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Midwest Aging Consortium

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Abstract Booklet

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Abstracts

55 abstracts presented at the 7th Annual MAC Research Symposium, listed alphabetically by presenting author.

1. Assessing the Therapeutic Promise of Mitochondrial Replacement in Combating Aging

Aida Adlimoghaddam | *Faculty* | *Southern Illinois University School of Medicine*

Aida Adlimoghaddam

Mitochondrial transfusion (MT) is a relatively new, highly innovative therapeutic approach that involves the injection of isolated healthy mitochondria into regions of the body with damaged or diseased tissue. The approach has recently seen a significant increase in activity due to the work by McCully et al., who successfully transplanted healthy heart mitochondria into ischemic regions of a pediatric patient's heart and improved clinical outcome. In essence, the effects of damaged mitochondria are diluted by injecting a large number of healthy mitochondria so that the increase in healthy mitochondria is beneficial, with ATP output maximized. The objective of this work is to reveal present gaps in our understanding of MT and outline how our research program intends to resolve these gaps in our understanding. Our goal is to gain insights into whether MT can enhance bioenergetics and improve memory and muscle function during normal aging. Despite the growing interest, the functional impact of MT across aging studies has not been thoroughly evaluated. To address this, we conducted MT in aged C57BL/6mice. Young and healthy mitochondria from the liver of C57BL/6 were injected intraperitoneally into the aged mice, and their aging was evaluated using a comprehensive set of behavioral, cognitive, and metabolic tests. We assessed memory, motor, muscle, and metabolic function using novel object recognition (NOR), Y-maze, rotarod, grip strength, and indirect calorimetry (IC), respectively. Molecular assays were used to measure the expression of mitochondrial protein markers, which are involved in oxidative phosphorylation. We found that MT improves metabolic, cognitive, and muscle function in aged mice. Improving energy level and memory, muscle function will be a huge advancement in the field. Given this, the project's outcomes hold clinical significance for AD treatment and will offer insight into other disorders with mitochondrial dysfunction.

2. Dysregulation of Astrocytic NR1D1 Signaling in Aging and Amyotrophic Lateral Sclerosis

Rachel Becker | *Graduate Student* | *University of Wisconsin Madison*

Rachel Becker, Haylee L. Hamilton, Sandhya Ramachandran, Matteo T. Meyer, Marcelo Vargas, Mariana Pehar

Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of upper and lower motor neurons. Aging is a primary risk factor, with ALS incidence rising exponentially with age. Neuroinflammation is linked to the aging process and contributes to the progression of neurodegenerative diseases. Astrocytes are key regulators of the innate immune response within the central nervous system. NR1D1 is a member of the nuclear receptor superfamily and functions as a transcriptional repressor. NR1D1 is considered a key regulator of fatty acid-binding protein 7 (FABP7) expression, a protein that is upregulated by astrocytes in multiple pathological conditions, including ALS. Accordingly, we showed that in astrocyte cultures, downregulation of NR1D1 increases FABP7 expression, while the overexpression of FABP7 induces a pro-inflammatory phenotype. Here, we investigate the potential role of dysregulated NR1D1 signaling in modulating neuroinflammation during aging and ALS. NR1D1 expression decreases in the cerebral cortex of 2-year-old

mice, when compared to young 3-month-old animals. This decrease in NR1D1 expression was observed in astrocytes and other cell types. Furthermore, we observed that FABP7 expression, a key target of NR1D1 regulation, increases in a subpopulation of astrocytes in the aging brain. Moreover, astrocytes with high FABP7 levels displayed higher expression of inflammatory markers. Decreased NR1D1 expression and upregulation of FABP7 were also observed in symptomatic ALS mice. Remarkably, astrocytic NR1D1 overexpression significantly delays disease progression in SOD1G93A-ALS mice. Together, our results suggest NR1D1 could play a role in the regulation of astrocyte-mediated neuroinflammation during aging and constitutes a potential therapeutic target in ALS.

3. Facile method for isolating fibrous caps from human and mouse atherosclerotic plaques

Bennett Childs | *Postdoctoral Trainee* | *Mayo Clinic*

Bennett Childs, Cheng Zhang, Quinn Strassheim, Nirad Banskota, Linna Cui, Madeleine Paulosky, Amit Dey, Maria Hernandez, Noemi Kedei, Fahad Shuja, Allison Herman, Nathan Basisty, Hu Li, and Darren Baker

Atherosclerotic cardiovascular disease (CVD) is one of the most common causes of age-related morbidity and mortality. Here, high plasma levels of low-density lipoprotein (LDL) drive arterial oxidized LDL accumulation, thereby initiating molecular and cellular events that lead to plaque formation. Single-cell and spatial transcriptomics and proteomics studies have been instrumental in revealing hidden cellular plasticity in atherosclerosis and in defining the molecular features of clinically dangerous lesions. Compared to these techniques, bulk "omic" approaches assess a greater fraction of the target tissue and are more economical. However, bulk characterizations suffer from lost spatial information. In atherosclerosis, molecular and cellular properties of the fibrous cap are predictors of poor outcome. Here, we present a method for separation of atherosclerotic plaque compartments (fibrous cap, plaque core, and media) in mouse and human samples using a partial dissociation technique to preserve the linkage between transcriptome/proteome and location. RNAseq analysis revealed expected as well as novel differences between vascular smooth muscle cell rich media and fibrous cap, including the identification of a fibrous cap-specific gene signature in the Ldlr-/- mouse model reproducible by TGF? stimulation. Furthermore, murine fibrous caps are viable ex vivo and contract in response to depolarizing stimulus. We demonstrate that fibrous cap microdissection is also effective in both fatty and calcified human atheromas and yields material suitable for proteomics. We propose that this approach has broad utility for "omic" characterization as well as functional assessments of fibrous cap, a key plaque structure whose properties are directly linked to cardiovascular risk.

4. Distinguishing the Molecular Programs of Acute Inflammation, Chronic Activation, and Cellular Senescence in Macrophages

Ting Fan | *Graduate Student* | *Mayo Clinic*

Ting Fan, Xu Zhang, Jolliffe, Alyssa, Amanda A Heeren, Thomas A White, Nathan K. LeBrasseur

Aging is associated with chronic inflammation and the accumulation of senescent cells; however, whether inflammatory activation and cellular senescence represent overlapping or distinct macrophage programs remains unclear. Here, we compared macrophage states modeling acute inflammation, chronic inflammatory stimulation, and DNA damage-induced senescence. Bone marrow-derived macrophages (BMDMs) were exposed to high-dose LPS for 24 hours to model acute inflammatory activation, prolonged low-dose LPS for 7 days to mimic chronic inflammatory stimulation, or etoposide to induce cellular senescence. Cellular states were characterized using imaging-based phenotyping, flow cytometry, secretome profiling, and transcriptional analyses. Aging relevance was examined using macrophages derived from young

and old mice, which recapitulated key features observed in vitro. Acute LPS stimulation was associated with inflammatory activation accompanied by increased cytokine secretion and immune signaling programs. Prolonged low-dose LPS exposure produced an intermediate phenotype exhibiting partial similarity to senescence while retaining inflammatory characteristics. In contrast, etoposide exposure was associated with enrichment of senescence-associated programs, including increased p16-related markers and cell cycle and stress-response pathways. Integrated analysis of transcriptional and secreted protein profiles revealed partial overlap across conditions but clear separation between inflammatory and senescence-associated cellular programs. Together, these findings indicate that acute inflammation, chronic inflammatory stimulation, and cellular senescence define related yet distinct macrophage states. Establishing this distinction provides a framework for understanding macrophage heterogeneity within the aging immune landscape.

5. Mapping Epithelial Stability States Reveals Plasticity Shifts in Aging and Fibrosis

Ahmed Ghobashi | *Postdoctoral Trainee* | *The Ohio State University*

Ahmed Ghobashi Natalia Vanegas Victor Peters Qin Ma Mauricio Rojas Ana L. Mora

Cellular plasticity enables adaptive tissue repair but can also drive pathological remodeling during aging and chronic lung disease. Despite its central role in lung homeostasis, plasticity remains difficult to quantify rigorously from single-cell transcriptomic data because most approaches infer variability without directly modeling the stability of gene regulatory programs that define cell identity. Here, we present PLASMA (Plasticity via Spin-glass Modeling Architecture), a computational framework that quantifies cellular plasticity by measuring the stability of gene regulatory network states in individual cells. Applying PLASMA to single-cell datasets from human alveolar epithelium across age and idiopathic pulmonary fibrosis (IPF), we uncovered distinct epithelial stability regimes associated with lung aging. Alveolar type I (AT1) cells exhibited highly stable regulatory states, consistent with terminal differentiation. In contrast, Respiratory Airway Secretory (RAS) cells and transitional AT2 populations displayed elevated regulatory instability, reflecting heightened plasticity. Notably, a subset of AT2 cells in aged and IPF lungs showed markedly increased plasticity compared to young healthy donors, indicating age-associated destabilization of alveolar identity. These high-plasticity AT2 cells were enriched for inflammatory and injury-responsive regulators, consistent with chronic stress adaptation. Together, our findings establish a quantitative framework for measuring cellular plasticity and demonstrate that aging is associated with destabilized epithelial regulatory states that may predispose the lung to fibrotic remodeling. These results position regulatory instability as a measurable hallmark of lung aging and suggest that restoring epithelial stability may represent a therapeutic avenue to mitigate age-related pulmonary fibrosis.

6. Sex-specific benefits of p21 knockout in PS19 tauopathy female, but not male, mice.

Sara Graves | *Postdoctoral Trainee* | *Mayo Clinic*

Sara I. Graves, Karthik B. Jeganathan, Darren J. Baker

The protein encoded by the Cdkn1a locus, p21(waf1/cip1), plays important roles in cell cycle inhibition, regulation of apoptosis, and DNA damage response. Upregulation of p21 is also one mechanism by which senescent cells enter and maintain a state of stable growth arrest. Accumulation of p21-positive senescent cells is seen in aging and age-related diseases, including neurodegenerative diseases like Alzheimer's disease. We have previously shown that preventing senescent cell accumulation via p16(ink4a)-based manipulations largely prevents tau pathology in a mouse model of Alzheimer's disease. However, direct evidence for p21 upregulation as a driver of tau pathology in either senescence-

dependent or senescence-independent contexts does not yet exist. This study tests the necessity of p21 expression for neurodegenerative disease phenotypes by genetically ablating p21 in a mouse model of tauopathy. We report sexually dimorphic attenuation of senescence-associated gene expression, neuroinflammation, neurovascular abnormalities, phosphorylated tau burden, and neurodegeneration with knockout of p21 in females, but not males. These results provide interesting clues into the sex-specific biology of tau-dependent disease.

7. Therapeutic potential of targeting astrocyte-mediated neuroinflammation in Alzheimer's disease

Haylee Hamilton | *Postdoctoral Trainee* | *The University of Wisconsin-Madison*

Haylee L. Hamilton1, Rachel S. Becker1,2, Sandhya Ramachandran1,2, Matteo T. Meyer1, Caelan Wright1,3, Marcelo R. Vargas4, Mariana Pehar1,5 1 Division of Geriatrics and Gerontology, Department of Medicine; 2 Neuroscience Training Program; 3 Cellular and Molecular Pathology Program; 4 Department of Neurology, University of Wisconsin Madison; and 5 Geriatric Research Education Clinical Center, William S. Middleton Memorial Veterans Hospital.

The prevalence of Alzheimer's disease (AD) and other dementias is expected to continue rising as the global population ages, with the number of individuals affected projected to at least double by 2050 in the absence of major advances in prevention or treatment. Although neuroinflammation is recognized as a major contributor to AD pathogenesis, clinical trials investigating anti-inflammatory agents that target specific signaling pathways have largely failed. We previously demonstrated that fatty acid-binding protein 7 (FABP7) is upregulated in astrocytes of AD patients and APP/PS1 mice, a commonly used AD mouse model, and promotes a proinflammatory astrocyte phenotype by simultaneously regulating multiple signaling pathways. Here, we evaluated the therapeutic potential of targeting astrocytic FABP7 in APP/PS1 mice. To do so, we used a viral vector approach to knockdown astrocytic FABP7 in 6-month-old APP/PS1 mice with established amyloid pathology. Five months post-injection, the downregulation of astrocytic FABP7 in APP/PS1 mice led to reduced astrocyte and microglial reactivity, particularly in the vicinity of amyloid plaques. Moreover, we observed a significant downregulation in the expression of inflammatory markers in astrocytes. Although there was no effect on amyloid pathology, the downregulation of FABP7 in astrocytes attenuated the accumulation of phosphorylated-Tau in neurons and dystrophic neurites. Lastly, we observed improved cognitive performance in behavioral tests, including prepulse inhibition and both contextual and cued fear conditioning. Together, our findings support targeting astrocytic FABP7 as a potential therapeutic strategy to mitigate the deleterious effects of astrocyte-mediated neuroinflammation in AD.

