THE CHRONOS CHRONICLE:
The Newsletter of the Aging Institute
Winter **2024**

Message From the Director

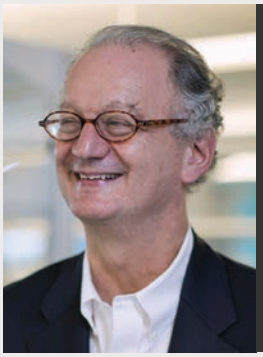
It turns out that aging is a bit like the weather. Namely, everyone always complains about it but no one ever does anything. Well — almost no one. One of the key points of emphasis for the Aging Institute is to leverage insights into aging biology to develop new therapies for age-related conditions. Some have called this the 'Geroscience Hypothesis.' That hypothesis posits that if we could develop drugs that target the basic biology of aging, these agents would be useful across a wide spectrum of age-related diseases. This make sense if you realize that age is perhaps the single greatest risk factor for the most crippling diseases of our time, including cancer, heart disease, and dementias.

In the following pages, you will learn a bit more about our progress on this exciting front. In particular, two of our investigators, **Bill Chen, PhD**, and **Stacey Rizzo, PhD**, have recently been awarded an $11.5 million grant from the National Institutes of Health to leverage aging biology to develop a drug for Alzheimer's disease. One of the biochemical changes that occurs as we age is that the level of a critical metabolite called nicotinamide adenine dinucleotide — or NAD for short — starts to decline. Evidence suggests that this decline in NAD contributes to a host of age-related problems, including weak muscles and impaired cognition.

Dr. Chen and Dr. Rizzo, along with other Aging Institute colleagues, are developing a drug that appears to boost NAD levels and restore the level of this metabolite back to its more youthful levels. With this new support from the NIH, their approach to help patients with cognitive decline will now be accelerated.

Similarly, another investigator at our Institute, **Yuan Liu, PhD**, was recently awarded a $3.1 million grant from the NIH to develop another geroscience approach. She is developing a drug that stimulates a process known as autophagy. This process is responsible for clearing away intracellular debris. Significant evidence suggests that as we age, autophagy slows down. Dr. Liu is trying to develop a drug to stimulate autophagy, so that old cells and tissues can clear their debris as well as they did when they were young.

You can learn more about these two approaches, as well as our annual Research Day and many other happenings at the Aging Institute, in the pages that follow. After reading, I hope you agree that we're making considerable progress in understanding and treating why and how we age. The weather on the hand — well, that's another story.



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

Faculty Spotlight: Shiori Sekine, PhD

by Ryan Houston, BS, Senior Laboratory Research Specialist, Sekine Lab

What made you want to become a scientist?

I was born in Tokyo, but my parents were originally from the countryside. In the summer, we would go swimming in the ocean near my mother's family home, and in the winter, we went skiing in the mountains near my father's family home. My parents were outdoorsy and often took me hiking, so I had many opportunities to be in contact with nature. We walked along the coast looking for seashells or climbed mountains looking for flowers and mushrooms. These experiences gradually nurtured the curiosity about living things inside me. I was excited to find something interesting in nature when I was a child. Now, I'm excited to see if I can find something beautiful and interesting inside a cell. Whenever I encounter the amazing molecular mechanisms in cells, I become curious to know more about them. My amazement, curiosity, and excitement at these discoveries gradually opened the path to me becoming a scientist.

What are your current research interests?

In my lab, we are dedicated to mitochondria biology research. I think mitochondria are fascinating, because they have so many functions. Besides their well-known role in ATP production, they serve critical roles in the synthesis of iron-containing cofactors, heat generation, and calcium ion buffering, and they even function as hubs for several intracellular signal transduction pathways, including cell death and the anti-viral response. Mitochondria are constantly exposed to various stresses in the process of performing these diverse functions. For example, reactive oxygen species are an inevitable byproduct of ATP production that damage mitochondria by oxidizing mitochondrial membrane lipids, proteins, and their DNA.

Recent research has revealed that mitochondria are equipped with several quality control strategies to cope with these stresses. These mechanisms are called the "mitochondrial stress response" or "mitochondrial quality control." We are exploring these. We are particularly interested in the molecular mechanisms by which mitochondria sense each stress and the downstream signaling pathways that are evoked in response. The dysregulation of the mitochondrial stress response ultimately results in several human disorders, including neurodegenerative diseases and cardiovascular diseases. Therefore, our understanding of the detailed molecular mechanisms of the mitochondrial stress response will provide novel therapeutic targets to treat these diseases. Our mission is finding a clue to enhance human health through basic mitochondrial biology research.

What important turning points did you experience in your career?

One of my dreams when I was a student was to give a name to one gene. Although that dream hasn't come true yet, I had an interesting experience that came very close to fulfilling this dream. When I was a graduate student in Japan, I worked on the mitochondria-localized protein phosphatase, PGAM5. I found that PGAM5 was cleaved in dysfunctional mitochondria by the mitochondria-localized protease PARL. At about the same time, Richard J. Youle, PhD, and his lab at the National Institutes of Health — where I would later work — reported that in healthy mitochondria, PARL cleaves the mitochondria-localized protein kinase PINK1. From the history of its discovery, PARL was named as presenilin associated rhomboid like. However, we now know that it is not really related to presenilin. For a long time, at least in mammals, PARL substrates were not known, but after our reports, PINK1 and PGAM5 became widely accepted as its substrates.

Sometime later, a review article on PARL published from the laboratory of Bart De Strooper, MD, PhD, a famous presenilin researcher, suggested that the acronym of PARL should be changed to **P**INK1/**P**GAM5-**a**ssociated **r**homboid **l**ike. Incidentally, this review was published after I joined Dr. Youle's lab, so he brought that review to me one day. It was only a suggestion, and unfortunately the name in the database hasn't been changed yet. But I was very happy. At the same time, I recognized one important thing — in science, if we can find something interesting and true, our discovery may catch the attention of a researcher somewhere in the world, and we can add something new to scientific history.

However, the biggest turning point in my research career was encountering two great scientists, Dr. Youle and **Toren Finkel, MD, PhD**, after moving from Japan to the U.S. In 2016, as a postdoc, I joined Dr. Youle's lab at the NIH, NINDS to learn more about mitochondria stress responses. I was interested in how PARL switches its substrates depending on the health status of mitochondria. To be honest, at that time, I never expected that I would eventually run my own lab in the U.S. My husband, **Yusuke Sekine, PhD**, was working in Dr. Finkel's lab, and our meeting with Dr. Finkel has changed our whole research career. Starting an independent laboratory outside of Japan has been a real surprise, but when I look back on my past, I see that each encounter is closely connected and that these connections on my research journey led me here: the Aging Institute.

What are the major differences in research environments between the U.S. and Japan?

