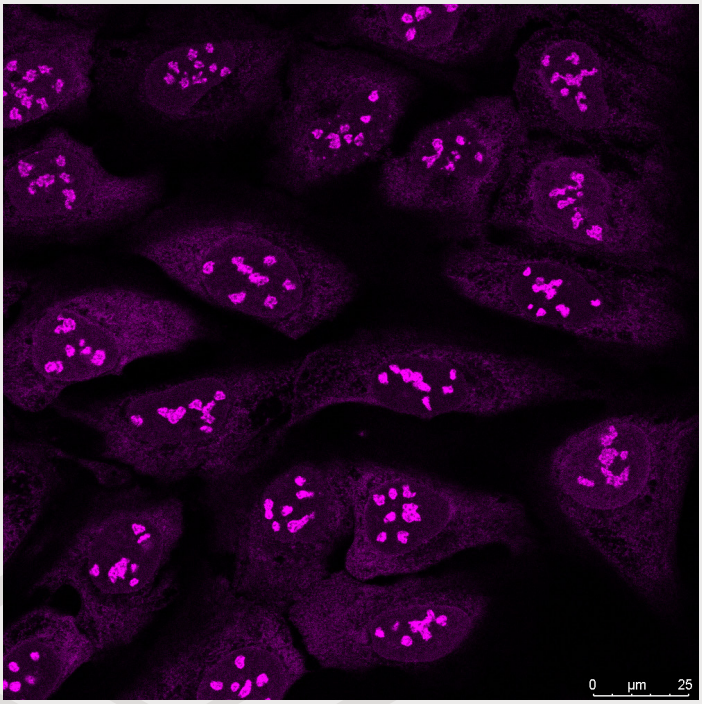
Summer **2021**

THE CHRONOS CHRONICLE:

The Newsletter of the Aging Institute

Message From the Director

In 1870, life expectancy in the United States was approximately 40 years. In contrast, some 150 years later, the average life expectancy has nearly doubled, to stand where it now does at roughly 80 years. Over that time period, if one looks at a graph of life expectancy, what one sees is that there has been a steady and consistent increase in average lifespan. Indeed, every decade since 1870, people in the US lived approximately 2 ½ more years than the decade before. However, a careful analysis of the curve does reveal a period of time when instead of going up, life expectancy in the US actually went down.



Nucleoli in U2OS cells marked by ribosomal RNA. Photo courtesy of Yusuke Sekine, PhD

That dip in the curve occurred in and around 1918, when the great Spanish flu swept through the US and the rest of the world. I am reminded of this event as we are currently dealing with, and hopefully emerging from, our own pandemic. Because of the quick work of scientists and physicians, it is likely that the impact of COVID-19 on overall US lifespan will be much more modest than what was observed in 1918. Nonetheless, the impact of this latest pandemic has been particularly devastating on the elderly. That reinforces our need to understand why we become less resilient as we age and how we can hopefully intervene to improve the age-dependent decline in physiological function. At its core, the Aging Institute seeks to address these and related questions. In that spirit, I hope you enjoy the second installment of our newsletter that highlights the multitude of work that is taking place at the Aging Institute.

Toren Finkel, MD, PhD
Director, Aging Institute, University of Pittsburgh/UPMC
Professor of Medicine, Division of Cardiology
G. Nicholas Beckwith III and Dorothy B. Beckwith Chair in Translational Medicine

AGING INSTITUTE

University of Pittsburgh and UPMC

Faculty Spotlight: Matthew Steinhauser, MD

By G.V. Naveen Kumar, PhD

Matthew Steinhauser, MD, is an associate professor in the Department of Medicine and the Aging Institute at the University of Pittsburgh School of Medicine and a practicing cardiologist at the UPMC Heart and Vascular Institute.

Dr. Steinhauser also directs the newly founded Center for Human Integrative Physiology at the University of Pittsburgh. He is an expert in the field of regenerative medicine as it relates to metabolic syndrome and cardiology. Dr. Steinhauser is also a leading expert in the field of imaging mass spectrometry and is currently conducting a study that investigates cancer metabolism using a technology called multi-isotope imaging mass spectrometry (MIMS). He has published more than 50 peer-reviewed publications in journals including *Nature, Cell*, and the *Journal of Clinical Investigation*.

During Dr. Steinhauser's Q&A, he highlights his achievements and goals with the intention to help guide trainees and young researchers as they move forward in their careers.

What inspired you to be a physician-scientist?

I was interested in being a doctor from a very early age. It was a combination of admiration for fictional doctors in novels and admiration for my uncle who was a physician. However, the idea of a research career wasn't on my radar until college. After my sophomore year at the University of Michigan, I got a summer job working in the lab of Dr. Steven Kunkel. When I accepted the position, I was naive and had no idea that he was a renowned inflammation researcher and an incredible person and mentor. His lab turned out to be a great training environment and a magnet for collaborations across the medical school. I gained access to incredible role models and fell in love with the idea that you could take care of patients and have a career in science.

What are your current research interests?

One area of focus is how aging and metabolic stress lead to pathological changes in fat tissue and how these changes set up patients for metabolic diseases, such as diabetes. My lab is very interested in caloric restriction, which has been shown to improve metabolic disease and promote longevity in animal models. We study fasting as an extreme form of caloric restriction because we believe that it is the cleanest way to understand the different physiological mechanisms that are activated with negative caloric balance. Whether one of these pathways can be targeted to improve aging is an open question that we are very interested in.

What are some important findings in your research career?

We often have fond memories about our formative experiences. Early in my career, when I was still a student in Dr. Kunkel's lab, I was involved in a project where we discovered a key immune regulator that was responsible for the predisposition to pneumonia with sepsis, which is a dangerous condition caused by infection in the blood. Aside from the scientific impact, it was during that project that I knew I'd caught the research bug.

