutions being most common, particularly in endometrial cancers. CBX1 mutations predominantly appeared in carcinomas. Interestingly, CBX1 expression was more prevalent in the brain than in the endometrium, suggesting a discrepancy between mutation sites and expression sites. Statistical analysis showed no significant age correlation with the mutations, indicating that these genetic changes could occur independently of age. Although there was no significant difference between mutations in conserved and non-conserved regions, significant mutations were found to cluster within conserved functional domains of the protein, highlighting their potential impact. The most notable mutation was a change from glutamic acid to lysine in a highly conserved region, which could lead to functional disruptions given the relevance of lysine to CBX1 activity. Our findings suggest that while CBX1 mutations are associated with cancer, they do not correlate with patient age. The primary recurrent mutations observed, particularly the glutamic acid to lysine change, may play a critical role in cancer development, especially in the endometrium. This study underscores the importance of mutations that alter protein structure in cancer progression and opens pathways for further research into the implications of CBX1 mutations in various cancer types, particularly those involving chromosome mis-segregation resulting from destabilized heterochromatin at the centromere.

60. Exploring the role of Sirt6 in the *Drosophila melanogaster* Alzheimer's Disease Model

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Abstract

In the quest to understand the molecular mechanisms of aging, the sirtuin family of proteins emerged as pivotal in protecting against age-related disease. Sirt6, a member of sirtuins, is a NAD^+-dependent histone deacetylase which promotes DNA repair, maintaining genomic stability. Previous studies have shown that Sirt6 overexpression extends the lifespan in mice, while Sirt6 knockout mice have a shortened lifespan and exhibit increased genome instability. Recent work suggests a possible neuroprotective role for Sirt6 in the context of Alzheimer's Disease (AD). Mice with brain-specific deletion of the Sirt6 gene display neurodegenerative phenotypes similar to AD patients. AD patients also exhibit reduced expression of Sirt6. In our work, we utilize *Drosophila melanogaster* to study the role of Sirt6 in AD. We report that *Sirt6* mRNA levels are reduced in the heads of flies expressing human Tau (hTau) protein, compared to controls. Climbing assay analysis shows a robust decline in climbing performance of hTau expressing flies with age, which was partially rescued by Sirt6 overexpression. Furthermore, Sirt6 overexpression extended the lifespan of Tau flies. Rough eye phenotype induced by hTau expression in the eye was more severe in flies with partial loss of Sirt6. Lastly, aged Sirt6 knockout flies exhibit increased DNA damage in the optic lobe, compared to age-matched control flies. Together, these results indicate a neuroprotective role of Sirt6 against Tau-induced neurotoxicity, as well as in the healthy aging brain.

61. The effects of maternal psychosocial stress during pregnancy on offspring heart health

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Background: Maternal health has a substantial effect on offspring health throughout their lifespan. The most common detrimental factor to maternal health is psychosocial stress. Psychosocial stress results from adverse experiences in everyday life and the inability to cope with them, resulting in anxiety and depression. Unfortunately, it is estimated that ~75% of mothers will suffer a stressful life event the year their infants are born, and upwards of 20% suffer depression. These already high levels of psychosocial stress have been exacerbated by the COVID-19 pandemic. While maternal psychosocial stress is known to lead to offspring psychiatric and behavioral issues; it is not known if maternal psychosocial stress during pregnancy affects offspring heart health. Thus, the **purpose** of this study was to investigate the effects of maternal psychosocial stress during pregnancy on offspring heart health throughout its lifespan. We **hypothesize** that maternal psychosocial stress during pregnancy will have an adverse effect on offspring heart health. **Methods:** In mouse studies, we used a well-established model of maternal psychosocial stress during pregnancy- restraint stress. Pregnant dams were stressed by placing them in a 50 mL conical tube (with air holes) for 2 hours / day on gestational days 10-17. Offspring heart health was assessed via echocardiography (Vevo 3100, MX550D transducer) throughout the lifespan. In human patients, maternal serum was tested for cortisol levels (i.e., stress hormone) via ELISA, with infant echocardiography obtained at 4-5 weeks-of-age. **Results:** In mice, we observed that offspring born to stressed dams exhibited poor heart health throughout their lifespan (from infant to old age). Specifically, we observed decreased heart performance (measured as stroke volume) with systolic and diastolic dysfunction (measured as strain and strain rate, respectively) and adverse remodeling (hypertrophy measured as left ventricular mass). Interestingly, in human patients, we observed that maternal serum cortisol levels correlated negatively with infant heart performance (measured as stroke volume). **Conclusion-** Our data indicate that offspring born to stressed dams exhibited poor heart health throughout the murine lifespan (from infant to old age) and in infant humans. These data indicate that maternal psychosocial stress during pregnancy is detrimental to offspring heart health.