There are many differences, but what I would like to emphasize the most is the difference in the environment surrounding young researchers and female researchers. Compared to Japan, the U.S. provides many more opportunities for young researchers to become independent. The financial support for these early investigators is

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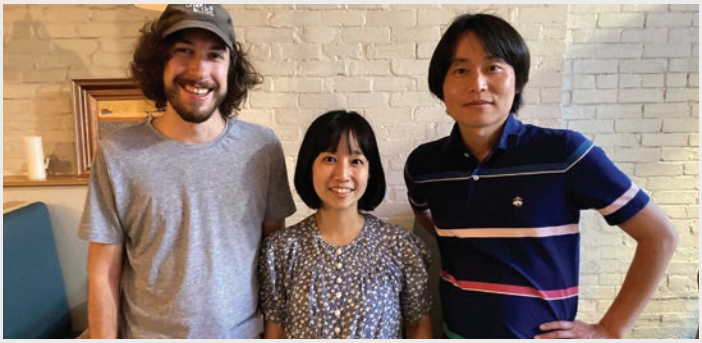
Faculty Spotlight: Shiori Sekine, PhD *(continued)*

enormous. The fact that there are so many female researchers in the U.S. is also very attractive. When I was a student at a Japanese university, there were few women in a doctoral course and only one female PI in the department. It was so sad not having enough role models around me. The Japanese government is now trying to increase the number of independent positions for young researchers and female researchers, but we are far behind the U.S.

The orientation of government-supported research is also different between the U.S. and Japan. In Japan, I think the government provides more opportunities for basic research, although the amount of funding is limited. On the other hand, I feel that the U.S. is more clinically-oriented. This can be challenging for basic research scientists like me, but I feel the U.S. system efficiently promotes research that can be directly returned to human society.

Mitochondrial iron-responsive pathway. Artwork: Shiori Sekine

In a recent article in Molecular Cell, Dr. Sekine and her colleagues revealed that mitochondria act as a signaling platform to activate integrated stress response (ISR) through DELE1-HRI pathway in response to iron deficiency. Under steady state, DELE1 is degraded by mitochondrial protease LONP1. Upon iron deficiency, DELE1 import is arrested, thereby stabilizing DELE1 on the mitochondrial surface to interact with and activate HRI. The image depicts how DELE1 (flower) escapes from the degradation by LONP1 (green caterpillar) and recruits HRI (butterfly) to the mitochondrial surface.



Sekine Lab Members (left to right): Ryan Houston, Shiori Sekine, PhD, Yusuke Sekine, PhD

What is the most enjoyable part of science for you?

What I appreciate most about cell biological research is the new perspective it gives you on the life around you. You can see the molecules working hard in cells under the microscope or on an immunoblot, and importantly, you can extend that to what is happening in your body now, at this moment. In addition, there are moments when it is exciting to think that the phenomenon we are seeing now may be a discovery for the first time in the world. Each molecule has supported our lives since we were born in this world, yet there are still many things left to be discovered. Cell biologists are given the opportunity to reveal them through our own ideas. Importantly, we can share our curiosity for these tiny events in cells with other researchers beyond national borders and through the ages. I always feel that experimental data are the common language of the world.

Outside of the lab, do you have any hobbies?

I love handcrafting things, whether it be pressing flowers, painting with watercolors, or sewing. Many of my artworks are inspired by nature. Pittsburgh is full of nature and the changing seasons always find ways to inspire me.

Do you have any advice for the next generation of scientists?

Big discoveries are not made in one day. Your hypothesis often ends up in failure. In such a situation, there are many times when you're at a loss. But you aren't alone in your struggle. The transition to the U.S. from a small country like Japan has allowed me to recognize there are so many researchers all over the world who are working hard to make great discoveries despite a lot of struggle. They strengthened my recognition that curiosity about life is universal, and that curiosity drives us through any hardship. Research is competitive, but at the same time, it is something special we can all pursue together regardless of race or language, and importantly, you are a member of this group. Above all, enjoy what is happening in the cells in front of you. Remember that those are necessary for your own life. Your mission is to spotlight them and uncover their roles. I'm still on my way to being fully established, so I always tell myself the same thing.

Trainee Spotlight: Zhihao Sun, PhD

Zhihao Sun, PhD, is a trainee under the supervision of **Shihui Liu, MD, PhD**, associate professor of Medicine in the Aging Institute. Dr. Sun joined the Liu Lab in 2021, and his research focuses on identifying and characterizing novel factors involved in inflammasome-mediated pyroptosis — a form of programmed cell death triggered by inflammation.

Inflammasomes are large protein complexes that detect and amplify signals from a variety of stimuli — such as exogenous pathogens that can be spread through contact with contaminated surfaces or infected people and endogenous danger signals that can be released by cell stress or damage — to activate inflammatory responses.

This inflammatory response results in the production of inflammatory cytokines and pyroptosis. Aberrant inflammasome signaling has been implicated in the development of multiple human diseases, including cardiovascular and metabolic diseases, cancer, and neurodegenerative disorders. To better understand underlying mechanisms, Dr. Sun used a genome-wide CRISPR knockout screen to isolate target DNA and identify novel drivers of inflammasome-mediated pyroptosis.

In his research, Dr. Sun found that phosphatidylethanolamine (PE) facilitates a critical step in rapid pyroptosis, Gasdermin D (GSDMD) mediated pore-formation. Dr. Sun and the Liu lab are also studying the role of the ubiquitin-proteasome system in pyroptosis.

Dr. Sun is originally from China and received his bachelor's degree from Qingdao University and his master's degree from Zhejiang University. For his master's degree, he studied pathway-specific regulators of antibiotic biosynthesis in *Streptomyces*. After graduating, Dr. Sun attended the University of Pittsburgh for his doctoral degree.

At Pitt, Dr. Sun studied protein quality control in the endoplasmic reticulum in the lab of Jeffrey L. Brodsky, PhD, Avinoff professor in the Department of Biological Sciences, and focused on how protein aggregation and ubiquitination dictates endoplasmic reticulum-associated degradation. Outside the lab, Dr. Sun enjoys swimming, biking, and spending time with his family and friends.

Join the Aging Institute – We are hiring!

Postdoctoral Associate positions are available in the following labs:

- **Dr. Aditi Gurkar**
- **Dr. Stacey Rizzo**
- **Drs. Shiori and Yusuke Sekine**
- **Dr. Jay Tan**
- **Dr. Bokai Zhu**

Other available positions at the Aging Institute:

- **Laboratory Research Technician III –** Dr. Stacey Rizzo Lab
- **Research Scientist –** Dr. Yuan Liu Lab
- **Laboratory Research Technician II –** Dr. Aditi Gurkar
- **Research Scientist –** Dr. Stacey Rizzo Lab
- **Laboratory Research Technician II –** Dr. Bokai Zhu
- **Research Scientist -** Dr. Andrey Parkhitko Lab
- **Laboratory Research Technician IV –** Dr. Yuan Liu Lab
- **Laboratory Research Technician III –** Dr. Yuan Liu Lab

Faculty Update: Shihui Liu, MD, PhD

Shihui Liu, MD, PhD, faculty member of the Aging Institute and the Division of Infectious Diseases at the University of Pittsburgh, was awarded tenure at the rank of associate professor of Medicine, effective June 1, 2023.

Dr. Liu joined the Aging Institute in June 2018 as a visiting associate professor and was appointed as an assistant professor of Medicine in January 2020. Before joining the University of Pittsburgh, he was a senior staff scientist at the National Institute of Allergy and Infectious Diseases in Bethesda, MD. Dr. Liu is honored by the conferral of tenure and the confidence that the University of Pittsburgh has in him to continue to make important contributions to the fields of Aging and Infectious Diseases.

One of Dr. Liu's long-term research interests is to reengineer bacterial protein toxins to target specific cell types, especially cancer cells.

