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Invited Contribution

The Fourth Annual Symposium of the Midwest Aging Consortium

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Abstract

The Midwest Aging Consortium (MAC) has emerged as a critical collaborative initiative aimed at advancing our understanding of aging and developing strategies to combat the rising prevalence of age-related diseases. Founded in 2019, MAC brings together researchers from various disciplines and institutions across the Midwestern United States to foster interdisciplinary geroscience research. This report summarizes the highlights of the Fourth Annual Symposium of MAC, which was held at Iowa State University in May 2023. The symposium featured presentations on a wide array of topics, including studies on slow-aging animals, cellular senescence and senotherapeutics, the role of the immune system in aging, metabolic changes in aging, neuronal health in aging, and biomarkers for measuring the aging process. Speakers shared findings from studies involving a variety of animals, ranging from commonly used species such as mice, rats, worms, yeast, and fruit flies, to less-common ones like naked mole-rats, painted turtles, and rotifers. MAC continues to emphasize the importance of supporting emerging researchers and fostering a collaborative environment, positioning itself as a leader in aging research. This symposium not only showcased the current state of aging biology research but also highlighted the consortium's role in training the next generation of scientists dedicated to improving the healthspan and well-being of the aging population.

Keywords: Aging, Biomarker, Gerontology, Immunity, Metabolism, Senescence

Over the past century, life expectancy has steadily increased across all age groups, leading to more people living longer and spending more years in old age. However, this longer lifespan has been accompanied by a significant increase in the occurrence of chronic diseases associated with aging (1). Therefore, the question of how to live those added years healthily is of great interest to everyone, and the importance of research into healthspan is increasing.

The process of aging is a complex biological phenomenon that encompasses numerous changes occurring both within cells and throughout the body's systems. These wide-ranging alterations collectively impact how an organism functions and determines its overall lifespan. An integrated approach, such as leveraging a wide array of techniques, and studying a broad range of model organisms, will help understand the complex interactions that drive aging and lead to the development of interventions that promote healthy aging and longevity.

The Midwest Aging Consortium (MAC) was founded in 2019, initially encompassing 6 states in the Midwest. It has since expanded to include institutions from 9 states, such as the Mayo Clinic, the University of Wisconsin-Madison, University of Minnesota, University of Iowa, University of Michigan, University of Illinois–Chicago, University of North Dakota, University of South Dakota, the Ohio State University, Southern Illinois University, Iowa State University, Wayne State University, Indiana University—Bloomington, Northwestern University, Kansas State University, Xavier University, Michigan State University, and the Marine Biology Laboratory.

Following a successful virtual meeting for the third annual MAC, as the coronavirus disease 2019 (COVID-19) pandemic subsided, the fourth annual meeting was held in person at Iowa State University from May 1 to May 2, 2023. The meeting was jointly organized by Dr. Hua Bai, Dr. Ping Kang, Dr. Marian Kohut, and Dr. Jennifer Margrett from Iowa State University; Dr. Maria Mihaylova, Dr. Mauricio Rojas, and Dr. Ana L. Mora from Ohio State University; Dr. Anne M. Bronikowski from Michigan State University; and Tim Rhoads from the University of Wisconsin-Madison. A total of 95 participants attended this fourth MAC meeting, where 26 talks and 46 posters were presented. Keynote lectures were given by Dr. Rochelle Buffenstein from the University of Illinois at Chicago and Dr. Jennifer Margrett from Iowa State University. This report will provide an overview of the presentations delivered at the fourth Annual Symposium of the MAC.

Learning From Slow-Aging Animals

Traditional models such as mice, flies, worms, and yeast have been invaluable in aging research but come with constraints due to their relatively short lifespans and the differences in their aging processes compared to humans. By exploring the unique biological mechanisms in slow-aging animals, researchers can gain deeper insights into the mechanisms facilitating longevity and disease resistance. In this context, **Dr. Rochelle Buffenstein,** a professor at the University of Illinois at Chicago, highlighted the mouse-size naked mole-rat (NMR, *Heterocephalus glaber*), as a powerful model for studying aging and cancer resistance in her keynote talk. NMRs exhibit exceptional longevity and resistance to age-related diseases. NMRs defy Gompertz's law, showing no increased risk of death even at advanced ages of up to 40 years (2,3). Dr. Buffenstein and her colleagues measured a range of physiological parameters in NMRs and found that they maintain stable cardiac function (4) and metabolic rates throughout their lifespan, resist various stresses such as hypoxia, toxins, and temperature variations, and display higher proteasome activity, better protein stability, and more efficient protein folding mechanisms compared to mice and humans. Additionally, NMRs exhibit a dampened immune or inflammatory response to ultraviolet radiation insults compared to mice. Dr. Buffenstein also showed that NMRs are resistant to developing cancer when subjected to oncogene transformation, DMBA treatment, or sunburn. To understand the detailed mechanisms, she performed transcriptomic analysis and found that NMRs were much more responsive than mice 24 hours after DMBA treatment. Most of their responses involved switching off cell cycle, DNA repair, and significantly downregulating immune function. Furthermore, p53 and antibiotic responses were upregulated in NMRs, while AKT signaling behaved oppositely in NMRs and mice. This signaling is regulated by PTEN, which is present at high levels in NMRs, decreasing mTOR signaling and slowing cell growth and proliferation. Genomic analysis revealed that NMRs have mutations in their RIPK3 and

MLKL genes, which are key proteins in necroptosis. In mice, increased necroptosis leads to more cell death, inflammation, and cancer. NMRs lack this process, making them resistant to cellular stress and giving them a high DNA repair capacity. Studying NMRs could provide valuable insights into avoiding age-related health decline and diseases in humans, offering new avenues for biomedical research and potential therapies for age-related conditions (5).

