MCGOVERN MEDICAL SCHOOL AT UTHEALTH HOUSTON'S
BROWN FOUNDATION INSTITUTE *of* MOLECULAR MEDICINE FOR THE PREVENTION *of* HUMAN DISEASES

IMMpact Report

FISCAL YEAR 2021

About the cover

The courtyard fountains are shown in the foreground of the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases.

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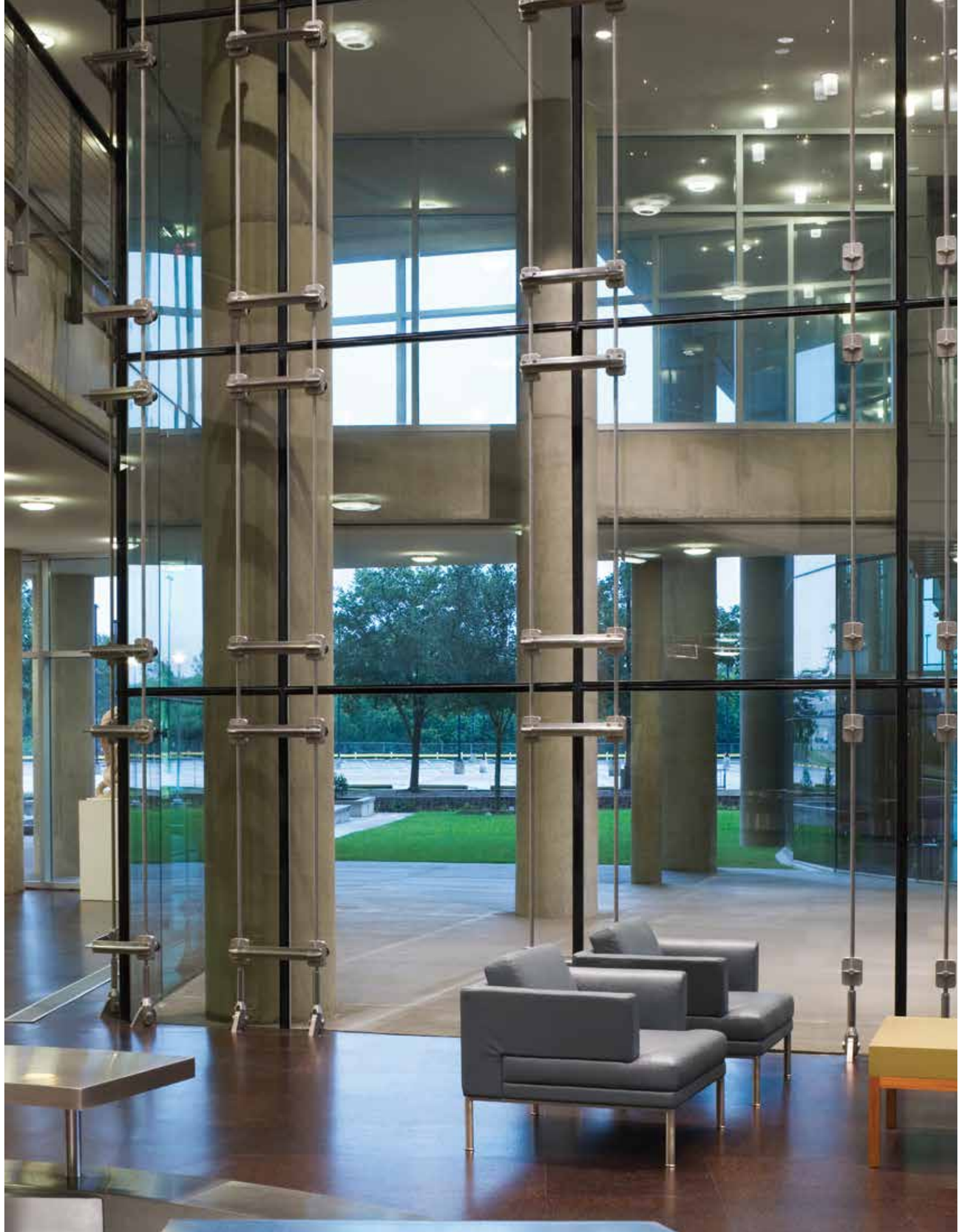

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Director's Message

The IMM has two major objectives:

1. Discovery is the highest priority for the IMM faculty. This is a major challenge, since diabetes, obesity, cancer, Alzheimer's, and cardiovascular diseases are unsolved medical problems that are not caused by single gene defects. Discoveries lead to new solutions.

2. New diagnostics and therapies are derivative of discovery and to the benefit of patients. The IMM focuses on these medical solutions. The IMM has organized talent in the Texas Therapeutics Institute specifically to achieve this goal of patient benefit from discovery.

I'm pleased to introduce the latest annual IMMpact report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). The IMM is a stand-alone research institute that is embedded within McGovern Medical School. The IMM mission is to deliver translational outcomes from research in molecular medicine that benefits patients. Inside the report you will find in-depth articles on four of our faculty and highlighted donors, plus an account from each IMM faculty member describing their research programs and recent progress.

There are many metrics that can be used to define research and institutional success, including grant funding, scientific publications, spin-off companies, and the capacity to recruit and retain stellar scientists from around the world. By all these metrics the IMM excels; I am especially pleased to report, that once again IMM faculty had remarkable success in garnering new grants from the National Institutes of Health, Department of Defense, Cancer Prevention and Research Institute of Texas (CPRIT) and other extramural funding agencies. Over the financial year just ended, our new grants and contracts matched last year, which was a best ever for new funding, capping increases in our extramural grant funding for each of the last 7 years. It is a testament to the outstanding quality and creativity of our scientists that the IMM remains so successful in attracting research funds. Among the many grants our faculty secured this year was a large $6M grant from CPRIT that was awarded to Dr. Jim Liu and his colleagues in the Center for Translational Cancer Research. This CPRIT grant is designed to help cancer researchers from all around Texas take advantage of unique expertise at IMM to progress biological therapeutics through early pharmacological evaluation. One example of such biologics is antibody-based drugs that are used to treat cancer, which will be the topic of the IMMpact symposium this year.

Nevertheless, full implementation of our mission remains heavily dependent on attracting support from alternative sources, including research charities and foundations, industry collaborations, and, most importantly, the continuing generosity of our friends and donors. Such funding is critical to allow our faculty to start innovative new projects and generate preliminary results that will in turn lead to new grant proposals. In this context, we are as always deeply appreciative of the strong work and dedication of the IMM advisory council, which plays a key role in the continued growth and development of the IMM. This year John Macdonald has stepped down as chair of the council after 3 years of stellar service, and we welcome Alan Baden in his stead.

This brings me to our annual IMM symposium: An illuminating and entertaining evening where you can hear exciting research stories directly from our faculty and discuss the implications for the future of medicine and health care. We had to cancel the symposium in 2020 at the start of the COVID pandemic, and for similar reasons did not hold one in 2021, but with vaccinations and boosters now widely available and case numbers dropping fast as I write these introductory comments, we will be going ahead with the 2022 symposium. This will be held at IMM on April 29, and will feature two talks on how antibody-based drugs fight cancer. If you want to hear more about this state-of-the-art technology and how IMM researchers are at the forefront of this emerging field of cancer research and treatment, please attend the symposium. The talks will be followed, as in years past, with a reception in the Dr. J.T. Willerson Discovery Hall. Full details can be found in this IMMpact report. I look forward to seeing you all there.

John Hancock, MA, MB, BChir, PhD, ScD
Executive Director, Institute of Molecular Medicine
John S. Dunn Distinguished University Chair in Physiology and Medicine

Mission

The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM) is a research institute that seeks to investigate the causes of human diseases at the cellular and molecular levels, using DNA and protein technologies to elucidate disease mechanisms. This development and progress are of particular interest for future planning in the increasingly important area of clinical research. The institute endeavors to design methods of rational therapy and, wherever possible, strategies for the prevention of human diseases.

Advances in molecular and cell biology have enormous potential for innovative medical research and the future practice of medicine with more novel therapies. These approaches have been most successfully used to determine the causes of infectious disorders and genetic diseases.

However, it is clear that molecular and cell biology will play a major role in clarifying the causes of many unsolved problems of modern medicine, such as heart disease, hypertension, vascular disorders, major mental illnesses, and inflammatory and immunologic diseases. The research of the institute's investigators is inspiring and promises to fulfill the mission of the IMM.

Because the applications of molecular and cell biology to medical practice are of major importance to product development in biotechnology and the pharmaceutical industry, the IMM has the potential and desire to form important links and collaborations between its own research activities and various industries to apply its discoveries and intellectual properties to pharmaceutical opportunities.

As an institute of McGovern Medical School, the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases strives to set the example for research excellence and collaboration locally, nationally, and internationally.

Our Locations

Fayez S. Sarofim Research Building

- Primary home of the IMM's faculty, administration, and support staff.
- Located adjacent to the The University of Texas Health Science Center at Houston (UTHealth) University Center Tower within the Texas Medical Center.
- Opened in 2006, the building encompasses 255,748 gross square feet.

South Campus Research Building – 3 (SCRB3)

- SCRB3 is a collaboration between The University of Texas MD Anderson Cancer Center and UTHealth, in cooperation with GE Healthcare and the Texas Enterprise Fund.
- Six-stories, 315,000 square-feet located on the South Campus of the Texas Medical Center.
- Opened in 2009, this facility houses Positron Emission Tomography, Magnetic Resonance Imaging, Optical Imaging Tracers, a Cyclotron, wet labs, and support offices.

The Denton A. Cooley Building – Texas Heart Institute at St. Luke's Episcopal Hospital

- The IMM occupies a 31,000 square-foot high-tech laboratory.
- Located in the Texas Medical Center.

Fayez S. Sarofim Research Building
1825 Pressler Street

Tuesday, April 26, 2022
4:30 pm

SAVE THE DATE

Arming antibodies: A new strategy to fight cancer

Drug payloads and designer antibodies: How to build homing anti-cancer missiles

Kyoji Tsuchikama, PhD
Assistant Professor
Texas Therapeutics Institute

Attacking colorectal cancer stem cells with weaponized antibodies

Kendra Carmon, PhD
Assistant Professor
IMM Center for Translational Cancer Research

go.uth.edu/IMMpact

The ADCs of drug delivery

For many, the last year could be described as challenging, chaotic, and overwhelming.

Thanks to a prestigious NIH grant, Kyoji Tsuchikama, PhD, associate professor in the Institute of Molecular Medicine's Texas Therapeutics Institute, describes the last year as "amazing."

The R35 grant from the National Institutes of Health provides research support for work within the mission of the National Institute of General Medical Sciences, allowing an investigator stable funding – up to $750,000 per year for up to 5 years. Such funding allows scientists to focus broadly on their work instead of spending valuable time applying for new grant support.

Tsuchikama's new R35 grant, "Chemical approaches for generating blood-brain barrier-permeable antibody conjugates" funds research to create a foundation for new antibody agents for brain diseases, including brain cancer, by creating a more efficacious and safer drug-delivery method.

The R35 grant recognizes the labs' previous research success with antibody-drug conjugates (ADCs) treating breast tumors and resulting in two publications in *Nature Communications* in 2018 and 2021. Now he is moving on to a more challenging area of the body.

"The brain," Tsuchikama explained, "is our most protected organ, and as such, delivering drugs to it is very difficult. We need a trick to facilitate reaching the brain. Some drugs are delivered directly to the brain but are invasive – our approach is developing new technologies to facilitate systemic drug delivery of effective antibodies to the brain or brain tumors by intravenous injection."

Tsuchikama's lab focuses on creating ADCs – an alternative to chemotherapy in the treatment of cancer – that target and kill tumor cells while not affecting healthy cells. An ADC combines antibodies to specific antigens on a tumor cell with anti-cancer agents via a chemical linker.

"In the same way we have worked on breast cancer tumors, we are developing novel ADC linker technologies to treat brain diseases, particularly glioblastoma," he said.

Glioblastoma is the most common occurring malignant primary brain tumor. With about 12,000 cases diagnosed in the United States each year, the average survival time is 12-18 months after diagnosis.

"Glioblastoma is the most aggressive lethal brain tumor, and poor delivery seems to be the issue for new drugs to treat it. We are in dire need of improving the drug delivery method," Tsuchikama said.

The lab is working to take advantage of its technology platform to fight not only cancer but also leverage it for antivirals and other diseases.

"We will try other drug combinations for other non-oncology fields. We have been receiving many requests for collaboration," Tsuchikama said, adding that they will be developing the ADCs for neurological disorders, such as Alzheimer's disease, as well as other cancers such as lung and pancreas.

"We are working in animal models now and our next step is to work with experts in each field to see if our molecular platform can work in their models," he said.

The lab is also funded by the Department of Defense and the Cancer Prevention Research Institute of Texas, a state agency that funds cancer research. Scientists from across the state and nation have reached out to Tsuchikama regarding collaborative projects.

"There is no limitation or restriction on our disease target. Our ADC technology is versatile and universal," he said.

> "We need a trick to facilitate reaching the brain. Some drugs are delivered directly to the brain but are invasive – our approach is developing new technologies to facilitate systemic drug delivery of effective antibodies to the brain or brain tumors by intravenous injection." — Dr. Kyoji Tsuchikama

Kyoji Tsuchikama, PhD, and his lab have flourished during the pandemic, utilizing new technology to target a variety of diseases.

Research on the clock

During the time of the pandemic, there has been a constant – disruption. Disruptions in our daily routines, in our interactions with others, and to our sense of time.

Time is central not only to our organized lives but also to our organized bodies. Circadian (i.e. 24-hour) rhythms govern our wake-sleep cycles and responses to light and dark.

Kristin Eckel-Mahan, PhD, associate professor in the IMM's Center for Metabolic and Degenerative Diseases, focuses her research on circadian rhythms and how their disruptions increase our risk for specific diseases or disorders.

"Historically, circadian rhythms were thought to be primarily controlled by the brain because part of the hypothalamus is directly responsive to light and dark. But the truth is that circadian rhythms occur at a cellular level and in almost all cells of the body. Rhythms occur at the level of gene expression and metabolic pathway activity at the cellular level," Eckel-Mahan explained.

People with disruption in their circadian clocks – such as those who work night shifts, or stay up too late – move their natural rhythm out of sync. This often involves an eating phase no longer coordinated with the light or active phase. Research shows those out of sync increase their risk of adverse cardiovascular events such as arrhythmias, obesity, and Type 2 diabetes.

Eckel-Mahan and her colleagues seek to understand other connections to disease and circadian disruptions, including connections with obesity and aging, gastric disorders, and cancer.

Working with colleague Mikhail Kolonin, PhD, Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research, Eckel-Mahan is looking at the role of the circadian clock in fat tissue and its changes during a 24-hour cycle. "In this study, we are trying to understand how the circadian clock preserves a healthy fat pad throughout the aging of an organism," she said, adding that they study tissue from gastric bypass surgery patients.

Studying the circadian clock's role in gastrointestinal function, Eckel-Mahan is partnering with Rick Wetsel, PhD, Hans J. Muller-Eberhard, MD, PhD, and Irma Gigli, MD, Distinguished Chair in Immunology, in an investigation of the complement cascade – part of the body's immune system. "Epidemiological studies show an increased incidence of irritable bowel disease and other gastrointestinal disorders that we think involves circadian disruptions of the complement cascade," she said. "Some of these immune response pathways undergo 24-hour changes in activity, and depending on when you apply a toxin, you can get a different immune response."

Timing treatments (often referred to as "chronotherapy") also may produce different responses based on our internal circadian rhythms, Eckel-Mahan said. "The time of drug delivery may improve efficacy and reduce toxicity – this is at the forefront of all of our research," she added.

Cancer is another area Eckel-Mahan and her colleagues are studying for clock connections. Working with scientists at MD Anderson Cancer Center, Eckel-Mahan is evaluating the circadian links associated with the natural flavonoid Nobiletin, a circadian modulating compound extensively investigated by Zheng (Jake) Chen, PhD, associate professor of Biochemistry and Molecular Biology, when applied to acute myeloid leukemia cells.

Her lab is also studying its effects in the context of hepatocellular carcinoma. "We have administered this compound *in vivo* in mice and have seen accelerated cell death and delayed tumor progression in response," she explained.

Other ongoing work involves early stage liver disease, including non-alcoholic fatty liver disease, which is a direct result of the obesity epidemic and serves as a risk factor for developing fibrosis and hepatocellular carcinoma

"We think that a high-fat diet decreases the expression of a tumor suppressor in the liver that has circadian activity. The result is circadian activation of proliferation genes that promote tumor formation and progression" she said.

Just one more reason to restore balance in our lives.

Kristin Eckel-Mahan, PhD, studies circadian rhythms and the impacts of their disruptions.

Engineering a biomedical career

After a decade teaching chemical engineering, a new Institute of Molecular Medicine recruit decided to dedicate 100 percent of his effort to a new professional challenge – biomedical research.

Alex Ge, PhD, associate professor with the Texas Therapeutics Institute, joined the IMM in July 2021, serving on the faculty at the University of California, Riverside, where he taught chemical engineering to undergraduate students, in addition to biochemical research.

"Chemical engineering is a well-established discipline," he admitted. "With biomedical research, there is more freedom, and it has more impact."

Ge earned his PhD in chemical engineering at McMaster University in Canada and completed postdoctoral training at The University of Texas at Austin.

"During my postdoc training, I worked with biomedical antibodies, and that is what sparked my vision to pursue this direction," he said, adding that the IMM was the perfect opportunity to develop antibodies in a supportive research environment.

An engineering background gives Ge a unique perspective for research.

"I like challenging targets," he said. "The engineer in me likes to take things apart to solve problems. Even when I was a boy I would take things apart, and I remember my mom reminding me to put things back together."

Ge's research is funded by the National Institutes of Health (NIH) and Department of Defense and focuses on creating antibodies for treatments in areas as various as cancer, pain, stroke, obesity, and snake venom.

His R21 grant from the NIH, considered a "try out" grant on an interesting topic with great need, concerns poisonous snake bites.

According to the NIH, between 7,000-8,000 people in the United States are bitten by a venomous snake each year, with up to 44 percent sustaining long-term injury and five dying. Current antivenom therapy, over a century old, is created by injecting poisonous snake venom into horses and harvesting the resulting antibodies, which are expensive, often ineffective, and may even cause major allergic reactions.

Ge and his lab are working on a new method to treat venomous snake bites with new antibodies.

"The action of venom among snakes is similar," Ge said, "but it contains a spectrum of toxins, so there is a level of complexity. We are starting with the most significant snake in North America, the rattlesnake."

One major toxic enzyme found in rattlesnake venom interferes with the bloodstream, kicking off a reaction cascade causing severe bleeding. Ge and his colleagues are aiming to specifically block this fatal snake toxin with antibodies developed in the lab.

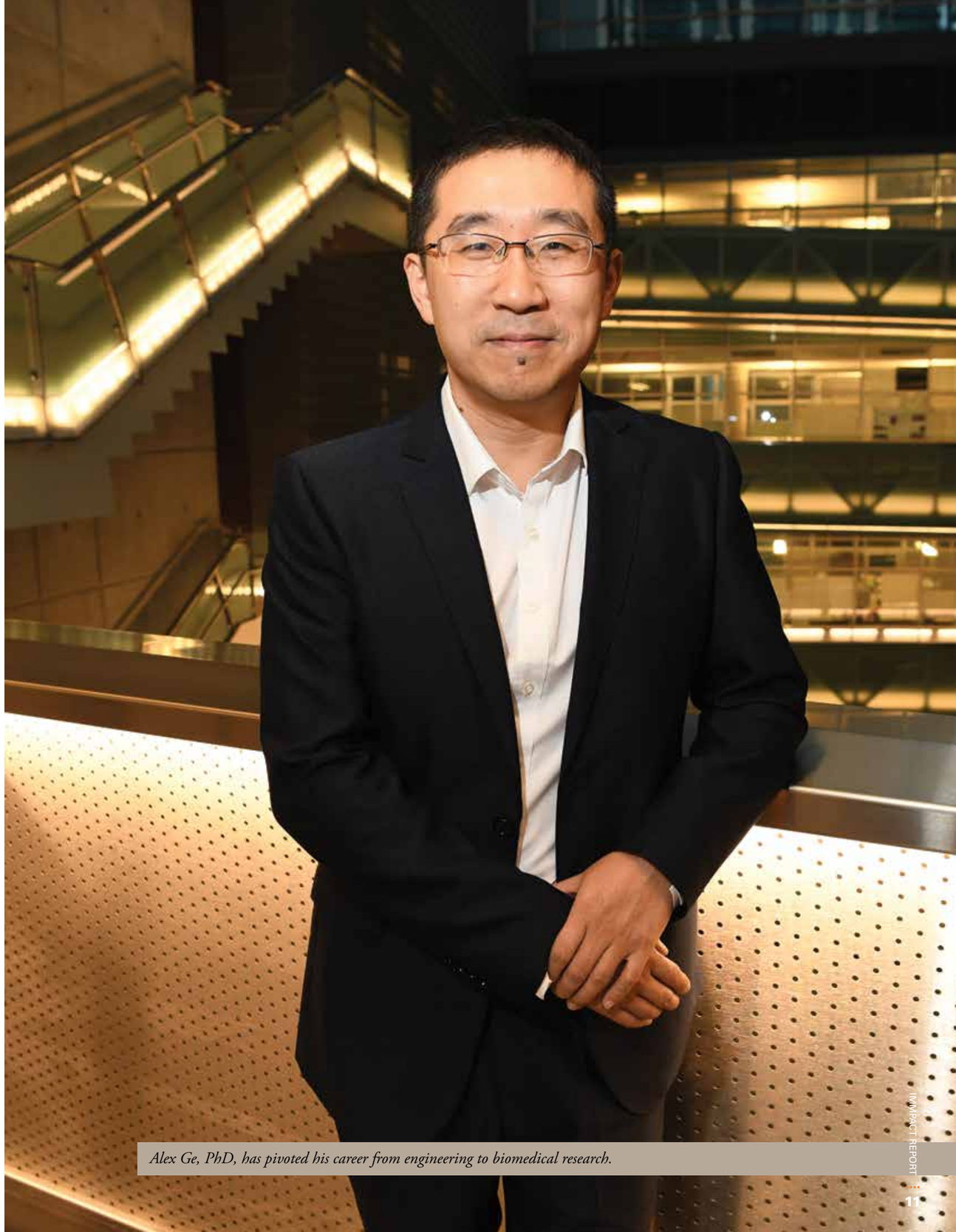
To get the venom for testing, Ge traveled to the only federally funded Viper Resource Center in the United States. Located in Kingsville, Texas, the National Natural Toxins Research Center is supported by the NIH and Office of Research Infrastructure Programs and houses many snakes.