62. Fresh Human Tissue Distributed from the Ohio State Biobank Enhances Pulmonary Research

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The Ohio State University Comprehensive Transplant Center Human Tissue Biorepository (HTB) was established in 2018 to provide researchers with IRB-approved access to high-quality, clinically-annotated normal and diseased human-sourced biospecimens. Many biobanks only store and distribute frozen biospecimens. Due to the demand of cellular needs from researchers, this can lead to inefficient sample utilization. To maximize efficiency, the HTB operates as a human tissue procurement and processing center by distributing cells and fresh tissue while continuing to bank samples and data. The HTB distributes to over 30 OSU-affiliated researchers, who then utilize innovative techniques to analyze biospecimens on cellular and molecular levels. In this way, the HTB optimizes sample utilization, creates a leaner enterprise, and assists researchers in studying the diseases and processes surrounding transplantation. We acquire “normal” non-transplantable organs from authorized deceased donors via our local organ procurement organization and diseased organs from consented transplant recipients via our IRB protocol and partnership with

pathology. Tissue and/or cells are then isolated from fresh organs and promptly distributed to researchers. Cryogenic and formalin-fixed samples are stored for future research. Additionally, we have developed a method of ex vivo bronchoaveolar lavage (BAL) to harvest alveolar macrophages (AMs) from airways. Finally, pre- and post-transplant BAL fluid, oral wash, urine, plasma, and peripheral blood-derived monocytes (PBMCs) are collected from lung transplant recipients at various timepoints pre- and post-transplant for longitudinal studies. The HTB has processed lungs from 173 normal donors and 198 transplant recipients and longitudinal samples from 365 transplant recipients. From donors, we delivered or banked fresh tissues, pulmonary lymph nodes, plasma, PBMCs, AMs, nasal epithelial cells, and bone marrow-derived monocytes. For recipient-explanted lungs, 33% are IPF, 20% are COPD, and 47% are from rarer diseases. For our longitudinal study, we captured 1546 BAL fluid, 1407 oral wash, 1028 urine, 2185 plasma, 530 PBMC, and 1100 transbronchial biopsy samples. Most longitudinal patients have at least 4 sample collection timepoints. Though many researchers rely on biobanks for frozen samples, the HTB distributes fresh biospecimens to meet the needs of our researchers. Our approach favors modern molecular and single-cell experiments, thus enhancing researchers' yield of more accurate and innovative data. For our institution, this translates to investigators being more competitive for grants and high-impact publications that precipitates scientific growth and better patient outcomes.

63. Characterizing effects of aging in cellular processes of *dSirt6* knockout flies.

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Abstract

Sirt6 is a nuclear localized member of the sirtuin family with NAD^+-dependent protein deacetylation and ADP-ribosylation. Through these enzymatic activities, Sirt6 regulates metabolism, DNA repair, and various age-related diseases. In mice and flies, reducing Sirt6 levels shortens lifespan, while overexpressing Sirt6 extends lifespan, indicating a conserved role for Sirt6 as a pro-longevity gene. Previous work from our lab found that, in flies, Sirt6 overexpression appears to extend lifespan in part through deacetylation of H3K9ac in the promoter region of genes involved in ribosome biogenesis, leading reduced expression and a subsequent reduction in protein synthesis. Although Sirt6 has been in the spotlight of many age-related studies, the complete mechanisms by which Sirt6 regulates lifespan are not yet fully understood. *Drosophila melanogaster* is an efficient tool to study the effects of genetic modifications during aging and expresses an ortholog of Sirt6, dSirt6. Here, we used *dSirt6* knockout (*dSirt6* $^{-/-}$) files to investigate specific cellular processes that are essential for regulation of lifespan by Sirt6. In agreement with previous studies, we find that *dSirt6* $^{-/-}$flies have reduced lifespan, compared to controls. Western blot and immunostaining analysis shows higher levels of H3K9ac, a primary deacetylase target of Sirt6, in *dSirt6* $^{-/-}$ flies. *dSirt6* $^{-/-}$ flies also have increased body weight compared to their control counterparts; however, deletion of *dSirt6* does not affect starvation resistance. Studies in mice indicate Sirt6 is transcriptionally up-regulated during fasting conditions. Surprisingly, we find that fasting flies for 24 hours significantly reduces *dSirt6* transcript levels. RT-qPCR results indicate that *dSirt6* $^{-/-}$ flies have reduced levels of 4E-BP mRNA, a repressor of translation initiation that is inhibited by mTOR. Conversely, *dSirt6* overexpression flies have increased levels of 4E-BP. Collectively, our findings reveal Sirt6 is required for normal lifespan in *Drosophila* and that Sirt6 may regulate aging and protein synthesis via transcriptional regulation of 4E-BP.