Studies using another slow-aging animal were introduced by **Dr. Anne M. Bronikowski**, a professor of integrative biology at Michigan State University, a member of the Nanovaccine Institute at Iowa State University, and Institute on Sex, Aging, Genomics, and Evolution (IISAGE). Dr. Bronikowski studies evolutionary solutions to aging by examining wild animals, particularly reptiles. Her research aims to identify genetic pathways that influence aging and longevity, which could offer insights for human aging studies. Dr. Bronikowski's team investigated the P53 and Insulin pathways in reptiles. They analyzed the P53 molecular pathway across 66 amniote species and discovered that P53 and its regulator MDM2 were the fastest-evolving genes. Specific amino acids in P53 showed signs of positive selection, especially in reptiles (6). They also examined the insulin signaling and TOR pathways, finding rapid evolution in the IGF-1, IGF-2, and IGF1R genes. These genes showed co-evolution in turtles and snakes, with positive selection targeting their hormone-receptor binding affinities (7). In a recent phylogenetic study on aging and longevity, turtles exhibit low, and even negative, aging rates despite their exceptional longevity (8). Dr. Bronikowski uses painted turtles (*Chrysemys picta*) as an aging model due to their unique traits, such as hypoxia resistance, the ability to endure prolonged subzero temperatures, and temperature-dependent sex determination. Long-term studies revealed that male turtles age faster than females, with methylation levels declining with age in both sexes. Males had higher overall methylation and expression of DNA methyltransferase (DNMT3). In addition, cellular basal metabolic rate and ATP-linked respiration decrease with age in both male and female turtles. Her research underscores the importance of comparative biology in understanding aging. By studying diverse species, we can identify key evolutionary adaptations in aging-related genes and pathways.

Cellular Senescence and Senotherapeutic Strategies

Cellular senescence is a lasting and irreversible condition in which cells cease to grow and are unable to divide, even under optimal conditions (9,10). This state can be induced by multiple factors such as DNA damage, telomere malfunction, oncogene activation, and organelle stress, and it is linked to aging and age-related diseases (11). Cells undergoing senescence display a unique characteristic known as the senescence-associated secretory phenotype (SASP). This phenotype is marked by the secretion of molecules that promote inflammation and break down the extracellular matrix. The SASP is particularly harmful as it can cause local and systemic inflammation, reinforce the senescence program, and induce senescence in healthy cells. Targeting senescent cells (senolytics) or senescent phenotype (senomorphics) has become key strategy for mitigating aging (12,13). At this fourth MAC, the discussion focused on cellular senescence in lung health and the mechanisms and strategies of senotherapeutics.

Senescence in Lung

Aging is a primary risk factor for lung diseases such as acute lung injury, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis (IPF). IPF is a progressive lung disease involving the accumulation of scar tissue, abnormal fibroblast proliferation, inflammation, and epithelial cell damage, leading to breathing difficulties and a decline in lung function. IPF fibroblasts exhibit senescent markers like SA-b-gal, p16, and p21 expression, and increased transcription of SASP genes (14). Significant advancements in understanding cellular senescence and its implications for age-related lung diseases were presented by 3 postdoctoral researchers from **Dr. Ana L. Mora** and **Dr. Mauricio Rojas** (**Mora-Rojas**) lab at The Ohio State University.

Dr. **Natalia-Del Pilar Vanegas,** a postdoctoral researcher in **Mora-Rojas** lab at the Ohio State University, aimed to identify the precise composition of secreted proteins in the SASP that contribute to the development and progression of IPF. She used a proximity-dependent biotin identification (BioID2) with an endoplasmic reticulum retention sequence (ER-BioID2 (15)) to tag and purify secreted proteins. Using this method with mass-spectrometry analysis, Dr. Vanegas identified specific secretions from IPF donors and replicative senescent (RS) human lung fibroblasts, as well as early passage human lung fibroblasts from young healthy donors. More than 1 504 human proteins were detected across the samples. Inflammation-associated proteins like SERPINB12, extracellular matrix remodeling proteins TIMP3 and COL5A2, and lipid metabolism and inflammation protein like FABP5 were exclusively detected in IPF samples. ER stress-associated proteins like ERAP2 were exclusively detected in RS. Bioinformatic protein interaction network analysis showed that both IPF and RS involve extracellular matrix organization. A notable finding was the shared protein collagen V (COL5A2), suggesting its significant role in IPF. Overall, the secretome profiles of RS and IPF fibroblasts demonstrated that they are driven by distinct molecular mechanisms, but also there are shared biological functions associated with IPF development. Additionally, this work demonstrated that ER-BioID2 could be a valuable strategy for investigating the effects of different triggers of senescence and testing novel senomorphic therapies.

Traditional in vitro (cell culture) and in vivo (rodent) models for studying lung senescence have limitations in mimicking human disease, single-cellular makeup, and fibrosis resolution (16). To overcome these limitations, **Dr. Lorena Rosas**, a postdoctoral researcher in the **Mora-Rojas** lab at The Ohio State University, leveraged human precision-cut lung slices (hPCLS) to establish human ex vivo models. hPCLS retains native lung tissue structure and cellular complexity when exposed to different agents to induce cellular senescence. Dr. Rosas examined 4 different conditions: bleomycin, active recombinant human TGF-β, rotenone, and IPF explant lungs in hPCLS to define specific signatures of the senescence phenotype. All models showed increased senescence markers (SA-β-gal, p21) and SASP markers (eg, GDF15). Interestingly, the models showed differences in the expression of p16, p53, and interleukin-6. Together, Dr. Rosas established various human ex vivo models of cellular senescence using hPCLS, which can be promising tools to identify physiologically relevant triggers for senescent cell formation in age-related lung diseases, such as IPF. These models can be used with advanced techniques such as single-cell RNA sequencing and spatial

transcriptomics, potentially leading to the testing of novel senotherapeutic therapies.