"When we walked into the room, all of the rattles started making noise – that signals that the rattlesnake wants to strike," Ge recalled, adding that all of the snakes were in cages.

Although the current research is focused on the rattlesnake, if successful, it could be applied to other poisonous snakes.

"Sometimes you need an engineering mind to consider new ways and new technologies," he said.

> "Chemical engineering is a well-established discipline. With biomedical research, there is more freedom, and it has more impact." — Dr. Alex Ge



Alex Ge, PhD, has pivoted his career from engineering to biomedical research.

Zhiqiang An, PhD, director of the Texas Therapeutics Institute, is pioneering new technology to fight COVID.

Responding to the pandemic

When you think of a first responder, a firefighter or emergency room physician may spring to mind. Yet the COVID-19 pandemic proved scientists at the IMM's Texas Therapeutics Institute (TTI) also could adopt this designation as they quickly mobilized their labs and colleagues to help fight a worldwide threat.

Confronting the pandemic, Zhiqiang An, PhD, director of TTI, pivoted his lab and through keen innovation and collaboration created a new product – a nasal spray to prevent and treat SARS-CoV-2.

The journey to the nasal spray began with a postdoc in An's lab, Zhiqiang Ku, PhD, whose background in virology helped him to create antibodies to COVID-19 in the earliest days of the pandemic, in February 2020. Simultaneously, An's colleague from The University of Texas Medical Branch at Galveston, Pei-Yong Shi, PhD, reached out about collaborating on an engineered COVID virus.

An and his lab decided to create a therapy using IgM antibodies instead of IgG antibodies, which are currently found in emergency-use authorization COVID treatment. The IgM antibodies are 10-valent (10-armed), meaning they can bind up to 10 viral spike proteins at once, versus the IgG antibodies, which are two-armed. More "arms" provides a stronger connection and more possibilities to attach to the virus.

"I knew the virus would mutate, and we needed our antibody to work in the future," An explained of the IgM choice.

In addition to using the IgM antibodies, An said he and his group carefully chose a nasal delivery to directly target the respiratory tract, thereby using less drug and in a more convenient manner than the current intravenous antibody treatments.

"Respiratory mucosal antibodies are key to clearing SARS-CoV-2 infection and reducing viral transmission, and IgM antibodies are nature's first line of defense against pathogens such as viruses," An explained. "The current emergency-use authorization antibodies, which are all IgG antibodies, are administered intravenously at high doses and don't directly target the main sites of infection."

Research published in the July 3, 2021 issue of *Nature* found that the team's IgM antibody nasal spray provided a broad coverage of COVID variants of concern and interest and was 230 times more effective at neutralizing SARS-CoV-2 than the IgG antibody they first tested.

"Our antibody can neutralize the virus," said An, holder of the Robert A. Welch Distinguished University Chair in Chemistry.

The nasal spray, which has been licensed to IGM Biosciences for drug development, is now being tested in humans in the United States and South Africa.

An said the nasal spray could be an effective alternative to the ubiquitous mask. "Say you were going to a party and wanted to be protected – you could use the nasal spray in advance. Or, if you were at an event and someone was sick, you could use the spray afterwards and be protected for up to two weeks," An said.

The nasal spray is just one of 10 drugs currently in development from TTI. With a focus on drug discovery and development, the institute's investigators and collaborators have garnered more than $25 million in research support from NIH, CPRIT, DoD, and the biotechnology/pharmaceutical industry in the last five years.

"TTI is an antibody drug discovery platform," An said. "Our lab is working on the cutting edge and being responsive – that is what we are here to do. We are here to immediately respond to public health emergencies and create drugs that can help."

Jeff Firestone and Kathy Smyth carry on the family legacy of the Nancy and Clive Runnells Foundation and the Pierce Runnells Foundation

Supporting the IMM – All In the Family

We hold dear our family traditions– from a daily moment at the dinner table to celebrating holidays. The Institute of Molecular Medicine is proud to have been a part of the Runnells' family tradition of philanthropic support for decades.

Nancy and Clive Runnells created the Nancy and Clive Runnells Foundation in 2000, which is built upon the strong family principles of giving back.

Clive's mother, Mary Withers Runnells, instilled the value of philanthropy in Clive at a young age, recalled his widow, Kathy Smyth.

"Clive had a strong belief in philanthropy. He often said, 'if you don't do something for others, you ain't worth a !!##!!!'" Smyth said.

Clive Runnells connected to the IMM back in 2004, when he reached out to Rick Wetsel, PhD, director of the IMM's Hans J. Muller-Eberhard and Irma Gigli Research Center for Immunology, whom he had read about in the *Houston Chronicle*.

"He asked me how much it would take to jump start my research, and I told him $100,000," recalled Wetsel, holder of the Hans J. Muller-Eberhard, M.D., Ph.D. and Irma Gigli, M.D. Distinguished Chair in Immunology. "I didn't know if I was asking for too much, or too little. He told me he would have to talk to his wife."

That started Wetsel's long friendship with the Runnells, who continued to support his stem cell research over the years.

"Clive loved getting to know the grant recipients personally and made many lasting friendships," Smyth said. "His primary interests were medical research and conservation."

The generous support made a great difference for Wetsel's research.

"Those philanthropic funds have made it possible to develop four of our own stem cell lines – two of which are approved by the National Institutes of Health," Wetsel said.

The Runnells' unwavering support of stem cell research was personal. Clive and his wife Nancy's son, Pierce, suffered a debilitating back injury due to a devastating skiing accident and died before the promise of stem cell therapy could be realized.

Clive and Nancy had instilled the value of giving back in Pierce, who, in turn, created the Pierce Runnells Foundation.

Pierce died in 2007, Clive died in 2019, and Nancy died in 2016, but their foundations live on in continued support of IMM research.

Today, four trustees work together on these two family foundations to continue the legacy of Nancy and Clive Runnells and their son Pierce Runnells. Smyth leads the Nancy and Clive Runnells Foundation, and Jeff Firestone oversees the Pierce Runnells Foundation.

The foundations continue to support stem cell applications, including preclinical studies of the role of stem cells for correcting cleft palate, headed up by Charles Cox, MD, holder of the George and Cynthia Mitchell Distinguished Chair in Neurosciences; macular degeneration, overseen by Wetsel; and an investigative trial of the use of stem cells in improving stroke outcome, led by Sean Savitz, MD, director, UTHealth Institute for Stroke and Cerebrovascular Diseases and Frank M. Yatsu, M.D., Chair in Neurology.

"Since being introduced to the IMM at an IMMPact Symposium, I have had the pleasure of visiting with some of these dedicated professionals, who have dedicated their lives to their important work," Smyth said. "I am grateful for what they do, and it is an honor and a privilege to be able to support their work."

The IMM Center for Cardiovascular Genetics, established in 2006, focuses on elucidation of molecular genetics, genomics, and pathogenesis of cardiovascular diseases with the objective of utilizing the discoveries to prevent and treat cardiovascular diseases in humans. The Center provides specialized clinical services to patients with genetic cardiovascular disorders at the Cardiovascular Genetic Clinic. The Center also has a Research Clinic, which is utilized for clinical research activities, including NIH- and industry-sponsored clinical trials.

Mission: To prevent and treat cardiovascular diseases in humans through identification and targeting of the pathogenic genes and pathways.

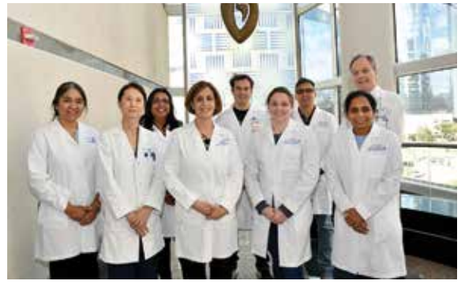
Faculty: Priyatansh Gurha, PhD, assistant professor; Sirisha M. Cheedipudi, PhD, instructor; AJ Marian, MD, professor

General theme of the research programs: The research programs at the Center start with human molecular genetic studies aimed at identifying the causal genes for human cardiovascular diseases. The focus is primarily on hereditary cardiomyopathies, which are important causes of sudden cardiac death and heart failure. Genetic analysis is performed by whole exome and genome sequencing. Genetic discoveries are then coupled with the genomic studies to identify differentially expressed coding and non-coding transcripts and dysregulated pathways, chromatin remodeling, and DNA methylation in cardiomyopathies. The integrated approach is used to identify the key dysregulated pathogenic pathways for preventive and therapeutic genetic and pharmacological interventions. The findings in the model systems are extended to human patients through pilot randomized placebo-control double-blind studies clinical trials. The findings provide the platform for large-scale multi-center efficacy clinical trials.

Research Programs:

The research programs are as follows:

I. Human molecular genetic studies of cardiomyopathies: We have a repository of several hundred cases and their family members with cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy (ACM). Pathogenic and causal variants are identified by whole exome sequencing in the probands and family members. These studies have identified new disease-causing genes and have advanced the genetic causes of heart failure. We



are actively recruiting additional probands and family members.

II. Genomics and epigenetic studies of human heart failure and mouse models of cardiomyopathies: The studies predominantly relate to DCM and ACM and include whole transcriptome analysis by RNA-Seq, DNA methylation analysis, and analyzing chromatin remodeling by ChIP-Sequencing. Specific epigenetic regulators of gene expression are identified and targeted in order to delineate their functions in the heart.

III. DNA damage response in human hereditary cardiomyopathies: We have detected increased double stranded DNA breaks (DSBs) in human hearts from patients with hereditary cardiomyopathies and in mouse models. Studies are ongoing to define genomic characteristics of the DSBs and to define the pathogenic role of DNA damage response pathways in heart failure.

IV. Therapeutic targeting of dysregulated pathways in cardiomyopathies: Dysregulated pathways identified through integrated genomics are targeted through genetic and pharmacological interventions in model organisms and their effects on survival, cardiac function, and clinical outcomes are analyzed. A major focus currently is on the canonical WNT and the Hippo signaling pathway.

V. Clinical Studies: The Center participates in investigator–initiated single center pilot clinical trials as well as industry-sponsored multi-center clinical trials in hereditary cardiomyopathy. An NIH-sponsored double-blind randomized pilot study (HALT-HCM) in patients with HCM was recently completed. The Center also participates in industry sponsored clinical trials in cardiomyopathies.

AJ Marian, MD
Center Director & Professor

AJ Marian, MD
Professor and Director of the Center for Cardiovascular Genetics
James T. Willerson Distinguished Chair in Cardiovascular Research

Molecular genetics, genomics, pathogenesis, and treatment of hereditary cardiomyopathies

Our long-standing research objectives have been to delineate the molecular genetics, genomics, and pathogenesis of hereditary cardiomyopathies in humans and apply the discoveries to prevent the evolving and reverse the established phenotypes of heart failure and sudden cardiac death. We have active research programs in three common forms of hereditary cardiomyopathies:

Arrhythmogenic Cardiomyopathy (ACM): ACM is an enigmatic form of hereditary cardiomyopathies that clinically presents with cardiac arrhythmias, heart failure and sudden cardiac death, particularly in the young. A unique feature of this disease is a gradual replacement of cardiac myocytes with fibro-adipocytes. There is no effective therapy for ACM.

Hypertrophic Cardiomyopathy (HCM): HCM is the most common form of hereditary cardiomyopathies, affecting ~ 1 in every 500 individuals in the general population. The affected individuals are typically asymptomatic and sudden cardiac death is often the first manifestation of this disease. HCM is the most common cause of sudden cardiac death in the young. While there are effective therapies to alleviate patient's symptoms, there is no effective therapy to prevent or reverse the disease process.

Dilated Cardiomyopathy (DCM): DCM is genetically the most heterogeneous form of hereditary cardiomyopathies and a major cause of heart failure and heart transplantation in the young. The affected individuals often present with symptoms of heart failure, cardiac arrhythmias and sometimes, sudden cardiac death. There are a number of effective pharmacological and non-pharmacological therapies for DCM, but currently there is no cure for DCM.

The overall approach integrates human molecular genetic studies through high throughput whole exome and genome sequencing to identify the causal genes and the pathogenic variants, followed by genomic studies including transcriptomics and epigenetics to define the molecular remodeling of chromatin in the presence of causal mutations. The aim is to identify the pathogenic pathways and intervene, genetically or pharmacologically, to prevent, attenuate, or reverse the phenotype, initially in mouse models and ultimately in human patients.

RESEARCH PROJECTS

- Identification of causal genes for heart failure and sudden cardiac death with a focus on oligogenic nature of the phenotype in small families and sporadic cases
- Delineation of the role of the mechano-sensing signaling pathways, namely the Hippo and the canonical WNT pathways, in the pathogenesis of hereditary cardiomyopathies.
- Defining and characterizing the role of DNA damage response pathway in hereditary cardiomyopathies and the beneficial effects of blocking the DNA damage sensor CGAS in attenuating the heart failure phenotype.
- Multi-center phase II/III double-blinded placebo-controlled clinical trials to test efficacy of a myosin heavy chain 7 ATPase modulator on improving symptoms, exercise tolerance and left ventricular outflow tract obstruction in patients with obstructive hypertrophic cardiomyopathy.

KEY PUBLICATIONS

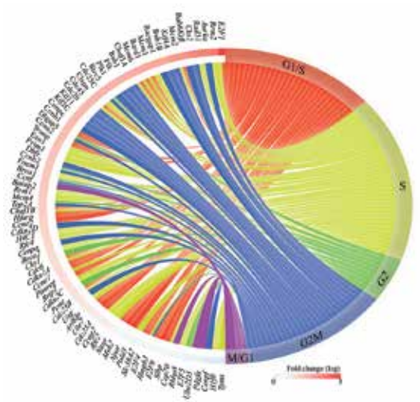
A combinatorial oligogenic basis for the phenotypic plasticity between late-onset dilated and arrhythmogenic cardiomyopathy in a single family. Pourebrahim K, Marian JG, Tan Y, Chang JT, Marian AJ.*J Cardiovasc Aging*. 2021;1:12. doi: 10.20517/jca.2021.15. Epub 2021 Sep 3.PMID: 34790974

Pharmacological suppression of the WNT signaling pathway attenuates age-dependent expression of the phenotype in a mouse model of arrhythmogenic cardiomyopathy. Cheedipudi SM, Fan S, Rouhi L, Marian AJ.*J Cardiovasc Aging*. 2021;1(3):10.20517/jca.2021.04. doi: 10.20517/jca.2021.04. Epub 2021 Jun 6.PMID: 34447973

The EP300/TP53 pathway, a suppressor of the Hippo and canonical WNT pathways, is activated in human hearts with arrhythmogenic cardiomyopathy in the absence of overt heart failure. Rouhi L, Fan S, Cheedipudi SM, Braza-Boïls A, Molina MS, Yao Y, Robertson MJ, Coarfa C, Gimeno JR, Molina P, Gurha P, Zorio E, Marian AJ.*Cardiovasc Res*. 2021 Jun 16:cvab197. doi: 10.1093/cvr/cvab197. Online ahead of print.PMID: 34132777

LAB MEMBERS

Research instructor : Sirisha C. Marreddy
Post-doctoral fellows : Leila Rouhigharabaei, PhD, Melis Olcum, Benjamin Cathright
Research technician : Saman Asghari
Research and clinical nurse : Yanli Tan, RN



Increased transcript levels of genes involved in cell cycle progression in cardiac myocytes isolated from the *Myh6-Mcm-Ctnnb1*^{6oF} (gain-of-function of the canonical WNT) mice. A. Circos plot depicting different phases of cell cycles and the differentially expressed genes assigned to each phase. B. Tabular listing of the differentially expressed genes in different phases of cell cycle and changes in their expression levels, shown as fold change.

Priyatansh Gurha, PhD
Assistant Professor

Molecular mechanisms and functions of Non-coding RNAs and epigenetic regulation in heart failure

The main objective of my research is to understand the molecular mechanisms that coordinately regulate gene expression and contribute to the pathogenesis of heart failure. Within this theme, we are studying the function of epigenetics and non-coding RNAs in proliferation, differentiation, and maturation of myocytes and how alteration of these interlinked processes eventually leads to cardiac dysfunction and failure. My previous studies have identified epigenetic dysregulation of miR-184 and its role in the pathogenesis of ACM. We have now begun to investigate how reprogramming of epigenetic code governs gene transcription and ensuing cardiac phenotype in heart failure (HF). Recently, we uncovered the role of DNA methylation and Lamin Associated Domain in Human HF and identified an epigenetic regulator KDM5A and B, and a novel cardiac myocyte enriched long intergenic non-coding RNA (lincRNA) in the phenotypic manifestation of HF. The role of KDM5A and B and CM enriched lincRNAs in the heart is unknown. We are using induced pluripotent stem cells (iPSCs) and several mouse models to investigate the tissue and cell type-specific contribution of these regulators in cardiac physiology and their contribution toward human HF.

RESEARCH PROJECTS

- Role of lncRNAs in the pathogenesis of cardiomyopathies and heart failure
- Identification and characterization of molecular mechanisms and functions of Lysine demethylase KDM5 in cardiomyopathies and heart failure.

KEY PUBLICATIONS

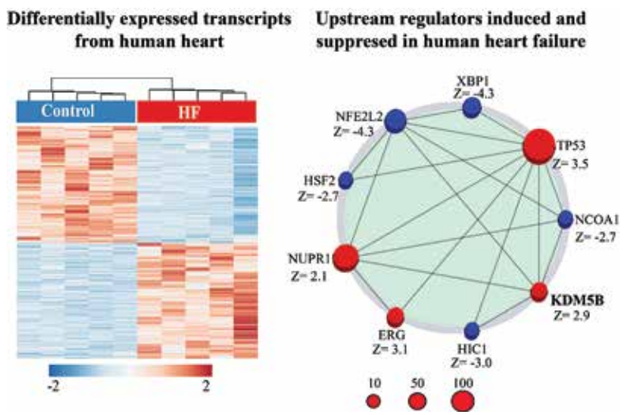
Coste Pradas J, Auguste G, Matkovich SJ, Lombardi R, Chen SN, Garnett Chamberlain K, Riyad JM, Weber T, Singh SK, Robertson MJ, Coarfa C, Marian AJ, Gurha P. Identification of Genes and Pathways Regulated by Lamin A in Heart. *Journal of American Heart Association*. 2020 Aug 18; 9(16): e015690. PMID: 32805188.

Marreddy Cheedipudi S, Matkovich SJ, Coarfa C, Hu X, Robertson MJ, Sweet ME, Taylor M, Mestroni L, Cleveland JC, Willerson JT, Gurha P#, Marian AJ#. Genomic Reorganization of Lamin-Associated Domains in Cardiac Myocytes is Associated with Differential Gene Expression and DNA Methylation in Human Dilated Cardiomyopathy. *Circ Res*. 2019 Feb 11. PMID: 30739589 (# Co-corresponding authors).

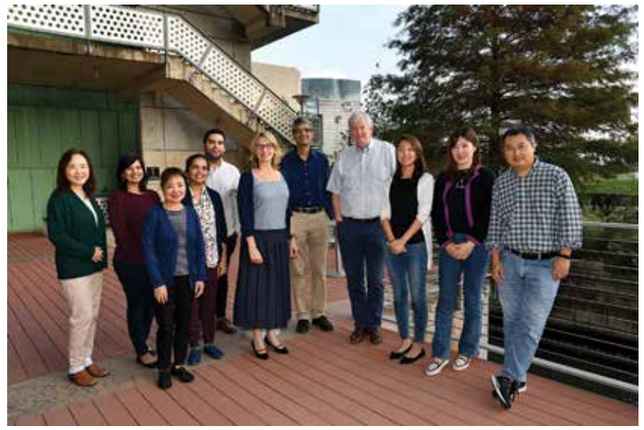
Gurha P*#, Chen X*, Lombardi R, Willerson JT, Marian AJ #. Knockdown of Plakophilin 2 Downregulates miR-184 Through CpG Hypermethylation and Suppression of the E2F1 Pathway and Leads to Enhanced Adipogenesis *In Vitro*. *Authors contributed equally *Circ Res*. 2016 Sep 2; 119(6):731-50 (# Co-corresponding authors).

LAB MEMBER

Post-doctoral fellow: Manisha Deogharia



Heat plot of differentially expressed transcript and dysregulated upstream transcriptional regulators in human heart failure (HF).



The Center for Human Genetics works to generate new understanding about genetic risk for common cardiovascular diseases and to use that information to identify pathways leading to new therapies for these diseases. High blood pressure is an amplifying element that drives cardiovascular disease risk from stroke, heart and kidney disease. These diseases emerge in middle and later life and so are interlinked with the normal processes of aging. The genetic variation that makes us unique individuals and that has been passed to us from our parents impacts our risk of these diseases. Our work targets the identification of genes that contribute to cardiovascular diseases and the mechanisms by which variation in these genes re-shape the biological pathways in which disease emerges.

An emerging concept developing in our laboratories is that an important element of chronic disease of the cardiovascular system is that these diseases involve a persistent state of inflammation. For example, in atherosclerosis the blood vessel wall is invaded by immune cells and the danger posed in atherosclerotic plaques may reflect the ongoing level of inflammation in them. We need a better understanding of these processes of "sterile inflammation" in which our immune systems become activated in response to the emergence of damage to our tissues. We need greater understanding of the genetic variants that determine whether these inflammatory responses subside or remain active or even advance. In order to adapt to the continuous and rapid mutation of pathogens like viruses and bacteria, our immune systems harbor extensive genetic variation. Such variation can provide us a head-start in responding to new or evolving pathogens. But it also can create risk of disease later in life. As our living standards have increased and our lives have lengthened, the advantages provided earlier in life can turn into threats to our health by increasing our risk of chronic cardiovascular and cerebrovascular disease.