The focus of Dr. Liu's research is on the roles of several medically important bacterial virulence factors, including anthrax toxins in bacterial pathogenesis. His lab investigates how these toxins initiate disease pathology by altering key signal transduction pathways — in particular the RAS and ERK pathways — and then leverages these mechanistic insights to develop novel therapies for the diseases caused by these pathogens. Dr. Liu is also interested in leveraging insights from his lab to develop novel anti-cancer drugs with high tumor specificity and new approaches to selectively eliminate senescent cells to address aging-related diseases.

One of Dr. Liu's long-term research interests is to reengineer bacterial protein toxins to target specific cell types, especially cancer cells. Many FDA-approved small molecule inhibitors for cancer therapies lack tumor specificity and can also gain entry into normal cells, causing off-target and sometimes fatal side effects.

There is a critical need, therefore, to develop tumor-selective inhibitors with high therapeutic index. In this regard, many bacterial pathogens have evolved potent protein toxins to disrupt specific pathways, such as the RAS-RAF-MEK-ERK pathway and NF-κB pathway involved in microbial pathogenesis. However, these pathways are also critical for cancer development.

Fortunately, these potent, naturally occurring toxins can also be structurally modified to achieve high tumor specificity. Dr. Liu and his colleagues are currently testing several of these potent and highly tumor-selective agents in mouse tumor models. These novel and highly tumor-selective agents hold promise to simultaneously target multiple cancer hallmarks with minimal toxicity to normal tissues, potentially meaning fewer negative side effects for future patients. Another one of Dr. Liu's long-term goals is to develop safe and potent therapeutics for treating human cancers with oncogenic RAS or BRAF mutations.

Dr. Liu's research is supported by the National Institutes of Health and institutional funds. Currently, he is a principal investigator on three active R01 grants from the National Cancer Institute and the National Institute of Allergy and Infectious Diseases.

Aging Institute Research Seminar Series: October 2023

Shin-ichiro Imai, MD, PhD: **"Achieving Productive Aging: The Inter-Organ Communication for Mammalian Aging and Longevity Control and anti-Aging Intervention"**

by Yusuke Sekine, PhD, Assistant Professor of Medicine at the Aging Institute and in the Division of Endocrinology and Metabolism

Dr. Imai's vision is that seniors can enjoy their lives, **stay physically and mentally healthy**, and **continue to contribute to society** with their years of experience.

In a seminar at the Aging Institute in October 2023, Shin-ichiro Imai, MD, PhD, Theodore and Bertha Bryan Distinguished Professor in Environmental Medicine at Washington University in St. Louis, presented recent advances in the understanding of how mammalian aging is regulated by the small molecule nicotinamide adenine dinucleotide (NAD+).

NAD+ is an essential coenzyme that supports various biological processes in living organisms. The levels of NAD+ decline in multiple tissues with aging, which may cause age-associated pathologies. Dr. Imai is a pioneering researcher whose findings regarding regulatory mechanisms of NAD+ biosynthesis and NAD+-dependent protein deacetylases (sirtuins) have placed NAD+ metabolism at the center of the aging and longevity research field.

Dr. Imai began his seminar with a set of chromatogram data he obtained more than 20 years ago while a postdoctoral fellow in the lab of Leonard Guarente, PhD, at MIT. The data showed NAD+-dependent deacetylation activity of yeast sirtuin protein Sir2, which had already been known to modulate yeast replicative lifespan (Imai et al, *Nature,* 2000). This landmark discovery opened the new field of NAD+ and Sirtuin biology in aging research. Since Dr. Imai started his laboratory at Washington University in 2001, his group has published a series of studies showing the importance of the mammalian sirtuin, SIRT1 and NAMPT — the rate-limiting enzyme in the NAD+ biosynthesis — in aging and longevity. Dr. Imai has developed an integrative concept termed the "NAD world" where the inter-tissue communication mediated by NAMPT, NAD+, and SIRT1, plays a pivotal role in the control of mammalian aging/longevity.

Dr. Imai's group generated brain-specific Sirt1-overexpressing transgenic mice, which exhibit a significant extension in median lifespan (~10% in males and ~16% in females) (Satoh et al, *Cell Metab*., 2013). They found that activity of SIRT1 and its substrate NK2 Homeobox1 in specific areas of the hypothalamus is important for neuronal activities, suppressing aging phenotypes and promoting longevity in mice. Furthermore, Dr. Imai's group identified the extracellular form of NAMPT (eNAMPT), which is secreted within extracellular vesicles (EVs) from adipose tissues, as a critical regulator of NAD+ metabolism and SIRT1 activity in the hypothalamus (Yoon et al, *Cell Metab*., 2015).

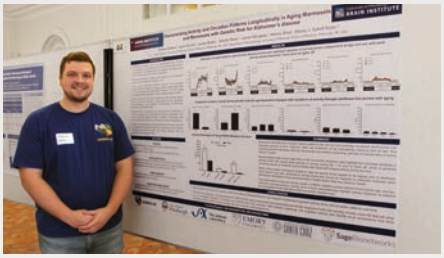
Plasma eNAMPT levels decrease with age in mice and humans, and a significant positive correlation was observed between the plasma eNAMPT levels and lifespan in mice (Yoshida et al, *Cell Metab*., 2019). Strikingly, injection of eNAMPT-containing EVs obtained from the plasma of young mice enhanced the lifespan of aged mice, and EV-injected mice exhibited improved health metrics, including higher activity compared to age-matched control mice. As a part of "the NAD world," these results suggest that the inter-tissue communication between adipose tissues and the hypothalamus through eNAMPT controls healthspan and lifespan in animals.

After presenting unpublished data showing a novel neuronal signaling pathway regulating adipose tissues and aging in mice, Dr. Imai concluded with presentation of a recent human clinical trial demonstrating the effect of nicotinamide mononucleotide (NMN), a NAD+ intermediate generated by NAMPT, on metabolic function in postmenopausal women with prediabetes (Yoshino et al, *Science*, 2021). Supplementation of NMN increased muscle insulin sensitivity, insulin signaling, and muscle remodeling in NMN-treated participants. The results support therapeutic potential for modulating NAD+ metabolism in humans.

Throughout his seminar, Dr. Imai emphasized a vision of "productive aging." Further understanding of the "NAD world" holds promise to advance productive aging.

Aging Institute Research Day

by Lauren Ward, MA, Executive Director of Corporate and Foundation Relations at the University of Pittsburgh



Geriatrics research is on the verge of a revolution, according to George Kuchel, MD, director of the University of Connecticut's Center on Aging and this year's keynote speaker at the Aging Institute's 15th annual Research Day, held on Nov. 1, 2023.

Dr. Kuchel noted that research today aims to push back the evolution of multiple diseases associated with aging. Current investigations now focus on identifying unique pathways that underlie disorders, including cancer, diabetes, and cognitive decline.

In opening the meeting, Aging Institute Director **Toren Finkel, MD, PhD**, emphasized that we must rethink prevailing approaches that focus on treating single diseases. Projects led by Aging Institute investigators as part of the SenNet Consortium, for example, will map genes and proteins associated with senescence and point to "senolytics," a new generation of anti-aging drugs. Moreover, big data analytics and large-scale functional screens, including leveraging methods developed here, are helping us target age-related cell pathways, according to Pitt faculty **Andrey Parkhitko, PhD, Yuan Liu, PhD, Sameneh Farsijani, PhD**, and **Megan Marron, PhD**, who spoke at the meeting.