8. NRIP1 as a Regulator of Autophagy and Metabolic Stress Adaptation in Breast Cancer Cells

Xiuqi Han | *Graduate Student* | *Southern Illinois University School of Medicine*

Xiuqi Han, Yumeng Huang, Yun Zhu, Andrzej Bartke, Rong Yuan

Background: Nuclear receptor interacting protein 1 (NRIP1) is a transcriptional coregulator implicated in aging-associated metabolic regulation and cancer progression. Previous studies have shown that NRIP1 deletion suppresses breast cancer development, yet the underlying cellular mechanisms remain unclear. Because autophagy is a central adaptive mechanism that enables cancer cells to survive metabolic stress, we investigated whether NRIP1 regulates autophagic flux and metabolic stress responses in breast cancer cells. Methods: Stable NRIP1 knockdown and overexpression MCF7 cell lines were generated. Cell proliferation and metabolic activity were assessed using direct cell counting and MTT assays, and migration was evaluated by wound-healing assays. Mitochondrial activity was evaluated by Seahorse XFp mito stress assay. Autophagy was analyzed using the tandem RFP-GFP-LC3 reporter under amino acid and glucose

deprivation, with and without chloroquine to distinguish autophagosome formation from autophagic flux. Results: NRIP1 knockdown significantly reduced proliferation, colony formation ability and migratory capacity in MCF7 cells, whereas NRIP1 overexpression enhanced these phenotypes. NRIP1 overexpression increased basal cellular OCR. Autophagy under resting conditions was reduced by NRIP1 knockdown but elevated by NRIP1 overexpression. Under amino acid starvation, NRIP1 knockdown cells showed impaired autophagosome formation and maturation, as well as diminished autophagic flux capacity. Conversely, NRIP1 overexpression enhanced autophagic throughput during prolonged starvation. Similar trends were observed under glucose deprivation. Conclusion: NRIP1 promotes autophagic and mitochondrial respiration in MCF7 cells; these processes may contribute to enhanced cell viability and justify further investigation of NRIP1 as a therapeutic target in breast cancer.

9. Measures of senescence across four non-human primate tissues

Chathurika Henpita | *Postdoctoral Trainee* | *University of Minnesota*

Chathurika R Henpita, Tra L Kieu, Rajesh Vyas, Paul D Robbins, Ricki J Colman, Laura J Niedernhofer

Aging is a complex process culminating in loss of organ function and organism resilience. One of the hallmarks of aging is cellular senescence, which is defined as the stable proliferative arrest that occurs in cells after many cell divisions or acute stress. Senescent cells are known to drive aging and chronic diseases of old age, and as a result are being targeted by senolytics that selectively kill senescent cells. Translating senolytics requires assays to prove that senolytics reduce senescent cell burden in easily accessible tissues. To address this need, we measured expression of multiple senescence-associated genes in archived rhesus macaque tissues to determine if senescence in skin is a good proxy for senescent cell burden in internal organs and organism frailty Expression of LMNB1, CCND1, and IL6 most robustly correlated with non-human primate frailty, particularly in the liver and kidney. However, the skin had much lower levels of the ten cell cycle regulatory and senescence-associated-secretory-phenotype gene expression than the internal organs, suggesting that at least with these markers, skin is not a good proxy for internal senescent cell burden and has a low dynamic range for detecting activity of senolytics in the preclinical model most genetically related to humans.

10. Astrocyte-microglia metabolic crosstalk underlies SGLT2i neuroprotection in Alzheimer's Disease (AD)

Dulmalika Herath Manchanayake | *Graduate Student* | *Wayne State University*

Manchanayake, DNH., Jayarathne, HSM., Suhail, H., Dehaan, L., Sullivan, R., Sadagurski, M.

Aging is the strongest risk factor for Alzheimer's disease (AD), yet current drugs fail to directly target aging-associated neuroinflammatory mechanisms driving disease progression. We recently demonstrated that the FDA-approved sodium-glucose co-transporter 2 (SGLT2) inhibitor Canagliflozin (Cana) attenuates AD pathology and improves cognitive performance in 5xFAD mice at 6 months of age, accompanied by reduced hippocampal microglial and astrocyte activation, suggesting SGLT2 inhibition as a promising AD intervention. However, whether these benefits are driven by systemic metabolic effects or direct central metabolic reprogramming remains unclear. Therefore, we fed male and female 5xFAD mice with Cana (180 ppm) from 3 months of age and evaluated longitudinally to 12 months. Cana improved glucose tolerance in both sexes and enhanced cognitive performance selectively in males. In the hippocampal CA3 region, Cana significantly reduced plaque-associated phagocytic microglial activation and suppressed inflammasome engagement, evidenced by reduced ASC speck formation and diminished NLRP3 localization within microglia. These

changes were accompanied by signatures of altered cellular metabolic tone and improved redox balance in astrocytes, suggesting that bioenergetic stress modulation underlies microglial inflammasome suppression. To assess central mechanisms, we generated an astrocyte-specific inducible Sglt2 knockout (Aldh1l1Cre-Sglt2 ko). Astrocytic Sglt2 ablation in adulthood protected against stress-induced neuroinflammation and recapitulated key anti-inflammatory features of Cana treatment, supporting a model whereby astrocyte SGLT2 activity regulates microglial inflammasome activation through metabolic-redox coupling. These findings identify astrocyte-microglia metabolic crosstalk as a mechanistic axis underlying SGLT2-mediated neuroprotection and highlight central metabolic reprogramming as a strategy to enhance resilience in aging and AD.

11. A deep-learning enabled assay of heat stress resistance in C. elegans identifies novel geroprotective drugs

Thomas Hodder | *Graduate Student* | *University of Minnesota*

Thomas Hodder Matthew Gill, PhD Chad Myers, PhD

Drugs that slow the rate of aging in model organisms have potential use as therapeutics for improving human healthspan. However, the number of validated anti-aging compounds remains low and thus there is a need for large-scale screening enterprises. In this respect, the nematode C. elegans is an attractive system for high throughput compound screens due to its scalability and shared biology with mammals. We considered two improvements to existing drug screens for increased lifespan in C. elegans. First, most high throughput survival screens rely on a single drug exposure early in adulthood, potentially limiting compound efficacy over a 2-3 week lifespan. Second, scoring survival often relies on manual assessment of live and dead worms, which is laborious and subjective. To address the first issue, we have examined acute heat stress resistance as a proxy for lifespan. This has the advantage that survival can be scored 48 hours after a single drug exposure, thereby maximizing drug efficacy. Second, we have integrated deep-learning models such as ResNet and Segment Anything into an image-based screening pipeline that produces an automated survival score based on the posture of the worm post-heat shock. After validating this automated score in a 96-well plate assay using known longevity interventions, both genetic and chemical, we screened a library of 2,782 drugs, identifying 31 compounds that reproducibly confer acute heat stress resistance. Follow-up experiments have identified 15 drugs that extend lifespan, 12 of which have not been previously reported to be geroprotective. In conclusion, we have developed an assay that significantly reduces the time required for screening by using heat stress resistance as a proxy for lifespan and by automating scoring of survival. We anticipate that this platform will not only lead to the identification of other promising geroprotective drugs, but it will also facilitate screening of other disease-related phenotypes in C. elegans.

12. Pre-Existing Cellular Senescence Accelerates KRas-Driven Lung Tumorigenesis in a Progeroid ERCC1-Deficient Mouse Model

Jiayi Hu | *Graduate Student* | *University of Minnesota*

Jiayi Hu, Mythili Dileepan, Mackenzie Plummer, Alan Mangen, Linshan Laux, Sara McGowan, Davis Seelig, Laura Niedernhofer

Cancer is an age-associated disease. There is an increased probability for people over 60 to develop invasive cancer compared to younger individuals. Senescent cells (SnCs) play a causal role in aging and multiple age-related pathologies. Increasing evidence suggests that SnCs and their senescent-associated secretory phenotype (SASP) promote tumorigenesis in several cancer types, including pancreatic cancer and breast cancer. Despite this, most preclinical

cancer studies rely on young adult mice (6-8 weeks old), roughly equivalent to early adolescence in humans, which does not accurately reflect the median age-at-onset of the disease being modeled. Although the use of young mice improves experimental efficiency, it limits the physiological relevance and translational validity. To investigate how pre-existing senescence and the SASP influence lung cancer progression in aged hosts, we generated a mouse model that combines an oncogenic KRas allele harboring the human G12D mutation that drives non-small cell lung adenocarcinoma with a mutation that drives accelerated aging (Ercc1-/?;LSL-KRasG12D;Rosa26-LSL-NLuc). Four-to-five-month-old ERCC1-deficient mice exhibit systemic levels of cellular senescence comparable to 24-month-old wild-type mice, providing a rapid and cost-effective model for the purpose of this study. We demonstrated that tumorigenesis is accelerated in the progeroid ERCC1-deficient mice compared to wild-type controls. SnCs within the tumor microenvironment were spatially mapped using RNAscope with probes detecting two senescence biomarkers, p16Ink4a and p21Cip1, as well as the SASP factor Tnfa. Lung cancer cells co-culture with endothelials cells induced to senescence exhibited increased proliferation. Since SnC and their SASP are therapeutics targetable, this innovative Ercc1-/?;KRasG12D mouse model can be extended to other cancer types and provides a physiologically relevant platform to evaluating therapeutic strategies targeting age-associated processes, including senotherapy. Overall, this work offers translational insights into the interplay between aging, cellular senescence and cancer, an increasing important area as both the aging population and cancer incidence continue to rise.

13. Transient Early-Life Protein Restriction Establishes Sex-Specific Developmental and Metabolic Programming

Yumeng Huang | *Graduate Student* | *SIU School of Medicine*

Yumeng Huang, Yun Zhu, Xiuqi Han, Asmita Sharma, Elham Mohebbi, Yaoyuan Zhang, Lisa Hensley, Rong Yuan, Andrzej Bartke

Early-life developmental conditions shape aging trajectories in accordance with the pace-of-life framework linking developmental process, metabolism, and lifespan. To determine whether transient nutritional modulation alters developmental processes or induces adaptive physiological reprogramming, genetically heterogeneous UM-HET3 mice were exposed to a low-protein diet (12.5%) from postnatal day 0-48, followed by lifelong control diet. Early-life protein restriction reduced developmental body weight and delayed sexual maturation in a sex-specific manner, while adult body composition and adiposity remained normal. Glucose tolerance and insulin sensitivity were preserved at both midlife and advanced age in males and females, indicating maintained systemic metabolic homeostasis. However, aged males exhibited an attenuated glycemic response to pyruvate challenge, suggesting that restrained hepatic gluconeogenesis. Indirect calorimetry further revealed persistently lower respiratory exchange ratio and reduced total energy expenditure, consistent with a durable shift toward lipid-based energy utilization and altered fuel selection. Cognitive function assessed by novel object recognition demonstrated intact discrimination performance in both sexes. Although female mice displayed modestly increased investigation of familiar objects, total exploration time and recognition indices were unchanged, indicating subtle modulation of exploratory strategy without cognitive decline. Together, these findings demonstrate that transient early-life protein restriction slows down developmental pace and induces coordinated, sex-dependent metabolic and behavioral reprogramming without cognitive dysfunction, consistent with an adaptive slower pace-of-life strategy that may influence long-term aging trajectories.