Another important point in my career was working on the development of a new biological imaging method called MIMS. In an early application of the technology, we tested and ultimately disproved a long-standing hypothesis that argued for the existence of immortal strands of DNA in intestinal stem cells that were thought to protect the small intestine from cancer with aging. I learned an incredible amount from that experience, including the importance of negative data. I also gained a love for developing new technologies and methods, which are critical to moving science forward.

How does aging affect fat?

We have found that the birth of new fat cells declines with age in both mice and humans, and most patients might view this as a good thing. After all, aren't we all trying to fight fat cells with better diet and exercise? However, functional fat cells are crucial for normal metabolic function. We have found that the decline in the ability to generate new fat cells is associated with diabetes, and if we use stem cells to create new, functional fat cells, we can prevent diabetes, at least in mice.

Of the wide-ranging effects of age on fat, one of the most exciting aspects has to do with the recognition that individual cells in fat age and can become senescent. Senescent cells are cells no longer capable of replicating, but they also release toxic byproducts that cause inflammation and may even accelerate the aging process in the whole body. If you think about it, for most of us, fat makes up a minimum of 15% to 20% of our entire body mass. So, if there is something bad happening in aging fat, it is likely to spill over and cause problems in the whole system.

continued >

Faculty Spotlight: Matthew Steinhauser, MD

(continued)

What are the highlights of your fasting study in humans?

With my collaborator, Dr. Pouneh Fazeli, we recruited research volunteers to fast for 10 days. These individuals were admitted to the research center at our hospital and consumed no calories for the full 10-day study. One thing that we gained a greater appreciation for is the fact that there are key differences between fasting mice and humans. Although both mice and humans must use the lipids stored in fat cells as an energy source while they are not consuming calories, we are finding interspecies differences in the timing and paths taken to achieve that goal. One difference is that the hormone FGF21, a hormone that is secreted in response to fasting in both humans and mice, is released at a different stage in fasting humans as compared to mice and seems to have different functions unrelated to the immediate fuel shifts that occur.

The other anecdotal fun fact that I am fascinated by is how well tolerated prolonged fasting is in humans. For most, if not all of our volunteers, the first day or two of the study were the hardest, and they had minimal complaints for the remaining days of the fast.

How does your background in regenerative medicine influence your thinking about aging fat and metabolic diseases?

For most of us, regeneration brings to mind the regrowth of a limb after amputation or the regeneration of heart tissue after a heart attack. Yet, tissues become dysfunctional after less dramatic events. Very subtle insults that accumulate in fat with aging can lead to cell death, inflammation, and metabolic dysfunction. If we look at this problem through a regenerative medicine lens, one can see an opportunity to regrow functional fat cells and reduce inflammation and scarring, which is not so different from the regenerative challenge of replacing heart tissue after a heart attack. What I like about these types of problems is that in theory, if you improve the cellular and structural composition of a tissue like fat with therapy, you may achieve a lasting effect.

What advice do you have for young researchers?

Go after questions in biology and medicine that are important. Work on things that excite you. Don't be afraid to learn new approaches and develop new methods, because important problems often go unsolved until a student or post-doc comes up with the right approach to the problem.

Pictured, Left to right: Naveen Kumar, PhD (the author of the interview) Matthew Steinhauser, MD, Tristan Pesaresi, BS, Taniá Amorim, PhD, Rosemary Andrews, MS, Natalie David, BS, and Varun Mandi, BA, BS.

What are your future goals?

My current thinking has been very much influenced by our experience with human fasting studies, where we've wrestled first-hand with the limitations of model organisms. Many critical metabolic control pathways discovered in model organisms don't translate into humans. So, I'm increasingly interested in working on problems simultaneously in humans and mice, rather than the more traditional sequential approach of doing discovery and validation first in model organisms before translational testing in humans. As part of this goal, I'm really excited about the new Center for Human Integrative Physiology that we are launching, where we hope to lead a series of multidisciplinary discovery projects in humans. We plan to incorporate principles of open science into these studies, hoping to catalyze bidirectional collaboration and translation between basic and clinical scientists, beyond the immediate goals of our own work.

Trainee Spotlight: Heather Ballance, PhD, Life in the Zhu Lab — Exciting Science 12 Hours at a Time

Heather Ballance, PhD, is excited to be working in the lab of Dr. Bokai Zhu at the Aging Institute. She joined at the lab's opening and is inspired by the momentum and growth of the lab over the past two years. As a PhD student at the University of Pennsylvania, her doctoral work focused on circadian rhythms. She characterized the 24-hour rhythmic expression of genes, including RNA-binding proteins, prion proteins, and other proteins that are prone to aggregation. Additionally, she characterized rhythms in the protein chaperones that prevent aggregation, and is curious about the interaction between the rhythms of aggregation-prone molecules and the rhythms of their chaperones. Dr. Ballance held her first post-doctoral position at Boston University, where she studied the role of aggregation-prone RNA-binding proteins such as TDP-43 and Tau in neurodegenerative conditions associated with aging, such as frontotemporal lobar degeneration and Alzheimer's disease.

Given her interests in circadian rhythms and RNA-binding proteins, she was excited to find a position in Dr. Zhu's lab, which contains the best of both worlds. Her current projects include the 12-hour rhythms of RNA-binding proteins in nuclear speckles, endoplasmic reticulum (ER) protein content, and cell-to-cell communication through protein secretion. Dr. Ballance is supported by the T32 training program in the Division of Geriatrics, Department of Medicine, and she is eager to move her projects on 12-hour rhythms from basic science into translational work on aging.