Chronological age is the number of years we've lived, while biological age reflects the health of our cells, including telomere length, mitochondrial health, and inflammation levels. **Dr. Paula Agudelo-Garcia,** a postdoctoral researcher in **Mora-Rojas** lab at The Ohio State University, focuses on transcriptional age, which examines gene expression changes to better understand biological age and predict chronological age (17). Dr. Agudelo-Garcia analyzed new and published single-cell transcriptomic data from lungs of 29 healthy donors from young (19–23 years old), middle-aged (29–49 years old), and aged (55–78 years old), and identified cell types, gene expression differences and relevant pathways. Dr. Agudelo-Garcia identified 22 distinct cell types in the lung. Among them, monocytes and FABP4 macrophages showed significant differences in age-associated gene expression. Pathways associated with aging and lung disease, such as IL-2, TGF-beta signaling, and wound healing pathways, were enriched. Consistently differentially expressed genes across cell types were used to calculate a LungAging Signature Score. She tested these signatures on her data and external data (18) and found that LungAging score can predict chronological age. She identified the top 20 genes important for prediction, associated with inflammation, DNA damage repair, and stress response, all related to aging. Dr. Agudelo-Garcia also mapped senescent populations in the lung using spatial transcriptomics, showing regions with high lung age and senescence scores overlapping with immune and AT2 cells. This work demonstrated the usefulness of lung age signatures in understanding aging and disease development.

Senotherapeutic Approaches

Senescent cells are marked by a persistent DNA damage response, the induction of cyclin-dependent kinase inhibitors, and a pro-inflammatory secretome referred to as the SASP. Furthermore, senescent cells exhibit chronic activation of the transcription factor NF-κB, a known mediator of the SASP. In the context of cancer, certain chemotherapeutics cause DNA damage and induce cellular senescence, which has been shown to contribute to cachexia—a debilitating syndrome characterized by systemic inflammation and skeletal muscle wasting. **Dr. Davis A. Englund,** formerly a postdoctoral researcher in **Dr. Nathan LeBrasseur's** lab at Mayo Clinic and currently an Assistant Professor at The University of Alabama at Birmingham, aimed to assess the extent to which targeting NF-κB alleviated senescent cell burden and the unintended consequences of genotoxic drugs. To do so, he utilized a small-molecule NF-κB inhibitor, called SR12343 (19). Mice treated with the chemotherapeutic cocktail FOLFIRI (fluorouracil, leucovorin, and irinotecan), experienced significant reductions in body weight, lean mass, and physical function which were associated with increased markers of cellular senescence and pro-inflammatory molecules. Administration of SR12343 concurrent with FOLFIRI treatment attenuated markers of senescence and inflammation in liver and skeletal muscle and improved signs of skeletal muscle pathology, including muscle atrophy and fibrosis. Moreover, SR1232 improved clinical manifestations of cachexia, including reductions in body weight, lean mass, and muscle strength. These results suggest that targeting senescent cells and the SASP alleviates skeletal muscle loss and dysfunction during chemotherapy, offering a potential therapeutic strategy to alleviate cachexia and improve patient outcomes during cancer treatment.

Another potential senotherapeutic target was explored by **Dr. Allancer Nunes,** a postdoctoral researcher in **Dr. Paul D. Robbins'** lab at University of Minnesota. Diets rich in bitter vegetables are associated with a significant increase in longevity and wellness. Genetic variation in taste receptors, particularly the G protein-coupled receptor bitter taste receptors (TAS2Rs), can influence food preferences and nutrient absorption, potentially affecting the aging process (20,21). However, the expression of TAS2Rs and the effects of their activation in senescent cells remain unknown. Dr. Nunes found that senescent human IMR90 and HUVECs have increased expression of TAS2R1. He then tested the effect of 2 TAS2R1 agonists—KDT-501, a synthetic isohumulone with anti-diabetic properties, and Xanthohumol, a flavonoid from the hops plant (*Humulus lupulus L.*)—on senescent cells. He measured the expression of senescence markers (eg, $p16^{INK4a}$ and $p21^{Cip}$ (1)), and SASP factors (eg, IL6, IL8, TGF-β1, and CXCL1) in senescent IMR90s and HUVECs. He showed that KDT-501 induced a senomorphic effect, suppressing senescence and reducing the expression of senescence-associated genes and inflammatory SASP factors. Xanthohumol also induced a senomorphic response in IMR90 cells but exhibited no senotherapeutic activity on HUVECs. These findings suggest that TAS2R agonists could offer a new class of senotherapeutic agents, providing a novel approach to managing aging and age-related diseases.

The Immune System in Aging

As people age, the immune system undergoes significant changes, collectively referred to as immunosenescence (22,23). This process is characterized by a decline in both innate and adaptive immunity, resulting in increased susceptibility to infections, diseases, and diminished vaccine efficacy (24). The accumulation of senescent cells can cause a state of chronic low-grade inflammation, termed inflammaging (13). This condition is characterized by an imbalance of pro-inflammatory and anti-inflammatory mediators, which persistently activate the immune system, contributing to age-related diseases.