14. Neuronal and metabolic regulation of stress resistance and longevity

Shijiao Huang | *Faculty* | *Kansas State University*

Shijiao Huang, Shelby Innes, Mingyi Liu, Andrea Miller, Amit Dutta

Organisms experience a variety of stresses throughout their lives: cellular stress that triggers molecular intracellular stress responses, physical stress that challenges the whole organism, and psychological stress that involves neurons and hormones. All types of stress perturb homeostasis, leading to either homeostatic imbalance or resilience. In contrast, appropriate activation of stress response pathways leads to improved health indices and lifespan extension. Cell non-autonomous longevity pathways involving neural signaling were initially identified in C. elegans, including insulin-like signaling, dietary restriction, mitochondrial unfolded protein response, ER unfolded protein response, heat shock response, hypoxic response, and are likely to be conserved in more complex organisms. Neural signaling has been reported to be involved in both stress response and regulatory of longevity. Our goal is to identify neural circuits and their regulated metabolic changes that are central in regulating stress resistance and longevity. We have identified an interneuron involved in the resistance to heat, ER, and mitochondria stress. Ablation of this neuron decreases the levels of total lipids and a poly saturated fatty acid. We also found that knockout of a neuropeptide specifically expressed in this neuron increases heat stress resistance. In addition, we also use neuroactive compounds as mechanistic probes to identify neuronal and metabolic targets of lifespan and healthspan extension. We showed that neurotransmitter antagonists extend lifespan, increase motility, and improve short-term and long-term memory in C. elegans. Total lipid levels and fatty acid profiles are remodeled by these neuroactive compounds. We will further explore whether lifespan and cognitive extension are achieved from conserved neurotransmitter-dependent or -independent mechanisms.

15. Neural circuit defects underlying dehydration risk in aged mice

Heeun Jang | *Faculty* | *Iowa State University*

Heeun Jang, Alexis Behne Sharma, Usan Dan, Jasmine Wong, Zachary Knight, Jennifer Garrison.

Chronic dehydration is a leading cause of morbidity for the elderly, but how aging alters the fluid homeostasis system is not well understood. Here, we used a combination of physiologic, behavioral and circuit analyses to characterize how fluid balance is affected by aging in mice. We found that old mice have a primary defect in sensing and producing the anti-diuretic hormone vasopressin, which results in chronic dehydration. Recordings and manipulations of the thirst circuitry revealed that old mice retain the ability to sense systemic cues of dehydration but are impaired in detecting presystemic, likely oropharyngeal, cues generated during eating and drinking, resulting in disorganized drinking behavior on short timescales. Surprisingly, old mice had increased drinking and motivation after 24-hour water deprivation, indicating that aging does not result in a general impairment in the thirst circuit. These findings reveal how a homeostatic system undergoes coordinated changes during aging.

16. Skeletal muscle remodeling contributes to enhanced healthspan in Ames dwarf mice

Matthew Johnston | *Graduate Student* | *University of North Dakota, Biomedical Sciences Department*

Matthew J. Johnston, Holly M. Brown-Borg

Ames dwarf mice exhibit a robust extension in lifespan relative to control littermates due to congenital disruption of growth hormone (GH) signaling. Although central to their longevity, the effects of postnatal GH deficiency on skeletal muscle remains largely unknown. This gap is significant as skeletal muscle is a regulator of systemic metabolism, inflammatory tone, physical resilience, and survival during aging. Whether remodeling of skeletal muscle contributes to lifespan

extension in this model is unknown. To address this question, we evaluated fitness performance, muscle architecture, metabolic phenotype, and inflammatory responses in Ames dwarfs and hormonally normal controls across three age groups (young, middle-aged, and aged), with the goal of defining how skeletal muscle health intersects with exceptional longevity. Fitness testing revealed preserved strength and coordination, with endurance capacity diverging sharply at 21 months of age, when dwarf mice outperformed controls in both absolute and relative running measures. Histological assessment of tibialis anterior muscles was performed to evaluate myofiber size, structural integrity, mitochondrial abundance, and fiber type composition. Aged dwarf mice resist hallmarks of sarcopenia observed in controls, including fiber atrophy, fibrosis, and functional decline. Ames dwarf myofibers are smaller in cross-sectional area yet significantly more numerous within the muscle, accompanied by a shift toward oxidative fiber types that favor endurance-oriented metabolism. Dwarf mice also exhibit an attenuated immune response following eccentric exercise, suggesting improved stress resistance. Collectively, our data suggest that lifelong disruption of GH signaling remodels skeletal muscle toward increased oxidative capacity, expanded fiber number, and diminished inflammatory reactivity.

17. Genetic Determinants of Brain Response to Caloric Restriction and Intermittent Fasting in Mice and Man

Catherine Kaczorowski | *Faculty* | *University of Michigan*

Catherine Kaczorowski, Arturo Rocha, Dave Bridges, Erica Guell

Dietary restriction is among the most effective non-pharmacological interventions to counteract aging-related diseases and promote longevity. This has been consistently demonstrated across species from mice to humans. However, emerging evidence indicates that dietary restriction may adversely affect brain health. In our study utilizing genetically diverse mice, which model the genetic heterogeneity of the human population, we found that severe 40% caloric restriction (CR) extended lifespan but caused memory deficits. Genetic factors accounted for up to 55% of this memory variation. This is caused - in part - by gene variants in Slc16a7, where we see large-effect variants at Slc16a7 locus associated with long-term memory in middle-aged diversity outbred (DO) mice. Consistent with these findings, single-nucleotide polymorphisms (SNPs) in SLC16A7 were associated with brain aging outcomes in a human clinical trial of dietary restriction, including changes in Alzheimer's Disease (AD) biomarkers. Specifically, where we found a protective allele improving AD biomarkers such as total tau and ?A42/40 ratio. SNPs in SLC16A7 were also associated with longitudinal cognitive decline in older adults enrolled in the Health and Retirement study. Further supporting a role for SLC16A7 in cognitive resilience, higher expression of SLC16A7 RNA in memory relevant brain regions is associated with better cognition in older adults with Alzheimer's neuropathology. An additional genetically diverse Alzheimer's mouse model has confirmed that the Slc16a7 locus is nominally associated with a global resilience score and can be used to test the causal role of Slc16a7 in aging and AD, evaluating new genes and possible pharmacological interventions.

18. Host Age Can Impose Age-Specific Cell-to-Cell Variation on Donor Cells

David Katz | *Postdoctoral Trainee* | *Mayo Clinic - Arizona*

David Katz, Jacob Fisher, and Jessica Lancaster

We report that within defined cell types, transcriptional cell-to-cell variation across multiple CD4+ T cell populations decreases with age. This pattern is evident in our single-cell RNA-sequencing data and is independently recapitulated in two public datasets analyzed using our cell-to-cell variation pipeline. We propose that inconsistencies in the literature

largely reflect failure to distinguish within-cell-type variation from (i) emergence of new cell types with age and (ii) increased inter-individual variability with age. Importantly, the age-associated reduction in within-cell-type variation is not strictly cell intrinsic. In heterochronic transfer experiments, young CD4+ T cells adopt reduced transcriptional variability when placed into aged hosts. These findings suggest that measuring within-cell-type cell-to-cell variation from single-cell RNA-seq data provides a simple and sensitive readout of regulatory signals that shape the T cell compartment with age. This analysis technique may also more broadly report on age-dependent regulation in other cellular systems.

19. PAI-1 Reduction as a Strategy to Extend Healthspan

Alireza Khoddam | *Graduate Student* | *Northwestern University*

Alireza Khoddam, Mesut Eren, Saul Soberanes, Johnson Yang, Brian Dinh, Helen Lyu, Anthony Kalousdian, Andrew Decker, Liz Lux, Ben Zywicki, Lisa Wilsbacher, and Douglas Vaughan

Plasminogen activator inhibitor-1 (PAI-1) is a marker of cellular senescence and is linked to age-related disease and mortality. Growing evidence suggests that PAI-1 is not just a biomarker, but a driver of biological aging. Reducing PAI-1 preserves tissue function, senescence-related signaling, and improves resilience to age-related stress. About ten years ago, we discovered that an Amish population with a heterozygous loss of function mutation in the gene encoding for PAI-1 live ten years longer than the rest of the Amish without the mutation. Inspired by this human "natural experiment", we have now expanded our knowledge on how PAI-1 may be driving biological aging in three domains: cardiovascular, immune, and cognitive. Together, these findings identify PAI-1 as a key regulator of aging and support PAI-1 reduction as a potential strategy to extend healthspan.

20. Uncovering the Impact of Senescence Liver and Aged Immune cells on Liver Cancer Progression

Tra Kieu | *Graduate Student* | *University of Minnesota-Twin Cities*

Tra Kieu1,2, Valentin Barthet3, Scott Lowe3, Laura Niedernhofer1,2. 1Masonic Institute on the Biology of Aging and Metabolism, University of Minnesota. 2Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota. 3Cancer Biology and Genetics Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center

Senescent cells (SnCs) accumulate with age and secrete tissue remodeling and immuno-modulatory factors known as the senescence-associated secretory phenotype (SASP). In the liver, SnCs exert context-dependent effects on hepatocellular carcinoma (HCC), functioning as both tumor suppressors and promoters. Despite growing interest in senotherapeutics as anticancer strategies, the heterogeneity of liver SnCs and their specific roles in tumor progression remains poorly defined. Here, we evaluate how distinct SnC populations contribute to tumorigenesis. We characterized senescence and SASP profiles across liver populations (hepatocytes and non-parenchymal cells (NPCs)) and immune cells (splenocytes or CD8+T cells). In vitro, transwell assay revealed that exposure to the secretome of aged hepatocytes, NPCs or splenocytes significantly increased HCC cells proliferation (Ki-67+ signal). Aged hepatocytes expressed numerous biomarkers of senescence including enlarged morphology, increased lysosomes, increased ??H2AX nuclear foci, and elevated cell cycle inhibitors (p16/p21) and SASP expression. Additionally, both aged hepatocytes and splenocytes exhibit robust pro-inflammatory secretome. To assess immune aging in vivo, we induced HCC in young wild-type (WT) mice after adoptive transfer of splenocytes from aged WT or accelerated immune aging (Vav-iCre?/?;Ercc1?/fl) donors. A single transfer induces systemic senescence and inflammation in young recipients, enabling subsequent hydrodynamic tail vein injection(MYC-GFP; Trp53-/-), to induce liver cancer in immune-aged hosts. We are still ongoing characterize how

different donor genotypes and immune cell populations undergo expression changes and influence the hepatic niches and tumor outcomes. Our next step involves spatial transcriptomics to map the interaction between donor-derived senescent immune cells and the tumor microenvironment. Supported by U01AG077921(Dr. Lowe).

21. Characterizing the effect of individual dietary non-essential amino acid depletion on metabolic health and aging

Bailey Knopf | *Graduate Student* | *University of Wisconsin-Madison*

Bailey Knopf, Isaac Grunow, Brady Anderson, Tareq Rihawi, Michelle Sonsalla, Reji Babygirija, Mariah Calubag, Yang Liu, Chung-Yang Yeh, and Dudley Lamming

Reducing dietary protein content in rodents has been shown to improve metabolic health and increase lifespan. Further studies have shown that reducing essential amino acids, which are required from the diet, can mimic the effects of protein restriction such as improved body composition, insulin sensitivity, and lifespan. While essential amino acids restriction has been extensively documented, the effect of non-essential amino acid (NEAA) restriction remains unknown. To investigate the role of NEAA restriction in metabolic health, we placed 8-week-old male C57BL/6J mice on individually depleted NEAA diets until 20 weeks of age. Our studies reveal that most NEAA depleted diets do not impact body weight or metabolic health. However, we observed one NEAA depleted diet, arginine depletion, significantly improved body weight and glucose handling in mice. These improvements may be driven by improved lipid homeostasis through reduced fat accretion, increased lipolysis, decreased lipid droplets in the liver, and increased thermogenesis. The molecular changes driven by arginine depletion may promote healthy aging. This study reveals that depletion of dietary arginine is beneficial for metabolic health and provides the groundwork for further exploration of how arginine metabolism interacts with metabolic dysfunction and contributes to aging.