When Dr. Ballance is not in the lab, she can be found hiking in one of Pittsburgh's many parks or just enjoying the flowers at local gardens. She also enjoys theater and evenings out with family and friends.

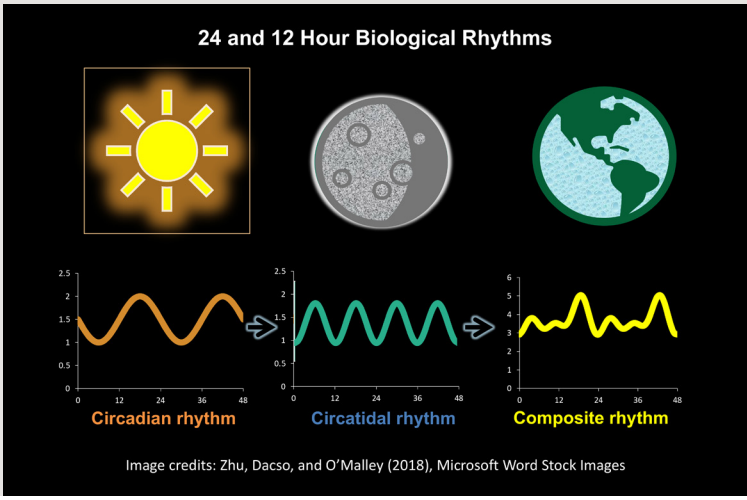


Image credits: Zhu, Dacso, and O'Malley (2018), Microsoft Word Stock Images

Faculty Update: Welcome Anita Saraf, MD

The Aging Institute welcomed **Anita Saraf, MD, PhD**, an assistant professor at the Heart Institute at UPMC Children's Hospital of Pittsburgh. Dr. Saraf is fellowship-trained in adult congenital heart disease (ACHD). She holds dual academic appointments in the Department of Pediatrics and the Division of Cardiology in the Department of Internal Medicine at the University of Pittsburgh School of Medicine. In addition to her position at the Heart Institute at UPMC Children's, she also is a member of the UPMC Heart and Vascular Institute. Dr. Saraf earned her doctorate in bioengineering from William Marsh Rice University in Houston, Texas, and her medical degree from Baylor College of Medicine. She then completed her internal medicine residency, cardiology fellowship, and ACHD fellowship at Emory University School of Medicine in Atlanta, Georgia.

Dr. Saraf's clinical practice encompasses the full spectrum of ACHD, with a special focus on Fontan procedure patients and women with ACHD. As a physician-scientist, Dr. Saraf became interested in studying biomarkers in ACHD during her medical training at Texas Children's Hospital and continued that line of research at Emory University during her residency and fellowship.

While at Emory University, Dr. Saraf and colleagues established a biorepository for Fontan patients to study biomarkers and biomarker expression in this complex single ventricle congenital heart disease. Dr. Saraf's research using data collected in the Fontan biorepository was the first to uncover that Fontan patients exhibit a chronically elevated inflammatory profile, together with a number of other cytokines as clinical markers of systemic dysfunction.

Dr. Saraf also is interested in determining how these biomarkers affect heart function. Her recent findings demonstrate that induced pluripotent stem cell-derived cardiomyocytes can model numerous aspects of effects of inflammatory markers such as TNF-α, including abnormal calcium transients and reduced contractility. Dr. Saraf's research at the University of Pittsburgh involves developing new translational models of congenital heart disease that may help discover new therapies to help ACHD patients live longer and healthier lives. At the Aging Institute, she works with Dr. Toren Finkel to understand the role that genetic mutations causing ACHD play in exacerbating cardiac dysfunction in a pro-inflammatory milieu. Dr. Saraf also has investigated and published findings related to neurocognitive function in ACHD and outcomes in pregnancy for ACHD patients.

UPMC Senior Services: News and Updates

Fighting COVID-19: From RRHC to RCAT

As of January 2021, the Regional Response Health Collaboration (RRHC) Program transitioned to the Long-Term Care Task Force (LTCTF), which includes Regional Congregate Care Assistance Teams (RCATs) to continue supporting long-term care facilities (LTCFs) battling COVID-19.

The RRHC program was a $175 million dollar project authorized by the Pennsylvania General Assembly, funded through the Federal Coronavirus Aid, Relief, and Economic Security (CARES) Act via the Pennsylvania LTCTF. It provided clinical, operational, and administrative support to protect residents in long-term care facilities from COVID-19. Federal funding for the RRHC program ended on December 31, 2020.

Moving forward in 2021, ten health systems that previously participated in the RRHC program continue to assist with rapid response when an outbreak is identified at a long-term care facility under RCAT. Since the launch of this collaboration in July 2020, the RRHC program, now RCAT, has successfully leveraged expertise and existing resources to provide 24/7 guidance and intervention to 127,000 residents in 2,000 long-term care facilities statewide.

UPMC: For additional information, contact **412-648-6714**.

AHN: For additional information, contact **866-496-1766**.

Caregiver Videos

Education and Consultative Services of UPMC Senior Services has developed a series of videos designed to support the specialized needs of family caregivers, supported by the insights and research findings of its Dementia Workgroup. UPMC Senior Services videos welcome caregivers to both the challenges and satisfaction of caring for a spouse, family member, or friend. They provide tips on how to adapt the home for caregiving safety, manage stress, and avoid burnout.

Find the videos att: **Caregiver Videos | UPMC Senior Services**.

Celebrating Senior Champions

2021 Celebrating Senior Champions

Mark your calendars for Thursday, Oct. 14, and join the celebration! The 13th Annual Celebrating Senior Champions Dinner and Auction will honor distinguished individuals and organizations for their outstanding accomplishments in creating a better life for seniors. The celebration will blend favorite elements from previous years with new ones, including a month-long raffle of 30 lavishly themed gift baskets. The highlight of the evening will be honoring the Champions who have made it their life's work and compassion to serve the senior population in our region. To reserve your ticket, contact Debra Panei, Director of Development for UPMC Senior Services, at **412-864-3524** or **PaneiD@upmc.edu**.