Progress in the laboratories of our investigators continues to yield exciting and important insights. Our human population geneticists, working under the direction of Dr. Myriam Fornage, are global leaders in their field and are making notable progress in the study of susceptibility to stroke and age-related decline in cognitive function. A significant fraction of sudden cardiac death results from rhythm disruptions that arise in genetic variation in the proteins processing the electrical activity within the heart. Our colleague, Dr. Ashish Kapoor, is an emerging leader in this field. Dr. Doris and his group have shown that kidney injury associated with increased blood pressure results from the emergence of auto-antibodies that damage tissues. His work has led to a recognition that we currently cannot assess – the role of complex hypervariable immune genes in disease risk – so he is using cutting-edge genome sequencing and assembly methods to overcome this limitation. Our understanding of the complexity of information storage and retrieval in the genome continues to expand. Our colleague Dr. Sidney Wang is addressing approaches to assess, extract, and exploit new levels of genomic complexity that will inform work in this field.

Common cardiovascular disease will eventually impact us or someone close to us. In the Center for Human Genetics, we have the opportunity to work for change, pushing forward the knowledge and moving toward new insights and new opportunities for disease prevention.

Peter A. Doris, PhD
Center Director & Professor
Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research

Peter A. Doris, PhD
Professor/Center Director
Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research

Genetics of cardiovascular end organ injury

High blood pressure is a frequent cause of renal injury, but the risk of renal disease in patients with high blood pressure is best predicted by family history, indicating a genetic predisposition. At present, we have almost no knowledge of why high blood pressure creates kidney disease in some people but not others. To try to fill this knowledge gap, we study a genetic model comprising inbred laboratory rats that have high blood pressure. The divergence of hypertensive renal disease risk seen in humans is also present in these rats. Some lines get progressive renal injury, and other lines don't. Therefore, this model provides a means to investigate what genetic differences can drive kidney disease. We can take what we have learned and conceive of treatment approaches to prevent disease and test them in the model.

What we have learned so far:

Genes influencing antibody formation affect the emergence of hypertensive renal disease.

We have identified important genetic variation in the immunoglobulin heavy chain gene, which encodes antibodies. We have also identified genetic deletion in the gene, Stim1. This is a key gene in lymphocyte function. T and B lymphocytes comprise the adaptive immune system. The mutation in Stim1 blocks normal T and B cell function and leads to antibody-mediated autoimmune disease.

Gut bacteria activate the hypertensive immune system and create antibodies that cause disease

When hypertensive rats unable to produce antibodies are raised without antibody replacement, they experience blood infection (sepsis). Blood culture indicates that the infecting bacteria are non-pathogenic bacteria that live in the gut. When antibiotics are given to hypertensive rats prone to injury, renal injury was markedly reduced. The bacteria induce antibodies to a common bacterial protein. This protein is highly conserved in mammals as well as bacteria. These antibodies may prevent this protein from functioning to protect the kidney from pressure-induced injury.

Genome sequencing to understand complex disease traits

We have recently begun an NIH-funded project to provide de novo assemblies of the genomes of inbred rat models of human cardiovascular disease. This project uses the most recently developed sequencing technologies and will include multi-tissue annotation of gene expression.

Key questions that are the focus of our current interest:

Do the pathogenic mechanisms active in rats give insight into renal disease in humans? Common genetic variants occur in humans that alter the control of antibody formation and may contribute to disease risk.

KEY PUBLICATIONS

Dhande, I.S., S.C. Kneedler, Y. Zhu, A.S. Joshi, M.J. Hicks, S.E. Wenderfer, M.C. Braun, P.A. Doris. Natural genetic variation in Stim1 creates stroke in the spontaneously hypertensive rat. *Genes and Immunity*. 2020. PMID 32300198

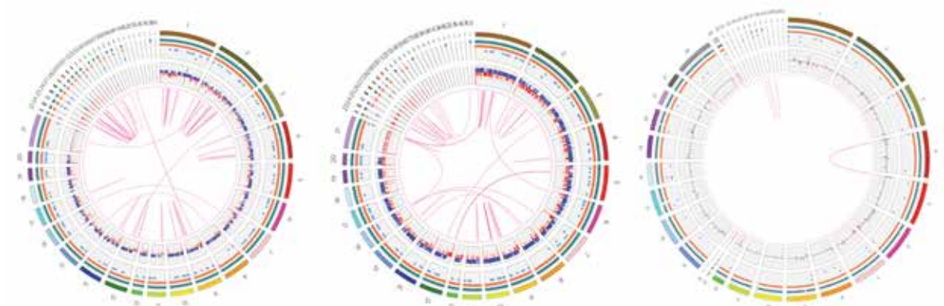
Dhande, I.S., Y. Zhu, S.C. Kneedler, A.S. Joshi, M.J. Hicks, S.E. Wenderfer, M.C. Braun, P.A. Doris. Stim1 polymorphism disrupts immune signaling and creates renal injury in hypertension. *J. Amer. Heart Association*. 9(5):e0141422019, 2020. PMID 32075490

Dhande, I.S., and P.A. Doris Genomics and inflammation in cardiovascular disease. *Comprehensive Physiology*, 11(4):1-22, 2021. PMID: 34570903

Dhande, I.S., M.C. Braun and P.A. Doris. Emerging insights into chronic renal disease pathogenesis in hypertension from human and animal genomic studies. *Hypertension,* 78(6): 1689–1700, 2021 PMID 34757770

LAB MEMBERS

Instructor: Isha S. Dhande, PhD
Research assistants: Yaming Zhu, MD; Aniket Joshi, BS



Improving the structural accuracy of the rat genome. These "circos" plots of show rat chromosomes that have been assembled by optical mapping. This method uses nanofluidics to identify the relationship between very long strands of DNA form the genome. Going from right to left we see that the newer genome alignment shows important differences from an older version that did not rely on optical mapping. The middle plot shows something very similar for one of the cardiovascular disease models we work with. The rightmost plot shows how improved genome assembly with optical mapping results in much better concordance indicating fewer genome errors.

Myriam Fornage, PhD
Professor
The Laurence and Johanna Favrot Distinguished Professorship in Cardiology

Molecular epidemiology of the aging brain

Throughout our lifetime, our brain changes more than any other part of our body. Beginning in midlife, aging brings about subtle changes in brain structure, chemistry, and function. These changes are detectable by neuroimaging techniques, such as magnetic resonance imaging (MRI), and are associated with a greater risk of future stroke, cognitive and functional impairment, dementia, and death. Current "omics" technologies provide us with high-dimensional information about the sets of biological molecules that make up cells, tissues, and organisms on a population scale. These include, for example, the entire genome sequence of a person and extensive data about the circulating metabolites and proteins in their blood. Our laboratory uses advanced computational techniques to make sense of this information with the goals to discover novel biomarkers for risk prediction and to enable informed preventive and therapeutic interventions to slow or reverse brain aging and cardiovascular disease.

High blood pressure, or hypertension, is a leading cause of stroke and mortality. While we have successfully identified many genetic factors influencing hypertension, few of these studies have considered the interaction of genes with environmental or more generally non-genetic factors (GxE interaction). The detection of GxE effects is important because it may allow us to more precisely predict individual disease risk in the context of potentially modifiable environmental, lifestyle, and behavioral risk factors. Psychological and social factors are known to influence blood pressure. We have therefore conducted genetic analyses taking into account the interaction effects of genetic variants with three psychosocial factors: depression, anxiety, and social support. We identified nine novel regions of the genome, which harbor genes implicated in the nervous system, the immune response, and in neuropsychiatric or stress-related disorders.

Besides genetic factors, we also study the link between other molecules, such as DNA methylation, proteins, and metabolites with disease of the aging brain. For example, we used data on almost 5,000 proteins in the blood of 4,110 older adults and analyzed their association with the risk of developing dementia over a period of five years. Using genetic data, we demonstrated a causal role for two dementia-associated proteins in Alzheimer's disease. These proteins have functions in the immune response and inflammation, underscoring the central role of the immune system in the risk of dementia.

RESEARCH PROJECTS

- Discovering DNA sequence variants influencing Alzheimer's disease, stroke and neuroimaging markers of brain aging.
- Discovering novel epigenetic (DNA methylation) variants that influence risk for brain small vessel disease and its related neurocognitive outcomes.
- Discovering novel genetic variants for high blood pressure using gene-lifestyle interactions and pathway analysis. In particular, discovering how depression and anxiety affects genetic risk of hypertension.
- Investigating the genetic determinants of cognitive function in diverse Hispanics/Latinos

KEY PUBLICATIONS

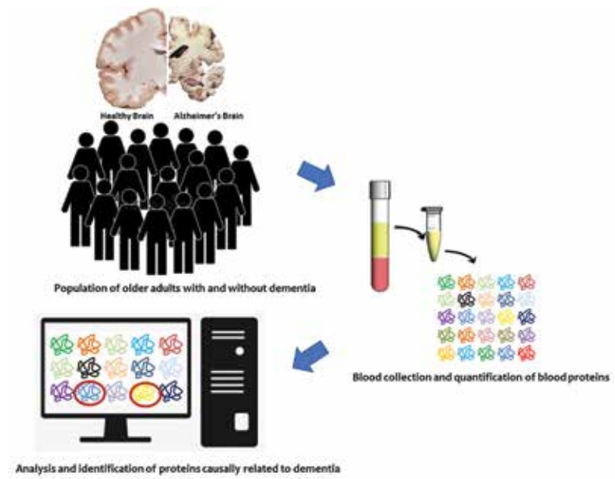
Sun D., Richard M., Musani S.K., et al., & Fornage M. Multi-Ancestry Genome-wide Association Study Accounting for Gene-Psychosocial Factor Interactions Identifies Novel Loci for Blood Pressure Traits. *HGG Adv.* 2021; 2:100013.

Walker K.A., Chen, J., Zhang, J., Fornage M. et al. Large-scale plasma proteomic analysis identifies proteins and pathways associated with dementia risk. *Nat Aging.* 2021; 1:473–489.

Hu Y., Haessler J.W., Manansala R., Wiggins K.L., et al. Whole-Genome Sequencing Association Analyses of Stroke and Its Subtypes in Ancestrally Diverse Populations From Trans-Omics for Precision Medicine Project. *Stroke.* 2021; Online ahead of print

LAB MEMBERS

Post-doctoral fellow: Yunju Yang, PhD
Graduate students: Songmi Lee (PhD program); Nitesh Enduru (MPH program)
Biostatisticians: Bin Shi, PhD; Emy Thomas, MS; Rui Xia, PhD
Research associate: Ping Wang, PhD



Novel proteomics analyses integrated with genetic analyses afford new opportunities to identify alterations of the blood proteome that are indicative of dementia and to identify candidate proteins that can serve as a biomarker for dementia diagnosis.

Ashish Kapoor, PhD
Assistant Professor

Role of non-coding cis-regulatory sequence variation in cardiac arrhythmias and sudden death risk

Despite the progress in the prevention and treatment of cardiovascular diseases in general, sudden cardiac death (SCD) remains a major public health problem. SCD, defined as a sudden and an unexpected pulseless condition due to a cardiac arrhythmia (when heart beats out of rhythm) without evidence of a non-cardiac cause, is the leading cause of deaths in the United States (~500,000 each year) and accounts for ~15% of all-cause deaths and ~50% of deaths from cardiovascular diseases. Moreover, in almost half the cases, SCD is the first sign of an underlying cardiovascular condition. Although many forms of heart disease can lead to SCD, the most common process underlying SCD is ventricular fibrillation (VF), an irregular and uncoordinated contraction of cardiac muscles of ventricles (lower chambers of heart) due to disorganized electrical signals. VF is usually fatal if not reversed by defibrillation immediately. Most of the existing cardiovascular risk factors are poor at predicting SCD, even in those individuals with a history of heart disease, clearly showing that other environmental and/or genetic factors are likely to play a role in developing VF and SCD. Indeed, from population- and family-level studies there is evidence for genetic susceptibility to SCD. However, studies to identify genetic factors underlying susceptibility to SCD directly have had limited success due to pooling of the very diverse forms of heart diseases leading to SCD into one group. Instead, we focus on the electrocardiographic QT interval, an intermediate observable characteristic/trait (phenotype) that predisposes to SCD. Electrocardiography, also known as ECG, measures the electrical activity of heart chambers and the QT interval in an electrocardiogram corresponds to the time taken by ventricles to depolarize (activated state) and repolarize (resting state) in every heart beat. In the general population QT interval varies across individuals and is a useful clinical marker as both prolongations and shortenings of the QT interval have been known to be associated with increased risk of cardiac arrhythmias and SCD. We are interested in identifying the genes that underlie this variation with the aim that understanding the genetic factors for QT interval variation will potentially impact our understanding of SCD risk and its management. Our studies have the prospect to identify the genetic causes for QT interval variation, some of which in turn could serve as potential therapeutic (drug) targets or potential biomarkers (genes and gene products) to identify individuals at high risk for SCD. What we as a community have learned so far is that many genes together contribute to QT interval variation and that majority of DNA changes leading to QT interval variation do so not by altering the form of the gene product rather by altering the amount of the gene product made by our heart cells. Starting with known genetic associations between DNA sequence variants and the QT interval in the general population, our work involves pinpointing the causes behind these associations to identify the underlying gene defects and how they impact QT interval.

RESEARCH PROJECTS

- Molecular characterization of QT interval genome wide association study (GWAS) signals to identify the underlying causal variants, genes, and their mechanisms.
- Evaluation of constitutive, cardiac- and nervous-system restricted *Nos1ap* null mice to understand its role in cardiac electrophysiology.
- Functional genomic approaches to understand cardiac gene expression regulation.

KEY PUBLICATIONS

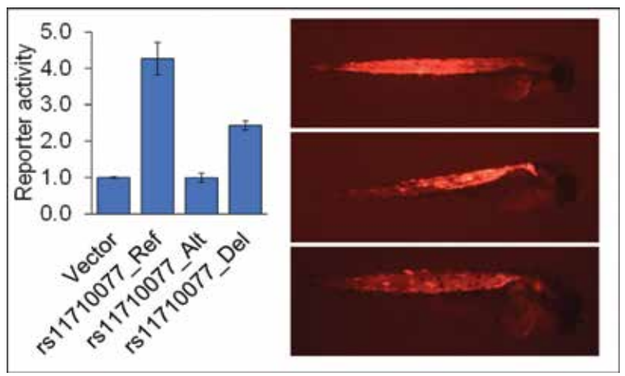
Kapoor A, Nandakumar P, Auer DR, Sosa MX, Ross H, Bollinger J, Yan J, Berrios C, HDRC, Chakravarti A. Multiple, independent, common variants at *RET, SEMA3* and *NRG1* gut enhancers specify Hirschsprung disease risk in European ancestry subjects. *Journal of Pediatric Surgery* 10.1016/j.jpedsurg.2021.04.010, 2021.

Kapoor A, Lee D, Zhu L, Soliman EZ, Grove ML, Boerwinkle E, Arking DE, Chakravarti A. Multiple SCN5A variant enhancers modulate its cardiac gene expression and the QT interval. *Proceedings of the National Academy of Sciences* 116:10636-45, 2019.

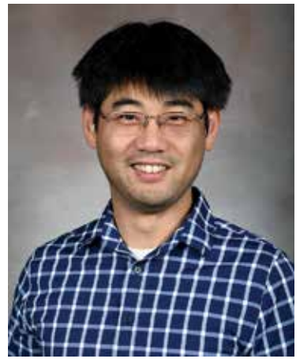
Lee D, Kapoor A, Safi A, Song L, Halushka MK, Crawford GE, Chakravarti A. Human cardiac cis-regulatory elements, their cognate transcription factors, and regulatory DNA sequence variants. *Genome Research* 28:1577-88, 2018.

LAB MEMBERS

Research assistant: Ernesto Sanchez, BS
Undergraduate trainees: Supraja Kadagandala



QT interval associated common noncoding variant rs11710077 at SCN5A acts as a cis-regulatory element with allele-specific *in vitro* luciferase reporter activity in HL1 cardiomyocytes (left). Representative images of *in vivo* transient enhancer activity of rs11710077 reference allele in 3 days old zebrafish larvae (right).



Sidney Wang, PhD
Assistant Professor

Deciphering the regulatory code: A functional genomics approach to protein translation

Regulation of gene expression is fundamental to a wide range of biological processes. From cell fate determination during development to malignant transformation during tumorigenesis, precise control of gene expression forms the basis of these processes. Our current understanding of gene regulation is, however, far from complete. Most published studies that profile gene expression are transcript-centric (i.e. they focus on measuring mRNA levels and levels of transcription factor binding). While these efforts revealed intricate networks of cooperativity amongst transcription factors in shaping complex biological processes, much of the post-transcriptional regulation are left unexplored. It remains unclear whether the process of protein translation is regulated by a network of factors to an extent of complexity similar to transcription regulation. We ask questions such as, "Do sequence specific RNA binding proteins (RBP) cooperate in controlling translation?" "Are there translational regulatory networks that orchestrate critical biological processes?" Our research program focus on addressing these questions in biological contexts that are relevant to human health. Our immediate goals are to develop novel tools to systemically study RBP binding; to investigate regulatory functions of upstream Open Reading Frames (uORFs); and to integrate these functional genomics annotations with results from genetic studies in order to fine map the regulatory variants and to provide mechanistic understanding for disease associated variants.

RESEARCH PROJECTS

- Regulation of protein translation by uORF in stress response. Translation regulation by uORF has long been hypothesized based on supports from studies of a handful of uORFs. We have reported a systemic survey of uORF impact on protein translation and identified genetic variants associated with this impact (Figure 1). We are further expanding this line of research in the context of stress response, where global scale changes in translational regulation are expected.
- Using RNA binding protein footprint sequencing to investigate translational regulation of protein synthesis. RNA binding proteins are known to regulate protein translation. We aim to develop a general and effective tool to facilitate research in this area.
- Identification of functional novel coding regions across multiple tissues. We have previously identified 7,273 novel coding regions from a single cell type using ribosome profiling data. While we provided evidence of active translation at these loci, the biological function and importance of these loci remains unknown. We are following up on this line of research by designing knockout screens to identify loci that are essential for cell survival. We are also expanding our efforts in identifying novel coding regions through performing ribosome profiling experiments in additional cell types and tissues.
- Gene expression buffering at the post-translational level. Gene expression at the transcript level are often assumed to propagate to the protein level. In a series of studies, we have demonstrated that, in our cell line model system, the variations observed at the transcript level is often buffered at the protein level through post-translational processes (Figure 2). In order to evaluate how general this observation is, we are now expanding our analysis to other tissue types and species.

KEY PUBLICATIONS

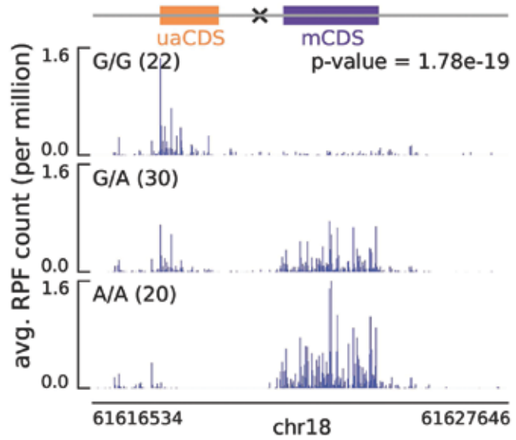
Raj A, Wang SH, Shim H, Harpak A, Li YI, Engelmann B, Stephens M, Gilad Y, Pritchard JK. Thousands of novel translated open reading frames in humans inferred by ribosome footprint profiling. *eLife.* 2016 May 27; 10.7554/eLife.13328

Wang SH, Hsiao CJ, Khan Z and Pritchard JK. Post-translational buffering leads to convergent protein expression levels between primates. *Genome Bio.* 2018 Jun 27;19(1):83.

Wang SH and Elgin SCR. The impact of genetic background and cell lineage on the level and pattern of gene expression in position effect variegation. *Epigenetics & Chromatin.* 2019; 12(70)

LAB MEMBERS

Post-doctoral fellow: Sandeep Bansal



Genotype of a genetic variant is associated with uORF regulation of protein translation at HMSD locus in HapMap LCL. Negative correlation in the levels of protein translation between the two Open Reading Frames at HMSD locus is clearly shown through stratifying ribosome profiling data by genotype.

The investigators of the Hans J. Müller-Eberhard and Irma Gigli Center for Immunology and Autoimmune Diseases are examining the molecular, cellular, and genetic bases of several different allergic, autoimmune, and infectious diseases.

These studies explore the nature, structure, and function of specific cell membrane receptors and their ligands in modulating immune and inflammatory responses.

In concert with the molecular studies, the Center's scientists have engineered mice with specific targeted gene mutations or deletions that are used as models for human disease. These animal studies have facilitated the identification of key gene products that play significant roles in regulating the immune system, as well as contributing to the pathogenesis of human disease.

Results from these research efforts have identified several therapeutic targets for the treatment of asthma, septic shock, and lupus erythematosus.

The Center recently established a robust research program focused on the development of stem cell therapeutics for the treatment of acute and chronic lung diseases and for genetic deficiencies that affect normal lung function as well as for major eye diseases, including macular degeneration and diabetic retinopathy.