22. Co-operation of clonal haematopoiesis mutations

Esra Gozde Kosebent | *Graduate Student* | *Mayo Clinic*

Esra Gozde Kosebent Nathalie Havranek Eszter Doma Karoline Kollman Kristina Kirschner

Clonal hematopoiesis of indeterminate potential (CHIP) is defined as the clonal expansion of hematopoietic stem and progenitor cells (HSPCs) in healthy aged individuals. This condition is highly characterized with myeloid bias. Clinically, CHIP is associated with an increased risk for hematological cancer, especially myeloid, and all-cause mortality, where age is a major risk factor. We recently presented a robust method to establish hematopoietic progenitor cells (HPCLSKs) from mice. This system allows us to establish state-of-the-art assays with infrequent progenitor cell populations and to mimic cell state heterogeneity. We hypothesize that CHIP-associated mutations lead to distinct molecular alterations that fundamentally reshape stem cell fitness and clonal dynamics. CHIP mutations introduced into HPCLSKs via retroviral overexpression produced distinct, mutation-specific cellular phenotypes. At the level of cell fate, ASXL1, SF3B1, SRSF2, and U2AF1 mutant clones drove apoptosis pathway with clonal disadvantage. Within the spliceosome and cell cycle pathway, SF3B1 and SRSF2 mutations upregulated genes related to spliceosome machinery and E2F targets, and bulk RNA-sequencing confirmed that all three splicing factor mutants (SF3B1, SRSF2, U2AF1) showed elevated expression of genes associated with oncogene-induced senescence and DNA methylation. In the inflammatory and cytokine signaling pathway, the epigenetic regulators DNMT3A and ASXL1 increased cytokine-cytokine receptor interaction-related gene expression, while TET2 mutant cells showed remodeling of actin cytoskeleton-related pathways by KEGG analysis,

DNMT3A, TET2, and ASXL1 collectively upregulated genes involved in inflammatory response and apoptosis. Regarding proliferative and translational programs, MYC target genes were broadly downregulated across all epigenetic mutants, with DNMT3A and ASXL1 clones additionally showing reduced expression of translation-associated gene signatures. Finally, at the level of genome integrity, TET2 mutants demonstrated a specific downregulation of DNA repair pathways. Together, these findings reveal that CHIP mutations reprogram distinct intracellular programs in a mutation-specific manner, providing an in vitro platform to dissect the mechanisms underlying the adverse clinical consequences of clonal hematopoiesis.

23. Longitudinal and life-phase specific signatures predict the first onset of age-related morbidity and mortality in rhesus monkeys.

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Aging is the greatest risk factor for a variety of chronic diseases, although age-point resolved biomarkers of healthy aging are limited. Caloric restriction (CR) without malnutrition sustains health into advanced age, but trajectories of aging and the impact of life-stage on health biomarkers are not known. Here we report a high-resolution, life-stage resolved, longitudinal analysis of plasma from rhesus monkey and identify novel biomarker models that capture age and health status. Using deep learning, age was the biggest driving factor for changes in plasma lipoproteins, lipids, and metabolites across the adult lifespan independent of diet. Taking a matrix approach, we show that the impact of age was non-linear. An inflection point identified in mid-life tracked with the first onset of age-related morbidity and occurred later in CR animals than in Controls. Predictive signatures of age-related morbidity were identified by machine learning, including circulating factors more informative than age itself. Causal network analysis identified connections among predictive variables, suggesting an underlying mechanism of reprogrammed metabolism. In a linear model, iterative LASSO identified different sets of age predictors by diet, suggesting that CR does not simply delay aging, it changes how aging occurs. This work is supported by NIH grant R01AG074503, Wisconsin National Primate Research Center and William S. Middleton Memorial VA Hospital, Madison, WI.

24. Dynamics of lipid metabolism in cellular senescence: SASP regulation and therapeutic opportunities for aging

Gung Lee | *Postdoctoral Trainee* | *Mayo Clinic*

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Cellular senescence is a major driver of aging, characterized by irreversible cell cycle arrest and onset of a pro-inflammatory secretory phenotype. Senescent cells accumulate with age, contribute to age-related diseases, and their

selective clearance has been shown to delay or alleviate age-associated pathologies. While their transcriptional programs are well characterized, the metabolic mechanisms, especially lipid metabolism, that sustain the inflammatory phenotype remain poorly understood. Our work identifies a coordinated lipid remodeling program as a central regulator of senescent cell behavior. Senescent cells undergo a metabolic shift that drives lipid accumulation and enrichment of signaling lipids, which in turn amplify inflammatory signaling through the activation of mitogen-activated protein kinase cascades. Using spatial lipidomics, we show that these lipid signatures are regionally enriched within aged tissues rather than uniformly distributed. Consistent with this, lipid-high senescent cells exhibit a heightened inflammatory phenotype, supporting a direct functional link between lipid remodeling and inflammatory output. Importantly, genetic or pharmacologic modulation of lipid metabolic pathways reshapes the senescent lipid profile and significantly reduces chronic inflammatory signaling. In aged mouse models, this intervention improves systemic outcomes, including physical function and cognitive performance. Together, these findings position lipid remodeling as an active driver of senescence-associated pathology and a promising therapeutic target for age-related disease.

25. Lifespan-extending interventions inhibited consistent expression patterns of IL-11 signaling across mouse livers and adipose tissues

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Abstract The emergence of a chronic, low-intensity systemic inflammatory state, commonly referred to as "inflammaging", is a defining characteristic of the aging process. This contributes to the pathogenesis of age-related disorders, such as neurodegenerative, cardiometabolic, and musculoskeletal dysfunction, alongside generalized physiological frailty and cancer. Inflammaging is multifactorial, involving an assortment of secreted molecules originating from multiple sources, and operates through established signaling mechanisms. Considering the multifaceted and complex nature of the aging process, there is a critical need to structurally delineate and reduce the complexity of inflammaging-related signaling networks. Recent studies have implicated interleukin-11 (IL-11), a cytokine within the IL-6 cytokine family characterized by pro-inflammatory and pro-fibrotic properties, as a crucial regulator of molecular pathways governing aging. IL-11 acts through the modulation of signaling nodes, including AMPK, ERK, JAK-STAT3, and mTOR. Previous studies indicate that IL-11 inhibition may result in enhancements in both health span and lifespan in animal models, with potential translational relevance in humans. In this study, we hypothesized that systemic changes in IL-11 signaling across liver and adipose tissues are correlated with lifespan-extending interventions (16?-hydroxyestradiol (OH_Est), Canagliflozin (Cana), Astaxanthin (Asta), Meclizine (Mec), Epicatechin (Epi), Halofuginone (Halo), and Mitoglitazone (Mit)). We identified that canagliflozin (Cana), started at 16 months of age, and 16?-hydroxyestradiol-estradiol (OH_Est), started at 12 months of age, each led to significant increases in lifespan in male UM-HET3 mice. In contrast, both interventions led to a significant decrease among their female counterparts. Astaxanthin, Meclizine, Epicatechin, Halofuginone, and Mitoglitazone were also found to extend the lifespan of UM-HET3 male mice. Our data showed consistent inhibition of IL-11 signaling in the liver and adipose tissue of mice with lifespan-extending interventions. We observed no alteration in IL-11 protein expression across liver samples, but saw a decrease in IL-11 levels in the inguinal adipose tissue of the slow-aging mouse models, without differences between sexes. In perigonadal adipose tissue from mice treated with Epicatechin, Halofuginone, Mitoglitazone, OH_Est, and Cana, IL-11 protein levels decreased in males, in contrast to the increase of IL-11 protein expression in females. No change in IL-11 protein levels was detected in the perigonadal adipose tissue of mice treated with Asta or Mec. These findings highlight that pharmacologic interventions may be mediated by reductions in inflammaging and inflammatory cytokine signaling.

26. Isoleucine restriction lowers LDL cholesterol in mice

Yang Liu | *Graduate Student* | *UW Madison*

Yang Liu, Dudley Lamming, Brian Parks

Excess cholesterol is a major risk factor driving many cardiovascular diseases, including atherosclerosis, heart attack and stroke. The risk of hypercholesterolemia increases with age, as metabolism changes. Drugs to lower cholesterol have issues like side effects and intolerance in patients, so we decided to seek for a dietary solution to lower plasma cholesterol using mice as a model. Protein restriction promotes metabolic health in diverse species. I found dietary restriction of one amino acid, isoleucine, decreases plasma low-density lipoprotein (LDL)-cholesterol in male C57BL/6J mice. I put young male wild type (WT) and low-density lipoprotein receptor knock out (LDLR-KO) C57BL/6J mice on either a control or a 67% isoleucine restriction diet for 3 weeks, then collected their plasma and tissues for analysis. I found 3 weeks on isoleucine restriction diet is sufficient to significantly decrease plasma LDL-cholesterol in WT mice, while this phenotype was blocked by the lack of LDLR, proving LDLR is required for isoleucine restriction to decrease plasma cholesterol. In WT mice, isoleucine restriction increased liver expression of NDRG1, a protein required for LDLR recycling. I'm looking forward to testing if isoleucine restriction diet alleviates pathologies in atherosclerosis mouse models. This project sheds light on the potential use of isoleucine restriction as an alternative treatment for certain patients. We hope to help develop dietary treatment to protect against cardiac risks for populations that are susceptible to hypercholesterolemia, especially the elder.

27. Age-Associated FOXP1 Decline Drives Impaired Lung Repair Following Viral Pneumonia

Ruihua Ma | *Faculty* | *Northwestern University*

Ruihua Ma

Older adults experience disproportionate morbidity and mortality from respiratory viral infections and ARDS, largely due to a failure in lung tissue regeneration. Alveolar repair is a tightly coordinated process requiring Alveolar Type II (AT2) cells to function as facultative stem cells while regulatory T (Treg) cells maintain a supportive, anti-inflammatory niche. Using a mouse pneumonectomy model, we found that aged lungs exhibit reduced AT2 cell abundance and impaired proliferation in vivo. Consistently, aged AT2 cells display diminished alveolosphere formation in vitro, indicating loss of stemness. In parallel, while young mice show robust Treg expansion after injury, aged mice fail to expand this population and instead display a skewing toward a spontaneously activated, effector-like phenotype. Single-cell RNA-seq identifies the transcription factor FOXP1 as significantly downregulated with aging. While FOXP1 is enriched in AT2 cells in both mice and humans, our data show a significant decline in FOXP1 levels within aged murine AT2 cells. In Treg cells, FOXP1 is preferentially expressed in resting populations but is markedly reduced in aged lungs, correlating with the loss of quiescence and acquisition of a dysregulated effector phenotype. Together, these findings support a model in which age-associated FOXP1 decline impairs both AT2 cell-intrinsic stemness and Treg-mediated support of the repair niche, collectively compromising lung regeneration in the aging population. Targeting FOXP1-dependent pathways may offer a novel strategy to restore lung repair and improve outcomes following viral pneumonia in the aging population.

28. Mitochondrial metabolism and epigenetic crosstalk in senescent cells.

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Chronic low-grade inflammation is a hallmark of aging and a major risk factor for morbidity and mortality in older adults. Senescent cells (SCs) promote tissue dysfunction and organismal aging in part through the senescence-associated secretory phenotype (SASP). However, the mechanisms contributing to the SASP are not fully elucidated. Our team has shown that mitochondrial dysfunction plays a central role in aging and SASP production. Understanding how mitochondrial dysfunction drives the SASP is essential for identifying new therapeutic targets to counteract the detrimental impact of SCs during aging. Here, we demonstrate that mitochondrial metabolism is a key regulator of the epigenetic regulation of the SASP, where mitochondria-derived metabolites, such as citrate and acetyl-CoA, fuel histone acetylation at SASP gene loci, promoting their expression and inflammation in SCs. Interfering with mitochondrial metabolism, by inhibiting SLC25A1 and citrate export, reduces histone acetylation and SASP expression. Moreover, we show that mtDNA/cGAS/STING signaling cooperates with this metabolic-epigenetic pathway to enable full SASP activation and inflammation. Finally, single-cell multiome analysis, in muscle, demonstrates that pharmacological inhibition of SLC25A1 in vivo reduces chromatin accessibility at SASP genes, decreases inflammation, and is associated with improved healthspan in aged mice, underscoring its therapeutic potential. Our data support the concept that the SASP is driven by crosstalk between mitochondrial metabolism and epigenetic changes and identify new targets to combat the pro-aging effects of SCs.