These same "omic" investigations should help scientists discover genetic differences and alterations in immunity that account for variations in our capacity for resilience as we grow older, as well as our different susceptibilities to infections and chronic disease. Speakers also presented how chrononutrition and fasting offer potential ways to slow age-related declines.

These new avenues of exploration and others were discussed by more than 150 attendees during a poster session featuring 51 projects. Twelve posters were selected by judges as outstanding for their promise to advance geroscience and translational medicine. The day concluded with a community forum and a series of talks by Aging Institute faculty, all focusing on similar themes. The 20 community attendees were eager to learn more about the Institute's work to advance healthy aging, and we look forward to more opportunities for community outreach in the future.

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Winners Research Day Nov. 1, 2023

Winners received a certificate and Pittsburgh-themed gift baskets from Baskets of Pittsburgh.

Basic Science

Faculty: Md Saifur Rahman
Metabolomics-Guided Translational Research for Uncovering the Pathogenesis of Type 2 Diabetes

Postdoctoral: Yu-sheng Yeh
Identification of Lysosomal Lipolysis as a Non-canonical Mediator of Adipocyte Fasting- and Cold-induced Lipolysis

Graduate: Jinrui Xun (Poster #1)
A conserved ion channel function of STING mediates non-canonical autophagy and cell death

Undergraduate: Ghanesh Gutta
DNA Damage and Neuronal Senescence: Differential Vulnerabilities of Neurons in Different Cortical Layers

Research Staff: William Lu
GRK2 activity promotes Aß generation by altering GPR3 signaling and intracellular trafficking

Population Research

Faculty: Samaneh Farsijani
The Relationship between ChronoNutrition Behaviors and Muscle Health in Older Adults

Postdoctoral: Thomas Kraynak
Do cardiovascular, metabolic, or inflammatory risk factors relate to brain age in late life?

Graduate: Jimmie E. Roberts
Fall & Fall Injury Associations with Fall-Risk Increasing Drugs (FRID) Use in Older Black and White Men and Women: the Health, Aging, and Body Composition Study

Clinical and Translational

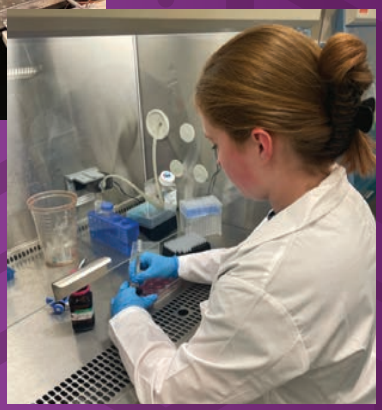
Postdoctoral: Jr-Jiun Liu
Assessing the Correlations Between Pathology and Hippocampal Volume in Neurodegenerative Diseases Using 7T Postmortem MRI

Graduate: Samuel Adida
Predicting Complications After Kyphoplasty in Elderly Patients with Vertebral Compression Fractures

Undergraduate: Jaehoon Noh
Assessing the Correlations Between LATE Pathology and Amygdala Volume in Neurodegenerative Diseases Using 7T Postmortem MRI

Research Staff: Taylor Lazzari
Big Five Personality Characteristics, Neuropsychological Performance, and Subjective Cognitive Functioning in Older Adults

UPMC Hillman Cancer Center Academy



The UPMC Hillman Cancer Center Academy is an award-winning summer learning program for high school and undergraduate students. In Summer 2023, the Aging Institute welcomed four UPMC Hillman Academy high school students and one undergraduate student from the Pittsburgh area: Vanessa Gonzalez-Rychener, Lucas Hertzel, Tabo Mkandawire, Sophia Song, and Lauren Weaver. These students worked full-time over the summer to complete individual research projects at the Aging Institute with oversight from faculty mentors and their labs. The students also had the opportunity to learn more about STEM educational and career opportunities.

- **Vanessa Gonzalez-Rychener** worked on a project entitled "Generation of Reporter Cell Line That Can Visualize Iron Responsive Molecule DELE1" under the mentorship of Shiori Sekine, PhD, and her lab, including senior laboratory research specialist Ryan Houston.
- **Lucas Hertzel** worked under the mentorship of Beibei (Bill) Chen, PhD, and his lab, including postdoctoral associate Travis B. Lear, PhD, and technician Áine Boudreau on his project, "Determining the Subcellular Location of TFEB Degradation During Viral Infection."
- **Tabo Mkandawire** worked on her project, "Pharmacological Activation of IRE1-XBP1s ER UPR for Host-directed Anti-coronaviral Therapeutic Development," under the mentorship of Yuan Liu, PhD, postdoctoral associate Travis B. Lear, PhD, and technician Áine Boudreau.
- **Sophia Song** worked on a project entitled "To Reveal the Mechanism of Clusterin Degradation Under Glucose-Deprived Conditions" under the mentorship of Yusuke Sekine, PhD, and his lab, including senior laboratory research specialist Ryan Houston.
- Undergraduate student **Lauren Weaver** worked under the mentorship of Xiaojun (Jay) Tan, PhD, and his lab, including Bo Lyu, PhD, on her project, "A Cytonuclear Translocation Reporter System Using Split-GFP." Lauren also provided mentorship and leadership of her own in her capacity as a resident advisor for the program.

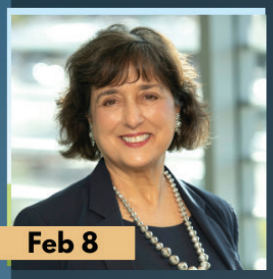
Thank you to our wonderful UPMC Hillman Academy faculty, staff, and students and to all of the Aging Institute faculty, staff, and students for a fantastic summer. You can find more information about the Aging Institute's participation in the Hillman Academy, as well as other Aging Institute training for high school and undergraduate students, on our **website**.

University of Pittsburgh and UPMC

AGING INSTITUTE RESEARCH SEMINAR SERIES

Visiting Speakers, 2023 - 2024

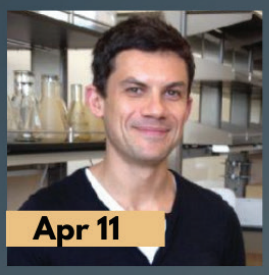
2nd Thursday of the Month | 4:00 pm - 5:00 pm
Bridgeside Point 1 | 100 Technology Drive | 5th Floor Conference Room
Zoom link: https://pitt.zoom.us/j/99688230409



Feb 8

Roberta Diaz Brinton, PhD

Professor, Department of Pharmacology ad Neurology
Professor Psychology and Evelyn F. McKnight Brain Institute
Director, Center for Innovation in Brain Science
University of Arizona



Apr 11

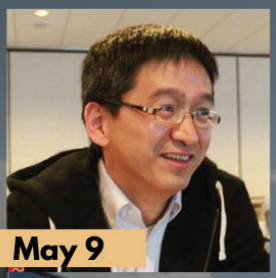
Roberto Zoncu, PhD

Associate Professor, Biochemistry, Biophysics and Structural Biology
University of California, Berkeley

Mar 14

Vittorio Sebastiano, PhD

Associate Professor (Research)
Obstetrics/Gynecology
Director, Transgenic Knockout and Tumor Model Service (TKTC)
Stanford University



May 9

Ling Qi, PhD

Chair, University of Virginia Department of Molecular Physiology/Biological Physics
University of Virginia

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UPMC LIFE CHANGING MEDICINE

Highlighted Manuscripts at the Aging Institute

Shihui Liu, MD, PhD, Zehua Zuo, PhD, Jie Liu, PhD, and Team Published Manuscript in *Proceedings of the National Academy of Sciences*

In a recent study, **Shihui Liu, MD, PhD**, associate professor of Medicine in the Aging Institute and the Division of Infectious Diseases at the University of Pittsburgh, and his lab engineered a robust genetic tool to define the precise role of each tumor stromal cell type in tumor development and progression. This innovative work, titled "ERK and c-Myc signaling in host-derived tumor endothelial cells is essential for solid tumor growth" was published in *Proceedings of the National Academy of Sciences* (PNAS) in early 2023. **Zehua Zuo, PhD**, research scientist, and **Jie Liu, PhD**, research associate professor, are the lead authors.