OUR 2021 HONOREES

GRAND CHAMPION
David A. Nace, MD, MPH, CMD

Regional Response Health Collaborative Program of Western Pennsylvania

COMMUNITY CHAMPION
Regional Response Health Collaborative (RRHC) Program of Western Pennsylvania

CAREGIVER CHAMPION
The Rev. Gaea Thompson, M.Div.

World-Renowned Expert Visits the Aging Institute

Joseph Takahashi, PhD, the Loyd B. Sands Distinguished Chair of the Department of Neuroscience and an Investigator of the Howard Hughes Medical Institute at UT Southwestern, gave a virtual seminar on April 8, 2021, on the interplay between circadian clocks and aging.

Dr. Takahashi, a true giant in the field of circadian clock, has made seminal discoveries toward understanding the genetic basis of the mammalian circadian clock. His numerous contributions include:

- The discovery and cloning of the first mammalian circadian gene clock in 1994 and 1997, respectively
- The demonstration of circadian clock in the regulation of metabolism and implication in diabetes in peripheral tissues, like the pancreas
- The comprehensive characterization of the changes in the chromatin and epigenetic landscape seen with mammalian circadian rhythms in the liver
- The importance of the circadian clock and timing in the regulation of aging

His remarkable accomplishments over decades of research have led him to receive many honors. These include election to the National Academy of Sciences in 2003, election to the National Academy of Medicine in 2014, and recipient of the prestigious Gruber Prize in Neuroscience in 2019.

During the seminar, Dr. Takahashi gave a very clear summary of the history of the discovery of the genetic and physiological basis of the circadian clock, which for trainees and faculty outside of the field was a very valuable learning experience. In the latter part of the talk, Dr. Takahashi presented his most recent unpublished work on the effects of different dietary regimens, including time restricted feeding on aging and circadian rhythms, which has great translational implications. It was a very productive and informative seminar for all faculty and trainees alike.

University of Pittsburgh and UPMC

AGING INSTITUTE RESEARCH SEMINAR SERIES

Visiting Speakers, Spring 2021

Virtual Presentations | 2nd Thursday of the Month | Noon to 1:00 p.m.

JAN 14

Aging and Cancer: Rival Demons?

Judith Campisi, PhD

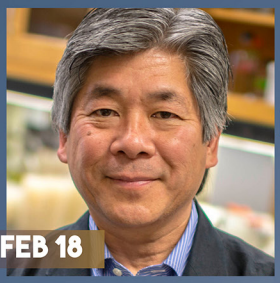
Professor,
Buck Institute for Research on Aging

APR 8

Circadian clocks and the importance of timing in aging and longevity

Joseph Takahashi, PhD

Professor and Chair, Department of Neuroscience
Investigator, Howard Hughes Medical Institute
Loyd B. Sands Distinguished Chair in Neuroscience
Peter O'Donnell Jr. Brain Institute University of Texas Southwestern Medical Center



FEB 18

Proteostasis Collapse in Aging and Neurodegenerative Diseases

Richard Morimoto, PhD

Bill and Gayle Cook Professor of Biology
Director, Rice Institute for Biomedical Research
Department of Molecular Biosciences
Northwestern University

MAY 13

Therapeutic base editing for progeria

Jonathan David Brown, PhD

Assistant Professor of Medicine
Division of Cardiovascular Medicine
Vanderbilt University Medical Center

MAR 11

Convergent mechanisms of longevity

Adam Antebi, PhD

Director, Max Planck Institute for Biology of Ageing, Cologne, Germany

JUN 3

Stress as a driver of aging

Alessandro Bartolomucci, PhD

Ancel Keys Biomedical Scholar in Physiology and Metabolism
Director, Physiology Core, Department of Integrative Biology and Physiology, University of Minnesota

Scan QR Code to register or visit
https://bit.ly/RegisterPittAging2020

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University of Pittsburgh | Department of Medicine, School of Medicine

UPMC LIFE CHANGING MEDICINE

Highlighted Manuscripts at the Aging Institute

Yuan Liu, PhD – Published Manuscript in *Nature Chemical Biology*

This photo was taken at the Annual Celebration of Innovation. These three faculty members were granted Pitt Innovator Award.

Pictured left to right: Yuan Liu, PhD, Toren Finkel, MD, PhD, Bill Chen, PhD, Ann Cudd, Pitt Provost and Senior Vice Chancellor.