29. Age-Associated Epithelial Fucosylation Drives CD8? T Cell Exhaustion and Senescence in the Colon Tumor Microenvironment

Ramkumar Mathur | *Faculty* | *University of North Dakota*

*Hakan Celik, Jacqueline Kim Correa, Kristian Herman, Reet Goyal, Leia Lauer, Sofie Robinson, Elise Foell and Ramkumar Mathur**

Immune dysfunction associated with aging is characterized by impaired antitumor immunity, leading to decreased responsiveness to immunotherapy. CD8? tumor-infiltrating lymphocytes (TILs) within the tumor microenvironment (TME) often become functionally impaired and enter states of exhaustion and cellular senescence. However, the factors driving exhausted/senescent T-cell states within the aging intestine are not well defined. We examine the contribution of epithelial fucosylation a glycosylation modification enriched on intestinal epithelial cells (IECs) to immune suppression during colon tumor progression. We found that epithelial fucosylation was enriched with age and tumor burden in human and mouse colon tissues. Tumors with elevated epithelial fucosylation had increased expression of immune checkpoints and enrichment for a unique CD8? T-cell population expressing exhaustion markers (PD-1, TIM-3) and senescence markers (p16, p21). Single-cell transcriptomics revealed age-associated epithelial cell signatures related to fucosylation that were enriched for genes associated with immune suppression, decreased apoptosis, and tumor progression within the aging TME. Functionally, pharmacologic inhibitors of fucosylation (2-fluorofucose or resveratrol) decreased colon cancer cell proliferation and migration and potentiated antitumor immunity when combined with immune checkpoint blockade. Overall,

these data identify epithelial fucosylation as an age-related contributor to CD8? T-cell exhaustion and senescence within tumors that may serve as a therapeutic target to improve responsiveness to immunotherapy in aging patients.

30. Examining Structural Stability of Intrinsically Disordered Protein Complexes and Sub-Complexes through Gas-Phase Unfolding and Ion Mobility-Mass Spectrometry

Rowan Matney | *Graduate Student* | *University of Minnesota*

Rowan Matney, Varun V. Gadkari

?-synuclein is an intrinsically disordered protein associated with Parkinson's disease. It is known to undergo a structural change en route to forming insoluble aggregates characteristic of Parkinson's disease progression; however, its transient oligomeric states are relatively understudied. To understand the mechanism of intrinsically disordered protein aggregation, it is essential to characterize these structural intermediates. In this work, we aim to characterize oligomers of ? -synuclein using native ion mobility-mass spectrometry (IM-MS). Native IM-MS allows intact proteins and protein complexes to be transferred to the gas phase while retaining their secondary, tertiary, and quaternary structure. Previous research has shown that collision induced unfolding (CIU) can be used to characterize the stability of the soluble oligomers of intrinsically disordered proteins. In this work, we first use surface induced dissociation (SID) to break oligomers into native-like subcomplexes in the gas phase. We then unfold those subcomplexes using CIU and compare their stability to their natively occurring counterparts. Current work with ?-synuclein shows that natively occurring monomer unfolds into a partially unfolded intermediate state; however, ?-synuclein monomer that has been dissociated (via SID) from a dimer (sub-complex ?-synuclein) seems to be unfolded upon dissociation and experiences compaction during CIU. This is in direct contrast to both previous research and to proof-of-concept studies performed on the protein standards transthyretin and streptavidin. Expanding this work to additional intrinsically disordered proteins and oligomeric states will further our understanding of the aggregation pathways for small oligomers in neurodegenerative diseases.

31. Sex dimorphism in aging and metabolic rejuvenation by AdipoRon

Cassandra McGill | *Postdoctoral Trainee* | *University of Wisconsin - Madison*

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Aging is the most significant risk factor for a broad spectrum of debilitating diseases, including cancer, diabetes, neurodegenerative disorders, and sarcopenia. A critical factor in the biology of aging is biological sex; many pharmacological interventions exhibit sex-dimorphic outcomes, emphasizing the need to consider biological sex as a variable in pre-clinical and clinical trials. Here, we investigate sex differences in aging metabolism and muscle physiology at both functional and molecular levels in mice. We identify similarities in aging physiology but detect substantial dimorphism at the cellular and molecular levels in terms of energy metabolism and cellular processes that are associated with functional decline. AdipoRon, an adiponectin receptor agonist, mitigates age-associated declines in muscle mass and integrity in males only; however, AdipoRon exerted systemic beneficial metabolic effects in both sexes. Our findings provide mechanistic insight into sex-specific drivers of sarcopenia and highlight the need for tailored interventions to treat or prevent sarcopenia.

32. Adiponectin receptor activation reduces hippocampal tauopathy

Eric McGregor | *Graduate Student* | *University of Wisconsin-Madison*

Eric R. McGregor, Aaron J. Berkowitz, Cassandra J. McGill, Mathew V. Jones, Rozalyn M. Anderson

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. Despite evidence that mitochondrial abnormalities coincide with functional decline in AD, effective therapeutic strategies targeting this relationship are lacking. Here, we investigate the therapeutic potential of mitochondrial targeting via the adiponectin receptor activator AdipoRon (AR) in a mouse model of tauopathy. Adiponectin is an adipose tissue-derived hormone linked to metabolic syndrome and aging, and adiponectin receptors are expressed in all brain cell types. We used primary neurons and ex vivo hippocampal slices from hTauP301S mice or their WT littermates to study the impact of tau pathology on metabolism and how tau-associated metabolic changes relate to electrophysiological properties. Accumulation of hippocampal phosphorylated tau was reduced by four months of daily AR treatment. Whole-cell patch-clamp experiments of CA1 pyramidal neurons identified tauopathy-associated defects in passive electrophysiological parameters and lower action potential firing rates. Four months of daily AR treatment prevented Tau-associated deficits in action potential firing and maintained passive parameters at wildtype levels. Voltage-clamp experiments assessed changes in inhibitory and excitatory synaptic activity in CA1 pyramidal neurons. Metabolic changes in the hippocampus will be investigated using immunohistochemistry to assess mitochondrial activity and density. Astrocytic response to AR will also be investigated. Collectively, these data reveal an intracellular network linking mitochondrial function to cellular maintenance processes and electrical function that can be targeted via adiponectin receptor activation.

33. ATGL-mediated lipolysis mitigates DNA damage via a p300-p53 signaling axis

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Rachel K. Meyer, Mahima Devarajan, Gavin Fredrickson, Will A. Hofstadter, Mara T. Mashek, Mari V. Reid, Douglas G. Mashek

Aging is associated with increased DNA damage, resulting in genomic stress, mutations, and senescence that drives the development of age-related disease. Conversely, fasting is a robust intervention for lifespan extension that mitigates DNA damage; however, the underlying mechanisms remain unclear. In addition to the impact on DNA damage, fasting also increases triglyceride breakdown, or lipolysis, catalyzed by adipose triglyceride lipase (ATGL), the rate-limiting enzyme in this pathway. Because fasting both increases lipolysis and mitigates DNA damage, we hypothesized that increasing ATGL-mediated lipolysis would replicate the effect of fasting on DNA damage. To investigate this hypothesis, we used viral and transgenic overexpression of ATGL in AML12 cells, IMR90 cells, and primary murine embryonic fibroblasts (MEFs) with acute etoposide exposure. ATGL overexpression significantly reduced lipid droplet accumulation following oleate treatment, confirming increased lipolysis in our model, and decreased yH2AX levels following etoposide across all cell types, indicating reduced DNA damage. Mechanistically, ATGL overexpression increased protein acetylation and expression of p53 gene targets; therefore, we next hypothesized that p53 acetylation may mediate the effect of ATGL. Indeed, pharmacological inhibition of the histone acetyltransferase, p300, as well as siRNA-mediated knockdown of p53, abolished the effect of ATGL overexpression on yH2AX following etoposide exposure, indicating that p300-mediated p53 acetylation is required for the effect of increased lipolysis on DNA damage. Overall, these data suggest a lipolysis-p300-p53 signaling axis that mitigates DNA damage in response to etoposide that can be leveraged in future studies to prevent age-related disease and off-target cell damage following chemotherapy.

34. Impact of dietary sodium intake on blood pressure and risk of Cancer and Cardiovascular Disease Risk

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Though interconnected, the mechanisms linking aging, blood pressure (BP), cardiovascular disease (CVD), and cancer remain incompletely understood. While elevated BP is widely viewed as a consequence of aging, emerging evidence suggests BP may itself influence the rate of biological aging and susceptibility to age-related diseases. Dietary sodium (Na?) intake is a major modifiable determinant of BP and may contribute to aging-associated physiological changes. This study examines whether chronic dietary modulation of Na? intake alters BP, aging-related traits, and disease risk, and whether these effects depend on somatotropic (growth hormone, GH) signaling. We hypothesize that high Na? intake promotes systemic inflammation, accelerates biological aging, and increases disease risk, whereas Na? restriction confers protective effects that vary according to GH signaling status. To test this, mice with distinct GH signaling and aging phenotypes are used: bovine GH-overexpressing transgenic (bGH-Tg) mice, which exhibit accelerated aging and reduced lifespan, and growth hormone receptor knockout (GHRKO) mice, which are GH-resistant and long-lived. Age- and sex-matched normal siblings serve as controls. Mice are maintained on low-, normal-, or high-sodium diets from weaning for six months. Longitudinal measurements include BP, glucose and insulin homeostasis, and body composition. At seven months of age, tissues are collected to assess markers of inflammation, cardiovascular stress, mTORC1 signaling, CVD- and cancer-related gene expression, and Aging Rate Indicators. Preliminary findings in bGH-Tg mice reveal sex-specific BP and metabolic responses to dietary Na?. Ongoing analyses will clarify how dietary sodium interacts with somatotropic signaling to influence BP regulation, biological aging, and disease risk.

35. L-carnosine mitigates obesity- and age-driven senescent cell accumulation in visceral adipose: Implications for obesity/age-acquired insulin resistance

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Enhanced senescent cell burden in visceral adipose tissue is an important driver of age- and obesity-associated metabolic dysfunction. Although the characterization of senescent cell populations and delineation of mechanisms underlying their accumulation in adipose tissue remain incomplete, there is a correlative association between lipid peroxidation and longevity/age-related pathologies. Importantly, there is increased generation of electrophilic lipid peroxidation products (e.g., 4-HNE) in visceral adipose depots of mouse models of aging and obesity. We demonstrated previously that these biogenic lipid electrophiles cause cellular senescence in vitro via Biogenic Lipid Induced Senescence (BLIS). To assess BLIS in vivo, we employed L-carnosine, a dipeptide carbonyl scavenger that sequesters biogenic electrophiles, in models of diet-induced obesity in young (6-month) and old (24-month) C57/BI6J mice. Single nuclei (sn)RNA sequencing was used to measure transcriptomic shifts in visceral adipose tissue from treated mice. In both young and old mice, L-carnosine markedly blunted accumulation of Cdkn1a-highly expressing cells, particularly in adipose stem and progenitor cells (ASPCs) and endothelial cell populations. In affected populations, L-carnosine was associated with blunted activity of

pro-inflammatory transcription factors IRF3/5, p53, and NF-?B, and restored activity of stress resistance transcription factor Foxo3. In young mice, we observed a concomitant improvement in insulin sensitivity. Taken together, our results suggest that biogenic electrophiles represent a mechanistic link between age, obesity, and adipose senescence. As Cdkn1a-highly expressing cells are implicated in development of downstream systemic insulin resistance, L-carnosine and similar carbonyl scavengers have potential as therapeutics for age- and obesity-associated insulin resistance.

36. The lifespan-extending drug, acarbose, differentially affects cognitive resilience in a heterogeneous mouse model of normal aging and Alzheimer's disease.