64. MAF1 Mutations in Aging and Cancer: A Molecular Analysis

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Abstract

MAF1, a key transcription factor, regulates RNA polymerase I, II, and III activity, affecting cellular balance by controlling the expression of tRNA, rRNA, and lipid synthesis genes. Disruptions in MAF1 can lead to dysregulated cellular metabolism, contributing to accelerated aging and heightened cancer risk. Our study focuses on the association between MAF1 mutations and their contribution to age-related cancer development, with particular attention to lipid metabolism's role in these processes. Using data from the Catalog of Somatic Mutations in Cancer (COSMIC), we identified prevalent MAF1 mutations in cancer samples, notably in the large intestine of individuals aged 71-80. These mutations primarily occur within coding regions, suggesting significant effects on MAF1's structure and therefore function in translational capacity and fat metabolism. Through in-depth analysis using the Cancer-Related Analysis of Variants Toolkit (CRAVAT) and computational modeling, we assessed these mutations' impact on RNA polymerase activity and their potential to impact aging through this mechanism. Our findings indicate that specific mutations in MAF1 lead to dysregulated Pol II and Pol III activity, which could exacerbate cellular stress and metabolic dysfunction, thereby accelerating cellular aging and increasing susceptibility to age-related cancers. The study reveals that MAF1 mutations not only contribute to cancer development but also play a crucial role in the aging process by affecting cellular homeostasis and metabolism. These insights underline the significance of MAF1 as a biomarker for aging and cancer susceptibility and highlight the potential for targeted therapeutic strategies.

65. Neural targeting of aging and neurodegenerative diseases

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Aging is the primary risk factor for multiple neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Same genetic or pharmacological interventions that extend lifespan are also protective against neurodegenerative diseases across species. However, the common mechanisms shared by aging and neurodegenerative diseases are not known. Since age-related decline of proteostasis is a common cause of AD, PD, and HD, and at the same time a major target of multiple longevity pathways including mitochondrial unfolded protein response (UPR), ER UPR and the heat shock response, it is likely that aging and neurodegenerative diseases share common mechanisms that can be targeted by drugs benefiting both aging and neurodegeneration.

Here we utilize a longevity gene surrogate reporter to examine neuromodulator drugs that extend lifespan and improve neurodegenerative disease models in *C. elegans*. AD, PD, and HD *C. elegans* models have severe motility defects and short lifespans and are great models for fast screening of drugs. We have identified 7 drugs that induce the longevity gene, and 3 drugs that improve motility and extend lifespan of *C. elegans*. We will utilize these drug hits as tools to interrogate the common genes and mechanisms responsible for the health benefits in delaying aging and improving neurodegenerative diseases.