Research interests include:

- Asthma and Sinusitis
- Diabetic Retinopathy
- Mucosal Immunology & Autoimmunity
- Microbial Infectious Disease
- Acute Lung Injury and COPD
- Lung Surfactant Deficiencies
- Macular Degeneration
- Pulmonary Regenerative Medicine

Rick Wetsel, PhD
Center Director & Professor
Hans J. Müller-Eberhard, MD, PhD and Irma Gigli, MD Distinguished Chair in Immunology

Rick Wetsel, PhD
Professor and Director of the Center for Immunology and Autoimmune Diseases
Hans J. Müller-Eberhard, MD, PhD and Irma Gigli, MD Distinguished Chair in Immunology

Innate immunology, inflammation, infectious diseases, and stem cell therapeutics for diseases of the lung and eye

Chronic diseases of the lung and eye are often the result of dysregulation of the immune and inflammatory response to pathogenic or toxic substances, resulting in the destruction of healthy tissue, establishment of debilitating pathologies due to fibrosis, and impairment of normal tissue repair mechanisms. However, the paucity of cellular and molecular knowledge regarding lung and eye immunity, inflammation, and repair processes has slowed the development of novel therapeutics that could be used for the effective treatment of chronic diseases of the lung and eye. Accordingly, our laboratory has for the past several years focused on delineating the key molecules that mediate the inflammatory and immune responses in the lung and eye during both normal and pathological conditions. Much of this research has involved studies of the complement system. The complement system is a major arm of the innate immune system and is well known for being the first line of defense against bacterial and viral pathogens. It is comprised of over 30 plasma proteins and cellular receptors. It has become evident in the past decade that the complement system is very important in biological functions other than killing bacteria and viruses. These other functions include tissue regeneration, polarization of immune cells including T-cells, and normal development of the central nervous system. In addition to these novel complement biological functions, dysregulation of the complement system has been discovered as a major cause of AMD and a major contributor to lung diseases such as asthma and COPD. To determine the overall importance and biological functions of complement, we have generated numerous "knock-out" mice in which the genes encoding specific complement proteins, regulators, and cell receptors have been selectively ablated by gene targeting and homologous recombination using mouse embryonic stem cells. The generation of these mice has facilitated the discovery of numerous biological roles of complement in the pathogenesis of various disease pathologies. For example, in studies using mice in which the C3a receptor was deleted, we discovered that the complement anaphylatoxin peptide C3a is an important mediator of key hallmarks of asthma, including airway hyperresponsiveness, and therefore may prove to be an excellent therapeutic target for the treatment of asthma. As part of this overall research program, we are investigating the therapeutic use of embryonic (hES) and induced pluripotent (iPS) stem cell derived cells for repair of damaged retina in AMD, for regeneration of the damaged lung epithelium in acute lung injury, and for cell based gene therapy for newborns born with genetic deficiency of surfactant protein B.

RESEARCH PROJECTS

- Determine how the function of vascular and lymphatic endothelial cells are impacted by complement during the immune response
- Generate "universal donor" embryonic stem cell lines that can be differentiated into transplantable cells that will not be rejected after transplantation
- Evaluate the therapeutic potential of gene corrected iPS cell-derived lung cells for surfactant protein deficiencies
- Develop hES-retinal pigment epithelial cells therapeutics for treatment of AMD

KEY PUBLICATIONS

Simmons KT, Mazzilli JL, Mueller-Ortiz SL, Domozhirov AY, Garcia CA, Zsigmond EM, Wetsel RA. Complement Receptor 1 (CR1/CD35)-expressing retinal pigment epithelial cells as a potential therapy for age-related macular degeneration. *Mol Immunol*. 2020:118:91-98. (PMID: 31862673).

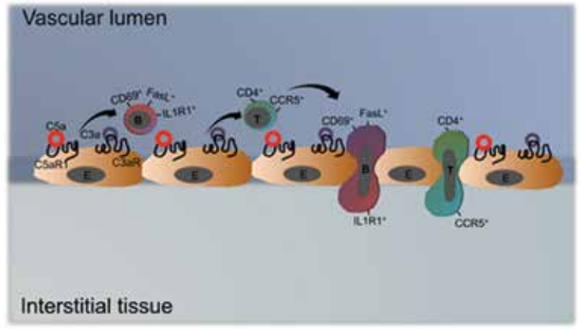
Shivshankar P, Li Y-D, Mueller-Ortiz SL, Wetsel RA. In response to complement anaphylatoxin peptides C3a and C5a, human vascular endothelial cells migrate and mediate the activation of B-cells and polarization of T-cells. 2020: *FASEB J*. 34:7540-7560. (PMID: 32301538).

Mueller-Ortiz SL, Shivshankar P, Wetsel RA. The second receptor for C5a, C5aR2, is detrimental to the host during systemic infection with *Listeria monocytogenes* in mice. *J Immunol*. 2019: 203: 2701-2711 (PMID:31597707). PMID:3159663

Mazzilli JL, Domoshirov AY, Mueller-Ortiz SL, Garcia CA, Wetsel RA, Zsigmond EM. Derivation and characterization of the human embryonic stem cell line CR-4: differentiation to human retinal pigment epithelial cells. *Stem Cell Res*. 2017: 18: 37-40 (PMID: 28395800).

LAB MEMBERS

Senior research scientist: Stacey Mueller-Ortiz, PhD
Senior research associate: Aleksey Y. Domozhirov, MS



Model illustrating how the vascular endothelium on stimulation by the complement anaphylatoxin peptides (C3a and C5a) activates B-cells and polarizes T-cells during an immune response. Endothelial cells shown in brown with letter E. T-cells and B-cells shown in green and purple, respectively. The elongated cells depict activated B-cells and polarized T-cells as they transmigrate through the endothelium.

Michael R. Blackburn, PhD

Executive Vice President & Chief Academic Officer, UTHealth
Dean and John P. McGovern Distinguished Professor of Biomedical Sciences
The University of Texas Graduate School of Biomedical Sciences at Houston
Professor, Department of Biochemistry and Molecular Biology
William S. Kilroy Sr., Distinguished University Chair in Pulmonary Disease

Adenosine signaling and the regulation of chronic lung disease

Inflammation and remodeling responses are prominent features of chronic lung diseases, such as asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and pulmonary hypertension. Although signaling pathways associated with the genesis of these diseases have been described, little is known about the signaling pathways that serve to regulate the chronic nature of these diseases. The major goal of my laboratory is to identify pathways that regulate the chronicity of these disorders with the intent of developing novel therapeutic strategies.

A central hypothesis of my laboratory is that the signaling molecule adenosine is an amplifier of lung inflammation and damage. Adenosine is generated in response to cell damage, and it is our belief that as adenosine levels increase in the lung they access pathways that serve to promote airway inflammation and remodeling. Adenosine signals by engaging specific adenosine receptors on target cells, such as inflammatory cells, fibroblasts, airway epithelial cells, and smooth muscle cells. Most of the projects in my laboratory focus on understanding the mechanisms by which adenosine signaling influences the activities of these cells in the context of lung inflammation and remodeling.

We make extensive use of genetically modified mice to examine the role of adenosine signaling in chronic lung disease. This includes knockout mice of components of adenosine metabolism and signaling. We also conduct mechanistic experiments in disease-relevant cell types and work extensively with human explanted lungs obtained following lung transplantation here in the Texas Medical Center. These translational approaches help us identify novel strategies for treating chronic lung disease.

RESEARCH PROJECTS

- Examining the role of A2B adenosine receptor expression on pulmonary macrophages during the progression of pulmonary fibrosis
- Investigation of adenosine transport in acute and chronic lung injury
- Novel regulation of mRNA polyA tails in the regulation of pulmonary fibrosis and Chronic Obstructive Pulmonary Disease
- Examination of the hypoxia as an amplifier of chronic lung disease
- Understanding novel mechanistic roles for IL-6 signaling in pulmonary fibrosis
- Systems biology approaches to understand the progression of chronic lung disease

KEY PUBLICATIONS

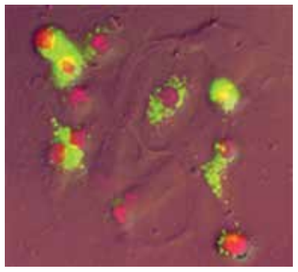
Philip, K.; Mills, T. W.; Davies, J.; Chen, N. Y.; Karmouty-Quintana, H.; Luo, F.; Molina, J. G.; Amione-Guerra, J.; Sinha, N.; Guha, A.; Eltzschig, H. K.; and Blackburn, M. R. (2017) HIF1A up-regulates the ADORA2B receptor on alternatively activated macrophages and contributes to pulmonary fibrosis. *FASEB J.* 31, 4745-4758. PMID: 12871304

Luo, F.; Le, N. B.; Mills, T.; Chen, N. Y; Karmouty-Quintana, H.; Molina, J. G.; Davies, J.; Philip, K.; Volcik, K. A.; Liu, H.; Xia, Y.; Eltzschig, H. K.; and Blackburn, M. R. (2016) Extracellular adenosine levels are associated with the progression and exacerbation of pulmonary fibrosis. *FASEB J.* 30, 874-883. PMID: 26527068

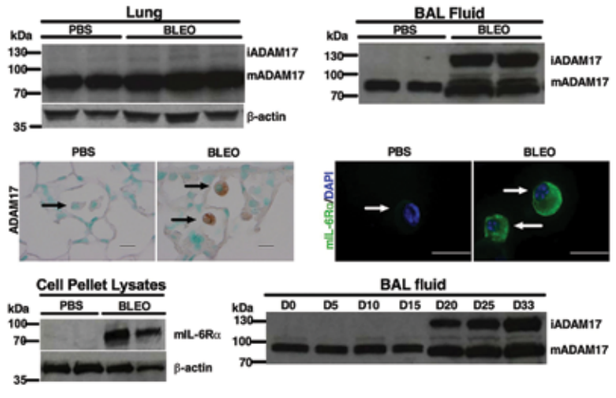
Karmouty-Quintana, H.; Philip, K.; Chen, N. Y.; Weng, T.; Molina, J. G.; Luo, F.; Davies, J.; Acero, L.; Le, Bao; Bunge, I.;Volcik, K.; Le, T.; Johnston, R. A.; Xia, Y.; Eltzschig, H. K.; and Blackburn, M. R. (2015) Deletion of ADORA2B from myeloid cells dampens lung fibrosis and pulmonary hypertension. *FASEB J.* 29, 50-60. PMID: 25318478

LAB MEMBERS

Assistant professor: Tingting Weng, PhD
Senior research scientist: Kelly Volcik, PhD
Research associate: Ning-Yuan Chen
Research scientist: Jose Molina, Sr.
Graduate student: Josh Ko, PhD



Primary type II alveolar epithelial cells isolated from genetically modified mice.



Increased expression (brown color) of proteinases in pulmonary macrophages in mice with pulmonary fibrosis (BLEO).

Amber Luong, MD, PhD

Professor, Center for Immunology and Autoimmune Diseases and Department of Otorhinolaryngology – Head and Neck Surgery; Vice Chair for Research, Department of Otorhinolaryngology – Head and Neck Surgery

Environmental triggers regulating innate immune responses in chronic airway inflammation

Over 40 million Americans suffer from chronic rhinosinusitis (CRS), which causes facial pain and pressure, nasal congestion, and obstruction. These symptoms ultimately drive conservatively 18-22 million physician visits yearly with an annual direct healthcare treatment cost of over $3 billion. In addition, patients suffering from CRS often are diagnosed with asthma, specifically those characterized by nasal polyps (CRSwNP). Together, CRS and asthma as chronic respiratory diseases represent some of the most prevalent chronic illnesses in the United States. Despite this healthcare burden, much remains unknown about its pathophysiology, and current treatment options, which typically involve recurrent surgeries and anti-inflammatory agents, are not curative. CRS represents an ideal human research model for studies in chronic inflammatory respiratory diseases. CRS patients often undergo surgery providing an opportunity to harvest critical diseased tissue and are seen regularly in clinic, which allows periodic evaluation of the patient and diseased mucosa.

Allergic fungal rhinosinusitis (AFRS) represents a unique CRSwNP phenotype typically presenting in patients in their twenties with nasal polyps and inflamed, often expanded, sinuses impacted with thick mucin laden with fungal hyphae and eosinophils, that result in dramatic CT sinus findings (see Fig 1). AFRS is a severe subtype of CRSwNP that uniquely can present with vision changes and intracranial complications with disease severity associated with lower socioeconomic status.

AFRS represents an excellent disease to model for understanding the role of fungi in the pathophysiology of sinonasal inflammation characterized by eosinophils and elevated Type 2 cytokines (e.g. IL-4, -5, and -13). One key characteristic of AFRS is the accumulation of eosinophilic mucin and filamentous fungi within inflamed sinuses. Based on our microarray data, one of the most differentially downregulated genes in AFRS as compared to other CRSwNP phenotypes is histatin 1 (HTN1). We confirmed these results in independent inflamed sinus mucosa and found that the other family member, histatin 3, also was significantly downregulated in AFRS (Fig 2). Given AFRS is characterized by an accumulation of fungal hyphae within eosinophilic mucin, a lack of antimicrobial peptides in AFRS potentially contributes to this phenotype.

We have shown that non-diseased sinonasal epithelial cells (SNECs) are capable of expressing antimicrobial peptides (AMPs) such as histatins, but that regulatory pathways governing AMP expression are impaired in AFRS. Our lab is currently focused on evaluating the role a key regulatory mechanisms of antimicrobial peptide expression in AFRS, IL-22 signaling, and therapeutic options for upregulating AMP expression or activity in treatment of CRS.

Respiratory epithelial cells represent the first line of defense against the environment for sinonasal mucosal. Recent studies have shown that epithelial cells serve an active role through regulation of cytokines and release of anti-microbials. Three identified epithelial cell derived cytokines, thymic stromal lymphopoietin, interleukin (IL)-25 and IL-33, have been linked to the type 2 immune response.

Our lab has focused on the role of IL-33 in orchestrating the type 2 immune response characteristic of CRS with nasal polyps. We confirmed that the receptor of IL-33 is upregulated in the diseased sinonasal mucosa of CRSwNP. We demonstrated an increased presence of innate lymphoid type 2 cells (ILC2) preferentially in CRSwNP patients relative to health controls. These ILC2 express ST2, the receptor for IL-33, and represent the major cell type producing IL-13 in response to IL-33 (see image 3). Interestingly, we found that fungal antigens, specifically *Aspergillus*, can stimulate respiratory epithelial cells to release IL-33.

Given the appreciation of the innate immunity and known data of the role of the adaptive immune response in CRS, we are currently interested in the distribution and ultimately in the function of innate lymphoid cells and T helper cells in various CRS subtypes.

In addition to antimicrobial peptides, my lab is interested in the molecular characterization of fungi-mediated signaling pathway(s) and the fungal component responsible for signaling in the inflammatory response in some CRS subtypes. This has led us to our recent interest of establishing a mouse model of eosinophilic upper and lower airway inflammation and the protocols to evaluate the sinus inflammation.

RESEARCH PROJECTS

- Characterization of immunologic and molecular defects contributing to pathophysiology of allergic fungal rhinosinusitis.
- Molecular signaling through respiratory epithelial cells of fungi alone and with other environmental triggers responsible for initiating and/or maintaining the characteristic Th2 immune response.
- Clinical characterization and identification of biomarkers for CRS subtypes.

KEY PUBLICATIONS

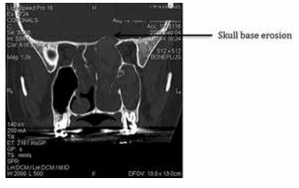
Tyler MA, Russell CB, Smith DE, Rottman JB, Dietz CJ, Hu X, Citardi MJ, Fakhri S, Assassi S, and Luong A. Large scale gene expression profiling reveals distinct type 2 inflammatory patterns in chronic rhinosinusitis subtypes. *J Allergy Clin Immunol,* 2017 Mar;139(3):1061-1064.

Dietz CJ, Sun H, Yao WC, Citardi MJ, Corry DB, Luong AU. Aspergillus fumigatus induction of IL-33 expression in chronic rhinosinusitis is PAR2-dependent. *Laryngoscope.* 2019 Oct;129(10):2230-2235.

Tyler MA, Lam K, Marino MJ, Yao WC, Schmale I, Citardi MJ, Luong AU. Revisiting the controversy: The role of fungi in chronic rhinosinusitis. *Int Forum Allergy Rhinol.* 2021 Nov;11(11):1577-1587.

LAB MEMBERS

Hua Sun, PhD; Yi-Dong Li



Bony erosion of skull base from accumulated eosinophilic mucin laden with fungal hyphae

The Center for Metabolic and Degenerative Diseases unites eight laboratories that collaborate to investigate aging- and obesity-associated diseases, including cancer. Mechanistic changes in brain activity, energy metabolism, vascular function, cell signaling, protein homeostasis, and cell fate determination that lead to pathophysiology are being interrogated in animal models and studies of clinical specimens. The primary interests include the crosstalk between brain and adipose tissue, as well as integrative physiology changes leading to dysfunction of organs, such as liver and skeletal muscle. Questions pursued by the Center's faculty include the following:

- Which cells stop dividing with age, leading to aging-associated disease?
- What are the mechanisms underlying circadian rhythms in adipocyte progenitor proliferation?
- Which cells of adipose and muscle tissue can be targeted for therapeutic purposes and how?
- How does lipid metabolism change during cancer progression and cachexia development?
- How does transient inflammation activate heat production by fat tissue?
- What are the mechanisms linking blood vessel formation with the nervous system?
- How do stress hormones regulate sugar and fat utilization in diabetes?
- What is the molecular basis of exercise benefits in metabolic and cardiovascular disease?
- How do the brain and peripheral clocks control energy balance and metabolism?
- How does the hepatic circadian clock protect against fatty liver disease and liver cancer?
- How does the brain control glucose homeostasis in diabetes?
- What are the functions of the genes mutated in neurodegenerative diseases?
- How does disruption of membrane trafficking and cell homeostasis cause neurodegeneration?
- How does stress accelerate the onset and progression of Alzheimer's disease?
- How does bearing children contribute to late-life onset of depression, anxiety, and dementia?

Collaboration among the Center's laboratories promotes research synergy, thereby increasing productivity and innovation. The Center's members collaborate with clinicians and epidemiologists to translate their discoveries for the benefit of patients with metabolic and degenerative diseases.

Mikhail Kolonin, PhD
Center Director & Professor
Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research

CENTER FOR **METABOLIC AND DEGENERATIVE DISEASES**

Mikhail Kolonin, PhD
Professor & Director, Center for Metabolic and Degenerative Diseases
Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research

Aging-associated cellular changes and their herapeutic targeting

My group is interested in the mechanisms underlying aging-related diseases and developing new approaches to target them. We focus on the role of pathogenic fibroblasts, which can be recruited from fat (adipose) tissue in the context of obesity, type-2 diabetes, muscle degeneration, and cancer. While white adipocytes store lipids to release them in times of energy scarcity, brown adipocytes burn lipids off to keep the body warm. In obesity, overgrown white fat becomes inefficient in holding lipids, hence causing diabetes, cardiovascular disease, and cancer. In contrast, active brown fat can prevent the onset of metabolic disease. The past year, we have published several reports on the mechanisms and role of fatty acid transport in the context of type-2 diabetes and cancer. Another research direction is focused on the role of inflammatory signaling and fat tissue remodeling in metabolic response to anti-diabetes drugs. Both white and brown adipocytes are continuously replaced as they undergo senescence, and their pools in fat tissue are maintained by adipose stem cells (ASCs). In obesity, ASCs over-proliferate and undergo replicative senescence, hence, aggravating aging. This was revealed by our studies in mice lacking telomerase (TERT) in ASCs. Currently, we are testing the role of replicative senescence in other types of stem cells and the effects on aging-associated neurological and muscular dysfunction. We have discovered that in chronic disease tissues recruit ASCs, which can fuel cancer and fibrosis progression. Taking advantage of our expertise in targeted therapeutics, we have developed the first experimental drug (D-CAN) targeting ASCs. Our publications demonstrate that D-CAN prevents obesity and suppresses cancer progression in mice. We have also applied ablation of ASC as a new therapeutic approach to Duchenne muscular dystrophy treatment. Recently, we reported a panel of peptides that can be used for non-invasive detection and imaging of metastatic cancer cells and their conversion to therapeutics blocking cancer progression.

RESEARCH PROJECTS

- Adipose stromal cells: heterogeneity, function in health, and targeting in disease
- Replicative senescence of progenitor cells and its role in aging-associated diseases
- Molecules mediating intercellular interactions and signaling in obesity and cancer
- Identification of tissue-specific drug targets

KEY PUBLICATIONS

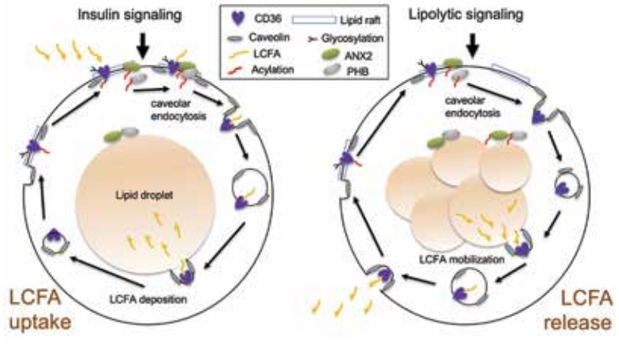
Gao Z, Daquinag AC, et al., Désaubry L, Kolonin MG. Prohibitin Inactivation in Adipocytes Results in Reduced Lipid Metabolism and Adaptive Thermogenesis Impairment. *Diabetes*. 2021.

Daquinag AC, Gao Z, Fussell C, Immaraj L, Pasqualini R, Arap W, Akimzhanov AM, Febbraio M, Kolonin MG. Fatty acid mobilization from adipose tissue is mediated by CD36 post-translational modifications and intracellular trafficking. *JCI Insight*. 2021.

Subramanian S., DaquinagAC AghaAmirAi, Ghosh S, Azhdarinia A, Kolonin MG, Characterization of peptides targeting metastatic tumor cells as probes for cancer detection and vehicles for therapy delivery, *Cancer Research*, 2021.

LAB MEMBERS

Post-doctoral fellow: Joseph Rupert
Sr. research scientists: Alexis Daquinag, Zhanguo Gao
Research assistant III: Yongmei Yu



A model of CD36-mediated outside-in and inside-out LCFA transport. Insulin signaling activates extracellular long chain fatty acid (LCFA) uptake mediated by acylated transporter CD36 at the cell surface in complex with co-transporters PHB and ANX2. Deacylation of CD36 enables caveolar endocytosis and lipid droplet trafficking of LCFA-bound CD36. In lipolytic conditions, CD36 deacylation, caveolar endocytosis, and lipid droplet trafficking enable CD36 loading with LCFA released from lipid droplets and mobilization from the cell.