Cancers are driven by a complex mixture of tumor initiating malignant cells with oncogenic mutations and a variety of host-derived tumor-enabling stromal cells. In the tumor microenvironment (TME), malignant cells and the surrounding stromal cells interact dynamically through direct interactions, as well as by indirect communications mediated by secreted molecules, such as growth factors, angiogenic factors, cytokines, chemokines, and extracellular vesicles. Given the critical roles of tumor stromal compartment in tumor initiation and progression, strategies to therapeutically target key tumor stromal cells in TME have emerged as promising approaches for cancer treatment.

However, anti-TME therapies currently in clinical evaluation usually target key factors involved in intercellular communications between tumor stromal cells and cancer cells. The limited efficacy of these anti-TME strategies is due in part to our poor understanding of the roles and relative contributions of the various tumor stromal cells to tumor development. Absent this knowledge, rationale for selection of therapeutic targets is challenging.

In this work, the Liu Lab described a versatile and tractable anthrax-toxin protein delivery system-based genetic platform, allowing for unambiguous evaluation of the contribution of each tumor compartment to tumor progression. Many bacterial pathogens have evolved potent protein toxins to disrupt specific pathways involved in host defense and metabolism.

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Fortunately, these naturally occurring toxins can be structurally modified to achieve high tumor specificity. Anthrax lethal toxin (LT), which targets the MEK-ERK pathway, represents such a toxin. LT is a typical A-B type toxin consisting of two proteins: a cellular receptor-binding and delivering component termed protective antigen (PA), and an enzymatic moiety denoted as lethal factor (LF) **(Figure 1)**. To target host cells, PA binds to the cell surface receptor CMG2 (capillary morphogenesis protein-2). This binding results in proteolytic activation of PA by a cell surface furin protease, yielding the active PA oligomer.

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Highlighted Manuscripts *(continued)*

Shihui Liu, MD, PhD, Zehua Zuo, PhD, Jie Liu, PhD, and Team Published Manuscript in *Proceedings of the National Academy of Sciences*

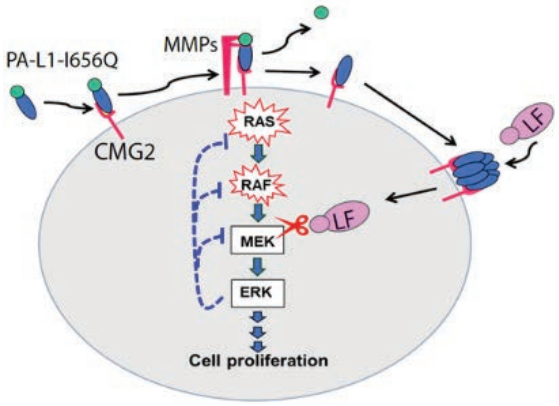

Fig. 1: *Anthrax toxin protein delivery system as a unique platform for cancer therapy with high specificity. Tumor specificity is achieved by engineering the delivering vehicle PA to rely on tumor-associated proteases (MMPs) for activation. Thus, LF and tumor stromal cells to inactivate MEK1/2, disrupting the ERK signaling.*

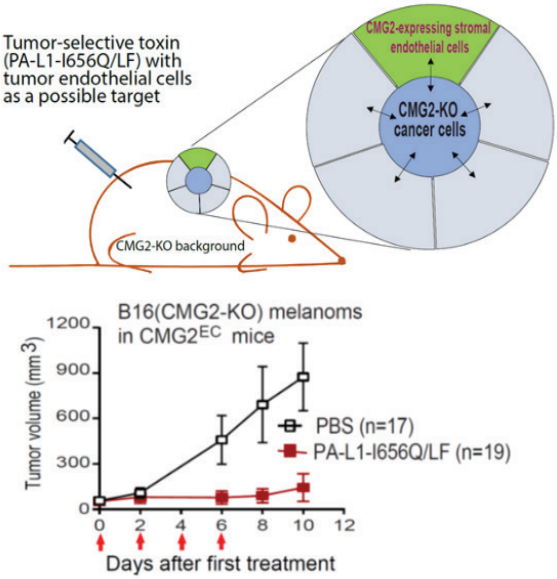

Fig. 2: *The mice with the toxin receptor CMG2 only expressed in endothelial cells was used to assess the role of this tumor stromal cell-type in the targeted therapy using the engineered toxin.*

The PA oligomer then binds and translocates LF into the cytosol of target cells to exert its cytotoxic effects. The unique requirement for PA proteolytic activation on the target cell surface provides a way to re-engineer PA, by modifying the protease cleavage site, so that it is activated by a tumor-associated protease rather than by furin. The team of researchers has successfully generated a tumor-selective PA variant (termed PA-L1-I656Q) that can be specifically activated by tumor-associated MMPs (matrix metalloproteinases), thereby achieving high tumor specificity in delivering the cytotoxic effector LF into tumor cells and tumor stromal cells **(Figure 1)**. Therefore, PA-L1-I656Q plus LF displays potent antitumor activity in a wide range of human tumor xenograft mouse models.

Since the antitumor activity of PA-L1-I656Q plus LF requires the presence of CMG2 receptor on target cells, the authors reasoned that, by genetically manipulating the expression pattern of CMG2 on cancer cells as well as on each tumor stromal cell type, they can selectively target the toxin to specific cell types and thereby unambiguously determine the specific contribution of each of these cell types to the toxin's antitumor activity. Cell-type specific expression of CMG2 can be conveniently achieved by using cell-type specific CMG2 gain-of-function as well as loss-of-function mice, as developed in the Liu Lab.

Thus, by examining the responses of implanted tumors lacking the CMG2 receptor to the engineered tumor-selective MEK inactivating toxin, the authors were able to define the role of each tumor stromal cell type, such as tumor endothelial cells, in tumor progression. Using this tumor-host genetic platform, the authors found that MEK-ERK signaling in tumor endothelial cells plays an essential role in tumor progression **(Figure 2)**. They also discovered that c-Myc, a master transcription factor controlling central metabolism, is a downstream effector of MEK-ERK, and that disruption of the MEK-ERK-c-Myc-bioenergetic axis in tumor endothelial cells efficiently inhibits tumor growth.