66. Acute Signaling Responses and Chronic Adaptations in Skeletal Muscle after Progressive Weighted Wheel Running in Female Mice

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Progressive Weighted Wheel Running (PoWeR) is a voluntary, high-volume exercise paradigm that elicits hypertrophy in select muscles of adult and aged mice. Despite growing interest in using PoWeR as a translational exercise model in aging, the impact on canonical signaling and hallmark adaptations to exercise has not been completely defined. To address this knowledge gap, adult female mice (4 months old) were allocated to short-term (n=8) or long-term PoWeR (n=10) and sedentary controls (n=8-10). Short-term PoWeR consisted of 5 days of unweighted acclimation to wheel running followed by 3 consecutive days of PoWeR. During the 3rd night, the wheel was locked after running ~5 km, and 1 hour later tissues were collected. During 8-weeks of PoWeR, mice averaged ~8km/day and muscles were collected ~24 hours after last exercise bout. PoWeR improved maximal exercise capacity, glucose tolerance and body composition compared to sedentary controls. PoWeR increased muscle weight in the soleus, plantaris, flexor digitorium longus (FDL), and tibialis anterior (TA). PoWeR increased cross-sectional area of all fiber types in the FDL and Type IIA fibers in the plantaris and soleus. PoWeR induced a more oxidative profile in FDL, plantaris, and soleus, as indicated by a concomitant increase in the percentage of type IIA fibers and/or decrease in Type IIB and IIX fibers. In the gastrocnemius, quadriceps, and triceps, PoWeR increased mitochondrial respiratory chain content and mitochondrial respiratory capacity. Short-term PoWeR stimulated mTORC1 and AMPK signaling in plantaris and FDL. We demonstrate that PoWeR increases conventional anabolic and metabolic signaling, leading to increased glucose tolerance, myofiber size, muscle mass, and mitochondrial bioenergetics consistent with what is observed in humans. We anticipate that PoWeR can be used as a tool to reveal the fundamental biology of aging that extends healthy longevity with exercise.

67. A PTER-dependent pathway of secondary taurine metabolism in physiology and aging

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Taurine is a conditionally essential micronutrient and one of the most abundant amino acids in humans. In endogenous taurine metabolism, dedicated enzymes are involved in biosynthesis of taurine from cysteine as well as the downstream derivatization of taurine into secondary taurine metabolites. One such taurine metabolite is N-acetyltaurine, an amidated conjugate of taurine and acetate6. While taurine N-acetyltransferase activity has been previously detected in mammalian cells, the molecular identity of this enzyme, and its potential physiologic relevance, have remained unknown. Here we show that the body mass index-associated orphan enzyme PTER (phosphotriesterase-related) is the principal mammalian taurine N-acetyltransferase/hydrolase. In vitro, recombinant PTER catalyzes bidirectional taurine N-acetylation with free acetate, as well as the reverse N-acetyltaurine hydrolysis reaction. Genetic ablation of PTER in mice results in complete loss of taurine N-acetyltransferase/hydrolysis activities and concomitant >2-10-fold elevation of N-acetyltaurine levels across tissues. Upon dietary or physiologic stimuli that increase taurine levels, PTER-KO mice exhibit lower body weight and reduced adiposity. These phenotypes are recapitulated by administration of N-acetyltaurine to wild-type mice. Lastly, the full anorexigenic and anti-obesity effects of N-acetyltaurine require functional GFRAL receptors. Together, these data uncover enzymatic control of a previously enigmatic pathway of secondary taurine metabolism linked to energy balance. Projecting forward, given the growing evidence of taurine in aging-associated diseases, imminent efforts will focus on determining how PTER/N-acetyltaurine pathway affects mammalian aging.

68. Response to Gradient Isoleucine Restriction Diet in C57BL6/J Mice

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Protein and some amino acid restriction diets promote metabolic health and longevity in diverse species. Our lab has previously found in young male C57BL/6J mice, the beneficial metabolic effects of low protein diet are principally mediated by Isoleucine. While it's still unknown what degree of Isoleucine restriction would elicit the most health benefit in mammals, as well as the corresponding molecular response.

To address the question, we put young male C57BL6/J mice on 40%, 55%, 67%, 82% isoleucine reduced or control diets. Body composition change on different degrees of Isoleucine restriction was monitored for 3 months, during which their metabolic health was measured, then the mice were euthanized and tissues were collected for molecular analysis. We found there's a sex difference in change of body composition and glucose tolerance on different degrees of dietary Isoleucine restriction, with males showing a wider window. Fasting plasma cholesterol decreased gradiently in response to different degrees of Isoleucine restriction, which is specific in males. In male mice, 55% Isoleucine restriction was sufficient to prevent gain of fat mass and improve glucose tolerance to a level similar to 67% degree restriction. Mice on 82% restriction rapidly lost both their fat mass and lean mass, while mice on 55% restriction was able to maintain their lean mass, unlike the mice on higher degree of restriction. Molecularly, in the liver, autophagy and downstream glucose and lipid metabolism genes were only drastically elevated in 82% restriction group. While in iWAT fat, Fgf21 and lipid metabolism genes expression were only significantly induced in 67% restriction group. The 55% and 67% restriction group did share some genetic regulation trend in common, including downregulation of Pparg in liver and upregulation of thermogenic genes in white adipose.