Rebecca Berdeaux, PhD
Associate Professor
Director, Graduate Program in Biochemistry and Cell Biology

Regulation of muscle nutrient use in type 2 diabetes regeneration

Skeletal muscles of people with prediabetes and type 2 diabetes lose efficiency of burning off dietary sugars and fats. This inefficiency leads to damage of the central cellular 'power plants' (mitochondria) and to higher blood sugar and fat deposits in liver. Our program is aimed at identifying new ways that this efficiency can be improved by targeting a specific family of enzymes known as kinases. Using genome editing, we are testing how a stress-induced kinase affects muscle mitochondrial function in type 2 diabetes. Our results indicate that we may have stumbled on a hidden route to stimulating efficient nutrient use by skeletal muscle and improve health of people suffering from type 2 diabetes.

RESEARCH PROJECTS
- Regulation of mitochondrial energetics by SIK1
- SIK1 activation of stress-induced mitochondrial fission
- Targeting hormone-activated pathways to boost muscle stem cell activity

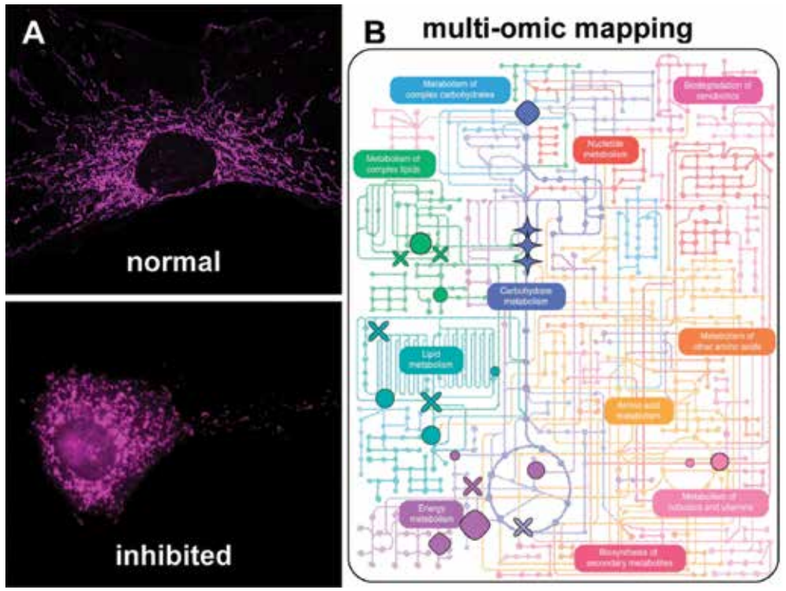
KEY PUBLICATIONS

Nishimori, S., O'Meara, M.J., Castro, C., Noda, H., Cetinbas, M., da Silva Martins, J., Ayturk, U., Brooks, D.J., Bruce, M., Nagata, M., Ono, W., Janton, C.J., Bouxsein, M.L., Foretz, M., Berdeaux, R., Sadreyev, R.I., Gardella, T.J., Juppner, H., Kronenberg, H.M., Wein, M.N. (2019) Salt inducible kinases dictate PTH1R action in bone development and remodeling. *J Clin. Invest.,* 129(12): 5187-5203.

Tang, C.C., Castro Andrade, C.D., O'Meara, M.J., Yoon, S.H., Sato, T., Brooks, D.J., Bouxsein, M.L., Martins, J.D.S., Wang, J., Gray, N.S., Misof, B., Roschger, P., Blouin, S., Klaushofer, K., Velduis-Vlug, A., Vegting, Y., Rosen, C.J., O'Connell, D., Sundberg, T.B., Xavier, R.J., Ung, P., Schlessinger, A., Kronenberg, H.M., Berdeaux, R., Foretz, M., and Wein, M.N. (2021) Dual targeting of salt inducible kinases and CSF1R uncouples bone formation and bone resorption. *eLife,* 10:e67772.

Hollstein, P.E., Eichner, L.J., Brun, S.N., Kamireddy A., Svensson, R.U., Vera, L., Ross, D.S., Rymoff, T.J., Hutchins, A., Galvez, H., Williams, A., Shokhirev, M.N., Screaton, R.A., Berdeaux, R., and Shaw, R.J. (2019) The AMPK-related kinases SIK1 and SIK3 mediate key tumor suppressive effects of LKB1 in NSCLC. *Cancer Discovery,* pii: CD-19-1261; doi: 10.1158/2159-8290.CD-18-1261.

LAB MEMBERS
Post-doctoral fellow: Antonio Soares
Graduate student: Muchen Liu
Research assistants: Elena Dyukova, Mark Rosenfeld



A) Mitochondrial structure in resting muscle cells becomes fragmented when mitochondrial activity is inhibited. We have discovered a potential new way that this process is regulated that has implications for type 2 diabetes. B) Visualization of a gene signature in mice lacking SIK1. By studying gene, protein, and metabolite patterns in muscles, livers, and brains of these mice, a new paradigm is emerging that explains how this enzyme coordinates energy use and storage.

Kristin Eckel-Mahan, PhD
Assistant Professor

Circadian rhythms in health and disease

The goals of my lab center on the importance of our internal 24-hour biological (i.e. circadian) clock in health and disease prevention. The circadian clock in an exquisite time-keeping system present in all cells of our body that drives daily rhythms in physiology and tissue-specific function. Examples of our internal clock at work include the sleep/wake cycle, variations in internal body temperature, and rhythmic hormone or neurotransmitter secretion. Our 24-hr. clock aligns to, and anticipates the rotation of, the earth on its axis. Recent evidence from large epidemiological studies reveals that chronic circadian disruption increases our risk of several diseases. Examples of circadian disruption include travel across time zones (jet lag), working a night shift or rotating shifts ("social jet lag"), and light contamination by white and blue light sources. In addition, some clock gene mutations lead to sleep disorders. Disruption of the circadian clock, genetically or environmentally, increases the risk for several diseases, including cancer and various metabolic diseases. We are trying to understand why circadian disruption produces these effects.

While the "central pacemaker" of our brain, the suprachiasmatic nucleus of the hypothalamus, keeps us entrained to our 24-hr. environment via light activation of the retina, other environmental factors can prominently control 24-hr. rhythms in several peripheral organs (for example, liver, kidney, adipose tissue, and muscle). Poor quality nutrients as well as food intake at the wrong time not only compromises tissue-specific function, but also promotes body-wide circadian clock "desynchrony". Collectively, these defects in circadian rhythms internally increase our risk of metabolic disease. The lab is currently trying to understand which environmental factors are most important for tissue-specific clock function and the mechanisms by which tissue-specific clocks protect against metabolic disease.

Chronic nutrient excess disrupts 24-hr. rhythms in several insulin sensitive tissues that become insulin resistant under conditions of prolonged lipid overload. Susceptible tissues include the liver, muscle, and adipose tissue. Our studies point to tight regulation of circadian lipid metabolism in the liver as well as diurnal proliferation of adipocyte precursor cells in the context of adipose tissue. These 24-hour activities are disrupted under conditions of nutrient challenge, predisposing an organism to metabolic disease, such as obesity and type II diabetes.

In addition to metabolic disease, circadian disruption is associated with an increased risk of cancer. We have identified the circadian function of a liver protein ("HNF4a"), which is altered in the context of specific liver cancers. Attempts to restore the circadian function in these cells causes tumor cell death and impairs tumor growth. We are using this information to determine whether liver tumors that show circadian dysregulation can be effectively treated with molecules that boost the circadian activity in these cells.

RESEARCH PROJECTS
- Mechanisms by which circadian disruption leads to obesity and type II diabetes
- Mechanisms linking circadian disruption to fatty liver and ultimately, liver cancer
- Mechanisms underlying circadian proliferation of adipocyte progenitor cells and their ability to promote adipose tissue health during aging

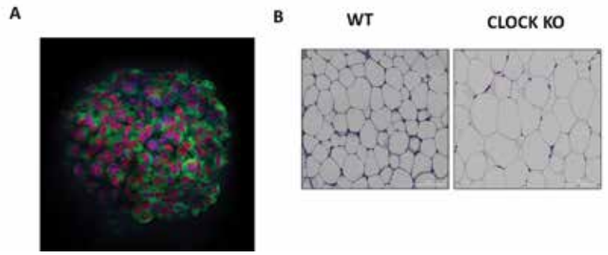
KEY PUBLICATIONS

Ribas-Latre A, Santos RB, Fekry B, Tamim YM, Shivshankar S, Mohamed AMT, Baumgartner C, Kwok C, Gebhardt C, Rivera A, Gao Z, Sun K, Heiker JT, Snyder BE, Kolonin MG, Eckel-Mahan KL. Cellular and physiological circadian mechanisms drive diurnal cell proliferation and expansion of white adipose tissue. *Nat Commun.* 2021 Jun 9;12(1):3482.

Van Drunen R, Eckel-Mahan K. Circadian Rhythms of the Hypothalamus: From Function to Physiology. *Clocks Sleep.* 2021 Feb 25;3(1):189-226.

Baharan Fekry, Aleix Ribas Latre, Corrine Baumgartner, Alaa M.T. Mohamed, Mikhail G. Kolonin, Frances M. Sladek, Mamoun Younes, and Kristin L. Eckel-Mahan HNF4α-deficient Fatty Liver Provides a Permissive Environment for Sex-independent Hepatocellular Carcinoma *Cancer Research* 2019 Nov 15;79(22):5860-5873

LAB MEMBERS
Research faculty and post-doctoral fellows: Baharan Fekry, PhD; Rafael Bravo Santos, PhD
Graduate students: Rachel Van Drunen (PhD student), Jamie Tran (MS student)



(A) A human-derived hepatocellular carcinoma (HCC) spheroid that, though expressing hepatocyte nuclear factor four alpha (HNF4α, red) protein, has reduced activity of the tumor suppressive isoform and increased expression of a fetal isoform of the protein. This expression, which can increase in the context of diet-induced obesity, can lead to sex-independent, early onset liver cancer. (B) Enlarged adipocytes in the visceral fat of mice lacking the circadian CLOCK protein (right) compared to their normal littermate controls ("WT", left).

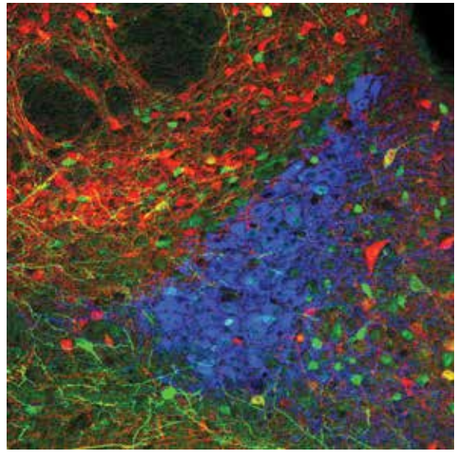
Nicholas Justice, PhD
Associate Professor

The impact of stress on psychiatric and neurodegenerative diseases

High levels of stress lead to persistent anxiety that can cause and contribute to the development of devastating mental illnesses, most commonly depression, generalized anxiety disorder, and addiction. Being constantly stressed also can dramatically impact the progression of diseases not directly caused by stress, in part due to elevated levels of Cortisol, the hormone released by the body in response to stress. Diseases that are particularly sensitive to stress include metabolic diseases like diabetes, high blood pressure and cardiovascular disease, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease. In addition, high levels of stress promote normal loss in memory that occurs with age. Our lab is focused on how our bodies perceive stress, react to stress acutely, and are impacted by stress exposure. We seek to understand how the responses of the body to stress change our physiology to negatively impact mental and physical health and accelerate the progression of age-related neurodegenerative diseases such as Alzheimer's disease.

RESEARCH PROJECTS

- At the IMM, we have developed many genetic tools that make it possible to perform experiments that test the function of previously uncharacterized and highly specific stress-responsive circuits in the brain. We can now manipulate activity of circuits while at the same time monitoring behavior and changes in physiological function in freely behaving animals. Our first discovery in the lab was a new class of hypothalamic neuron that we demonstrated is critical for controlling behavioral, autonomic, and hormonal stress responses. As we have continued our studies of this new class of neurons, we have found they play additional roles by transmitting signals from Corticotropin Releasing Factor (CRF) neurons to other neurons to influence behavioral and autonomic features of the stress response.
- In our lab at the IMM, we discovered unprecedented neural connections from CRF neurons in the hypothalamus to key neural circuits in the basal ganglia, a brain region that controls movement. The most common neurodegenerative disease associated with dysfunction of basal ganglia circuits is Parkinson's disease, in which patients experience tremors, uncontrolled movements, and the inability to initiate movement. Interestingly, many Parkinsonian patients report that their symptoms increase when they are stressed. We are currently performing experiments to test how stress responsive circuits transmit this information to basal ganglia circuits to alter dynamics of movement. We hope to use the information we gain to develop therapeutic strategies to treat debilitating movement-related symptoms caused by neurodegenerative diseases and other diseases that impact the ability to control movement.
- Understanding how the stresses of reproduction lead to acute and chronic mental illness in mothers. We identified a new mechanism by which the stress-released neuropeptide, CRF, directly influences Oxytocin secretion, which occurs only in postpartum mothers who have recently given birth. We are currently experimentally testing how the maternal specific CRF signaling to Oxytocin neurons relays information about stressful threats, in many cases uniquely suffered by postpartum mothers to alter maternal behavior. Our investigation into CRF-Oxytocin signaling in postpartum mothers has introduced a new field of study to the lab: The importance of the stress response during reproduction, and how this impacts the health of mothers and their offspring.



CRF neurons (red) and noradrenaline neurons (concentrated in the Locus Coereleus; blue) orchestrate a coordinated change in behavior, physiological state, and autonomic tone in response to stress to promote survival.

KEY PUBLICATIONS

Hunt AJ, Dasgupta R, Rajamanickam S, Jiang Z, Beierlein M, Chan CS, Justice NJ (2018) Paraventricular hypothalamic and amygdalar CRF neurons synapse in the external globus pallidus. *Brain Struct Funct* 223:2685–2698.

Jiang Z, Rajamanickam S, Justice NJ (2018) Local Corticotropin-Releasing Factor Signaling in the Hypothalamic Paraventricular Nucleus. *J Neurosci* 38:1874–1890.

Justice NJ (2018) The relationship between stress and Alzheimer's disease. *Neurobiol Stress* 8:127–133.

LAB MEMBERS

Research scientist: Shivakumar Rajamanickam, PhD
Post-doctoral fellow: Lierni Ugartamendia, PhD

Vihang Narkar, PhD
Associate Professor
George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research

Gene regulation in metabolic, vascular, and degenerative diseases

Our laboratory broadly studies transcriptional regulation of metabolic and vascular homeostasis using nuclear receptors as model signaling molecules. Currently, we are investigating the cellular and physiological functions of orphan nuclear receptors (e.g. estrogen-related receptors) and their co-regulators (e.g. PGC1's). We use a wide-ranging approach, including genetically engineered mice, murine disease models, high-throughput gene expression analysis (e.g. RNA-sequencing, ChIP-sequencing), pharmacology, cell signal and *in vitro* systems in our studies. These tools are being used to investigate the role of ERR's and PGC1's in (I) cellular processes such as genome-wide gene orchestration, mitochondrial biogenesis and angiogenesis; (II) physiological phenomenon such as exercise adaptation and whole-body metabolism; as well as (III) diseases such as obesity/diabetes, peripheral arterial disease, and muscular dystrophies. Our ongoing work has uncovered the therapeutic role of estrogen-related receptors (ERR's) via metabolic and angiogenic regulation in peripheral arterial disease (PAD), and in Duchenne muscular dystrophy (DMD). Similarly, our studies on peroxisome proliferator activator receptor delta (PPAR-delta) have yielded insights in to exercise mimicking cellular mechanisms that can be harnessed to boost metabolism, protect against obesity, and prevent diabetes. On the other hand, we have also uncovered the detrimental role of nuclear receptor co-activator PGC1-beta in PAD and muscle degeneration via regulation of anti-angiogenic, apoptotic, and autophagic pathways. Our work spanning the area of metabolic vascular syndromes that include obesity, diabetes, and its cardiovascular complications has been published in journals including *Cell, Cell Metabolism, Cell Reports, Circulation Research, eLife, Faseb Journal, Nature Communications* and *Science.*

RESEARCH PROJECTS

- Transcriptional regulation of muscle metabolism, vascularization, mass, and fitness by nuclear receptors.
- Nuclear receptor target discovery for muscle recovery in peripheral arterial disease, Duchenne muscular dystrophy, obesity and diabetes.
- Role of nuclear receptors in blood vessel growth and diabetic retinopathy.

KEY PUBLICATIONS

Sopariwala DH, Likhite N, Pei G, Haroon F, Lin L, Yadav V, Zhao Z, Narkar VA. Estrogen-related receptor α is involved in angiogenesis and skeletal muscle revascularization in hindlimb ischemia. *FASEB J.* 2021 May;35(5):e21480. doi: 10.1096/fj.202001794RR. PMID: 33788962

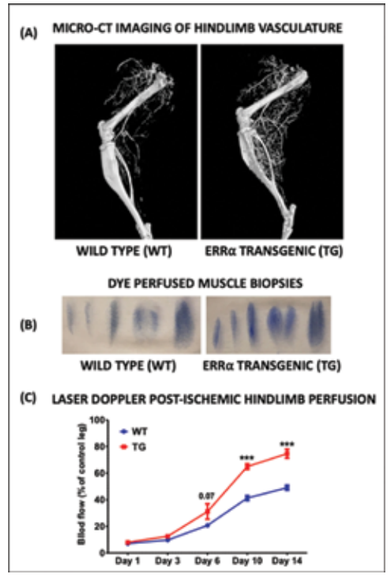
Estrogen-related receptor α is involved in angiogenesis and skeletal muscle revascularization in hindlimb ischemia. Sopariwala DH, Likhite N, Pei G, Haroon F, Lin L, Yadav V, Zhao Z, Narkar VA. *FASEB J.* 2021 May;35(5):e21480. doi: 10.1096/fj.202001794RR. PMID: 33788962

TAK1 preserves skeletal muscle mass and mitochondrial function through redox homeostasis. Roy A, Sharma AK, Nellore K, Narkar VA, Kumar A. *FASEB Bioadv.* 2020 Aug 7;2(9):538-553. doi: 10.1096/fba.2020-00043. eCollection 2020 Sep. PMID: 32923988 Free PMC article.

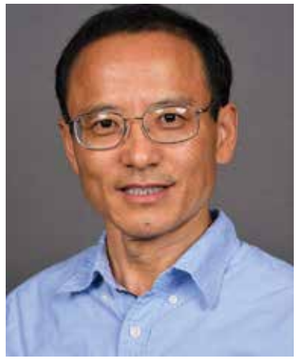
Interleukin-13 drives metabolic conditioning of muscle to endurance exercise. Knudsen NH, Stanya KJ, Hyde AL, Chalom MM, Alexander RK, Liou YH, Starost KA, Gangl MR, Jacobi D, Liu S, Sopariwala DH, Fonseca-Pereira D, Li J, Hu FB, Garrett WS, Narkar VA, Ortlund EA, Kim JH, Paton CM, Cooper JA, Lee CH. *Science.* 2020 May 1;368(6490):eaat3987. doi: 10.1126/science.aat3987. Epub 2020 Apr 30. PMID: 32355002

LAB MEMBERS

Post-doctoral fellow: Dr. Danesh Sopariwala
Research assistants: Sean Barnes, Andrea Guzman
Undergraduate trainees: Lisa Lin
New members joining soon: Dr. Hao Nguyen (post-doctoral fellow)



Neo-angiogenic role of ERRα in the skeletal muscle. ERRα overexpression boosts neo-angiogenesis and vascularization (A-B) in the skeletal muscle, as well as promotes ischemic reperfusion (C) in hindlimb ischemia.



Kai Sun, MD, PhD
Associate Professor

Adipose tissue remodeling, metabolic health, and cancer development

My laboratory investigates and discovers novel factors that regulate the dynamics of adipose tissue remodeling during obesity development. The long-term goal of our research is to address the clinical significance of these factors in human obesity, diabetes, cardiovascular diseases, and cancer growth.

In the past years, we have revealed that high fat diet-induced obesity shapes a hypoxic microenvironment that initiates the local fibrosis and inflammation in adipose tissue. The unhealthy adipose tissue further leads to systemic insulin resistance and cardiovascular dysregulation. Intriguingly, we found that VEGF-A-induced angiogenesis ameliorates the pathological changes by suppressing the local hypoxia and stimulating sympathetic innervation in both white and brown adipose tissue. Our study further reveals that the hypoxia-induced MT1-MMP facilitates the healthy expansion of adipose tissue by stimulating angiogenesis in combination with VEGF-A and leptin, thus relieving the pathological conditions. Furthermore, MT1-MMP cleaves collagenous proteins to increase the ECM flexibility in adipose tissue.

Most recently, we analyzed the dynamics of lipid droplet-associated proteins during adipose tissue remodeling by mass spectrometry. We have sucessfully identified several novel proteins that translocalize onto lipid droplets and the interface of endoplasmic reticulum (ER)-lipid droplets in response to different stimuli. Particularly, one of the identified protein named Carboxyl Esterase 1 (CES1) targets lipid droplets upon β-adrenergic-stimulation where it exerts the lipolytic function on the lipids. Meta-analysis of clinical data reveals that CES1 levels are significantly increased during the development of certain types of cancer and are tightly correlated with the death rates, suggesting that CES1 might be a novel target to treat the cancer. We are now applying state-of-the-art tools and techniques to ellucidate the mechanisms governing the functions of the novel factors and investigating their potential implication in metabolic health and cancer therapy.

RESEARCH PROJECTS
- Hypoxia induced pathological changes in adipose tissue.
- Sympathetic innervation in adipose tissue and energy expenditure.
- Reversibility of adipose tissue fibrosis by novel anti-fibrotic therapies.
- Dynamics of lipid droplets, metabolic regulation and tumor development .