Zuo Z, Liu J, Sun Z, Cheng YW, Ewing M, Bugge TH, Finkel T, Leppla SH, Liu S. ERK and c-Myc signaling in host-derived tumor endothelial cells is essential for solid tumor growth. Proc Natl Acad Sci *USA*. 2023 Jan 3;120(1): e2211927120.

Highlighted Manuscripts at the Aging Institute

Stacey J. Sukoff Rizzo, PhD: Published "Bridging the rodent to human translational gap: Marmosets as model systems for the study of Alzheimer's disease."

Alzheimer's disease is a devastating neurodegenerative disorder with no cure. Recent FDA-approved therapies slow down accumulation of proteins in the brain known as amyloid plaques, but don't prevent cognitive decline or treat memory loss.

Aging Institute faculty member and Associate Professor of Neurobiology **Stacey J. Sukoff Rizzo, PhD**, is focused on understanding primate-specific mechanisms that drive divergence from healthy aging towards inception and progression of memory loss and Alzheimer's disease. In a recent paper published in the journal *Alzheimer's & Dementia: Translational Research and Clinical Interventions*, Dr. Rizzo and colleagues describe how closely studying non-human primates from birth and throughout their lifespan will reveal the peak in learning and memory in adulthood and the inflection point at which memory begins to decline with aging.

As our closest relatives on the evolutionary tree, non-human primates share much of the genetic, molecular, cellular, structural, and functional organization with the human brain and have remarkable similarities in developmental, postural, physiological, and immune functions. Important to the study of aging and Alzheimer's disease, non-human primates share the identical protein sequence of amyloid with humans, and they also demonstrate age-related cognitive decline.

Dr. Rizzo and colleagues received a five-year, $32.5 million grant from the National Institutes of Health in 2022 to establish a consortium studying common Marmosets As Research Models for Alzheimer's Disease (MARMO-AD). Common marmosets (*Callithrix jacchus*) are a small new world species of non-human primates.

MARMO-AD

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As part of the MARMO-AD consortium, the marmosets will be studied using the same assessments used to diagnose Alzheimer's disease in human patients, including non-invasive blood biomarkers, MRI and PET neuroimaging, and comprehensive cognitive assessments of learning and memory using touchscreen devices.

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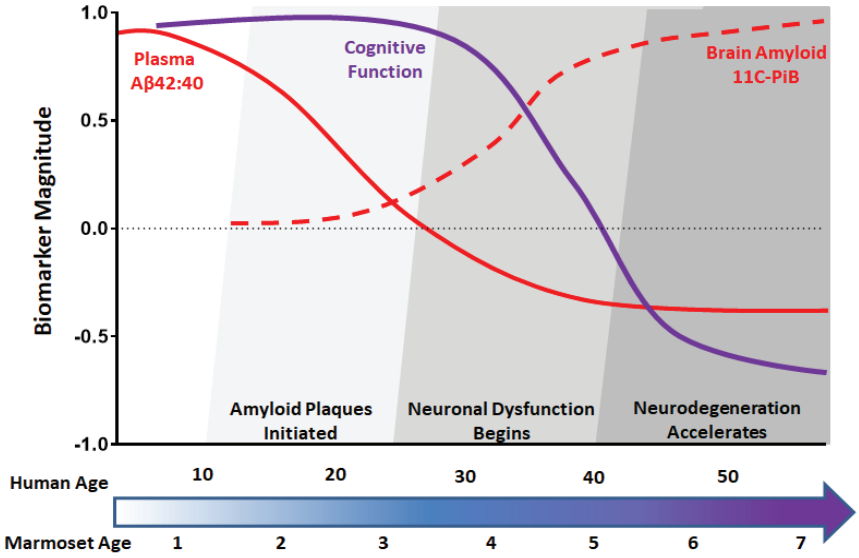
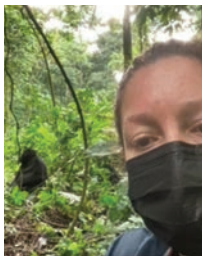
Highlighted Manuscripts *(continued)*

Stacey J. Sukoff Rizzo, PhD: Published "Bridging the rodent to human translational gap: Marmosets as model systems for the study of Alzheimer's disease."



As shown in the predicted phenotypic trajectory of the marmosets in this study, the marmoset to human age ratio is 1:8. Based on prior research, Dr. Rizzo and her colleagues expect to see an accumulation of amyloid beta in marmosets aged four years and older (approximately 32 years old in humans). By monitoring the marmosets as they age, Dr. Rizzo will observe the effects of amyloid beta accumulation and reduced plasma amyloid beta on cognitive function.



Photos of mountain gorillas taken by Dr. Rizzo on her recent trip to the Bwindi Impenetrable Forest in Uganda to study primate behavior.

The marmosets will be tracked from birth throughout their lifespan which will allow Dr. Rizzo and her colleagues to define the earliest changes that signal disease onset with aging, including biomarkers that precede cognitive decline and neurodegeneration. Marmosets have an age equivalent to humans of approximately 1:8 years, which means that by eight years of age they will present with similar signs of aging — including amyloid plagues in the brain — as humans in their mid-sixties. Importantly, by studying marmosets before aging and cognitive decline, the team will be able to identify changes that happen before amyloid plaques and neurodegeneration begin, which will allow discovery of novel interventions to protect against Alzheimer's disease.

As an extension of her research in understanding whether Alzheimer's disease is primate-specific or uniquely human, Dr. Rizzo recently traveled to Uganda to the Bwindi Impenetrable Forest to study the behavior of mountain gorillas. Since all primates, both human and non-human alike, exhibit the same amyloid pathology in the brain, studying behaviors of non-human primates in their natural habitat may also help reveal uniquely human aspects of Alzheimer's disease, including evolutionary mechanisms that make humans more susceptible and non-human primates more resilient to this disease.

Sukoff Rizzo SJ, Homanics G, Schaeffer DJ, Schaeffer L, Park JE, Oluoch J, Zhang T, Haber A, Seyfried NT, Paten B, Greenwood A, Murai T, Choi SH, Huhe H, Kofler J, Strick PL, Carter GW, Silva AC. Bridging the rodent to human translational gap: Marmosets as model systems for the study of Alzheimer's disease. Alzheimers Dement (N Y). 2023 Aug 21;9(3):e12417. doi: 10.1002/trc2.12417. PMID: 37614242; PMCID: PMC10442521.

Highlighted Manuscripts at the Aging Institute

Andrey A. Parkhitko, PhD, Published Review Manuscript in *Nature Aging*

Andrey Parkhitko, PhD, is an assistant professor in the Aging Institute and Division of Endocrinology and Metabolism at the University of Pittsburgh School of Medicine. Dr. Parkhitko was recently the lead author of a review article published in *Nature Aging*. Together with Marc Tatar, PhD, from Brown University and Elizabeth Filine from Harvard Medical School, Dr. Parkhitko discussed different combinatorial interventions that can extend health and lifespan across different species.