Our findings suggest that induction of liver autophagy is likely not the driver of metabolic health improvement in young mice on Isoleucine restriction. 55% Isoleucine restriction is enough to elicit physiological improvements as good and fast as 67% restriction in male mice, while the common molecular mechanism underlying the physiological effect needs further identification.

69. Dietary context determines the interaction between a low isoleucine diet and rapamycin

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A realistic and comprehensive anti-aging strategy should account for the biological state and the possibility of using multiple treatments simultaneously. In this study, we combined two longevity-extending approaches in a low isoleucine (Low Ile) diet and rapamycin administration, finding that not only there is a significant diet-drug interaction on the metabolic state of male C57BL/6J mice, this phenomenon was altered in the context of a Western diet and diet-induced obesity. Under standard dietary conditions, a Low Ile diet enhances metabolism in several ways, which were all summarily blocked by the co-administration of rapamycin. Physiologically, this includes Low Ile-induced weight loss, increases in energy expenditure, and improvements in glucose tolerance. Molecularly, we identified rapamycin treatment to block the upregulation of genes regulating lipid lipolysis in the inguinal white adipose tissue, while genes of thermogenesis and lipogenesis remain elevated. However, in the context of a Western diet, a reduction in dietary isoleucine remains effective in improving several aspects of metabolic health despite rapamycin co-treatment. In addition to significant reduction in body weight, rapamycin-induced glucose intolerance and insulin insensitivity were significantly ameliorated by the dietary intervention. In this model, rapamycin was unable to suppress gene expression associated with lipolysis. We further demonstrate that rapamycin, by itself, reduces bodyweight significantly in diet-induce obese mice fed either a Western diet or a High Fat diet. These surprising results indicate that practical considerations of how a treatment will perform in conjunction with other anti-aging therapies and within different environmental contexts are necessary for the successful translation of a geroprotector.

70. Assessment of biological aging in people with multiple sclerosis using epigenetic clocks and p16INK4a

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Background: Emerging evidence suggests accelerated biological aging in people with multiple sclerosis (MS). It is unknown how multiple different mechanisms of biological aging are associated with MS. Epigenetic clock algorithms derive from quantification of DNA methylation patterns and track closely with age-related phenotypes. $p16^{INK4a}$ is a tumor suppressor activated in response to conditions that trigger cellular senescence and increases logarithmically with age.

Objectives: To assess epigenetic age acceleration (EAA) and $p16^{INK4a}$ in people with MS.

Methods: We prospectively measured multiple epigenetic clock-based predictors and $p16^{INK4a}$ expression in people with MS and age- and sex-matched controls without MS. We also examined the effects of anti-CD20 therapy on EAA and $p16^{INK4a}$ and explored correlations between $p16^{INK4a}$ with chronological age and EAA. EAA was measured using multiple epigenetic clock algorithms to assess methylation patterns in peripheral blood T lymphocyte (PBTL) DNA. EAA was determined as the fitting residual between clock-based prediction and regression on chronological age and technical variables for the controls, and as prediction residuals for MS patients. $p16^{INK4a}$ expression was measured in PBTL, and extracted RNA samples were analyzed using a custom Nanostring CodeSet.

Results: In this ongoing study, preliminary data was obtained from 95 participants (30 relapsing-remitting MS, 29 secondary progressive MS, 10 primary progressive MS, and 27 controls). Of these groups, EAA was highest in people with secondary progressive MS using the Horvath (mean=6.1, SD=8.8), Hannum (mean=8.3, SD=10.8), GrimAge (mean=2.3, SD=4.9), and PhenoAge (mean=8.9, SD=12.9) clocks. No expressive differences were observed in EAA for people with MS receiving anti-CD20 therapy versus other disease-modifying therapies or no treatment. $p16^{INK4a}$ expression showed moderate positive correlation with chronological age in controls (r=0.44) but not in MS (r=0.05). Positive correlations between $p16^{INK4a}$ and different epigenetic clock measurements of EAA were observed in controls and to a less extent in MS.