Shannon Moore | *Faculty* | *University of Michigan*

Shannon J. Moore, Geoffrey G. Murphy

Aging is associated with declines in both physical health and cognitive function, and is the greatest risk factor for developing neurodegenerative diseases, like Alzheimer's disease (AD). The geroscience hypothesis suggests that successfully slowing the aging process could broadly and concurrently improve multiple age-related deficits; thus, the Interventions Testing Program seeks to identify "anti-aging" interventions, using lifespan extension as a proxy for delayed aging. A number of lifespan-extending interventions have been identified, with subsequent studies showing their efficacy to ameliorate several physiological aspects of aging; however, their impact on cognitive function - a crucial aspect of aging - has remained largely unexamined. To address this gap in knowledge, we investigated whether a successful lifespan-extending drug, acarbose, was also effective in improving cognitive function in a genetically heterogenous mouse model of "normal aging" (using HET3 mice) and AD (by incorporating the 5xFAD transgene onto the HET3 background; AD-HET3). Using the Morris water maze, learning and memory were assessed at three timepoints (young adult, middle age, and aged) in individual cohorts of mice treated with acarbose (1000 ppm in chow, started at 4 mo of age) or maintained on standard "control" chow. We found that acarbose effectively promoted cognitive function in normal aging but failed to ameliorate cognitive impairments that emerge with age in AD-HET3 mice. These results suggest that there are at least partially overlapping mechanisms that mediate lifespan and cognitive function, but that additional, distinct mechanisms contribute to the unique pathological processes underling AD-specific cognitive deficits.

37. Dietary isoleucine restriction improves metabolic health in a dose-dependent manner in genetically heterogenous mice

Amirah Nieves | *Graduate Student* | *University of Wisconsin-Madison*

Amirah Nieves Medina1,2,3, Michelle Sonsalla1,2, Bailey Knopf1, 2, 3, Mariah Calubag1, 2, 3, Yang Liu1, 2, Ricki Colman1, 3, Dudley Lamming1,2,3

Aging and obesity are significant risk factors for the development of diabetes and other age-related diseases. Protein restriction promotes health and longevity in mice; lower consumption of dietary protein is also associated with positive outcomes in humans. We have found that the key mediators of these benefits are the three branched-chain amino acids (BCAAs), leucine, isoleucine (ile), and valine. While restriction of all three BCAAs promotes metabolic health in C57BL/6J male mice, restriction of ile alone recapitulates the benefits of a protein restricted diet in mice. To better determine the translatability of ile restriction and the level of restriction necessary for metabolic benefits, here we investigate the metabolic impact of different levels of dietary ile (55% and 67% ile restriction) in response to a high-fat, high sucrose Western diet (WD) in genetically heterogeneous strain (HET3) of mice after diet-induced obesity. After switching to an

isoleucine restricted western diet, our results reveal improved body composition such as significantly lower body weight due to decreased fat mass and adipose percentage. Ile restriction significantly enhanced glucose tolerance and insulin sensitivity compared to control diets, but we see a greater impact on 67% ile restriction compared to 55% level. Intriguingly, the metabolic benefits also seem to be dose-dependent, with a significantly increased energy expenditure and reduction in respiratory exchange ratio at 67% ile restriction. Together, this data indicates that reducing dietary ile restores metabolic health in genetically heterogeneous mice with pre-existing obesity, promoting weight loss and leanness.

38. Extra-oral Bitter Taste Receptors: New Targets for Suppressing Cellular Senescence

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Genetic variation in taste receptors, particularly the bitter taste receptors (TAS2Rs), can modulate food preferences, and regulate food absorption and processing, which could modulate biological pathways affecting the aging process. However, there are 35 Tas2rs in mice and 25 in humans, heterogeneously expressed in many different tissues, suggesting they have roles in multiple biological processes in addition to taste. Given that TAS2Rs are activated by certain senotherapeutics such as the bitter flavonoids fisetin and quercetin, we examined the effects of TAS2Rs activation in senescent human IMR90s and HUVECs, and senescent murine 3T3-L1, as well as in diverse tissues from aged C57BL/6 mice. We demonstrated that treatment of senescent human and mouse cells with the specific TAS2R1/Tas2r108 agonist, KDT-501, conferred a senomorphic effect, suppressing markers of senescence and the inflammatory SASP. siRNA knockdown of G alpha-gustducin, a G protein important for all TAS2Rs signaling, abrogated the senomorphic effect. Additionally, pharmacologic inhibition of PLCB2 and IP3R, which are important for TAS2R signal transduction, also partially alleviated the effect of KDT-501. Importantly, we demonstrated that long-term treatment of aged mice with KDT-501 significantly downregulated markers of senescence and SASPs (e.g., p16INK4a, p21Cip1, Tgf-B1, Mmp-3, and Il-1B) in visceral adipose tissue and small intestines, reduced adipose weight, improved glucose tolerance as well as increased the circulating level of GLP-1. Overall, these results suggest a correlation between TAS2Rs activation and senescence as well as suggest that TAS2R agonists represent a new and promising class of senotherapeutics.

39. Age-Dependent Histopathological Changes in the Tibialis Anterior Muscle of Ames Dwarf Mice Following High-Fat Diet Exposure

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Jaspreet Kaur Osan, Landon Schumacher, Sharlene Rackozy, Holly Brown-Borg

Western diets are rich in saturated fats, and chronic consumption is strongly associated with metabolic dysfunction and the development of metabolic dysfunction-associated steatotic liver disease (MASLD). Prolonged high-fat diet (HFD) exposure has also been linked to cognitive decline, coronary heart disease, type 2 diabetes, and obesity. Obesity can impair physical fitness and may contribute to skeletal muscle dysfunction; however, the impact of chronic HFD exposure on muscle pathology, particularly in long-lived models, remains incompletely understood. Ames dwarf mice exhibit growth hormone deficiency and extended lifespan and demonstrate metabolic resilience, including reduced lipid droplet accumulation in the liver following HFD exposure. In this study, we utilize young, middle-aged, and old mice exposed to HFD for 10-16 weeks to evaluate age-dependent effects on skeletal muscle pathology. Preliminary Oil Red O staining

suggests increased lipid droplet accumulation in young wild-type (WT) mice following high-fat diet (HFD) exposure, whereas Ames dwarf mice exhibited lipid deposition under both standard and HFD conditions. We will quantify centrally located nuclei (H&E) and fibrosis (Sirius Red) and extend the analysis to middle- and old-aged cohorts to assess age-dependent effects. We hypothesize that HFD will induce lipid accumulation, inflammation, and fibrosis in WT muscle, while Ames dwarf mice will display relative protection consistent with enhanced metabolic resilience. This study will determine whether the metabolic protection observed in the liver of Ames dwarf mice extends to skeletal muscle and whether this protection is maintained across aging.

40. Delayed Neuromuscular Aging in Female Ames Dwarf Mice

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Painter, Taylor; Johnston, Matthew; Rakoczy, Sharlene; Brown-Borg, Holly

The Ames dwarf mouse possesses a point mutation that results in deficient anterior pituitary signaling and a 50% lifespan extension. This longevity is also accompanied by an increase in healthspan, with recent studies suggesting a resistance to age-induced sarcopenia and frailty. Female Ames mice demonstrate significantly improved muscle function (shown by grip strength, rotarod, and endurance running) compared to wildtype counterparts across the lifespan. In young animals (4-8 months), the number of strides needed to reach exhaustion is significantly higher ($F(1, 15) = 7.157$, $p = 0.0173$) in dwarf mice. Middle-aged (10-14 months) dwarf mice exhibit significantly higher grip strength ($F(1, 22) = 7.89$), $p =0.0102$). Grip strength ($F(1, 16) = 12.40$, $p=0.0028$), rotarod ($F(1, 15) = 6.165$, $p=0.0253$), and number of strides to exhaustion ($F(1, 15) = 7.157$), $p=0.0173$) are all significantly better in aged (18+ months) dwarf mice compared to age-matched wildtype mice. Histological analysis indicates that this maintained muscle function is supported by delayed deterioration of the neuromuscular junction. Early results suggest this is due to significantly lower levels of endplate fragmentation and higher levels of synaptic contact at the junctions of aged dwarf mice. These patterns imply that maintenance of the neuromuscular junction may be crucial to preventing sarcopenia onset in older animals.

41. Splicing factor engagement and regulation of RNA processing during caloric restriction

Timothy Rhoads | *Faculty* | *University of Wisconsin-Madison*

Timothy W. Rhoads, Spencer A. Tye, Josef P. Clark, Anshit Singh, Rozalyn M. Anderson, Andrew J. Engeler

Caloric Restriction (CR) without malnutrition delays aging and the incidence of age-related diseases. A substantial body of work suggests that metabolic reprogramming is a critical aspect of the mechanisms of CR; however, the details of the regulation of metabolism during CR and how this might lead to enhanced lifespan are still unclear. Emerging evidence points to RNA processing as a candidate mechanism for metabolic regulation during CR: specific splicing factors are required for the full dietary restriction lifespan benefit in C. elegans, and exon usage changes were widespread across the network of factors responsive to CR in non-human primates. However, details as to how these observations influence lifespan, especially in mammals, are still unclear. Given the role of alternative splicing in proteoform diversity and the adaptability of the cell, we hypothesize that RNA processing may serve as an integrative link between the various mechanisms engaged by CR and eventual longevity regulation. To examine this, we used deep short-read RNA seq of mouse cortex brain tissue from animals at 3 different ages maintained on either control or 30% CR from 2 months of age. We computationally identify splicing events that are altered by CR, finding that the gene expression response to CR is highly similar across ages, the statistically significant alternative splicing events were largely distinct to each age group.

Clustering and network analysis revealed splicing factors with expression patterns that correlated strongly with distinct alternative splicing event patterns observed in each age group, thereby identifying candidate longevity regulators.

42. Spatial Mapping of Human Hearts Reveals Distinct Fibrosis-Senescence Interactions in Health Aging and Disease

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Jhonny Rodriguez-Lopez,Natalia-Del Pilar Vanegas, Seo-Yoon Moon, Victor Peters, Jose Lugo-Martinez, Ana L. Mora, and Mauricio Rojas.

Cardiac fibrosis, a hallmark of heart failure, represents a complex pathological process that has long challenged therapeutic intervention. The contribution of cellular senescence to heart aging and fibrotic tissue remodeling in the context of disease remains poorly understood. We investigated the heterogeneity of myocardial tissue composition, the subtypes of senescence niches, and the spatial organization and co-localization with fibrotic tissue remodeling in human healthy and diseased hearts.The right and left atria, ventricles, and ventricular septum from healthy individuals and patients with a history of MI and heart failure were analyzed using scRNA-seq and Visium spatial transcriptomics. Sixteen tissue sections were profiled at spot resolution. Spatially resolved transcriptomes were analyzed to assess histologic-transcriptional concordance and to locate senescence-associated domains within the myocardium. Bioinformatics technologies and the SCTransform method were applied to normalize the data. We applied our in-house-developed MAPLE bioinformatics framework, which combines deep learning and Bayesian modeling to integrate multiple spatial transcriptomic datasets. Additionally, deconvolution analysis was performed, pairing single-cell RNA sequencing profiles with each sample and calculating senescence scores using the SenMayo and SenNet gene sets.Distinct clustering patterns were identified between healthy hearts and those affected by cardiomyopathy with severe heart failure or ischemic heart disease, such as myocardial infarction (MI). Cardiomyocytes were the predominant cell population across all regions. However, fibrotic tissue remodeling was markedly enhanced in diseased hearts. It was most prominent in subjects with MI and structural cardiac damage. In these hearts, tissue remodeling regions were enriched with CTHRC1-positive fibroblasts and SPP1-positive macrophages, accompanied by an accumulation of endothelial cells, indicating extracellular matrix reorganization. These cellular changes aligned with a fibrotic microenvironment, which was absent in non-ischemic control hearts. Notably, fibrotic remodeling involved changes in gene expression, such as increased activity in Fibrosis and STAT3 pathways, along with higher levels of matrix metalloproteinases (MMPs) and collagen deposition. Furthermore, remodeling areas of remodeling in MI and cardiomyopathy samples showed markers of senescence, suggesting that cellular senescence plays a mechanistic role in driving fibrosis and tissue remodeling in diseased hearts.Spatially resolved transcriptomic studies revealed changes in cardiac cell composition and heart microenvironment in both healthy and diseased hearts. Spatial transcriptomics allowed the visualization of tissue remodeling, including fibrotic niches with senescence signatures, providing deeper insight into the cardiac fibrotic process and aiding in the identification of novel molecular targets

43. BAX Activation by BTSA1.2 Drives Senolysis of CTHRC1-positive Fibroblasts to Alleviate Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a progressive age-related lung disease characterized by excessive extracellular matrix (ECM) deposition and irreversible loss of lung function. Although its cause remains unknown, growing evidence suggests that cellular senescence plays a central role in its progression. Senescent, apoptosis resistant CTHRC1-positive fibroblasts drive fibrosis by producing excess collagen and promoting extracellular matrix remodeling. Current therapies slow progression but do not eliminate profibrotic cell populations or reverse established fibrosis. In contrast, senolytic strategies have shown promise in reducing fibrotic burden in preclinical models. This study investigates whether pharmacologic BAX activation using BTSA1.2 can selectively induce apoptosis in senescent, apoptosis resistant CTHRC1-positive fibroblasts. Single cell RNA sequencing (scRNA seq) of IPF lung tissue revealed an expanded population of CTHRC1-positive fibroblasts exhibiting elevated senescence scores and upregulation of BAX associated genes. In IPF derived human lung fibroblasts (hLFs) and precision-cut lung slices (hPCLS), BTSA1.2 induced dose dependent BAX activation, mitochondrial depolarization, and reduction of anti apoptotic proteins (BCL-XL, BCL-2, MCL-1). CTHRC1-positive fibroblast abundance decreased in parallel. Immunofluorescence confirmed colocalization of CTHRC1 and the senescence marker p21 in hPCLS, and BTSA1.2 treatment reduced senescence markers (p53, p21, p16) and fibrotic markers, including fibronectin, vimentin, and collagen. In a bleomycin induced fibrosis mouse model, oral BTSA1.2 improved survival, promoted weight gain, and demonstrated robust senolytic and antifibrotic activity compared to Navitoclax. Collectively, these findings show that BTSA1.2 effectively eliminates senescent CTHRC1-positive fibroblasts and mitigates fibrosis, establishing pharmacologic BAX activation as a promising therapeutic approach for IPF.