The impact of aging on immunity, specifically in terms of implications for respiratory viral infection and vaccination, as well as intervention strategies, was presented by **Dr. Marian L. Kohut**, a kinesiology professor and a member of the Nanovaccine Institute at Iowa State University. Older adults have higher risks of respiratory infections from influenza and COVID-19, likely due to immunosenescence and inflammaging. Dr. Kohut shared her studies of influenza-infected aged mice, which showed impaired recovery from influenza, with higher viral loads and prolonged inflammation. Similarly, her studies on older adults exhibit that aging diminishes the antibody and T-cell responses to influenza and SARS-CoV-2 vaccinations. To mitigate the negative effects of aging, Dr. Kohut studied the effect of physical exercise on age-related immunity (25). She demonstrated that exercise training in aged mice reduces inflammatory mediators and viral loads, enhancing immune responses. Likewise, in humans, a year of exercise training improves antibody responses to flu vaccines and reduces pro-inflammatory cytokines, suggesting that exercise can effectively enhance immunity through immunometabolic manipulation. Her pathway analysis from transcriptome data of leukocytes and epithelial cells lining the airways suggests that exercise upregulates early viral detection pathways and type I interferon responses. Blocking interferon signaling by

treating exercised mice with anti-IFNα antibody eliminated the protective benefits of exercise. Dr. Kohut and colleagues at the Nanovaccine Institute are developing influenza vaccines for underserved populations including older adults. They are utilizing nanoparticles as antigen carriers combined with various adjuvants to boost immune responses. The study tests different adjuvants for their ability to activate dendritic cells while maintaining a balanced inflammatory response, focusing on TLR and NLR agonists. Further experiments aim to identify adjuvants that perform better in aged populations by assessing mitochondrial function, dendritic cell activation, and cytokine profiles.

The immunological state and metabolism are inextricably linked. A significant amount of evidence demonstrates that metabolic pathways are tightly associated with cell signaling and differentiation, causing different subsets of immune cells to adopt unique metabolic strategies depending on their state and environment (26). In this context, **Siddhant Kothadiya**, a graduate student in **Dr. Rizia Bardhan**'s lab at Iowa State University, presented his work aimed at understanding how the immune response to COVID-19 vaccination leads to metabolic reprogramming. The study, carried out in collaboration with **Dr. Marian L. Kohut**, involved human participants who received either Pfizer or Moderna vaccines, with samples collected at 3 different time points: before vaccination, 2 weeks after the first dose, and 1 week following the second dose. Raman spectroscopy, a well-established optical method, provides highly specific chemical profiles of metabolites, such as lipids, fatty acids, phospholipids, amino acids, and proteins (27). Using Raman spectroscopy, the study captured the chemical footprint of multiple metabolites in patient sera and found significant metabolic changes in response to vaccination. Kothadiya identified 40 metabolites, including carotenoids, fatty acids, lipids, and sugars, that altered in response to vaccination. He visualized high-dimensional Raman data using t-SNE (t-distributed stochastic neighbor embedding), uncovering distinct "immunometabolic signatures." These signatures varied significantly among patient cohorts based on innate host factors (age, stress), external factors (prior infection), nutritional factors (BMI, exercise), and vaccine factors (type, time point). This study suggests that age has a strong impact on the immunometabolic response to vaccination, with BMI, prior infection, and vaccination timing also influencing metabolic reprogramming.

As we age, T cells can experience a number of changes that can impact their function and overall immune responses. **Dr. Christina D. Camell**, an assistant professor at the University of Minnesota, discussed the role of exhausted $CD8^+$ T cells as a significant contributor to immunosenescence. Aging leads to $CD8^+$ T-cell exhaustion, which is marked by upregulated expression of inhibitory receptors like programmed cell death 1 (PD1), a decrease in effector function, and increased levels of inflammatory factors. PD1 diminishes T-cell receptor activity by promoting SHP2-dependent dephosphorylation across several pathways (28). Therefore, blocking PD1 with monoclonal antibodies can enhance $CD8^+$ T-cell responses but may also lead to harmful over-activation of the immune system. To investigate these possibilities, Dr. Camell employed a mouse model of normal microbial experience (NME), where older specific pathogen-free mice demonstrate 100% mortality upon exposure to various microbes typically found in pet store mice (29). NME revealed that older mice showed increased expression of multiple inflammatory pathways and higher frequencies of exhausted $CD8^+$ T cells expressing combinations of PD1, TOX, and CXCR5. Anti-PD1 treatment in these older mice improved survival without altering acute inflammatory responses or the expression of senescent cell markers. The survival advantage from anti-PD1 treatment relied on $CD8^+$ T cells rather than $CD4^+$ T cells, as the depletion of $CD8^+$ cells eliminated the protective effect, whereas the depletion of $CD4^+$ cells had no impact. Furthermore, $CD8^+$ $PD1^+$ T cells from anti-PD1 treated older mice showed increased cytotoxic capacity through enhanced granzyme B production. These findings suggest a promising new strategy for decreasing infection susceptibility in older adults by targeting exhausted $CD8^+$ T cells with PD1 checkpoint blockade immunotherapy (30).

The T-cell compartment, especially naïve T cells, is particularly affected by aging, leading to a reduction in their numbers (31). This reduction is more pronounced in naïve $CD8^+$ T cells compared to naïve $CD4^+$ T cells. **Dr. Ines Sturmlechner,** a postdoctoral researcher working in **Dr. Jörg Goronzy**'s lab at Mayo Clinic, investigated the mechanisms for the preferential protection of human naïve $CD4^+$ T cells against age-related loss (32). Dr. Sturmlechner found that TRIB2 is more abundant in naïve $CD4^+$ than $CD8^+$ T cells. Knocking down *TRIB2* in naïve $CD4^+$ T cells increased their proliferation in response to homeostatic cytokines, suggesting that TRIB2 represses T-cell proliferation and maintains the quiescent state in these cells. AKT activation is needed for T-cell homeostatic proliferation, and higher AKT activation leads to the exit from T-cell quiescence (33). Accordingly, *TRIB2* deficiency enhanced AKT phosphorylation in naïve $CD4^+$ T cells to a level typically seen in naïve $CD8^+$ T cells, indicating that TRIB2 suppresses AKT activation to maintain the T-cell quiescence of naïve $CD4^+$ T cells. *TRIB2*-deficient cells were also more susceptible to losing their naïve T-cell traits and to undergoing virtual T-cell differentiation, a phenomenon common during the aging process (34). Furthermore, this study discovered that *TRIB2* transcription was controlled by the lineage-determining transcription factors ThPOK and RUNX3. Ablation of these genes diminished the differences in proliferation between naïve $CD4^+$ and $CD8^+$ T cells in human cells and during lymphopenia in mice. With age, ThPOK levels decreased in naïve T cells, reducing TRIB2 expression and leading to increased AKT activation and consequently higher T-cell homeostatic proliferation. This work suggests TRIB2 as a critical player in maintaining naïve T-cell homeostasis and provides a model explaining why naïve $CD4^+$ T cells are more resilient to age-related loss compared to naïve $CD8^+$ T cells. Considering that no therapeutic strategies currently exist to preserve the naïve T-cell compartment during aging, the identification of TRIB2's central role may facilitate such efforts as TRIB2-stabilizing small molecules have been described (35).