KEY PUBLICATIONS

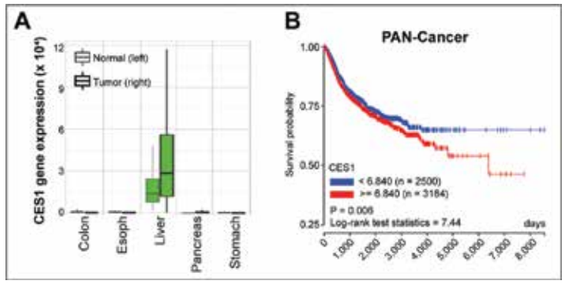
Yang L, Li X, Tang H, Gao Z, Zhang K, Sun K. A Unique Role of Carboxylesterase 3 (Ces3) in β-Adrenergic Signaling-Stimulated Thermogenesis. *Diabetes*. 2019 Jun; 68(6):1178-1196.

Li X, Zhao Y, Chen C, Yang L, Lee HH, Wang Z, Zhang N, Kolonin MG, An Z, Ge X, Scherer PE, Sun K. The critical role of MMP14 in adipose tissue remodeling during obesity. *Mol Cell Biol*. 2020 Mar; 40(8): e00564-19.

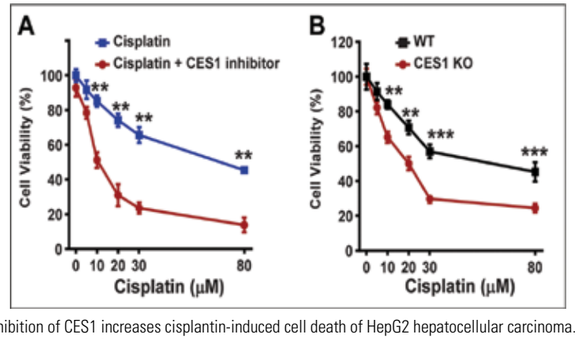
Li X, Yang L, Mao Z, Pan X, Zhao Y, Gu X, Eckel-Mahan K, Zuo Z, Tong Q, Hartig SM, Cheng X, Du G, Moore DD, Bellen HJ, Sesaki H, Sun K. Novel role of dynamin-related-protein 1 in dynamics of ER-lipid droplets in adipose tissue. *FASEB J*. 2020 Jun; 34(6):8265-8282.

LAB MEMBERS

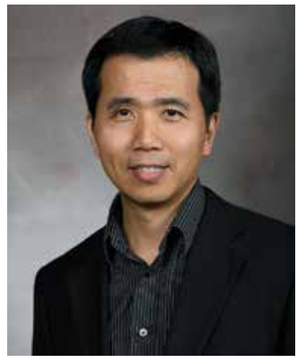
Post-doctoral fellows: Xin Li, MD, PhD; Gang Li, PhD
Research assistant II: Shuyue Wang, MS



CES1 levels are corelated with the death rate of cancer by meta-analysis. A: CES1 is enriched in the liver and its levels are significantly increased in the liver tumors. B: CES1 levels are positively correlated with the death rate of cancer.



Inhibition of CES1 increases cisplatin-induced cell death of HepG2 hepatocellular carcinoma. A: Inhibition of CES1 by its specific inhibitor WWL229 dramatically increases cisplatin-induced HepG2 cell death. B: Knockout of CES1 in HepG2 cells leads to higher efficiency of cisplatin-induced cell death.



Qingchun Tong, PhD
Professor
Cullen Chair in Molecular Medicine

Brain control of metabolism

The current obesity epidemic and its associated metabolic syndrome have imposed unprecedented challenges to society and medicine but with no apparent effective therapeutics. Our research is directed to understand the fundamental mechanistic insights on key driving causes for defective feeding and body weight regulation, therefore providing conceptual and effective targets for prevention and treatment of eating disorders, obesity, and its associated diabetes.

Toward these goals, we employ various animal models in combination with state-of-the-art techniques, including electrophysiology, optogenetics, chemogenetics, and *in vivo* live imaging. Cre-lox P, Flp-FRT mouse genetics is used to achieve neuron-specific manipulations in the brain. Various adenoassociated viral vetors (AAV) harboring genes that exhibit Cre- or Flp-dependent expression or inactivation will be stereotaxically delivered to specific brain regions of Cre- or Flp-expressing neurons, achieving neuron expression of foreign tool genes, leading to specific manipulations of neuron function. Example foreign genes include specific channels that either activate or inhibit neurons. In addition, virus-based tracing is used to map specific neural projections and their implications in physiology and behaviors. We are also exploring to use CRISPR/Cas9 technology to achieve neuron-specific gene deletion in adult mice. These advanced techniques ensure our studies are effective and conclusions are insightful.

One major direction in the lab is to identify and map novel neurocircuits underlying emotion control of feeding. Emerging evidence suggests that feeding abnormalities are associated with defects in control of emotion and clinical drugs that reduce symptoms of psychiatric disorders cause obesity development. Using unique animal models coupled with behavioral analysis and optogenetics, we aim to delineate important neurons and neural pathways that underscore interactive regulation of feeding and emotion. This line of research is highly significant to current clinical treatments for obesity, mental and eating disorders.

RESEARCH PROJECTS
- Novel neurons and neural pathways for feeding regulation and its relation with emotional states
- Brain efferent pathways controlling peripheral metabolism
- Brain mechanisms mediating blood hormone action on energy and glucose, and their involvement in obesity and diabetes pathogenesis
- Chronic stress and obesity development
- Oligodendrocyte myelin and diet-induced obesity

KEY PUBLICATIONS

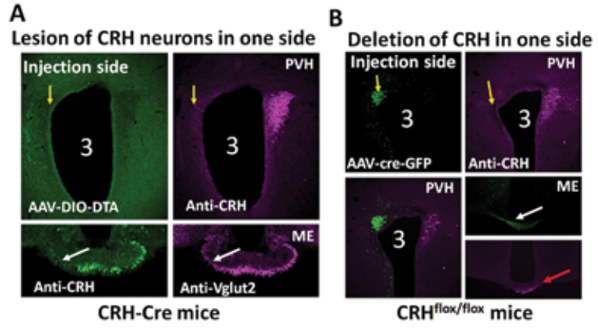
A neural basis for brain leptin action on reducing type 1 diabetic hyperglycemia. Fan S, Xu Y, Lu Y, Jiang Z, Li H, Morrill JC, Cai J, Wu Q, Xu Y, Xue M, Arenkiel BR, Huang C, Tong Q. *Nat Commun*. 2021 May 11;12(1):2662. doi: 10.1038/s41467-021-22940-4. PMID: 33976218.

Paraventricular hypothalamus mediates diurnal rhythm of metabolism. Kim ER, Xu Y, Cassidy RM, Lu Y, Yang Y, Tian J, Li DP, Van Drunen R, Ribas-Latre A, Cai ZL, Xue M, Arenkiel BR, Eckel-Mahan K, Xu Y, Tong Q. *Nat Commun*. 2020 Jul 30;11(1):3794. doi: 10.1038/s41467-020-17578-7.PMID: 32732906.

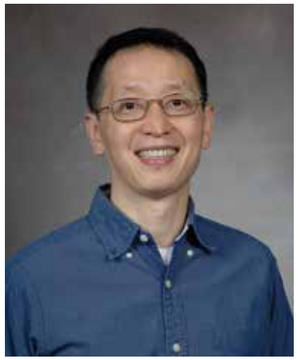
Profound and redundant functions of arcuate neurons in obesity development. Zhu C, Jiang Z, Xu Y, Cai ZL, Jiang Q, Xu Y, Xue M, Arenkiel BR, Wu Q, Shu G, Tong Q. *Nat Metab*. 2020 Aug;2(8):763-774. doi: 10.1038/s42255-020-0229-2. PMID: 32719538.

LAB MEMBERS

Assistant prrofessor: Yuanzhong Xu
Post-doctoral fellows: Yanyan Jiang, Zhiying Jiang
Graduate students: Jing Cai, Jessie Morrill
Research assistant: Claire Young



The Figure illustrates genetic approaches to alter the HPA axis, the major neuroendocrine pathway for the brain to control metabolism and stress responses, through manipulation of hypothalamic corticotropin releasing hormone (CRH) neurons. A) Genetic approach of CRH lesion. Injection of conditional AAV-DIO-DTA virus to one side of the hypothalamus of CRH-Cre mice. Cre-mediated DTA (diphtheria toxin) expression killed CRH neurons in the injected side as well CRH fibers in the median eminence (ME). B) Genetic approach of CRH deletion. Injection of conditional AAV-Cre-GFP to one side of the hypothalamus of floxed CRH mice. Cre-mediated deletion of the CRH gene eliminated CRH expression in the injected side as well as in Cre-GFP positive CRH neuron fibers in ME.



Sheng Zhang, PhD
Associate Professor
Becker Family Foundation Professor in Diabetes Research

Molecular mechanisms of neurodegenerative diseases

As we live longer and enjoy unprecedented longevity, we also become increasingly vulnerable to aging-related neuronal degenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD), among others. These incapacitating brain diseases inflict unbearable emotional and financial tolls to patients and their families, becoming a pressing threat to our society. However, by now there is little effective prevention and treatments against these maladies.

To address these challenges, we are studying how to keep neurons happy and healthy during normal aging. Our senses, reasoning, and responses are realized through neurons and their functional connections inside our body. However, unlike other cells, such as those from skin and blood that are constantly dividing and being replenished, neurons face unique challenges: once they are born and mature into interconnected functional units, they mostly lose the ability to reproduce and no longer can be replaced for the rest of the life. To achieve longevity, these long-lived neurons have their own self-maintenance machineries to stay health and ward off internal crisis and external insults for decades to come.

The self-maintenance machines include *chaperones* that help proteins to stay in shape, and different internal clearance machineries such as *proteasomes, autophagy* (meaning "self-eating" in Greek) and *lysosomal* systems to clean up and recycle worn-out or toxic cellular materials. In neurodegenerative diseases, these protective machineries become inefficient or nonfunctional, leading to excessive buildup of toxic wastes (known as aggregates, tangles or plaques) inside the brain, causing eventual neuronal death.

Using genetic, biochemical, and cell biology tools in different model systems from invertebrate *Drosophila* to cultured human cells and mouse animals, we are studying how these self-maintenance machines recognize and efficiently clear away internal toxins while spare and protect normal cellular constituents. Our eventual goal is to be able to command these innate protection machineries to fight against devastating brain degenerative diseases.

Currently we are especially focusing on the following questions:

(1) Chaperone Hsp110 on neuronal function and survival

Chaperone Hsp110 is one of the most abundant proteins in the brain. It helps other proteins to fold into proper shapes to function properly. It is also a major component of a potent molecular machinery called disaggregase that dismantles tightly packed protein aggregates.

(2) Biogenesis of autophagosomes and other specialized cellular organelles and their dysfunction in brain diseases

Cells produce many specialized cellular organelles, such as the autophagosome, lysosome-related organelles, and synaptic vesicles. Autophagosomes are garbage bags produced by a cell during autophagic process to collect unwanted or harmful cellular components for their eventual disposal and recycling. These specialized organelles control many aspects of neuronal function and survival, while their disruptions are linked to a spectrum of disorders, including AD, PD, HD, and schizophrenia.

(3) Huntington's disease gene Huntingtin

Huntingtin is important for neuronal survival and is involved in autophagy, but its exact roles in versatile cellular pathways remain to be fully elucidated.

RESEARCH PROJECTS

- Mechanisms of protein folding and cellular clearance pathways in brain degenerative disorders
- Normal functions of Huntingtin and its perturbation in Huntington's disease
- Biogenesis of autophagosomes and lysosome-related organelles

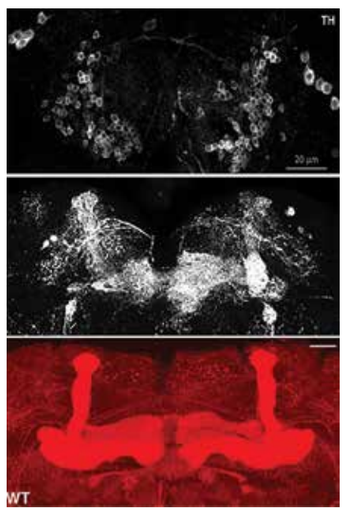
KEY PUBLICATIONS

Xu Y, Zhang S and Zheng H. (2019): "The cargo receptor SQSTM1 ameliorates Tau pathology and spreading through selective targeting of pathological Tau". *Autophagy* 15(4): 583-598.

Mao DX, Lin G, Senturk M, Zuo ZY, Tepe B, Zhang S, Arenkiel, BR, Sardiello M, and Bellen HJ. (2019): "Vap proteins are required for autophagic and lysosomal degradation". *Autophagy* 15(7): 1214-1233.

LAB MEMBERS

Instructor: Shiyu Xu, PhD
Graduate students: Yue Yu, Amanda Solbach
Research assistants: Xin Ye, PhD; Mrs. Lili Ye



An animal model for Parkinson's disease
The images show the cell bodies of dopamine neurons (top image) in *Drosophila* brain. Neurotransmitter dopamine (middle image), the messenger these neurons produce and use to communicate, are enriched in a structure called Mushroom bodies (bottom image), the learning and memory center in the brain.

The Mission of the Center for Molecular Imaging (CMI) is to develop and translate new medical imaging technologies, molecular imaging agents, and companion diagnostics to accelerate discoveries.

The CMI houses a diverse, interdisciplinary team of scientists and engineers who develop and use multi-modality molecular diagnostics and imaging techniques, including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and near-infrared fluorescence (NIRF) to enable new understandings of disease and chronic conditions. Sponsored industry, philanthropic, and federal research funding focuses upon autoimmune disorders, neuroinflammation, cancer metastases, hemo- and lymph-vascular diseases, and lymphedema. The team has experts in instrumentation, imaging agent development, inverse imaging algorithms, animal models of human disease, and translational science that effectively moves inventions and discoveries, "bench to bedside," and when discoveries are made in the clinic, from "bedside back to bench."

A highlight of the CMI is the basic science/clinical translational team that engages clinicians at UTHealth and at partnering institutions on the Texas Medical Center and in the Houston suburbs. These FDA-approved clinical studies enable visualization of the peripheral-lymphatic as well as the brain-lymphatic system using photonics technologies for better diagnosis and directed treatments. Conditions such as vascular anomalies, congenital heart disease, peripheral vascular disease, bacterial infections, breast cancer, and head and neck cancer are under investigation using our investigational imaging technologies. Earlier translational activities further explore visualization of brain function in neonates, and in preclinical models of human disease, cerebrospinal fluid outflow into the lymphatics, and intraoperative detection of lymph node metastases and tumor margins. Our team focuses upon translating new NIRF/molecular imaging agents using validated standards that can be applied across difference photonics device platforms.

In addition to having an assembly of faculty-driven independent basic science and clinical research projects, the center synergistically operates a "collaboration" center where clinicians and researchers partner to effectively apply imaging diagnostics to investigate and translate novel therapeutics and diagnostics.

Eva Sevick-Muraca, PhD
Center Director & Professor
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research

Eva Marie Sevick-Muraca, PhD
Professor and Director of the Center for Molecular Imaging
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research

Modulation of immune responses through the lymphatics

Over the past two decades, my research program has focused upon the development of near-infrared fluorescence imaging agents, instrumentation, and algorithms, as well as the validation against nuclear imaging technologies. Teaming with clinical collaborators and Center faculty, we have translated near-infrared fluorescence imaging in over 700 infants, children, and adults on the Texas Medical Center. These studies have resulted in discoveries mainly focused upon the lymphatic vasculature; a system involved in cancer and other chronic conditions, but has largely escaped medical attention.

One focus of my research program is to harness the lymphatics to attenuate inflammation or elicit disease-targeted immune responses. Regional T-cell and B-cell responses are mounted in lymph nodes, which receive antigens and antigen-presenting immune cells through afferent lymphatic vessels to educate T-cells and B-cells in order to produce antigen-specific T-cells and antibodies directed against these antigens. Once activated, these antigen-specific T-cells and antibodies leave the lymph node through efferent lymphatic vessels, drain into the circulatory blood system, and instill systemic immunity. Numerous immunotherapies target the molecular signaling associated with T-cell and B-cell activation in the lymph nodes. Cancer checkpoint blockade immunotherapy is one such therapy that has revolutionized cancer treatments for some patients, but the majority of patients experience disappointing responses or immune-related adverse events. We believe that these therapies can be more efficient if (i) they were delivered into the regional lymphatics, rather than through i.v. infusion, (ii) their systemic exposure through the blood stream could be minimized, and (iii) if they could be combined with vaccination strategies that would direct antigen-specificity. In CPRIT and NIH funded studies, we are using anti-CTLA-4 (analogous to human drug, Yeroy) and anti-PD-1 (analogous to human drugs, Keytruda or Opdivo) in mouse models of melanoma to show that anticancer activity is increased and adverse immune responses are decreased with lymphatic, as opposed to conventional intravenous, infusion. When combined with cancer vaccination strategies, we expect further improvements. Our team is working with the University Hospital of Bern and M.D. Anderson Cancer Center to develop preclinical evidence needed for clinical translation of our discoveries in cancer patients.

The second focus of my research program is harnessing the lymphatic drainage of cerebrospinal fluid (CSF) to combat neuroinflammation that arises from brain hemorrhage. Two patient populations are adversely impacted: preterm infants and traumatic brain injury patients. In both of these patient populations, hemorrhage activates the NLRP3 inflammasome creating pro-inflammatory cytokines that Aldrich and Sevick (2013) have shown arrests lymphatic contractility. We hypothesize that impaired lymphatic contractility could reduce CSF outflow from the brain, leading to debilitating hydrocephalus in pre-term infants, and chronic neuroinflammation and cognitive decline in adults. Dr. Banghe Zhu and I are collaborating with UTHealth Pediatric Neurosurgery to develop and use imaging that enables us to explore the relationship between CSF outflow and inflammasome biomarkers in infants within the NICU. Our goal is to identify new approaches to quell inflammasome activity in order to prevent hydrocephalus and improve outcomes. Pediatric hydrocephalus affects 6 in 10,000 live births and has a mortality rate before hospital discharge of 13%. In the adult traumatic brain patients at TIRR, we are evaluating impairments of CSF outflow and using physiotherapies to improve CSF outflow. If successful, we expect quicker cognitive recovery and less chronic neuroinflammation to result in the patient population.

Finally, my team also manages a collaborative facility that houses custom preclinical fluorescence imaging equipment and a small animal PET/CT Siemens Inveon scanner. The facility is available to industry and academic investigators to conduct preclinical imaging studies and is currently used in cancer, surgery, and neuroscience applications.

RESEARCH PROJECTS

- Lymphatic delivery of checkpoint blockade inhibitors for more effective immunotherapy
- Assessing CSF flow dynamics in pediatric hemorrhagic hydrocephalus
- Imaging lymphatic/CSF drainage from the head and neck in persons with traumatic brain injury

KEY PUBLICATIONS

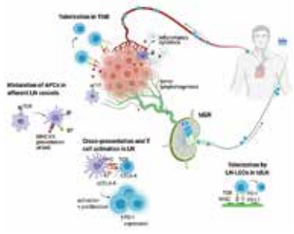
Kwon, S., Morena-Gonzalez, I., Taylor-Presse, K., Edwards Iii G, Gamez, N., Calderon, O., Zhu, B., Velasquez, F.C., Soto, C., and Sevick-Muraca, E.M., Impaired peripheral lymphatic function and cerebrospinal fluid outflow in a mouse model of alzheimer's disease," *J Alzheimer's Disease,* 69(2): 585-593, 2019 PMID 31104026

Kwon, S., Velasquez, F.C., Rasmussen, J.C., Greives, M.R., Turner, K., Morrow, J.R., Hwu, W.J., Ross, R.F., and E.M. Sevick-Muraca, "Nanotopography-directed lymphatic delivery of checkpoint blockade immunotherapy for improved anti-tumor responses," *Theranostics,* 22:9(26):8332-8343, 2019 PMID: 31754400

Aldrich, M.B., Rasmussen, J.C., Fife, C.E., Shaitelman, S.F., and E.M. Sevick-Muraca, "The development and treatment of lymphatic dysfunction in cancer patients and survivors," *Cancers,* 12(8), 2280, 2020 PMID: 32823928.

LAB MEMBERS

Post-doctoral fellows: Carolina Mantilla-Rojas, PhD
Research assistants: Fred Christian Velasquez and Janelle Morton



Tumor drainaing lymph nodes (tdLN) receive antigen (Ag) and antigen presentng cells that cross present to niave T cells to generate tumor specific T-cells in the absence of CTAL-4 signaling. T-cells leave via efferent lymphatic vessels and can be tolerized against tumoer antigens with PD-1 signalling in the lymphatics and in tumor microenvironment (TME).

Melissa B. Aldrich, MBA, PhD
Associate Professor

Imaging in immunology

Imagine receiving a cancer diagnosis, enduring surgery, radiation, and chemotherapy, and ringing the celebratory end-of-treatment bell, only to develop a devastating side effect of cancer treatment: lymphedema (LE), which manifests as a permanently swollen arm, leg, neck, or trunk. LE requires constant compression garment wear, meticulous skin care, and specialized massage. LE patients suffer discomfort, depression, cellulitis bouts, and there is no cure—only palliative treatment that includes 24/7 compression bandaging and specialized massage. Studies have shown that, if caught early in development, LE treatment can reverse the disease. Near-infrared fluorescence lymphatic imaging (NIRF-LI) delivers high-resolution, "see-through-the-skin," low-cost images of lymphatic vessel architecture and pumping. In disease states such as LE, NIRF-LI imaging can provide information for early diagnosis and evaluation of treatment efficacy. I lead a five-year prospective and longitudinal study using NIRF-LI surveillance of breast cancer patients to identify early LE development and biomarkers that could suggest pharmacological treatment. This study has shown that NIRF-LI allows very early detection of lymphatic dysfunction, well before obvious arm swelling appears, well before a clinical diagnosis of LE is typically delivered. I have presented the first lymphatic-visual (NIRF-LI) evidence at an international conference showing that, when NIRF-LI detects lymphatic dysfunction and early LE treatment is given, LE is reversible. This study also has shown that certain plasma cytokines are elevated in breast cancer patients destined to develop LE a year later, providing a prognostic tool to enable early identification of at-risk patients for pre-habilitation treatment referral.