44. Reactivating Developmental Signaling Rejuvenates Aged Liver Progenitor Cells

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Rahagir Salekeen, Samuel Peters, Linshan Laux, Laura Niedernhofer

Age-associated regenerative failure promotes chronic fibrosis, cirrhosis, and hepatocellular carcinoma in the liver, ultimately compromising systemic metabolic homeostasis. Effective liver repair depends on activation of liver progenitor cells (LPCs). LPCs proliferate and re-establish hepatocyte identity through coordinated developmental signaling programs that are normally re engaged after injury. However, LPC function declines sharply with age, and the mechanisms underlying this impairment remain poorly defined. Our preliminary studies revealed aged LPCs exhibit reduced hepatocyte specification and are primed towards alternative epithelial identity states, suggesting imbalance in key differentiation signaling. ScRNA-sequencing of LPCs from young and aged mouse livers demonstrated suppression of canonical Wnt signaling, diminished hepatocyte lineage gene expression, and enrichment of stress response associated programs in aged LPcs. Time lapse microscopy further confirmed functional impairment in LPCs marked by defective cell cycle entry and reduced activation of downstream Wnt effectors such as ?-catenin in response to mitogenic cues. These signatures were also conserved in ScRNA-Seq and spatialomics datasets derived from aged and fibrotic clinical liver samples. Importantly, stimulating the Wnt pathway through exogenous Wnt3a ligands and GSK-3? antagonists restored ?-catenin expression, enhanced proliferative competence, and promoted hepatocyte lineage differentiation in aged LPCs. Ongoing experiments are evaluating whether Wnt activation improves liver repair capacity, preserves tissue architecture, and limits fibrosis progression in aged animals undergoing chronic injury. Together, these findings indicate that age associated regenerative decline reflects insufficient reactivation of developmental signaling and suggest targeted developmental pathway activation as a promising therapeutic strategy to preserve tissue homeostasis and systemic health during aging.

45. A next-generation hyperpolarized 13C imaging toolbox to quantify metabolic remodeling and anti-aging drug response in brain aging

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Scofield, Sydney; Suhail, Hamid; Sutherland, Divine; TomHon, Patrick; Chekmenev, Eduard; Sadagurski, Marianna.

Metabolic inflexibility is a hallmark of brain aging and a driver of neuroinflammation, yet tools to dynamically measure metabolic flux across aging remain limited. Here, we establish a molecular imaging and flux-analysis platform that integrates hyperpolarized (HP) 13C magnetic resonance imaging (MRI) with rapid bench-top nuclear magnetic resonance (NMR) spectroscopy to quantify cellular metabolism. Our novel next-generation toolbox enables real-time interrogation of metabolic pathways, mechanistically linking bioenergetic remodeling to aging. Our approach utilizes SABRE-SHEATH hyperpolarization, a rapid and cost-effective method to generate highly polarized 13C-labeled metabolic probes. Unlike conventional dynamic nuclear polarization, this technology supports rapid, repeated, non-radioactive measurements, enabling longitudinal assessment of metabolic dynamics. Using HP [1-13C]pyruvate, we quantify dynamic pyruvate-to-lactate conversion as a readout of glycolytic flux and redox balance in vivo in the brain and ex vivo in freshly-isolated astrocytes. Additional probes in our toolbox, including ketoleucine, allow assessment of mitochondrial TCA cycle and amino acid-derived metabolism. We demonstrate the feasibility, sensitivity, and reproducibility of this approach. In vivo imaging reveals a shift in brain glycolytic flexibility consistent with metabolic resilience. Complementary ex vivo analyses demonstrate astrocyte-specific shifts in metabolic flux, indicating bioenergetic reprogramming. Importantly, treatment with the anti-aging drug Canagliflozin enhances metabolic flux in primary astrocytes, revealing increased pyruvate utilization, further validating the rapid capacity to detect pharmacologically-induced bioenergetic remodeling in cells during aging and intervention. By integrating whole-brain imaging with cell-type-resolved flux analysis, our novel platform provides a powerful tool to define how metabolic resilience can be quantified and therapeutically modulated across aging.

46. Impact of Early Life Intervention on Innate Immune Development: Pace of Life Directed Aging Research

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Asmita Sharma, Yun Zhu, Andrzej Bartke, Rong Yuan

The pace-of-life theory reveals a negative correlation between developmental speed and lifespan. Numerous studies suggest that early life metabolic interventions have the potential to delay aging extend lifespan. However, it remains uncertain if these interventions affect development and lifespan at the expense of immune function. Early-life growth hormone administration for short-term negates the longevity characteristics of Ames dwarf and juvenile UM-HET3 mice exposed to metformin delay sexual maturation while enhancing innate immune functions, emphasizing the immune system's sensitivity to early metabolic signals. To investigate how diet-induced modulation influences innate immune development, genetically heterogeneous UM-HET3 mice were fed on a low-protein diet (LPD) from postnatal day 0 to 48. Developmental pace was assessed using daily growth parameters and the timing of sexual maturation. Immune features were assessed cross-sectionally at 2 months of age. Following Lipopolysaccharide (LPS) stimulation, flow cytometry analysis of whole blood leukocytes showed preexisting sex-related differences in the expression of CD14 and TLR4. LPD had minimal changes in baseline receptor density, but still allows acute LPS responsiveness. ELISA assays revealed that cytokine secretion (IL-6, TNF-?) under both baseline and LPS-stimulated conditions, indicating preserved inflammatory response capability. In view of these results, limiting early-life protein restriction slows growth rate and delays sexual maturation without affecting innate immune function. This indicates an immune developmental adaptation rather than immune dysfunction. Our ongoing studies on different time points aim to clarify how we can establish the connections between early dietary interventions, immune aging and lifespan.

47. Nutrient-responsive acetylation of a nuclear RNA helicase DDX39B fine-tunes mitochondrial substrate choice and metabolic flexibility

Anshit Singh | *Graduate Student* | *UW-Madison*

Anshit Singh, Spencer A. Tye, Andrew J. Engeler, Timothy W. Rhoads

Genes encoding mitochondrial OXPHOS subunits are split across the nuclear and mitochondrial genomes (mtDNA encodes 13 of ~90 subunits), requiring precise coordination that declines with age. Caloric restriction (CR), a robust longevity intervention, remodels metabolism and emerging evidence implicates altered mRNA processing in these adaptations. We focus on DDX39B, a nuclear RNA helicase hyperacetylated upon CR, and hypothesize that DDX39B acetylation functions as a rheostat that modulates mitochondrial substrate choice metabolic flexibility. To test this, we generated DDX39B single-amino-acid mutants K162Q (acetyl-mimic) and K162R (non-acetyl-mimic) in HepG2 cells. We probed these cells using respiratory analyses, mitochondrial assays, and bulk transcriptomics to determine whether DDX39B acetylation-state alters cellular metabolism. Respiratory assays revealed that the K162Q cells exhibit increased mitochondrial respiration and spare respiratory capacity (SRC), while the K162R cells reduced both. Inhibition of beta-oxidation using etomoxir in K162Q cells largely blunted their advantage. When cultured in low-glucose (5mM), only K162R cells showed a dampened profile. Substrate-specific respiration assays reveal that K162Q cells have an enhanced ability to use palmitoyl-CoA, whereas K162R cells are unable to utilize this fuel for respiration. Transcriptomic analysis showed extensive changes in genes involved in fatty-acid uptake, and utilization suggesting that changes in DDX39B acetylation reprograms metabolic flux. Our study uncovers a novel role for DDX39B as a metabolic rheostat that modulates mitochondrial fuel utilization and metabolic flux. These insights advance our understanding of mito-nuclear communication and highlight DDX39B as a key player in cellular energy metabolism, with implications for aging and metabolic disorders.

48. Aging, Senescence, & the Metastatic Niche

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Brighton Strasiotto, Wanrui Wang, Emily Cronberger, Jing Yang, Jeffery Johnson, Camila Mercado-Figueroa, Claire King, Diya Percy, Sydney Vaughn, Sharon Stack

Senescent cell accumulation is a hallmark of aging, and has been shown to contribute to aging-related diseases, such as cancer, by creating a permissive tumor microenvironment due to secretion of pro-inflammatory and pro-growth factors. Senolytics have been utilized in several age-related diseases with successful mitigation of pathologies in aged patients. Specifically, age is a major risk factor for ovarian cancer, however, the accumulation of senescent cell populations in the peritoneal cavity and therapeutic targeting of this population in the aged host has not been evaluated. We hypothesize that clearance of senescent cells will abrogate the effects of aging in ovarian cancer progression and metastasis by limiting inflammation and reducing age-associated changes in the extracellular matrix, which promote biological aging. We utilize the proven senolytic combination of Dasatinib and Quercetin (D+Q) to create a comprehensive peritoneal aging timeline and evaluate the ability of D+Q therapy to prevent age-associated changes in the peritoneal cavity. Utilizing a clinical adaptation of a 31-item frailty index, we, non-invasively, quantified the biological aging of mice. From which, we have demonstrated that there is an acceleration of biological aging in female mice during menopause. Subsequent studies implemented D+Q prior to the onset of menopause, demonstrating a reduction in biological aging compared to vehicle-treated controls. These results have profound implications for the implementation of senolytic drugs as age-preventing treatments in a clinical setting, in order to delay aging and offset the costs associated with aging.

49. A Reproducible Statistical Method to Test Compression of Morbidity: Insights from a Mouse Model for Life?Course Health Research

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Deependra K. Thapa, Wasiuddin Najam, Erik Parker, Daniel L. Smith, James F. Nelson, Steven N. Austad & David B. Allison

Background: A life?course perspective emphasizes that early-life exposures - including nutritional, behavioral, and environmental factors - shape trajectories of health, functional capacity, and longevity in adulthood and older age. Many interventions are considered to improve both healthspan and lifespan, but it is unclear whether these interventions improve healthspan relative to lifespan; that is, whether they achieve "compression of morbidity" (CoM). Here, we present a rigorous method to test CoM by jointly analyzing morbidity and survival, using adult-onset dietary interventions (caloric restriction and intermittent fasting) as a case example with mouse data from Di Francesco et al. (2024). Methods: We operationalized healthspan as vitality (defined as 1 ? frailty index). We calculated the average rate of vitality decline by fitting exponential decay models to individual vitality trajectories and compared this rate with the rate of survival decline estimated from the Cox proportional hazards model. We hypothesized that the difference between the rates of vitality decline and survival decline would be smaller in the intervention group than in the control group. Results: The results showed that the interventions improved survival and slowed vitality decline, but survival benefits outpaced health benefits. As a result, interventions failed to compress morbidity. Instead, these interventions, particularly chronic caloric restriction, suggested a potential expansion of morbidity, as evidenced by a greater difference between the rates of vitality decline and survival decline in the intervention group than in the control group. Conclusions: Interventions intended to promote both healthspan and lifespan may extend survival without proportionate preservation of late?life health. From a life?course perspective, future research should examine whether earlier, developmentally appropriate initiation of interventions alters later?life healthspan-lifespan trade?offs. While we do not claim that these nutritional interventions categorically fail to achieve CoM, we provide a reproducible analytical framework for formally testing the CoM hypothesis and informing future evaluations of healthspan-lifespan relationships. Di Francesco et al. (2024) Dietary restriction impacts health and lifespan of genetically diverse mice. Nature. 634(8034):684-92.