Conclusion: Our interim findings show increased EAA in a subset of people with secondary progressive MS. Lack of expected correlation between $p16^{INK4a}$ and chronological age in MS requires further investigation to determine associations between aging biomarkers and MS disease outcomes.

71. Evaluation of Inflammation Markers in Patients with Idiopathic Pulmoanry Fibrosis in Bogotá

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Abstract
Introduction IPF is a chronic, progressive, and irreversible lung disease. Although the underlying mechanisms of the disease are not fully understood, it has been described as an age-related disease, as it leads cells to a state of senescence characterized by an atypical inflammatory phenotype, increasing susceptibility in older adults to respiratory infections and diseases. Likewise, the decrease in immunovigilance functions, responsible for recognizing, monitoring, and controlling defective or senescent cells, favors pathological remodeling and fibrosis in tissues.

Objective To identify proinflammatory cytokines in the plasma of patients with Idiopathic Pulmonary Fibrosis residing at high altitude in Bogotá.
Methods Peripheral blood samples were taken from 13 patients over 60 years old diagnosed with IPF and healthy counterparts. Plasma separation was performed and stored at -20°C. Proinflammatory cytokines were measured using the Human TH1/TH2 Cytometric Bead Array (CBA). This work was approved by the ethics and research committee of the Colombian Pneumological Foundation.

Results Patients with IPF showed an increase in cytokines such as IL-4, IFN-γ, and IL-6 compared to healthy older adults.

Conclusion Inflammaging has been associated with the development and coexistence of multiple noncommunicable chronic diseases that have a higher incidence after 65 years of age. The involvement of adaptive immunity has been described in the pathogenesis of IPF as an imbalance in the Th1/Th2 lymphocyte response. Additionally, stimulation by IL-4 favors the phenotypic change to M2 macrophages and Th2 cells along with the stimulation of pro-fibrotic environments by ILC-2, which recruit fibroblastic cells. Further studies are needed to identify other markers of immunosenescence that correlate with IPF.

Keywords: Idiopathic Pulmonary Fibrosis; Inflammatory Cytokines; Aging; Inflamm-aging.

72. NRIP1 Knockout Reduces Senescence and Suppresses Differentiation of Visceral Adipose Tissue-derived Mesenchymal Stem Cells

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Visceral fat tissue undergoes intricate transformations with aging, marked by increased volume, accumulation of senescent cells, elevated secretion of inflammatory cytokines, *etc.*, which compromise its function and contribute to the onset of aging-related metabolic disorders. Nuclear Receptor Interacting Protein 1 (NRIP1), identified as a novel aging gene implicated in the tradeoff relationship between female sexual maturation and lifespan regulation, has emerged as a key metabolic regulator. Previous findings from our research indicated that NRIP1 deletion reduces senescent cell accumulation in adipose tissue *in vivo*, concomitant with increased female mouse lifespan, promoting the investigation of whether the NRIP1 deletion could improve the viability and functionality of adipose-derived mesenchymal stem cells (ADMSCs). In the current study, we characterized ADMSCs from young and old mice (C57BL/6J, 6 vs. 20 months females), revealing that aging significantly diminishes ADMSC proliferation and differentiation capability while accelerating senescence and increasing proinflammatory cytokine secretion. Comparing ADMSCs from visceral fat pads of *Nrip1* knockout and wild-type mice (Nrip1-/- vs. Nrip1+/+, 6 months, female), our results demonstrated the deletion of NRIP1 could significantly reduce proliferation and differentiation of ADMSCs, but significantly decrease apoptosis, delay senescence, and reducing inflammatory cytokine production. Further analysis revealed that upon NRIP1 deletion cell cycle arrest in the G0/G1 phase. Importantly, *Nrip1* deletion reduces the expression of the molecular markers of cellular senescence, P16 and P21/P53. These findings underscore the pivotal role of NRIP1 in regulating ADMSC viability and senescence. However, it remains unclear whether the observed suppressed proliferation and differentiation contribute to the delayed senescence. Furthermore, the potential of targeting NRIP1 to enhance the function of visceral fat in old age and thereby ameliorate metabolic aging needs further investigation.