Metabolic Changes in Aging

Dietary Restrictions and Interventions

Caloric restriction is a well-recognized nonpharmacological method to prolong lifespan and enhance healthspan (36,37). Reducing protein intake can mimic the benefits of caloric restriction, promoting metabolic health and extending the lifespan of mice (38–40). Many of the benefits of protein restriction are mimicked by specific restriction of the 3 branched-chain amino acids (leucine, isoleucine, and valine), which is sufficient to enhance the metabolic health of both

lean and diet-induced obese mice, and which extends lifespan and healthspan of male mice (41–43). It was recently proved that reduced consumption of isoleucine alone is sufficient to improve metabolic health, increase healthspan, and increase lifespan in both male and female mice, with greater benefits accruing to males (44,45). Rapamycin is recognized for its robust effects in extending lifespan in diverse species (46), but it also has significant metabolic side effects, including insulin resistance and glucose intolerance (47). **Dr. Chung-Yang Yeh**, a postdoctoral researcher in **Dr. Dudley W. Lamming**'s lab from the University of Wisconsin-Madison who has recently found that restricting isoleucine starting in late-life promotes healthy aging (48) explored the potential of combining rapamycin with isoleucine restriction (IleR) based on the hypothesis that this combination might ameliorate the side effects of rapamycin and provide synergistic benefits. 9-week-old C57BL/6J mice were subjected to IleR and received daily rapamycin injections (4 mg/kg; i.p.). Unexpectedly, Dr. Yeh found that rapamycin prevented many of the metabolic benefits induced by IleR, such as improved glycemic control, increased energy expenditure, and elevated levels of the beneficial energy balance hormone FGF21. However, in inguinal white adipose tissue, adipose thermogenesis and lipogenesis genes remained highly elevated by IleR. Interestingly, rapamycin selectively inhibited the adipocyte lipolysis genes typically upregulated by IleR. Dr. Yeh highlighted the previously underestimated importance of the inguinal white adipose tissue lipolysis program in regulating metabolism and healthspan, suggesting it may be a key feature of various geroprotective treatments.

Dr. **Shijiao Huang**, formerly a postdoctoral researcher in **Dr. Scott Leiser's** lab at the University of Michigan and now an assistant professor at Kansas State University, presented her postdoctoral research on the induction of the longevity gene *fmo-2* in *C. elegans (Caenorhabditis elegans)* as a tool to identify drug mimetics for different longevity pathways (49). Flavin-containing monooxygenases (FMOs) are highly conserved enzymes involved in xenobiotic and endogenous metabolism and play a significant role in enhancing longevity in nematodes, with a plausible similar role in mammals (50,51). Dietary restriction (DR) and hypoxia each converge on induction of *fmo-2*, which is necessary for each pathway to extend lifespan in *C. elegans*. Unlike DR and hypoxia, overexpression of *fmo-2* alone extends lifespan and improves healthspan with no obvious negative effects. This makes *fmo-2* induction an attractive potential target for small-molecule drugs to stimulate the health-promoting effects of longevity pathways (50). Dr. Huang utilized an *fmo-2* fluorescent transcriptional reporter in *C. elegans* to evaluate a collection of 80 compounds previously demonstrated to enhance stress resistance in mouse fibroblasts (52). She identified 10 drugs that increase *fmo-2* expression by more than twofold, and 9 out of those 10 drugs were found to extend lifespan in *C. elegans*. After a thorough analysis of the drug candidates in conjunction with either hypoxia or DR, she discovered that 2 inhibitors of mitochondrial respiration chain complex interact with the hypoxia pathway to increase *fmo-2*, while 2 DRD2 (Dopamine Receptor type 2) antagonists engaged with the DR pathway to promote *fmo-2* induction. These findings indicate that mitochondria play a role in the hypoxia-mediated induction of *fmo-2*, while dopamine signaling is implicated in the DR-mediated induction of *fmo-2*. This research highlights that *fmo-2* induction could be a valuable approach for discovering and understanding the mechanisms behind potential longevity compounds.

Lipid Metabolism in Aging

Lipids play a crucial role in cell proliferation and maintenance, intracellular signaling, and energy metabolism. Impaired regulation of lipid utilization is linked to accelerated aging and genomic instability (53). **Dr. Wilber Escorcia**, an assistant professor at Xavier University, investigates the genomic and physiological effects of disruptions in 2 lipid regulator genes, SREBPF1 and DGAT1, which are commonly mutated in human cancers. Dr. Escorcia utilized the Catalogue of Somatic Mutations in Cancer database to connect mutation profiles with the mean age at the time of sequencing (MATS), primary tumor histology, and tissue distribution. His analysis revealed that these mutations predominantly affect the large intestine, liver, skin, and stomach tissues. Additionally, he observed that mutations in the HLH domain of SREBPF1 potentially interfere with gene expression, while those in the MBOAT domain of DGAT1 likely disrupt enzyme function. These mutations were associated with specific aging trajectories in different tissues, suggesting cellular environments that foster accelerated aging and cancer development. The research extended to model organisms like fission yeast (*Schizosaccharomyces pombe*) to explore the functional homology and impact of lipid deregulation. Knockouts of Dga1 (DGAT1/2 ortholog) and Ser1 (SREBPF1 ortholog) in yeast showed abnormal lipid levels, especially under sub-lethal DNA damage from UV-C light, leading to altered cell fitness, viability, and cell cycle dynamics. These findings suggest that disruptions in lipid metabolism contribute to genomic instability and accelerated aging, offering insights into the dysregulated metabolic and physiological conditions of prematurely aged cells in human cancers.