50. p21 Activation in Endothelial Cells Drives Cardiovascular Dysfunction and Early Death

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Pedro Versuti Del Cioppo Vasques, Satsuki Yamada, Bennett Childs, Raul Fierro Velasco, Andre Terzic and Darren Baker

Endothelial senescence has been observed in a variety of contexts including neurological and cardiovascular disease, and tumorigenesis, but its causal role in systemic aging remains unclear. To test whether endothelial senescence is sufficient to drive organismal decline, we generated mice with endothelial cell-specific overexpression of p21, a key mediator of senescence. Although initially intended for studies of natural aging, these mice exhibited markedly reduced survival and a severe premature aging phenotype. Strikingly, endothelial p21 overexpression was associated with features consistent with cardiac dysfunction. These findings demonstrate that endothelial-specific induction of senescence is sufficient to drive rapid systemic deterioration and early mortality, highlighting its pathogenic potential in aging-related disease.

51. Cytosolic mitochondrial double-stranded RNA is a driver of the SASP in senescent cells

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Senescent cells drive age-related tissue dysfunction partially via the induction of a chronic senescence-associated secretory phenotype (SASP). Importantly, we have shown that mitochondria are key drivers of the SASP, suggesting that targeting mitochondria could potentially reduce the harmful effects of SASP that contribute aging and age-related diseases. Mitochondrial double-stranded RNA (mtdsRNA), when present in the cytosol, is known to be especially immunogenic and trigger an inflammatory response. Here, we show that senescent cells contain increased levels of cytosolic mtdsRNA together with increased expression of cytosolic RNA sensors. Furthermore, we found that transfection of cells with mtdsRNA triggers an inflammatory response and CRISPR/Cas9 deletion of RNA sensors decreases the SASP in senescent cells. Our data demonstrates that deletion of BAX and BAK macropores, known for their role in mitochondrial outer membrane permeability, suppresses mtdsRNA leakage and the SASP. In addition, we observed that in a mouse model of diet-induced metabolic dysfunction-associated steatohepatitis (MASH), which displays increased burden of senescent cells, expression of RNA sensors is upregulated in the liver. Finally, we showed that hepatocyte-specific deletion of BAX and BAK significantly reduced the expression of RNA sensors in the liver of diet-induced MASH mice. BAX/BAK deletion also decreased the expression of the senescence marker p21, diminished MASH-induced inflammation and immune infiltration, and improved markers of fibrosis in the liver of MASH mice. Our results suggest that cytosolic mtdsRNA is a major contributor to the SASP in senescent cells. We also provide proof-of-concept evidence that targeting mechanisms involved in cytosolic mtdsRNA leakage may be a novel therapeutic intervention for the treatment of MASH.

52. A self-reinforcing niche between lung microfold cells and activated lymphocytes drives persistent tertiary lymphoid structure formation during recovery from viral pneumonia in aged hosts

Milica Jovisic | *Staff* | *Northwestern University*

Milica Jovisic, Christopher Petit, Isha Hawkins, Radmila Nafikova, Maxwell Schleck, Duc Phan, Alexandra Osborn, Geddy Rerko, Emily Kao, Hiam Abdala-Valencia, Lisandra Vila-Ellis, Nikolay Markov and Luisa Morales-Nebreda

Viral pneumonia remains the leading infectious cause of death in the United States, with advanced age representing the strongest predictor of poor outcomes. While the severity of acute lung injury is often comparable across age groups, recovery trajectories are highly heterogeneous, and the mechanisms driving impaired resolution in aged hosts remain poorly defined. Emerging evidence suggests that persistent activation of T cells within the alveolar microenvironment contributes to immunopathology and defective epithelial repair. To investigate the spatial and cellular determinants of age-related susceptibility, we performed high-resolution spatial transcriptomic profiling of murine lungs during recovery from influenza A virus (IAV) infection. Young (2–4 months) and aged (18–22 months) mice were infected with IAV and analyzed at 30 days post-infection using the 10x Xenium platform with a 302-gene panel. Downstream analyses, including clustering and niche inference, were conducted using Seurat, NicheCompass, and Squidpy. We identified a marked expansion of tertiary lymphoid structures (TLS) in aged lungs compared to young controls. Spatial niche analysis revealed that hyperactivated T cells, characterized by IFN/TNF-driven transcriptional programs, form a self-reinforcing signaling circuit with a subset of epithelial cells exhibiting microfold (M) cell–like features and elevated Ccl20 expression. This epithelial–immune interaction propagates inflammatory signaling to adjacent endothelial, stromal, and perivascular cells, promoting tissue remodeling and persistence of inflammation. Collectively, our findings demonstrate that persistent,

spatially organized immune–epithelial crosstalk sustains TLS formation and impairs lung repair in aged hosts. These results position TLS as a potential mechanistic driver of defective recovery and a candidate therapeutic target in age-related lung disease.

53. Mechanisms of defective Wnt signaling in T cell differentiation of older adults

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Qiankun Yang, Abhinav Jain, Ines Sturmlechner, Yaroslav Fedyshyn, Yunmei Mu, Cornelia M. Weyand, Jorg J. Goronzy

With increasing age, the ability of the immune system to induce long-lasting memory in response to infections and vaccination declines, contributing to the increased morbidity and mortality of older adults to infections. Memory depends on the induction of long-lived, stem-like memory T cells. Stemness is tightly linked to the transcription factor TCF1. Expression of TCF1 in activated CD4 T cells is reduced with age, which accounts for the immune memory defects in older individuals. TCF1 is a target of Wnt signaling, i.e., Wnt signaling upregulates the availability of ?-catenin that increases the enhancer activity of TCF1, thereby inducing TCF1 transcription as well as regulating many TCF1-dependent genes. Here, we examined the mechanisms that contribute to the downregulation of TCF1 expression with age. We found that BCL9L, the scaffold protein of the Wnt enhanceosome, is decreased in naïve CD4 T cells from older individuals. Deficiency in BCL9L impaired Wnt signaling, thereby reproducing many of the fate decisions in T cell differentiation that are characteristic of the aging host. Expression of follicular helper T cell lineage-defining transcription factor BCL6 was decreased, whereas expression of Th1 and Th9 lineage-defining transcription factors T-BET and IRF8 was increased. Development of germinal center B cells (GL7+ FAS+) was impaired in C57BL/6 mice receiving Bcl9l-silenced OT-II cells compared with control mice following ovalbumin challenge. Through screening of a kinase inhibitor library, we found that ROCK inhibitor Fasudil can reverse aging-associated phenotypes in activated naïve CD4 T cells of old adults. BCL9L expression is regulated by ETS1, and Wnt signaling is completely lost in ETS1-silenced human naïve CD4 T cells. Our data suggest that decreased BCL9L expression in naïve CD4 T cells accounts for the dysregulation of T cell differentiation in older adults, potentially leading to impaired humoral immune response.

54. A diet-drug interaction reveals hepatic mTORC1 to mediate diet-induced FGF21 expression and energy expenditure

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Chung-Yang Yeh, Lucas Chini, Isaac Freichels, Lexie Borgelt, Brynn Vogt, Alyssa Clark, Ted Wong, Huixian Qiu, Heidi Pak, Reji Babygirija, Michelle M. Sonsalla, Michaela Trautman, Mariah F. Calubag, Yang Liu, Fan Xiao, Bailey Knopf, Isaac Grunow, Teresa Liu, Ryan Marshall, Cara L. Green, and Dudley W. Lamming

The restriction of the branched-chain amino acid isoleucine enhances metabolism and extends longevity in mice, but much of its molecular mechanisms have remained elusive. Here, we discovered that the pharmacological agent rapamycin unexpectedly blocks the physiological effects of an isoleucine restriction diet through its inhibition of hepatic mechanistic target of rapamycin complex 1 (mTORC1). Intending to understand how two lifespan-extending treatments would interact, we concurrently administered rapamycin (4 mg/kg/day; i.p.) and a diet low in isoleucine (-66%) to 8-week-old male and female C57BL/6J mice. Surprisingly, rapamycin blocked the diet's benefits on body composition, glucose tolerance, energy expenditure, and the hepatic hormone FGF21. While some of these interactions were altered in the dietary context of a Western diet, the overall effect of rapamycin on isoleucine restriction was clearly inhibitory. Rapamycin

is an inhibitor of the mechanistic target or rapamycin complex 1 and complex 2 (mTORC2). Using a constitutive knockout model, we ruled out mTORC2's involvement in the expression of the diet's effects. AAV-mediated knockout of mTORC1 in the liver identified mTORC1 as a necessary mediator for the low isoleucine diet to upregulate FGF21 and enhance energy expenditure. Finally, we demonstrated that rapamycin blunts the activation of the precursor pathway for FGF21 expression in the HepG2 human liver cell line. This suggests that nutrient sensing in the liver is sufficient to initiate this process and the same molecular pathway may be conserved in humans. This finding is a significant step forward towards understanding how a longevity-extending dietary intervention exerts its physiological influence.

55. Low-protein diet alters maternal mammary remodeling without significant changes in offspring gland development

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Yaoyuan Zhang, Yun Zhu, Xiuqi Han, Andrzej Bartke, Rong Yuan

Pace-of-life theory proposes that early nutritional cues coordinate growth, reproductive investment, and long-term physiological strategy. As a nutrient-sensitive organ, mammary gland provides a model to test whether early protein restriction reshapes maternal function and offspring development. In genetically heterogeneous UM-HET3 mice, dams were maintained on control diet (CD; 25% protein) before parturition and were then maintained on CD or switched to low-protein diet (LPD; 12.5% protein) during lactation. Offspring nursed by LPD dams remained on LPD after weaning until postnatal day 48. We evaluated (1) the impact of lactational LPD on maternal mammary morphology and milk output and (2) its effects on mammary gland development in female offspring, given our prior finding that early-life LPD delays puberty. Carmine-alum whole-mount analysis showed that while CD mice exhibited parallel increases in mammary size and body weight, LPD virgins showed minimal differences. However, LPD breeders exhibited reduced body and gland weight but significantly increased ductal invasion and branching density, indicating a dense epithelial network within a smaller gland. Reduced weaning weights in LPD-nursed pups suggested impaired lactational capacity. These findings indicate that early protein restriction may uncouple mammary epithelial expansion from somatic growth, reflecting altered reproductive energy allocation rather than simple developmental delay. Future studies will test whether LPD alters milk production, epithelial proliferation, and mTOR/AMPK signaling. Because delayed reproductive development is associated with extended lifespan, and both puberty timing and breastfeeding history influence breast cancer risk, these findings provide a reproductive-tissue dimension to pace-of-life regulation and its implications for aging and cancer susceptibility.

56. Maintenance DNA methylation is necessary for age-related alterations in regulatory T cell transcriptional and DNA methylation signatures

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CD4+FOXP3+ regulatory T (Treg) cells maintain self-tolerance, orchestrate immune responses during inflammatory stimuli, and facilitate tissue function and repair. Treg cell lineage identity, stability, and function are dependent on the establishment of specific DNA methylation patterns maintained by the epigenetic regulator UHRF1, which is necessary for Treg cell function. Aging disrupts DNA methylation patterns necessary for Treg cell-mediated lung repair in a cell-autonomous manner. Nevertheless, whether UHRF1-mediated maintenance DNA methylation is necessary for age-

related Treg cell transcriptional and methylation programs is unknown. Here, we performed transcriptional and DNA methylation profiling on young and old Treg cells isolated from chimeric mice with Treg cell-specific loss of UHRF1. We observed cell-autonomous, age-related alterations in transcriptional and methylation signatures that were dependent on UHRF1. Maintenance DNA methylation is required for age-related alterations in Treg cell transcriptional and DNA methylation programs.