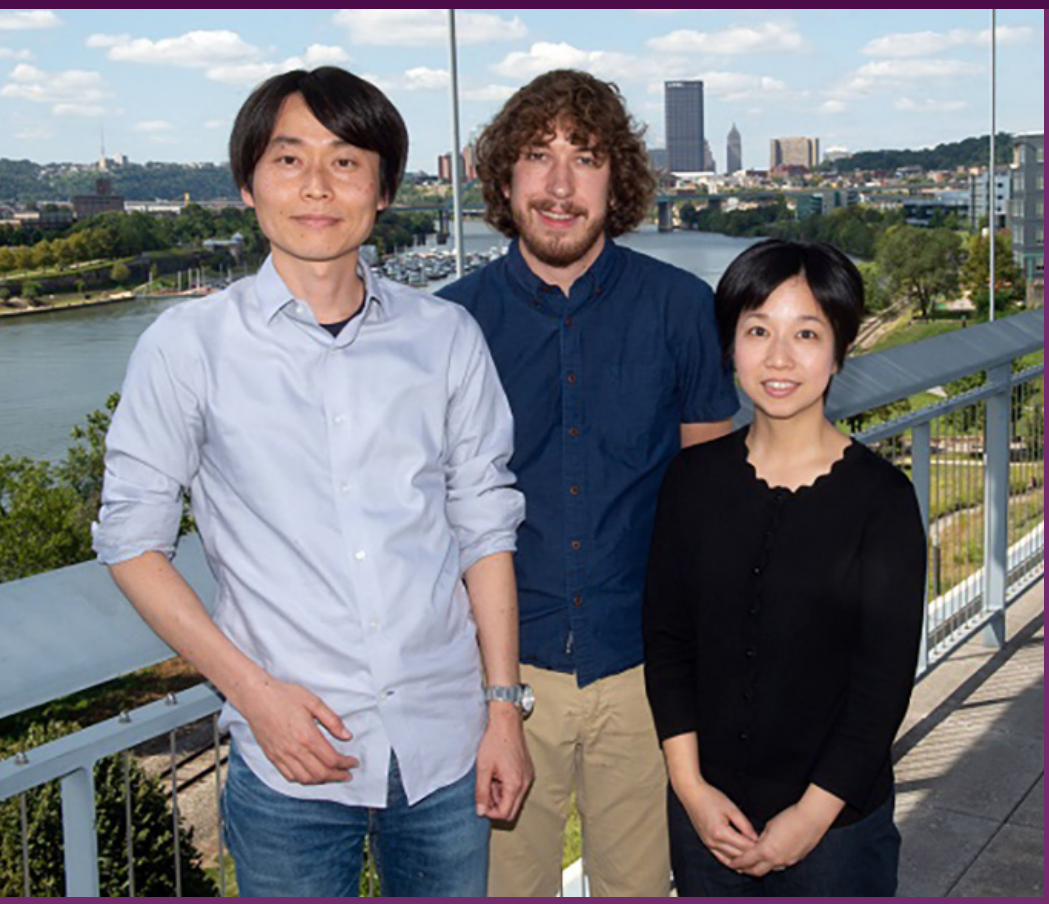
Yuan Liu, PhD, recently published a manuscript entitled "A Fbxo48 inhibitor prevents pAMPKα degradation and ameliorates insulin resistance" in the prestigious journal *Nature Chemical Biology*. It is known that the 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a master regulator of metabolic signaling pathways, sensing fluctuations in cellular energy supply to acutely modulate the balance of catabolic and anabolic processes. During starvation, hypoxia, and disease states such as type 2 diabetes (T2D), AMPK orchestrates the cell's global response to energetic stress in order to enact beneficial physiological changes. Pharmacologic AMPK activation therefore provides a highly attractive and widely studied therapeutic option for multiple disorders. In fact, the most widely prescribed diabetes drug, metformin, is believed to work, at least in part, through AMPK activation in order to improve insulin sensitivity and ameliorate hyperglycemia in patients. However, better and more potent AMPK activators have long been sought. Over the last few decades, many large pharmaceutical companies have tried to develop new AMPK activators. Unfortunately, these efforts have not yielded much success. In her recent study, Dr. Liu approached this problem from a completely new perspective.

In particular, she found that the active form of AMPK protein is often rapidly degraded. Through next generation RNA sequencing analysis, her team found an orphan ubiquitin E3 ligase subunit Fbxo48 mediated this degradation. The *in silico* screen of 3 million compounds identified a small molecule compound, BC1583, that blocked active AMPK degradation and elevated its protein level. A structure activity research campaign further developed an orally available novel compound BC1618 that enhanced active AMPK protein level and activity. Through activating AMPK, BC1618 stimulated autophagy in cells, and decreased liver glucose production, and in turn improved whole body insulin sensitivity in mice. Besides publication in *Nature Chemical Biology*, this recent discovery was also highlighted in *Nature Reviews Drug Discovery*.

Currently, BC1618 is licensed to Generian Pharmaceuticals, a Pittsburgh-based biotech startup founded by Toren Finkel, MD, PhD; Bill Chen, PhD; and Yuan Liu, PhD. The most advanced AMPK activatorGT200 is slated to enter phase I clinical trials by 2022.

1. Liu Y, Jurczak MJ, Lear TB, Lin B, Larsen MB, Kennerdell JR, Chen Y, Huckestein BR, Nguyen MK, Tuncer F, Jiang Y, Monga SP, O'Donnell CP, Finkel T, Chen BB, and Mallampalli RK. A Fbxo48 inhibitor prevents pAMPKalpha degradation and ameliorates insulin resistance. Nat Chem Biol. *2021;17:298-306.*

Yusuke Sekine, PhD – Published Manuscript in *PLoS Biology* – Selected as an Editor Top Pick for 2020



Pictured left to right: Yusuke Sekine, PhD, Ryan Houston, BS, and Shiori Sekine, PhD

Yusuke Sekine, PhD, Assistant Professor at the Aging Institute, has worked to understand the molecular mechanisms underlying cellular responses to various environmental stresses and metabolic fluctuations. Dr. Sekine and his team have recently uncovered a novel role of the nucleolus, an organelle in the nucleus, in regulating stress responses toward alterations in the level of acetyl-CoA, a key metabolite in cellular nutrient metabolism. The study was recently published in *PLoS Biology*. Collaborators include **Toren Finkel, MD, PhD**, and **Shiori Sekine, PhD**, in the Aging Institute, in addition to other research groups at the University of Pittsburgh and the National Institutes of Health and Cell Signaling Technology.