Molecular Mechanisms of Longevity in Response to Metabolic Changes

Insulin signaling is essential for controlling development, stress resistance, and lifespan in *C. elegans*. The daf-2 gene in *C. elegans* encodes a tyrosine kinase receptor with shares sequence and structural similarities with both the insulin and insulin-like growth factor-I (IGF-1) receptors found in mammals (54). **Dr. Matthew S. Gill**, an associate professor of biology at the University of Minnesota, characterized the expression and function of DAF-2B, an alternatively spliced truncated isoform of the daf-2 gene. DAF-2B contains the extracellular ligand-binding domain but is missing the intracellular signaling domain, allowing it to bind insulin without transducing a signal (55). Dr. Gill found that overexpression of DAF-2B enhances dauer formation, improves L1 starvation survival, and extends lifespan by sequestrating insulin peptides away from full-length receptors and activating the stress-responsive transcription factor DAF-16 (55). Utilizing a novel splicing reporter, he observed the ratio of daf-2b to the full-length daf-2 receptor was higher in starved L1 larvae than in those that were fed. Additionally, the genetic loss of daf-2b resulted in reduced survival in arrested first-stage larvae (L1), highlighting the critical role of DAF-2B in starvation survival for L1 larvae (56). Through an RNAi screen, Dr. Gill identified several splicing factors, such as rsp-6, that regulate daf-2b splicing and dauer formation. He also identified mutants with increased DAF-2B expression in adult worms.

One of these mutants, sdbu-1, increases DAF-2B and dauer formation. Future work will focus on understanding the splicing regulation and mechanisms of DAF-2B action.

Sirt6 is a member of the sirtuin family, which consists of highly conserved NAD^+-dependent protein deacetylases that perform various functions, including tumor suppression, DNA repair, metabolic regulation, and aging process (57). Although overexpression of Sirt6 has been demonstrated to prolong lifespan in mice, the underlying cellular mechanisms are not yet fully understood. **Dr. Jackson R. Taylor**, formerly a postdoctoral researcher in **Dr. Stephen L. Helfand's** lab at Brown University and currently an assistant professor at Cleveland State University, presented his postdoctoral research on the mechanisms of lifespan regulation by Sirt6 in *Drosophila melanogaster* (58). Dr. Taylor studied the *Drosophila* ortholog of Sirt6, known as dSirt6, and investigated its role in longevity regulation. He found that dSirt6 is a nuclear protein associated with chromatin and possesses NAD^+-dependent histone deacetylase activity. Overexpression of dSirt6 significantly prolongs lifespan in both male and female flies while decreasing protein synthesis rates. Dr. Taylor discovered that overexpression of dSirt6 leads to the downregulation of gene expression related to ribosome biogenesis, including the target genes of dMyc. He observed that dSirt6 overexpression partially rescues the lifespan shortening caused by dMyc overexpression. Moreover, haploinsufficiency of dMyc does not further extend lifespan in flies overexpressing dSirt6, indicating that dSirt6 overexpression acts upstream of dMyc in lifespan regulation. This research provides a new understanding of how Sirt6 overexpression contributes to an extended lifespan.

Organelle Homeostasis in Aging

There has been a steadily increasing number of children born to mothers over 35 years old in the US since the 1970s (59). Advanced maternal age is linked to declines in offspring health, lifespan, and stress resistance across many species. Despite this, little is known about molecular mechanisms controlling negative maternal effects. **Dr. Sovannarith Korm,** a postdoctoral researcher in **Dr. Kristin Gribble's** lab at the Marine Biological Laboratory, discussed how maternal age affects offspring lifespan, health span, and mitochondrial homeostasis in the rotifer *Brachionus manjavacas*. Rotifers are tiny aquatic invertebrates with a lifespan of 2–4 weeks, characterized by a high reproductive rate and lack of postnatal care, making them suitable for studying maternal aging effects. Dr. Korm found that offspring of older mothers (11 days old) in a specific rotifer strain have decreased lifespan, reduced reproduction, smaller mitochondria, lower mitochondrial DNA copy numbers, and impaired ATP production and phototaxis. Interestingly, old-mother offspring show better heat resistance. Metabolomic analyses reveal distinct early-life differences in metabolic pathways but similarities at late ages between offspring of older and younger mothers. Specifically, in early-life, old-mother offspring have increased metabolites in aminoacyl-tRNA biosynthesis, cystein/methionine metabolism, glutathione metabolism, and decreased metabolites in galactose metabolism, glycolysis/gluconeogenesis, amino/nucleotide sugar metabolism, with significant changes in purine metabolism. This work provides potential targets for further mechanistic studies on maternal metabolic effects and the impact of maternal age on offspring health and lifespan.