I also lead a three-year CPRIT-funded clinical study of reparative lymphatic microsurgeries, which are gaining in popularity for LE patients for whom traditional palliative care fails. These surgeries' outcomes have not been objectively assessed to determine if and to what extent lymphatic vessel anatomy and pumping improve. This new study, after a delay due to COVID, is now in full swing, and will use NIRF-LI to show whether the surgeries actually improve lymphatic drainage in affected limbs, and may suggest ways to improve outcomes, including decreasing cellulitis risk that plagues LE patients.

I am very active in the LE community, and I was recently appointed to the Scientific and Medical Advisory Council of the Lymphatic Education & Research Network (LE&RN), an international organization of researchers, physicians, therapists, and patients, dedicated to advancing lymphatic health. I also chaired the committee that established standards and vetted applications for LE&RN's Centers of Excellence designation, which now enable patients to locate health institutions with lymphatic expertise. To date, over 35 leading institutions, including MD Anderson Cancer Center, Stanford University School of Medicine, and Beth Israel Deaconess/Harvard Medical School, and centers in Australia, Europe, Japan, and Taiwan, are part of this program.

I have previously participated as a team member imaging treatment responses to head and neck LE, which affects ~90% of head and neck cancer patients. Other past studies include rheumatoid arthritis drug delivery optimization, a case study of chylothorax in a neonatal heart surgery patient, and imaging of lymphatics in lipedema, a fat disorder that affects ~11% of women.

RESEARCH PROJECTS

- Longitudinal study of breast cancer-related LE
- Longitudinal study of reparative microsurgeries for LE
- Imaging of lymphatics in lipedema
- Imaging of neonatal chylothorax and pediatric lymphovenous anomalies

KEY PUBLICATIONS

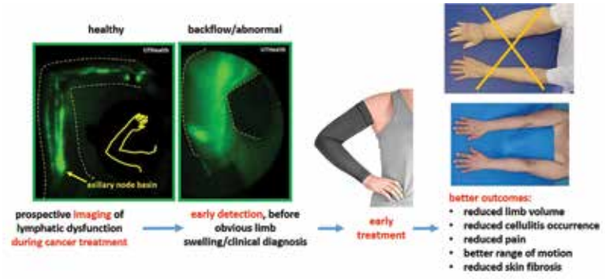
Aldrich M.B., Rasmussen J.C., Fife C.E., Shaitelman S.F., Sevick-Muraca E.M. The development and treatment of lymphatic dysfunction in cancer patients and survivors. *Cancers* (Basel) 12:2280, 2020.

Pham K., Balaguru D., Tammisetti V.S., Guevara C., Rasmussen J.C., Zvavanjanja R., Hanfland R., Sevick-Muraca E., Aldrich M.B. Multimodality lymphatic imaging of postoperative chylothorax in an infant with Noonan syndrome: a case report. *Eur J Med Res* 25:55, 2020.

Chang, D., Dayan, J., Fried, P., Patel, K., Repicci, W., Rockson, S. Singhal, D, Aldrich, M.B. Establishing standards for centers of excellence for the diagnosis and treatment of lymphatic disease. *Lymphat Res & Biol*, 19:4-10, 2021.

LAB MEMBERS

Medical student: Kay Pham
Graduate student: Anna Vang
Research assistant: Meghan McWain



Prospective, "see-through-the-skin" near-infrared fluorescence lymphatic imaging allows early detection of lymphedema development and early treatment. Catching lymphedema before the appearance of obvious arm swelling, with permanent changes to lymphatic anatomy, results in better outcomes, including lymphedema reversal.

John Rasmussen, PhD
Assistant Professor
Carolyn Frost Keenan Professor in Cardiovascular Disease Research

Device translation for lymphatic imaging

The lymphatic system is a vital, yet poorly understood, component of the circulatory system. As blood flows through the arteries and veins, water leaks from the vessels, entering the small gaps between the tissue cells. As the water moves through the tissues, it picks up cell waste, foreign contaminants, proteins, etc. and the resulting solution is taken up by the lymphatics, processed for immune response, and is ultimately returned to the veins. In addition, the lymphatics provide a pathway for the absorption of nutrients from the gut. However, because the lymphatics are typically small and primarily transport clear fluids, they are difficult to distinguish from the surrounding tissues, either with our eyes or using traditional clinical imaging modalities such as scintigraphy, X-ray, MRI, and ultrasound. Over the past few years, my research has focused upon the development and translation of near-infrared fluorescence (NIRF) optical imaging as a way to noninvasively image and characterize human lymphatics and quantify their contractile function in health and disease using microdose amounts of a fluorescent contrast agent.

One of our primary focuses is the relationship between the lymphatics and the blood circulatory system. Patients with advanced chronic venous disease often co-develop lymphedema, a condition of chronic swelling with fibrotic tissue changes and poor immune response. We recently imaged a group of patients with early venous disease, and observed a degradation of lymphatic anatomy as evidenced by the appearance of segmented lymphatic vessels and increased incidence of dermal backflow, or abnormal movement of contrast agent into the dermal tissues, as venous disease progressed. In addition, the lymphatic pumping rate initially increased to compensate for the increased venous load (C3 disease), but then decreased by nearly half as the disease continued to progress to C4 disease. A better understanding of the role of the lymphatics in early vascular disease may enable the development of more efficacious therapeutic approaches.

A second focus is the relationship between neuroinflammation and lymphatics. Cerebrospinal fluid (CSF) drains into the peripheral lymphatics and the interruption of this drainage has been implicated in the development of neurological disorders and poor recovery from traumatic brain injury. We recently completed a small study assessing the impact of gravity on the lymphatic drainage in the head and neck region. Under head-down-tilt conditions, which mimic the microgravity conditions of space, we observed impaired cervical lymphatic drainage indicating that gravity aids CSF drainage into the lymphatics under normal, upright positions. This impaired lymphatic drainage, in microgravity, may contribute to fluid shifting from the body to the head in space, results in chronically high cranial pressures that can damage the optical nerves of astronauts. We recently initiated a new study assessing the impact of lymphatic treatment on patient recovery from traumatic brain injury.

A third focus of my research program entails devising new methods for documenting lymphatic drainage in longitudinal studies. Current near-infrared imaging systems, provide two-dimensional fluorescence images that are difficult to anatomically relate to the areas of the body being imaged, especially in longitudinal studies seeking to track changes in lymphatic dysfunction with treatment. As part of our continued technology development, we are incorporating novel computer vision technologies, such as depth imaging, into our imaging system to enable the mapping of lymphatics in three-dimensional space. In addition, we seek to further the development of the technology by improving device sensitivity, automating different aspects of the hardware, and developing analytical tools to facilitate lymphatic image processing and analysis, all with the ultimate goal of answering new biological and clinical questions not addressed by other technologies.

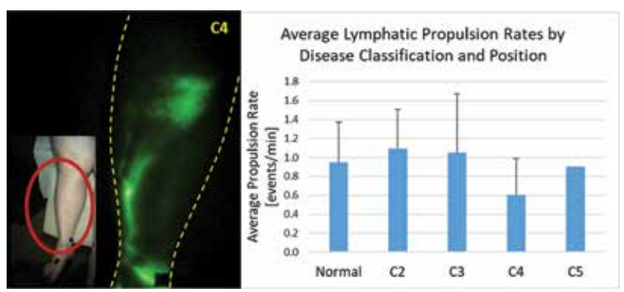
RESEARCH PROJECTS
- Understanding the role of lymphatics in the development of peripheral venous disease
- Understanding the role of lymphatics in the development and recovery of neurological conditions
- Incorporation of three-dimensional imaging technologies into lymphatic imaging

KEY PUBLICATIONS

Rasmussen, J.C. (corresponding author), Zhu, B., Morrow, J.R., Aldrich, M.B., Sahihi, A., Harlin, S.A., Fife, C.E., O'Donnell, T.F., and E.M. Sevick-Muraca, "Degradation of Lymphatic Anatomy and Function in Early Venous Insufficiency," *Journal of Vascular Surgery: Venous and Lymphatic Disorders,* 9(3):720-730.e2, 2021.

Rasmussen, J.C., Kwon, S., Pinal, A., Bareis, A., Velasquez, F.C., Janssen, C.F., Morrow, J.R., Fife, C.E., Karni, R.J., and E.M. Sevick-Muraca, "Assessing lymphatic route of CSF outflow and peripheral lymphatic contractile activity during head-down tilt using near-infrared fluorescence imaging," *Physiological Reports*, 8(4):e14375, 2020.

Aldrich, M.B., Rasmussen, J.C., Fife, C.E., Shaitelman, S.F., and E.M. Sevick-Muraca, "The Development and Treatment of Lymphatic Dysfunction in Cancer Patients and Survivors," *Cancers*, 12(8):2280, 2020.



Example NIRF images overlaid on three-dimensional point clouds representing (left) a phantom and (right) the lymphatics in the head and neck of a subject.

Banghe Zhu, PhD
Assistant Professor

NIR optical imaging of brain network dysfunction and CSF outflow

For over 25 years, advancements in Functional Near-Infrared Spectroscopy (fNIRS) imaging have shown promise as a valuable brain-imaging tool. Imaging from fNIRS allows examination of brain metabolism that is comparable to the BOLD fMRI signal. The fNIRS signal maps total hemoglobin (HbT) as well as oxygenated (HbO) and de-oxygenated (HbR) hemoglobin; this map approximates brain activation and deactivation acting as a proxy for localized glucose metabolism, similar to BOLD fMRI. Diffuse optical tomography (DOT) is an extension of fNIRS that combines the multi-channel data acquisition with imaging reconstruction techniques to provide images of neural related hemodynamic changes. Brain DOT could alter our understanding of dysfunction in the pediatric brain, a critically important step to positively modifying disabling movement disorders associated with cerebral palsy and devastating cognitive dysfunction associated with childhood-onset epilepsy. Our lab focuses on developing a transcranial NIR optical imaging system, called Cap-based Transcranial Optical Tomography (CTOT) able to image whole brain hemodynamic activity in an awake child. With recent advances to couple fast read-out scientific CMOS (sCMOS) devices and with optical switching of detector fiber optics, rapid dynamic CTOT mapping should be possible, which would then enable evaluation of functional connectivity in awake infants. In addition, we adapted CTOT imaging system called fCTOT (fluorescence-based CTOT) for mapping of cerebrospinal fluid (CSF) dynamics in a closely sized model of pediatric patients. With 20 second temporal resolution, we dynamically imaged the ICG-laden flow through the lateral, third, and fourth ventricles and into the subarachnoid space before its exit from the central neural system through the lymphatics and subarachnoid granulations. These results demonstrate the feasibility of imaging CSF in the pediatric population using fCTOT imaging technique and provide to guide surgical management or to improve upon the treatment of post-hemorrhagic hydrocephalus.

RESEARCH PROJECTS
- Develop fast CTOT imaging system for functional brain mapping
- Develop NIR fluorescence tomographic imaging system for CSF mapping
- Investigate the relationship between CSF and peripheral lymphatic function

KEY PUBLICATIONS

Rasmussen, J.C., Zhu, B., John R. Morrow, Melissa B. Aldrich, Aaron Sahihi, Stuart A. Harlin, Sheila Coogan, Caroline E. Fife, Thomas F. O'Donnell Jr, and Eva M. Sevick-Muraca, "Degradation of Lymphatic Anatomy and Function in Early Venous Disease," *Journal of Vascular Surgery: Venous and Lymphatic Disorders;* Vol 9, Issue 3, May, 2021.

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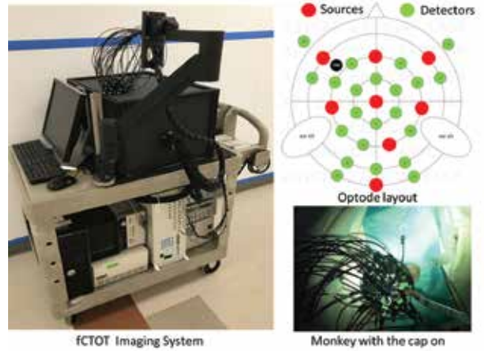
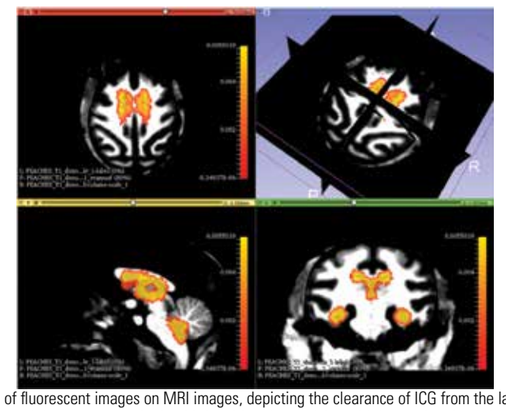
Zhu, B., Kwon, S., Rasmussen, J. C., Litorja, M., and Sevick-Muraca, E. M., "Comparison of NIR versus SWIR fluorescence imaging of indocyanine green using SI-derived metrics of image performance," *IEEE Transactions on Medical Imaging,* Vol. 39, No. 4, April, 2020



A fCTOT imaging system for mapping of CSF dynamics in normal cynomolgus macaques.



Overlays of fluorescent images on MRI images, depicting the clearance of ICG from the lateral ventricles and the appearance in the third and fourth ventricles with increasing time after administration.

The faculty, research staff, and trainees of the Center for Stem Cell and Regenerative Medicine (CSCRM) are focused on experimental studies of the biological properties of stem cells in both health and disease. The interest in stem cells is motivated by their essential role in both normal development as well as in maintenance of tissues and organs throughout life.
One of the hopes of regenerative medicine is that this fundamental understanding of stem cells may be effectively translated into therapies in which healthy stem cells, or their derivatives, can be employed to replace cells and tissues lost as a consequence of normal aging, injury, or disease.

There are at least two distinct classes of stem cells under active investigation within the Center for such therapeutic applications. The first class of stem cells of significant interest to Center investigators is induced pluripotent stem cells (iPSCs). iPSCs are patient-specific stem cells that can be generated from easily obtained cells from any individual and, in principle, may be specifically guided into the various cell types and tissues present within the human body. The second class consists of tissue-resident stem cells; such cells, present throughout life in various organs such as bone marrow, intestine, and lung are involved in active regeneration of cells and tissues lost due to normal cell turn-over or injury.

For patients presenting with genetically inherited disease, Center faculty are utilizing recently developed gene editing technologies to correct the disease-causing mutations in either iPSCs or tissue-resident stem cells. The goal of these studies is development of therapies that include correcting the mutations in a patient's own stem cells, then delivering either the corrected stem cells or cells/tissues derived from them back into the same patient.

Finally, there is increasing evidence for the presence within cancers of cells having specific properties typically associated with stem cells. Center faculty are interrogating the role of such cells in the initiation and maintenance of cancers of the blood such as mantle cell lymphoma and multiple myeloma.

In the pages following you will find examples of Center faculty exploring the potential therapeutic value of stem cells for repairing tissues such as spinal cord, brain, muscle, lung, and blood, as well as elucidating the role of stem cells in cancer. Competitive grant funding for these studies has been received from various sources including the National Institutes of Health, Department of Defense, Cancer Prevention Research Institute of Texas, Cystic Fibrosis Foundation, and others. Importantly, philanthropic funds made available to Center investigators, either in the form of endowed chairs, gifts, or pilot grants, have been and continue to be essential in seeding the early stage advances required for demonstrating proof of principle and eventual external grant funding.

If I may provide any additional information, please do not hesitate to contact me.

Brian R. Davis, PhD
Professor and Director
The C. Harold and Lorine G. Wallace Distinguished University Chair

CENTER FOR **STEM CELL AND REGENERATIVE MEDICINE**

Brian R. Davis, PhD
Professor and Director
C. Harold and Lorine G. Wallace Distinguished University Chair

Genetic correction of stem cells for treatment of inherited lung and blood diseases

My laboratory has as its primary objective the sequence-specific genetic correction of mutations in the chromosomal DNA of primary tissue-resident stem cells and/or induced pluripotent stem (iPS) cells derived from patients with inherited disorders affecting the lung or blood system. This is being pursued with the ultimate goal of developing stem cell-based therapeutic approaches. The ultimate objective is the delivery back to patients of their own lung or blood stem cells, only differing from the original stem cells by the genetic correction of the relevant mutation.

The first major project in the laboratory focuses on the site-specific correction of gene mutations responsible for inherited blood disorders such as the Wiskott-Aldrich Syndrome (WAS), a primary immune deficiency. In 2016, we demonstrated proof of principle for a methodology capable of correcting nearly all the mutations responsible for WAS in iPS cells. We are seeking to extend this methodology to patient-specific blood stem/progenitor cells that may be readily obtained from patients. In the past year, we have made significant progress in optimizing the efficiency of correction in blood stem/progenitor cells and have demonstrated that this methodology restores WAS protein and WAS protein-dependent function in cells carrying WAS mutations.

We are also presently utilizing the similar gene-correction methodologies to correct the CF mutations in tissue-specific stem cells directly obtained from CF patients. We have demonstrated highly efficient correction of the CF airway basal cells with functional restoration of CFTR channel activity. We are now working to make this approach even more universal in the range of mutations that may be corrected – and to extend the methodology for correction of airway basal cells directly in the airways of CF individuals. We have also utilized DNA sequence-specific nuclease-mediated homology directed repair to correct the most common genetic mutations in iPS cell lines derived from patients with cystic fibrosis – and have demonstrated genetic and functional correction in lung epithelial cells derived from these corrected iPS cells. We have recently reported the ability to specifically derive early lung progenitors and then airway basal stem cells for purposes of molecular and functional characterization as well as transplantation. We are currently employing CF patient-specific iPS cell-derived lung epithelium for testing sensitivity to specific CF drugs -- in order to facilitate a personalized therapeutic approach.

RESEARCH PROJECTS

- Correction of blood stem cells from Wiskott-Aldrich Syndrome patients
- Correction of airway basal stem cells from cystic fibrosis patients *in vitro* and *in vivo*
- Derivation and expansion of airway basal stem cell from cystic fibrosis patient-specific iPS cells.

KEY PUBLICATIONS

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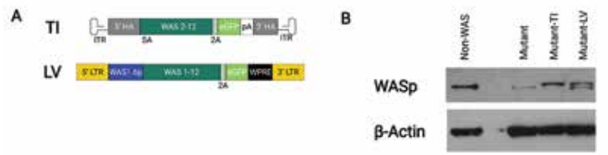
S. Suzuki, A.M. Crane, V. Anirudhan, C. Barilla, N. Matthias, S.H. Randell, A. Rab, E.J. Sorscher, J.L. Kerschner, S. Yin, A. Harris, M. Mendel, K. Kim, L. Zhang, A. Conway, B.R. Davis. Highly efficient editing of Cystic Fibrosis patient-derived airway basal cells results in functional CFTR correction. *Molecular Therapy* 2020 28: 1684-1695.

F.J. Hawkins, S. Suzuki, M.L. Beermann, C. Barilla, R. Wang, C. Villacorta-Martin, A. Berical, J.C. Jean, J. Le Suer, T. Matte, C. Simone-Roach, Y. Tang, T.M. Schlaeger, A.M. Crane, N. Matthias, S.X.L. Huang, S. Randell, J. Wu, J.R. Spence, G. Carraro, B.R. Stripp, A. Rab, E.J. Sorscher, A. Horani, S.L. Brody, B.R. Davis, D.N. Kotton: Derivation of Airway Basal Stem Cells from Human Pluripotent Stem Cells. *Cell Stem Cell*, 2021, 28: 79-95.

LAB MEMBERS

Post-doctoral fellows: Dr. John M. Avila, Dr. Cristina Barilla
Research instructor: Dr. Shingo Suzuki
Research staff: Dr. Cuong Q. Le, Samantha Winkler



Comparison of Targeted Integration (TI) and Lentiviral (LV) Approaches for Restoration of WAS protein (WASp) in B-cell Lines. (A) Donor constructs employed for TI (Adeno-associated virus) or LV transduction of mutant WAS B-cell line. (B) Western blot demonstrating restoration of WASp in WAS B-cell line having undergone TI.

Laura A. Smith Callahan, PhD
Assistant Professor

Tissue engineering approaches for the treatment of CNS injuries

The research in my laboratory focuses on developing biomaterials to be used in clinical treatments for spinal cord injury, traumatic brain injury, and stroke. The laboratory uses an interdisciplinary approach involving techniques from cell, molecular, and stem cell biology, chemistry, and material science. Utilizing engineering approaches, the laboratory seeks to optimize scaffold design for the expansion of clinically relevant cell sources for use in stem cell therapy and to support the cells after implantation into patients.

By examining cell-material interactions, we seek to understand which aspects of the native extracellular matrix facilitate tissue repair and integration with the surrounding host tissue. Once optimal composition, architecture (porosity, feature size, fiber alignment, etc.), mechanical properties, and bioactive signaling peptide concentrations have been identified using combinatorial methods, they are integrated into advanced hybrid matrices. These matrices maximize the advantages of both synthetic (consistency in fabrication and cellular response) and natural (native bioactive signaling) polymers, while mitigating their disadvantages, namely lack of bioactive signaling and batch to batch inconsistency in scaffold properties and cellular response, respectively. When combined with additional bioactive signaling and controlled architecture, these hybrid matrices can begin to emulate the native tissue microenvironment and support tissue development far better than traditional matrices. Preliminary studies have focused on formulating matrices to facilitate the extension of axons from the host across spinal cord lesion cavities in subacute rat models so spinal cord injury.

In order to advance biomaterial cell support matrices to wide spread clinical use, protocols for the expansion and differentiation of clinically relevant cell sources, also, need to be optimized. Human induced pluripotent stem cells (hiPSC) offer a potentially autologous cell sources for the treatment of traumatic injuries to the central nervous system. However, the number of viable cells for transplant produced from current differentiation protocols is extremely low. Both biochemical and mechanical properties of the cell culture surface have been shown to significantly affect cellular differentiation but have not been studied significantly in respect to hiPSC differentiation. The laboratory seeks to extend our knowledge of three dimensional culture systems to optimize two dimensional cell culture surfaces for differentiation of neural stem cells and oligodendrocyte progenitor cells from hiPSC. Preliminary studies have focused on the covalent tethering of proteins to the surface of hydrogels with containing a Young's Modulus gradient to study the effect of mechanical properties on hiPSC lineage choice.