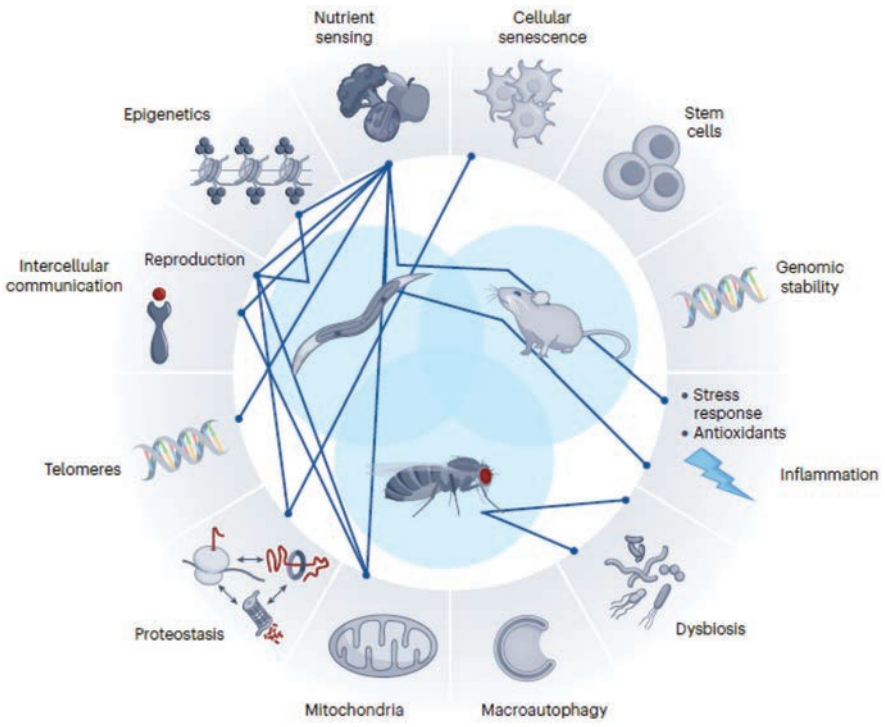
Understanding and manipulating the molecular mechanisms of aging holds promise for interpreting and treating age-related pathologies, improving quality of life, and extending healthy lifespan. Although multiple genetic and pharmacological manipulations are known to extend longevity in different species, it is unlikely that monotherapy — the use of a single drug to prevent aging and age-related diseases — will be effective for dramatic lifespan extension.

Additionally, most age-related diseases currently lack effective treatment. There is still very limited knowledge on how different interventions can synergize to extend health and lifespan, as well as the mechanisms driving synergy.

In this review, Dr. Parkhitko and his co-authors summarized different modes of cooperative interactions between age-related pathways and discussed various strategies that can be applied to simultaneously target lifespan-extending pathways in different organisms. In some cases, the authors describe how the combined manipulation of two or more targets additively increases lifespan.

In other cases, orthogonal targeting of different hallmarks of aging may maximize lifespan extension. Due to the high level of conservation of pro-longevity pathways in general, translating findings from model systems to humans will likely identify "druggable" targets relevant to multiple human pathologies associated with aging. This process could help researchers better understand how to combine therapies to slow the aging process and prevent age-related diseases.

Figure: Overview of interactions between different processes (hallmarks) of aging.



Parkhitko AA, Filine E, Tatar M. Combinatorial interventions in aging. *Nat Aging*. 2023 Oct; 3(10):1187-1200. doi: 10.1038/s43587-023-00489-9. Epub 2023 Oct 2. PMID: 37783817.

Highlighted Grants at the Aging Institute

Yuan Liu, PhD, is leading a new R01 grant called "Host defense small molecule development for COVID-19 treatment by targeting lysosome."

Yuan Liu, PhD, assistant professor at the Aging Institute and in the Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine at the University of Pittsburgh School of Medicine, was recently awarded an R01 grant from the National Institute of Allergy and Infectious Diseases (NIAID) for her project titled, "Host defense small molecule development for COVID-19 treatment by targeting lysosome."

With this new grant, Dr. Liu aims to first elucidate the molecular mechanism of how DCAF7-mediated TFEB protein degradation regulates SARS-CoV-2 infection and then develop a small molecule DCAF7 inhibitor to boost host anti-coronaviral response. SARS-CoV-2 is prone to mutations and rapidly evolves variants different from ancestral strains that can circumvent existing vaccines and antivirals. There is unmet need for alternative host-directed therapies targeting the broad-spectrum of coronaviruses, including current and future novel SARS-CoV-2 variants.

The autophagy-lysosomal degradation pathway is a cellular housekeeping process that is highly conserved across eukaryotes and functions to clean up intracellular accumulation of unwanted organelles and protein aggregates, as well as invaded pathogens. Transcription factor EB (TFEB) is a master transcriptional activator of autophagy and lysosomal biogenesis, and there is a growing appreciation that augmenting TFEB activity might be of significant benefit as a strategy to boost pathogen clearance and reduce inflammation.

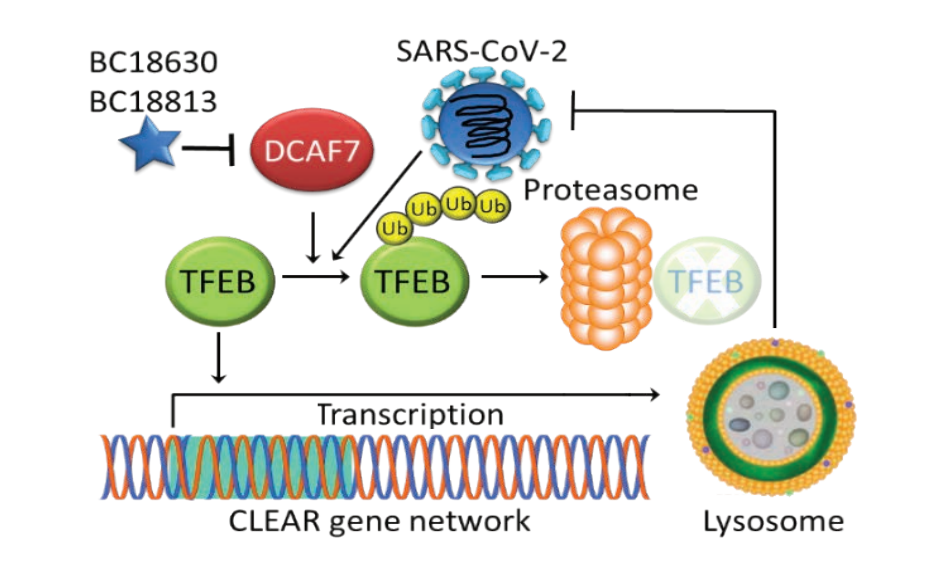


Fig. 1: *HCoV infection triggers the targeted degradation of the master transcription factor TFEB. This process is regulated by the ubiquitin E3 ligase DCAF7. Small molecules that inhibit DCAF7 maintain TFEB levels and augment lysosomal-based viral clearance.*

Dr. Liu's Lab discovered that TFEB protein translocates from the cytosol to the nucleus in response to coronaviral infection. With prolonged infection, the initial increase is followed by a decline in nuclear TFEB protein. Through a proteomic analysis, the Liu Lab identified that the ubiquitin E3 ligase DCAF7 interacts with TFEB protein and mediates its disposal through proteasomal degradation.