The nutrient metabolism has been shown to be a critical factor in aging and lifespan. Acetyl-CoA is generated through the metabolism of various nutrients, including glucose, thus the level of acetyl-CoA is tightly linked to the overall nutrient availability in the cell. This metabolite is used as a building block for essential biomaterials such as ATP, fatty acids, and cholesterol. Furthermore, acetyl-CoA is used for modification of proteins (acetylation), which enables cells to regulate protein activity and function in accordance with cellular metabolic states. Modulating the acetyl-CoA metabolism and protein acetylation has been shown to affect the longevity of model organisms, but details for this effect have not been fully understood.

To understand the molecular basis for how cells respond to changes in the acetyl-CoA level, the recent manuscript details how Dr. Sekine's team developed a genetically modified cell line that enables selective and acute manipulation of the acetyl-CoA level by simple exchange of the culture media. Using this cellular system and multiple quantitative omics analyses, they have identified the nucleolus as a hub that links a decline in the acetyl-CoA abundance to the activation of the stress response through the transcription factor p53. While the nucleolus is generally known as a site for ribosome biogenesis, this finding highlights an important role of the nucleolus in the response to energetic stress. Furthermore, this study demonstrated that alterations in protein acetylation by class IIa histone deacetylases (HDACs) play a crucial role in regulating the nucleolus and the nucleolus-mediated stress response. The nucleolar stress response has been observed in various human diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer. Furthermore, recent observations have suggested that the nucleolar size and activity might be a sensitive biomarker of aging.

The experimental system developed by Dr. Sekine will allow for further investigation of novel interactions between acetyl-CoA metabolism, protein acetylation, and the nucleolar stress response, and thereby provide novel therapeutic strategies for nucleolus-related diseases and aging itself.

Reference: Houston R, Sekine S, Calderon MJ, Seifuddin F, Wang G, Kawagishi H, Malide DA, Li Y, Gucek M, Pirooznia M, Nelson AJ, Stokes MP, Stewart-Ornstein J, Mullett SJ, Wendell SG, Watkins SC, Finkel T, and Sekine Y. Acetylation-mediated remodeling of the nucleolus regulates cellular acetyl-CoA responses. PLoS Biol. *2020;18:e3000981.*

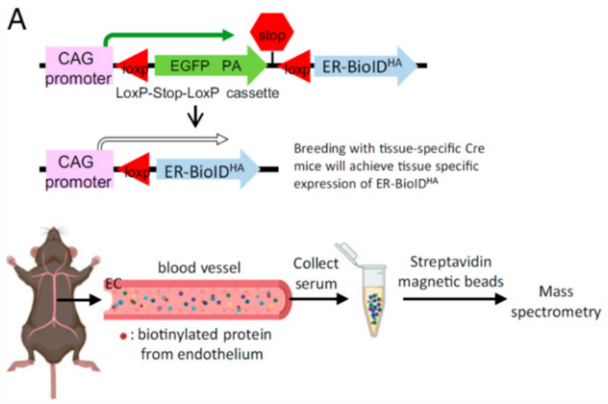
Jie Liu, PhD – Published Manuscript in the PNAS

Jie Liu, PhD, is a research associate professor at the Aging Institute and the Division of Cardiology at the University of Pittsburgh School of Medicine. She was lead author of a recent study from the Aging Institute, published in PNAS earlier this year (Liu et al., 2021), that looked at the complex mixture of proteins in serum that originate from a wide range of cells and tissues. Presently, it is impossible to know what set of proteins a tissue contributes to the circulating proteome. Identifying such information would undoubtedly be useful as there is a growing realization that the abundance of certain serum proteins might provide unique insight into human health.

A recent study from the Aging Institute has provided a genetic platform to address this important issue. This innovative work was published in Proceedings of the National Academy of Sciences of the United States of America (PNAS) earlier this year (Liu et al., 2021).

In this work, Dr. Liu and her colleagues explored the properties of biotinylation of nearby proteins by a biotin protein ligase called BioID2 to identify the whole set of proteins secreted by certain cells (called the secretome), in both cultured cells, as well as in a mouse. To identify the secretome in cultured cells they first made ER-BioID^{HA}, an epitope tagged version of BioID2 that constitutively localizes to the lumen of the endoplasmic reticulum (ER). ER-BioID^{HA} can tag other surrounding proteins by adding biotin groups. Given that most membrane and secreted proteins transit through the ER, expression of ER-BioID^{HA} provides a method of biotinylation of those proteins destined for secretion. Those biotin-labeled proteins can be easily captured by streptavidin magnetic beads and identified by mass spectrometry. Dr. Liu and her colleagues found that this strategy could reliably reveal the unique secretome of the tested cells. Realizing that ER-BioID^{HA} expression could also provide an in vivo method for detecting protein secretion, they decided to further apply this approach in a whole organism and generated the so-called "secretome mouse," a transgenic mouse line in which ER-BioID^{HA} expression can be achieved in a cell type-specific manner by crossing this line with a tissue-specific Cre-recombinase. In theory, this approach allows generation of any tissue-specific secretome mouse with biotin-labeled secreted proteins only derived from this certain cell type or tissue. Dr. Liu and her colleagues focused on characterization of two of these tissue-specific secretome mice in which ER-BioID^{HA} is only expressed respectively in endothelial cells and skeletal muscles.

They successfully identified the unique endothelial and muscle secretome from the plasma of these mice. The lists include well-known proteins previously identified as deriving from the endothelium and skeletal muscle, as well as new proteins as potential endothelium or muscle tissue makers. Interestingly, they found that a number of the serum proteins identified as deriving from muscle altered their abundance with exercise. Among these was the well-known muscle protein myostatin, whose abundance decreased with exercise. This observation agrees with the known physiological effects of exercise on myostatin levels.



Generation and characterization of the secretome mouse. Diagram of the floxed transgene used. Cre expression leads to tissue-specific expression of ER-BioID^{HA}.