Dr. **Jinoh Kim**, a postdoctoral researcher in **Dr. Hua Bai's** lab at Iowa State University, focuses on the cellular stress response induced by impaired organelle-specific protein import. Specifically, Dr. Kim studies peroxisomes, which are essential for cellular redox balance, oxidizing very long-chain fatty acids, and synthesizing plasmalogen (60). Several lines of evidence suggest that peroxisomal function and peroxisomal protein import declines with age (61–63). However, the cellular response to peroxisomal import impairment is not well established. To uncover the evolutionarily conserved cellular response to peroxisomal dysfunction, Dr. Kim conducted a comparative transcriptomic analysis on fruit flies (*Drosophila melanogaster*) with tissue-specific peroxin knockdown and human HEK293 cells expressing dominant-negative $PEX5^{C11A}$ (64). The findings reveal that defective peroxisomal import upregulates the integrated stress response and downregulates ribosome biogenesis in both flies and human cells. Functional analysis confirmed that impaired peroxisomal import induces eIF2α phosphorylation and ATF4 expression. In addition, she demonstrated that peroxisomal import stress decreases the expression of rRNA processing genes and inhibits early pre-rRNA processing. This inhibition leads to the accumulation of 47S precursor rRNA and a reduction in downstream rRNA intermediates. This work contributes to understanding the fundamental biology of the aging pathway by highlighting conserved cellular responses to peroxisomal dysfunction.

Neuronal Health in Aging

Aging is the most significant risk factor for neurodegenerative diseases such as Alzheimer's disease (AD). AD is the leading cause of more than 80% of dementia cases in elderly people (65), characterized by the accumulation of intracellular neurofibrillary tangles, extracellular amyloid plaques, and neuroinflammation. **Dr. Mariana Pehar,** an assistant professor from the University of Wisconsin-Madison presented her lab's recent work on the role of fatty acid-binding protein 7 (FABP7) in astrocyte-mediated neuroinflammation in AD (66). Astrocytes are crucial in the regulation of neuroinflammation, and FABP7 is part of a family of conserved proteins involved in regulating lipid metabolism, energy balance, and inflammation. Interestingly, Dr. Pehar found that FABP7 expression, which is largely restricted to astrocytes and radial glia-like cells in adulthood, is upregulated in AD conditions in both mice and human patients. Transcriptome analysis of human iPSC-derived astrocyte cultures after FABP7 overexpression revealed the induction of a pro-inflammatory phenotype involving NF-κB signaling activation. Astrocyte-specific silencing of FABP7 reduced NF-κB activation induced by LPS and other inflammatory stimuli in vitro. Together, this work suggests that FABP7 could be a valid therapeutic target to decrease neuroinflammation in the context of AD.

Dr. **Magdalena Blaszkiewicz**, a senior scientist in **Dr. Kristy L. Townsend**'s lab at The Ohio State University, presented her work on 17α-Estradiol (17α-E2), as a potential intervention for maintaining metabolic and nerve function with aging. Sex differences play a critical role in the onset and severity of many diseases and likely interact with genetics to influence the presentation of phenotypes like peripheral neuropathy. Dr. Townsend's lab previously demonstrated that female mice are protected from peripheral neuropathy and neurodegeneration until reproductive senescence, likely due to higher estrogen levels (67). In this study, Dr. Blaszkiewicz employed

genetically diverse HET3 mice to study the effects of 17α-E2 on metabolic/adipose functions and peripheral nerve health during aging. 17α-E2 is an Intervention Testing Program (ITP)-validated longevity treatment that is non-feminizing, and although lifespan extension with 17α-E2 was seen in male mice only, healthspan effects were not evaluated across both sexes. The HET3 model, a mix of 4 inbred strains, offers reproducible genetic variability, making it more relevant to human disease and allowing for statistical comparison of phenotype data to individual genetics (68). Interestingly, while rapamycin showed no positive effects, 17α-E2 treatment improved metabolic and nerve functions in middle-aged male mice (51 weeks), such as increased grip strength, contractility, and neuromuscular function, and improved intraepidermal innervation in older male mice (84 weeks). Although these metabolic and neural outcomes varied by genetic strain, this work highlights the promising role of 17α-E2 in preserving metabolic and nerve health throughout the aging process. Studies on the healthspan effects of 17α-E2 on female mice are currently underway.

Mitochondrial dysfunction is a hallmark of aging, and acetyl-CoA, a central metabolite in cellular metabolism, plays a crucial role in various processes including the TCA cycle, protein acetylation, and cholesterol biosynthesis. **Eric McGregor,** a graduate student of **Dr. Rozalyn M. Anderson**'s lab at University of Wisconsin-Madison, explored the role of acetyl-CoA flux in controlling growth and metabolism in neurons. SLC33A1 (AT-1), an ER membrane transporter, shuttles acetyl-CoA into the ER lumen, impacting protein quality control. Overexpression of AT-1 in mice results in a rapid aging phenotype with a maximum lifespan of about 4 months, and specifically in the brain, it leads to neural defects such as impaired memory and altered social behaviors (69,70). In humans, alterations in the expression of AT-1 are associated with neurological disorders like autism spectrum disorder and spastic paraplegia. McGregor further investigated the metabolic response to upregulated acetyl-CoA flux in primary neurons and brain tissues from transgenic mice, finding that increased AT-1 activity elevates PGC-1α, a master regulator of mitochondrial function, and the expression of genes associated with the electron transport chain and TCA cycle, cellular respiration, and mitochondrial membrane potential, potentially compensating for the loss of cytosolic acetyl-CoA. Additionally, increased branching and outgrowth of dendrites in transgenic neurons indicate a hyperactive growth state linked to metabolic changes. This work underscores the regulatory role of acetyl-CoA flux in metabolic shifts with structural and functional consequences in neurons. Future research aims to explore the mechanisms of inter-organelle communication, particularly between the ER and mitochondria, to uncover new insights into intracellular networks relevant to multiple neurological disorders.