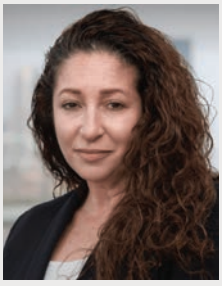
They then conducted an *in silico* screen of a 3 million compound library followed by a medicinal chemistry campaign to develop two lead small molecules, BC18630 and BC18813. These small molecules protect TFEB from DCAF7-mediated protein degradation and thus activate autophagy-lysosomal gene expression. These agents also markedly attenuate human coronavirus (HCoV) infection, including SARS-CoV-2. The preliminary studies thereby provide a novel avenue for generating effective, host-centric COVID-19 therapeutics that are likely to protect against current and future variants.

With this R01 funding support from NIAID, Dr. Liu and her lab will continue to explore the biological impact of inhibiting DCAF7 and augmenting TFEB, which will guide further chemistry development and optimization of on-target versus off-target effects. They will leverage the high throughput drug screen platform at the Aging Institute to initiate a new screen to identify novel DCAF7 inhibitor classes; improve oral bioavailability, potency, and safety profiles; and advance commercial development of an off-the-shelf strategy for future human coronavirus pandemics.

Dr. Liu hopes that this study will enhance our understanding of and strategies to combat coronaviruses and their strains, as well as produce therapeutics that will protect people from future coronaviruses pandemics.

Highlighted Grants *continued*

Bill Chen, PhD, and Stacey Rizzo, PhD, received an NIH U01 award to study how elevating NAD+ levels in the brain may improve aging and prevent Alzheimer's disease.



Aging Institute colleagues **Bill Chen, PhD**, professor of Medicine in the Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine and deputy director for Drug Development at the Aging Institute, and **Stacey Rizzo, PhD**, associate professor of Neurobiology and deputy director for Pre-Clinical Studies at the Aging Institute, are the recipients of a new National Institutes of Health-funded U01 award from the National Institute on Aging. For this new award, Dr. Chen and Dr. Rizzo will develop therapeutics for aging and Alzheimer's disease prevention.

The single greatest risk factor for Alzheimer's disease (AD) is chronological age. As the global population ages, the incidence of memory loss, cognitive decline, and frank AD cases is expected to rapidly increase. This age-dependent increase in AD diagnosis, along with concerns for memory loss and cognitive decline, has fueled intense interest in the development of strategies to slow or reverse aspects of brain aging.

As we age, there is a decline in energy metabolism in the brain. One enzyme involved in energy metabolism, called nicotinamide adenine dinucleotide (NAD+), has been shown to decline in aging brains and is also associated with cell death and cognitive impairment. Studies in animal models of aging and AD have shown that restoring youthful NAD+ levels improves cognitive function. Although NAD+ supplements are available at grocery and health food stores, they metabolize very quickly and don't easily get into the brain.

The Aging Institute Drug Discovery group has discovered novel molecules that activate an enzyme in the brain called nicotinamide phosphoribosyltransferase (NAMPT), which regulates NAD+ levels. These novel molecules can be taken orally and achieve sustained augmentation of NAD+ levels in the brain. Studies in animal models of aging and AD are in progress to test the efficacy of these compounds to improve cognitive function.

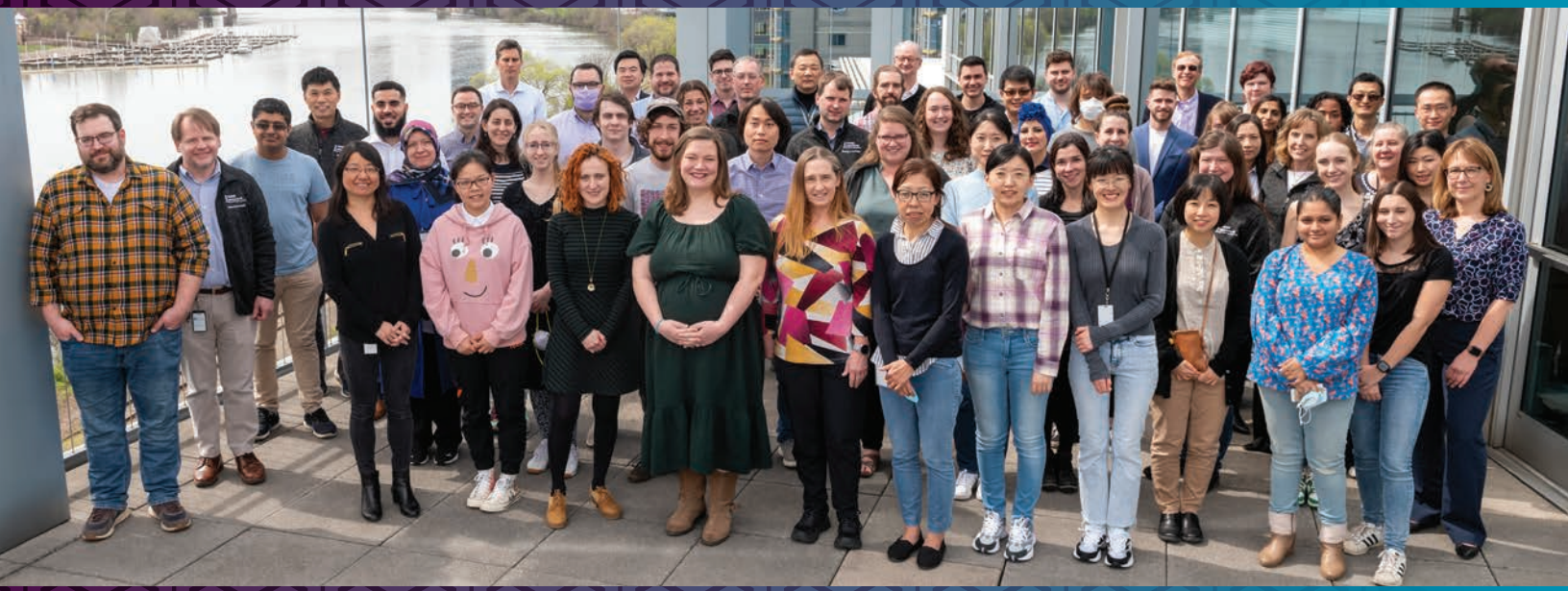
Dr. Rizzo and Dr. Chen have assembled a team of experts in drug discovery, Alzheimer's disease biology, and preclinical translational studies, including Aging Institute colleagues, **Toren Finkel, MD, PhD**, and **Yuan Liu, PhD**, and University of Pittsburgh Alzheimer's Disease Research Center colleagues, **Oscar Lopez, MD**, and **Tommy Karikari, PhD**. Together, the team aims to advance these compounds into clinical trials within the next five years.

Additional **New Grants** at the Aging Institute

Principal Investigator	Grant Title	Grant Type	Sponsor
Aditi Gurkar	Spatial acetyl-CoA metabolism as a regulator of hallmarks of aging	R56	National Institutes of Health
Andrey Parkhitko	Using methioninase as a methionine restriction mim		National Academy of Medicine
Matthew Steinhauser and Pouneh Fazeli	Beneficial reprogramming of lipid metabolism with intermittent fasting	R01	National Institutes of Health
Xiaojun (Jay) Tan	Lysosomal quality control through lipid remodeling	R35	National Institutes of Health

Co-Investigator	Grant Title	Grant Type	Sponsor
Andrey Parkhitko	Methionine Cycle as a Mechanistic Hub for the Hallmarks of Aging	R01	National Institutes of Health/ Brown University

Thank You from the Aging Institute Team



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