This tissue-specific in vivo secretome would serve as a rational entry point for subsequent biomarker development. The ability to understand what a cell or tissue secretes under basal conditions or following a physiological stimulus should provide significant biological insights. This platform might also aid in the rational design of disease biomarkers. For example, crossing the secretome mouse, where expression is restricted to astrocytes or neurons, with mice predisposed for Alzheimer's disease may allow for the generation of early serum or cerebrospinal fluid biomarkers for this condition. Similar approaches could be employed to develop cancer biomarkers to potentially allow early diagnosis of conditions such as pancreatic tumors.

References: *Liu J, Jang JY, Pirooznia M, Liu S, and Finkel T. (2021). The secretome mouse provides a genetic platform to delineate tissue-specific in vivo secretion. Proc Natl Acad Sci U S A* 118.

Highlighted Grants at the Aging Institute

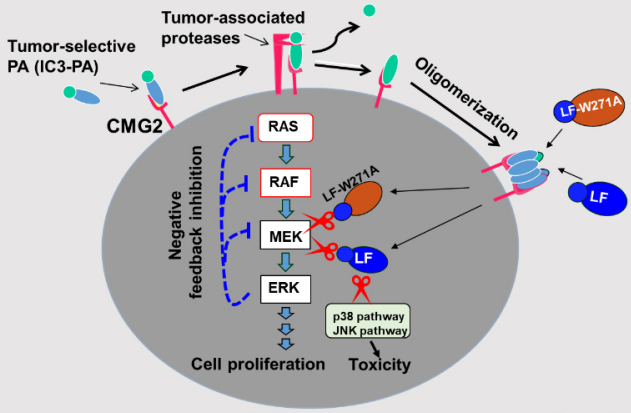
Shihui Liu, MD, PhD, received a new R01 grant for developing therapies for selectively targeting the MEK-ERK pathway in cancer cells and the tumor stromal compartment.

Dr. Liu and his co-investigators, **Jie Liu, PhD** and **Toren Finkel, MD, PhD** from the Aging Institute, and **Lin Zhang, PhD**, from the UPMC Hillman Cancer Center, were awarded a new five-year R01 from the National Cancer Institute for developing new therapies for cancer.

Oncogenic activation of pathways linked to cell proliferation and survival often underlie human tumorigenesis, and their incidence dramatically increases as we age. Cancer driver mutations in the RAS-RAF-MEK-ERK pathway occur in 46% of all human cancers, with mutations in the KRAS and BRAF being the most frequently observed. This has inspired the successful development of many small-molecule inhibitors of BRAF and MEK (such as Trametinib and Cobimetinib). These FDA-approved agents have benefited patients with metastatic melanomas; however, their therapeutic index is very low because they also kill normal cells, sometimes causing fatal side effects. Therefore, there is a critical need to develop additional highly tumor-selective inhibitors specifically targeting these pathways. In this regard, many bacterial pathogens have evolved potent protein toxins to disrupt specific pathways involved in microbial pathogenesis, which are also essential for tumor development. Fortunately, these potent, naturally occurring toxins can be structurally modified to achieve high tumor specificity. One of them, anthrax lethal toxin, which targets MEK, has the potential to be made into a potent anti-cancer drug.

In this proposal, Dr. Liu, who has been working on bacterial toxin-based tumor targeting for 15 years, and other co-investigators with complementary expertise, will develop a potent and highly tumor-selective MEK inactivator by engineering an anthrax toxin protein delivery system. Their preliminary data has demonstrated that the highly tumor-selective MEK inactivator they are developing has a potent anti-tumor activity for a wide range of solid tumors. This anti-tumor agent has a high tumor specificity because it has been engineered in such a way that it can only be activated by tumor-associated proteases, such as MMPs and urokinase, and is only toxic to tumors and not normal tissues.

In this grant, Dr. Liu and his colleagues will comprehensively examine this toxin-based MEK inactivator's anti-tumor activity in several animal tumor models, reveal the underlying mechanism of action, and determine the toxin's *in vivo* biodistribution and off-target toxicity.



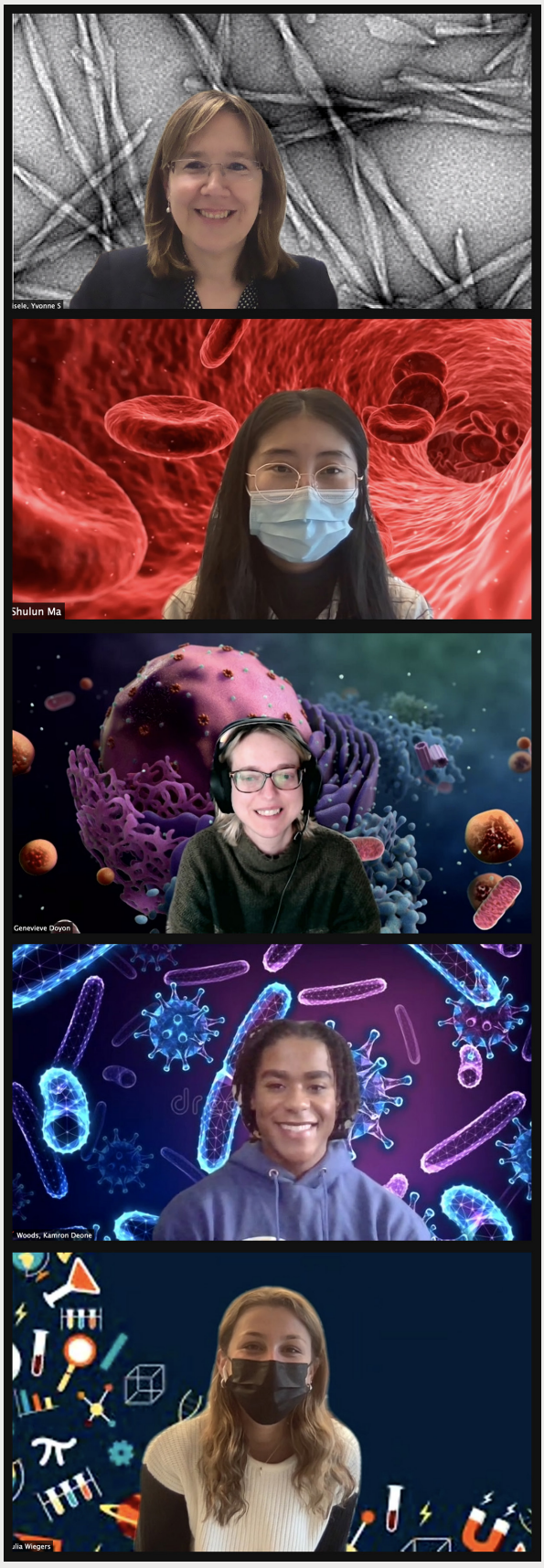
Anthrax toxin protein delivery system as a unique platform for cancer therapy with high specificity. Tumor specificity is achieved by engineering the delivering vehicle PA to bind to the CMG2 receptor and then relying on tumor-associated proteases (MMPs and urokinase) for activation. Thus, the payloads LF and LF variants can be selectively delivered into both tumor cells and tumor stromal cells to inactivate MEK1/2 for selective tumor targeting.