Lukas Stilgenbauer, a graduate student of **Dr. Marianna Sadagurski's** lab at Wayne State University, focuses on targeting the microglial endoplasmic reticulum (ER) stress response to address neuroinflammation and metabolic disease. The ER stress response and its effectors, particularly IRE1α, regulate inflammation and proteostasis. Aged animals show an increased ER stress response and inflammatory activity in microglia, leading to neuroinflammation and metabolic issues (71). Additionally, Dr. Sadagurski's lab previously reported that an IRE1α inhibitor blunted ER stress and inflammation induced by stress in microglial cells (72). To further investigate the role of ER stress response via IRE1α in adult microglia, Stilgenbauer developed tamoxifen-inducible, microglia-specific IRE1α knockout mice. He found that IRE1α deletion in microglia reduces inflammatory cytokine production and protects against diet-induced obesity by enhancing energy expenditure. This work suggests that targeting microglial IRE1α could reduce pro-inflammatory cytokines, offering potential health and lifespan benefits. Future research aims to age the knockout mice to assess long-term effects and further analyze the inflammatory response.

Aging is the primary risk factor for neurodegeneration; however, environmental and modifiable factors, such as diet, also contribute. **Alicia Taylor**, a graduate student of **Dr. Elizabeth McNeill**'s lab at Iowa State University, discussed the effects of a high saturated fat diet on neuronal health, focusing on microRNAs and neurodegeneration, using *Drosophila melanogaster*. High-fat diets in mammals are linked to poor neuronal health, cognitive decline, and neuroinflammation (73). However, the mechanisms connecting high-fat diets to these outcomes are not fully understood. Taylor found that a high-fat diet reduced fruit flies' lifespan and climbing ability and increased neurodegeneration, evidenced by brain vacuolization. Additionally, she observed that microRNAs, which regulate gene expression, inversely responded to high-fat diets as their target heat shock proteins involved in inflammation and aging. This regulation suggests a potential role for microRNAs in modulating these processes, which influence neurodegeneration. These findings validate Drosophila as a model for investigating diet-induced neurodegeneration and highlight the role of microRNAs in this process.

Biomarkers for Measuring the Aging Process

Dr. Richard A. Miller, a pathology professor from the University of Michigan, shared his research on identifying Aging Rate Indicators. Dr. Miller introduced the concept of Aging Rate Indicators as measures of how quickly an individual is moving toward phenotypic changes typical of old age, akin to a speedometer, whereas traditional biomarkers of aging measure the extent of aging, like an odometer (74). Dr. Miller examined various physiological and molecular changes shared by slow-aging mice, including 4 mutants (Snell dwarf, Ames dwarf, PAPPA-KO, and GHR-KO), one dietary intervention (caloric restriction), and 4 pharmacological treatments (rapamycin, acarbose, 17α-E2, and canagliflozin). Interestingly, these mice exhibited shared physiological traits, such as elevated GPLD1 in the liver and plasma, increased UCP1 in brown and white adipose tissue, high levels of FNDC5 in muscle and irisin in plasma, and elevated BDNF and DCX in the hippocampus, indicating enhanced cognitive function, metabolic health, and neuronal activity. Additionally, shared molecular traits include increased cap-independent translation, diminished activity of mTORC1, and downregulation of 2 MAP kinase cascades: the ERK1/2 pathway, which controls mRNA translation, and the p38 pathway, which modulates inflammation. Dr. Miller aims to identify reliable Aging Rate Indicators and explore their upstream regulators, which control multiple tissues simultaneously, and downstream regulators, which connect to age-related diseases. Future directions involve validating these indicators in normal mouse populations and collaborating on human studies to correlate accessible tissue measures with internal tissue health and overall aging.

As a perspective on the convergence of phenotypes during aging, **Dr. Thomas Stoeger,** an assistant professor at Northwestern University, presented the road trip hypothesis. Based on Dr. Stoeger's recent work (75), there is a correlation between transcript length and aging. Empirical evidence indicates a negative correlation between transcript lengths and their expression changes with age, where short transcripts are generally upregulated, and long transcripts are downregulated. This phenomenon is consistent across various tissues and species, implying a universal transcriptomic response to aging. Dr. Stoeger's road trip analogy illustrates how increased failure rates disproportionately impact longer transcripts, similar to the decreased likelihood of completing longer road trips with an old vehicle. Furthermore, Dr. Stoeger superimposed length-correlated changes onto a map created by Replogle et al. (76) to depict global dependencies of cellular transcriptomes on the function of individual genes. He identified length-correlated change as a latent factor aligning with the first 2 dimensions on the map, suggesting the transcriptomic phenotype of aging could be a default phenotype resulting from the loss of function of cellular components.

Conclusion

The Fourth Annual Symposium of the MAC has once again highlighted the consortiums' significant contributions to the field of aging research. The symposium underscored the critical need for interdisciplinary collaboration to address the complex biological mechanisms of aging and to develop strategies for mitigating age-related diseases. By bringing together researchers from diverse institutions across the Midwestern United States, the MAC continues to foster a rich environment for scientific exchange and innovation. The 4th MAC focused on emerging investigators and early-career researchers and underscored the consortium's dedication to promoting new talent and fresh perspectives, showcasing its commitment to cultivating the next generation of geroscientists. Through its collaborative efforts and strong focus on training and support for emerging researchers, the MAC is well-positioned to drive our understanding of aging and develop interventions that can significantly enhance the health and well-being of the aging population. The findings and discussions from this symposium provide a solid foundation for future research and underscore the importance of continued investment in aging biology.

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Conflict of Interest

D.W.L has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases. The other authors declare no conflict.

Author Contributions

J.K.: Writing-original draft, Writing-review/editing. R.B., A.M.B., N.P.V., L.R., P.A.G., A.L.M., M.R., D.A.E., N.K.L., A.N., P.D.R., M.L.K., S.K., R.B., C.D.C., I.S., J.J.G., C.Y.Y., D.W.L., S.H., S.F.L., W.E., M.S.G., J.R.T., S.L.H., S.K., K.E.G., M.P., M.B., K.L.T., E.R.M., R.M.A., L.S., M.S., A.T., E.M., T.S., H.B.: Writing-review/editing.

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