Dr. Liu and his colleagues have previously established a genetic system allowing anthrax toxin receptor CMG2 gain-of-function or loss-of-function in various specific cell types in the tumor microenvironment. In this proposal, they will use this unique system to determine the physiological consequences of MEK inhibition in selected tumor stromal cell types, including tumor endothelial cells, tumor-associated macrophages, cancer-associated fibroblasts, B-cells, and regulatory T-cells. These studies will provide critical insights for a future refinement of this toxin system, as well as other small-molecule approaches that treat age-related malignancies.

Yvonne Eisele, PhD, received grant funding from The Pittsburgh Foundation to further improve the diagnosis and outcome of cardiac transthyretin-related amyloidosis.

Cardiac transthyretin-related amyloidosis (ATTR) is an increasingly recognized cause of heart failure that typically affects people 60 years or older, although earlier onset is possible. The disease is caused by misfolding of a protein named transthyretin, a protein that normally circulates in the blood but can misfold and form amyloid fibrils that deposit in the heart or other organs. Over time, amyloid deposition in the heart leads to a restrictive cardiomyopathy and eventually to heart failure. While the disease is not widely known, many in Pittsburgh are aware of it since Mayor Richard Caliguiri's tragic passing from this disease in 1988 while in office. Thought to be rare and untreatable in Mayor Caliguiri's days, it is now recognized that the disease affects several hundred thousand people in the United States and many more worldwide. Fortunately, new FDA-approved therapeutics have become available within the last three years that improve survival and quality of life for these patients. However, these therapeutics are most efficacious in early stages of the disease, but diagnosis can be challenging and a window of opportunity may be missed. The new project in the Eisele laboratory aims to improve this.

By serendipity, it was found recently that radiotracer 99m-technetium pyrophosphate (informally often referred to as 'PYP') is able to detect cardiac ATTR with high specificity and sensitivity when accompanied by additional clinical tests. This is a noninvasive, nuclear imaging technique now routinely used in the clinic for this purpose. Interestingly, PYP is a repurposed bone tracer and the molecular target underlying its specificity for cardiac ATTR is unknown. Therefore, the first aim of this project is to investigate if PYP binds directly to transthyretin-type amyloid fibrils owing to their unique structure or if PYP binding in cardiac ATTR is related to other factors that are present in affected heart tissue. Both possibilities have important implications for further refining diagnostic testing, as well as for a better mechanistic understanding of the disease. Secondly, we hypothesize that transthyretin-type amyloid fibrils elicit a specific cellular response in the heart that can be detected in the blood. Blood-based biomarkers can be highly informative for diagnosis, as well as for monitoring disease progression and response to therapy. To date, this is a vastly underexplored opportunity for this disease. The Eisele laboratory is fortunate to be part of the Multidisciplinary Cardiac Amyloidosis Center at UPMC and the University of Pittsburgh, directed by Dr. Prem Soman, that comprises patient care, clinical, and basic/translational research, education, and a patient and family support group (https://cardiacamyloidosis.pitt.edu). Blood samples from the center will be analyzed by state-of-the-art methods like unbiased mass spectrometry for this part of the project.



Pictured from top to bottom: Yvonne Eisele, PhD, with lab members Shulun Ma, B. Med, Genevieve Doyon, BS, Kamron Woods, and Julia Wiegers.

Additional **New Grants** at the Aging Institute

Principal Investigator	Grant Title	Grant Type	Funding Agency
Toren Finkel, MD, PhD	Biomarker Development	—	Generian Pharmaceuticals, Inc.
Gang Li, PhD	Functional Interpretation of Alzheimer's Loci Across Cell Types, Age & DNA Damage	—	PA Department of Health
Shihui Liu, MD, PhD	Developmental therapy for selectively targeting MEK-ERK pathway in cancer cells and tumor stromal compartment	R01	NIH
Andrey Parkhitko, PhD	Studying Methionine Flux and Its Role in Aging and Neurodegeneration	R00	NIH
Stacey Rizzo, PhD	Preclinical investigation of common mechanistic links between aberrant protein aggregation and blood-brain barrier	RF1	NIH

Thank You from the Aging Institute Team

Contact the Aging Institute

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