RESEARCH PROJECTS

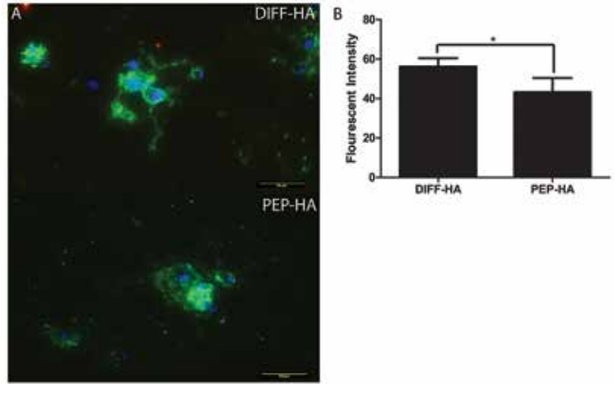
- Optimization of substrates and matrices to direct human induced pluripotent stem cells to neural progenitor cells to therapeutic linages using combinatorial approaches.
- Modulation of cellular environment *in vivo* to promote cell therapy survival, integration with the host and maturation toward functional mature cell types after central nervous system injury.

KEY PUBLICATIONS

Perera TH, Lu X, Howell SM, Kurosu YE, Smith Callahan LA. Combination of IKVAV, LRE and GPQGIWGQ Bioactive Signaling Peptides Increases Human Induced Pluripotent Stem Cell Derived Neural Stem Cells Extracellular Matrix Remodeling and Neurite Extension. *Advanced Biosystems*. 8: e2000084, 2020.

Perera TH, Lu X, Smith Callahan LA. Effect of laminin derived peptides IKVAV and LRE tethered to hyaluronic acid on hiPSC derived neural stem cell morphology, attachment and neurite extension. *Journal of Functional Biomaterials*.11: 15, 2020.

Perera TH, Howell SM, Smith Callahan LA. Manipulation of Extracellular Matrix Remodeling and Neurite Extension by Mouse Embryonic Stem Cells using IKVAV and LRE Peptide Tethering in Hyaluronic Acid Matrices. *Biomacromolecules* 20: 3009-3020, 2019.



Interaction of optimized laminin derived peptides signaling (IKVAV and LRE) on extracellular matrix secretion by human induced pluripotent stem cell derived neural stem cells (hNSC). A) Fibornectin staining (green) surrounds hNSC nucleus (blue) on hyaluronic acid matrices with (PEP-HA) and without (DIFF-HA) peptide signaling. Scale bars= 50μm. b) Quantification of fibronectin staining intensity. Fibronectin is associated with inflammation and fibrotic scarring, so reduced expression is beneficial to establishing new neural concentration during regenerative therapies.

Charles Cox, Jr., MD
Professor
George and Cynthia Mitchell Distinguished University Chair

Cellular therapies for neurological injury

Our current research program focuses on the use of cellular therapies for neurological injuries, principally traumatic brain injury (TBI). We have been interested in the modulation of the innate immune response to TBI and how cellular therapies have been successful without significant engraftment in the brain long term. Cell-cell interactions in the peripheral reticuloendothelial system have resulted in Treg upregulation and modulation of the microglia/macrophage phenotype in the brain. We use these types of data to help us determine dosing regimens (number of cells, type, and route of delivery, as well as timing), which may be very specific to the pathophysiology in question. We use *in vivo* models of injury and *in vitro* test beds.

Our team directs the Griffin Stem Cell Laboratory and the Hoffberger Stem Cell Laboratory, which are cGMP and cGTP cell processing facilities that enable us to translate discovery into treatments. These facilities allow clinical grade cell production for use in our clinical protocols.

RESEARCH PROJECTS

- Development of Phase 1 and 2 Clinical Trials using non-ESC stem/progenitor cells for traumatic brain injury.
- IND-enabling studies using APCs for traumatic brain injury.
- Amniotic fluid derived MSCs for the treatment of neurological injury associated with congenital heart disease and cardiopulmonary bypass/hypothermic circulatory arrest.
- Novel delivery systems for stem cells in neurological injury.
- Imaging of microglial activation *in vivo*.

KEY PUBLICATIONS

Jackson, M.L.; Srivastava, A.; Cox, C.S. Preclinical progenitor cell therapy in traumatic brain injury: a Meta-Analysis. *J Surg Res* 214:38-48, 2017. PMID: 28624058

Liao, G.P.; Aertker, B.A.; Kota, D.J.; Prabhakara, K.S.; Smith, P.A.; Hetz, R.A.; Xue, H.; Bedi, S.; Olson, S.D.; Cox, C.S. Assessing blood brain barrier permeability in traumatic brain injury research. *ADMET & DMPK*. 3(3):182-189, 2015.

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LAB MEMBERS

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Giselle Ortiz – Research Assistant
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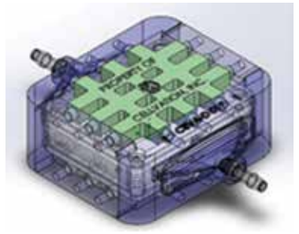
Fabio Triolo, PhD-GMP Center Director
Sufira Kiran, GMP-QA Director
Deepa Bhattarai-Manager, Lab
Sehreen Ali-QA Coordinator
Dannelys Perez-Bello-Sr. Research Associate
David Vo-Sr. Research Associate
Max Skibber-Research Assistant
Christina Willingham-Senior Program Manager
Melisa Walker-Program Manager



Development of a novel bioreactor for stem cell production.

Radbod Darabi, MD, PhD
Associate Professor

Disease modeling, gene correction, and therapeutic potential of human induced pluripotent stem cells (iPSCs) for skeletal muscle disorders

Skeletal muscle is the largest tissue in human body and is responsible for maintaining body posture, movement, and storage of key nutrients such as glycogen. Due to the presence of muscle stem cells, skeletal muscle has tremendous regenerative potential. Nevertheless, there are many disorders that can surpass this regenerative capacity and affect skeletal muscle health. These disorders include genetic disorders due a gene defect such as muscular dystrophies, volumetric muscle loss (VML) injuries due to accidents, or combat injuries and muscle wasting during chronic disorders, such as heart or kidney failure, or during the aging process. Unfortunately, these disorders often lead to varying degrees of muscle dysfunction and long-term disability and without any definitive cure.

Here at the IMM and Center for Stem Cell & Regenerative Medicine (CSCRM), we utilize human induced pluripotent stem cells (iPSCs) for disease modeling, site-specific gene correction, and skeletal muscle repair. iPSCs can be reprogrammed from adult skin or blood cells and can efficiently differentiate into all cell types in the human body. In addition, since iPSCs are derived from the same patients, they are immune-compatible. Therefore, iPSCs are generally considered as one of the ideal candidates for personalized stem cell therapy in many degenerative disorders.

Our lab is specialized in generation of human iPSCs from patients, site-specific gene correction, *in vitro* disease modeling as well as *in vivo* studies in animal models to evaluate therapeutic potential of human iPSCs. We use patient skin biopsy to obtain fibroblasts and reprogram them into iPSCs. In addition, in case of genetic muscle disorders, we use advanced gene targeting methods such as CRISPR/Cas9 system to target the gene defects and restore them into normal. We also derive different cell type progenitors from human iPSCs. These include skeletal muscle stem cells and endothelial progenitors, which can be used for regenerative applications.

So far, we have established few innovative methods for derivation of engraftable muscle stem cells from human iPSCs and demonstrated their application for skeletal muscle repair in different animal models, such as dystrophic or muscle injury mouse models. In addition, one of the recent directions of research with our MDACC collaborators is to use long non-coding RNA (lncRNA) molecules as a therapeutic agent to alleviate the severity of Duchenne and Becker muscular dystrophies.

The ultimate goal of our lab is to develop a stepwise and progressive strategy toward clinical application of human iPSCs for skeletal muscle disorders. Our research program is currently funded by awards from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) of the NIH and Department of Defense Office of the Congressionally Directed Medical Research Programs (CDMRP).

RESEARCH PROJECTS

- Disease modeling and gene correction of muscular dystrophies using CRISPR/Cas9 system
- Therapeutic potential of human iPSCs in volumetric muscle loss (VML) injuries
- Therapeutic potential of long non-coding RNAs (lncRNA) in Duchenne and Becker muscular dystrophies

KEY PUBLICATIONS

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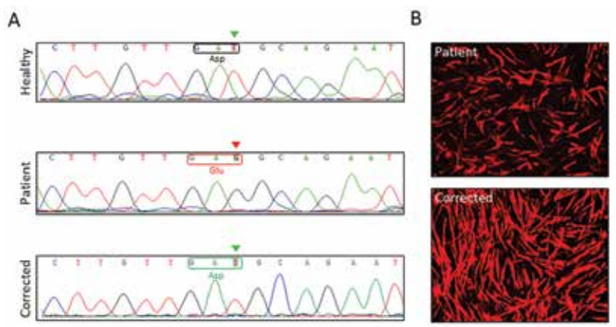
LAB MEMBERS

Instructor: Jianbo Wu



Gene correction of iPSCs from a patient with Limb-Girdle Muscular Dystrophy (LGMDR21). (A) Arrows shows healthy (green arrows) vs. mutated (red arrow) DNA code before and after correction. (B) Images demonstrate improved skeletal muscle formation ability (red staining for muscle) of the cells after gene correction.

Pramod Dash, PhD
Professor and Chair, Department of Neurobiology and Anatomy
Nina and Michael Zilkha Distinguished Chair, Neurodegenerative Disease Research

Concussion and stress-related disorders

Concussion (also known as mild traumatic brain injury, mTBI) has emerged as a major health problem, striking not only athletes participating in contact sports but persons of all ages and sexes. According to the Centers for Disease Control, approximately 2.6 million Americans sustain a brain injury each year, of which 87% can be classified as concussion. Recently, due to the increase in longevity and the number of falls in our older population, the incidence of concussion is on the rise in older Americans. As a person can sustain a concussion without ever losing consciousness, and many of these people never seek medical attention, the above statistics may only represent a fraction of actual concussion cases. Currently, there is no objective way to assess if brain injury has occurred after a concussion.

It has been recently appreciated that concussion is not a singular event but rather is a progressive disease with long-lasting consequences. It remains unknown when, or if, the brain returns to its pre-injury state. As the brain remains vulnerable to a second injury, continued research is required to understand the molecular, cellular, and structural changes that occur following concussion in order to develop treatments, which can offer functional improvement. To this end, we have been examining the influence of repeated brain injury in both humans and in animal models.

One of the consistent pathologies associated with both clinical and experimental traumatic brain injury is axonal injury, especially following concussion. Several lines of experimental evidence have demonstrated a role for NAD+ metabolism in axonal degeneration. One of the enzymes that metabolizes NAD+ in axons is Sarm1 (Sterile Alpha and TIR Motif Containing 1), and its activity is thought to play a key role in axonal degeneration. We have been examining the role of Sarm1 in axonal injury and cognitive outcome after repeated mild closed head injury (rmCHI). Our results indicate that rmCHI elicited white matter damage is markedly reduced in mice lacking the Sarm1 protein (Sarm1-/- mice). Further, we have found that the activation of astrocytes and microglia is also attenuated in the areas with white matter damage, suggesting a reduction in inflammation. Associated with these improvements, injured Sarm1-/- mice were found to perform significantly better in both motor and cognitive tasks.

RESEARCH PROJECTS

- To identify how concussion alters neural communication.
- To investigate neurovascular function after concussion.
- To investigate the consequences of mitochondrial plasticity and altered brain energy metabolism after concussion.

KEY PUBLICATIONS

Underwood E, Redell JB, Zhao J, Moore AN, Dash PK. A method for assessing tissue respiration in anatomically defined brain regions. *Sci Rep.* 10:13179, 2020.

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Maynard ME, Redell JB, Kobori N, Underwood EL, Fischer TD, Hood KN, LaRoche V, Waxham MN, Moore AN, Dash PK. Loss of PTEN-induced kinase 1 (Pink1) reduces hippocampal tyrosine hydroxylase and impairs learning and memory. *Exp Neurol.* 323:113081, 2020.

Broussard JI, Redell JB, Zhao J, Maynard ME, Kobori N, Perez A, Hood KN, Zhang XO, Moore AN, Dash PK. Mild Traumatic Brain Injury Decreases Spatial Information Content and Reduces Place Field Stability of Hippocampal CA1 Neurons. *J Neurotrauma* 37:227-235, 2020.

COLLABORATORS/LAB MEMBERS

Dr. James McCarthy: executive vice president and chief physician executive, Memorial Hermann
Dr. Paul Schulz: associate professor of Neurology; director, Dementia and Memory Disorders group
Dr. Summer Ott: associate professor of Orthopedic Surgery; director, Concussion Program at Ironman Sports Medicine Institute
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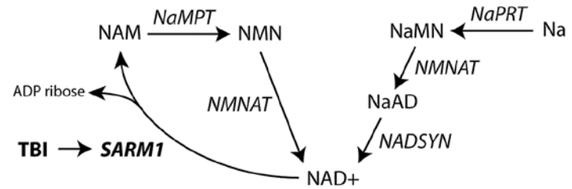
Dr. John Redell: assistant professor-research of Neurobiology and Anatomy
Dr. Jing Zhao: assistant professor-research of Neurobiology and Anatomy
Dr. Nobuhide Kobori: assistant professor-research of Neurobiology and Anatomy
Dr. Erica Underwood: instructor for Neurobiology and Anatomy
Post-doctoral fellows: Dr. Rebecca West
Research assistants: John Broussard, PhD, Kimberly Hood, MS, Anthony Moore, BS



Simplified pathway for NAD metabolism. NAD+ is synthesized from two metabolic pathways: a de novo synthesis pathway from Na (and amino acids) or a recycling pathway. Sarm1 is a NAD+ consuming enzyme. Our results indicate that in the absence of SARM1, axonal injury is reduced suggesting that depletion of NAD+ contributes to axonal damage after repeat concussion.

Sarah Xuelian Huang, MBBS, PhD
Assistant Professor

Human pluripotent stem cells for lung regeneration and disease modeling

My laboratory is interested in applying human pluripotent stem cells to study the molecular mechanisms of lung cell fate specification in the context of both normal and pathological conditions. The long-term goal is translation of the acquired knowledge into prevention and treatment of currently not curable lung diseases. Lung diseases are among the leading causes of death globally. Lower respiratory infections, chronic obstructive pulmonary disease, and lung cancer together account for approximately 9 million deaths annually worldwide. Despite the huge lung disease burden, we still have very limited understanding of the pathogenic mechanisms responsible for these diseases, and consequently there is a lack of successful therapeutic approaches.

Recently, a human pluripotent stem cell (hPSC)-based model has emerged as a novel system for studies of human diseases. The need for such a system stems from the limitations of the existing animal experimental models, which fall short in demonstrating concordance with human studies. In addition, experimental approaches utilizing primary human adult lung cells are inadequate in large part due to the limited availability of lung tissue from healthy subjects.

Realization of stem cell therapy in lung diseases relies on the successful generation of clinically applicable cell types. As a first, critical step in this direction, we have previously developed a step-wise differentiation strategy that directs human pluripotent stem cells to become different types of upper (airway) and lower (alveoli) respiratory lung epithelial cells at large quantities (Huang et al. Nat Biotechnol 2014, Nat Protoc 2015). As a proof of principle, the generated cells have been applied for lung development or disease studies by us and other research groups. Currently, we are working on culture conditions that can direct the human pluripotent stem cell-derived early lung progenitors toward an enriched population of either airway epithelial cells or distal alveolar cells. The availability of each of these enriched airway- and alveolar- fated cells provides a valid platform for studying lung diseases originate in both airway and alveolar. Examples include influenza virus infection induced severe infection and acute respiratory destress syndrome that affects the lower respiratory of the lung; and lung cancers that can arise in both the airway and alveoli cells depending on the subtype.

RESEARCH PROJECTS

- Use patient hiPSC differentiated lung and airway epithelial cells to study normal development and pathogen infection
- Understanding the basic mechanisms of lung lineage specification from NKX2.1+SOX2+SOX9+ human lung progenitors using molecular, genetic, and epigenetic approaches
- Understanding the molecular and epigenetic regulation of hPSC-derived airway basal stem cell competence

KEY PUBLICATIONS

Edwin J. Ostrin, Danielle R. Little, Kamryn N. Gerner-Mauro, Elizabeth A. Sumner, Ricardo Ríos-Corzo, Elizabeth Ambrosio, Samantha E. Holt, Nicolas R. Forcioli-Conti, Haruhiko Akiyama, Sam M. Hanash, Shioko Kimura, Sarah X.L. Huang, Jichao Chen. Beta-Catenin maintains lung epithelial progenitors after lung specification. *Development* (2018) 145, dev160788.

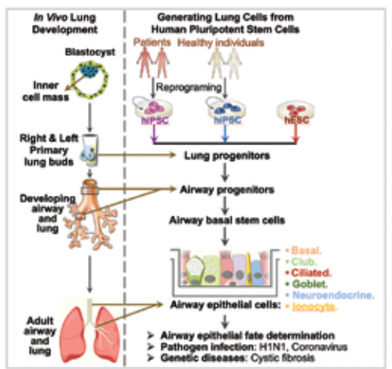
Hye Kyung Lim, Sarah X.L. Huang, Jie Chen, Gaspard Kerner, Olivier Gilliaux, Paul Bastard, Kerry Dobbs, Nicholas Hernandez, Nicolas Goudin, Mary L. Hasek, Eduardo Javier García Reino, Fabien G. Lafaille, Lazaro Lorenzo, Priya Luthra, Tatiana Kochetkov, Benedetta Bigio, Soraya Boucherit, Flore Rozenberg, Catherine Vedrinne, Michael D. Keller, Yuval Itan, Adolfo García-Sastre, Marie Celard, Jordan S. Orange, Michael J. Ciancanelli, Isabelle Meyts, Qian Zhang, Laurent Abel, Luigi D. Notarangelo, Hans-Willem Snoeck, Jean-Laurent Casanova, Shen-Ying Zhang. Severe influenza pneumonitis in children with inherited TLR3 deficiency. *J Exp Med.* 2019 Sep 2;216(9):2038-2056.

Finn J. Hawkins, Shingo Suzuki, Mary Lou Beermann1, Cristina Barilla, Ruobing Wang, Carlos Villacorta-Martin, Andrew Berical, J.C. Jean, Jake Le Suer, Taylor Matte, Chantelle Simone-Roach, Yang Tang, Thorsten M. Schlaeger, Ana M. Crane, Nadine Matthias, Sarah X.L. Huang, Scott H. Randell, Joshua Wu, Jason R. Spence, Gianni Carraro, Barry R. Stripp, Andras Rab, Eric J. Sorsher, Amjad Horani, Steven L. Brody, Brian R. Davis and Darrell N. Kotton. Derivation of Airway Basal Stem Cells from Human Pluripotent Stem Cells. *Cell Stem Cell*, 2021January 7; 28, 1–17.

Jiawen Chen, Seyed Mohammad Mir, Meghan R Pinezich, John D O'Neill, Brandon A Guenthart, Matthew Bacchetta, Gordana Vunjak-Novakovic, Sarah X L Huang, Jinho Kim. Non-destructive vacuum-assisted measurement of lung elastic modulus. *Acta Biomater.* 2021 Sep 1;131:370-380.

LAB MEMBERS

Research assistants: Shnutez Doddipalli, Nicole Acosta



Schematic illustration of human pluripotent stem cells-derived airway epithelial cells for modeling airway development and diseases.

Momoko Yoshimoto, MD, PhD
Associate Professor

Development of hematopoietic stem cells and innate-like B cells in the mouse embryo

The hematopoietic stem cells (HSCs) that produce all types of blood cells in the body are first generated in the aortic region of the mouse embryo at embryonic day (E) 10-11. Interestingly though, there are multiple waves of blood cell production prior to the emergence of the first HSC from endothelial cells (referred to as hemogenic endothelial cells: HECs) and these blood cells include erythro-myeloid, T- and B- lymphoid cells. Using lineage tracing mouse models and transplantation assays, we have recently found that innate-like B-1 lymphocytes, multi-potent progenitors, and the first HSCs are produced simultaneously from HECs. Furthermore, we found paradigm-shifting results that fetal-derived B-progenitors persist into adult life much longer than previously expected. We are elucidating 1) what molecular signals determine the divergent point between innate-like B-1a biased progenitors, multipotent progenitors, and multi-potent HSCs in HECs, 2) how fetal-derived B-progenitor develop in the perinatal period, and 3) how HSC-precursors mature into adult-repopulating HSCs in a limited time window of embryonic development.

B-1 cells are unique murine innate immune cells that are distinguished from conventional adoptive B cells (B-2 cells). B-1 cells localize in the peritoneal and pleural cavities and secrete natural antibodies without T cell help, displaying important roles in the first line of defense against various infections, atherosclerosis, and autoimmunity. Although B-1a cells are fetal derived and B-1 specific progenitors have been found in the fetal liver, how and in which organ B-1 progenitors develop into mature B-1a cells remains unknown.

By utilizing lineage tracing mouse models combined with knockout mouse models, and single-cell RNA-sequencing, we are elucidating the biological and molecular mechanisms that are responsible for the cell fate decision and maturation of fetal derived B-lymphocytes.

Knowledge obtained from above projects will help us to improve the system where HSCs are produced from human iPSCs *in vitro*, which may be utilized for cell therapy to the patients with hematological disorders and leukemias.

RESEARCH PROJECTS

- Lineage tracing for HSC-independent and/or HSC-dependent B-1 cell development from embryos to adults.
- Identify important molecules for HSC maturation in the mouse embryo utilizing single-cell RNA-sequencing.
- Examining the contribution of fetal-derived B cells to IgA secreting cells in the lamina propria of intestine.
- Producing human B-1 cells from human iPSCs.

KEY PUBLICATIONS

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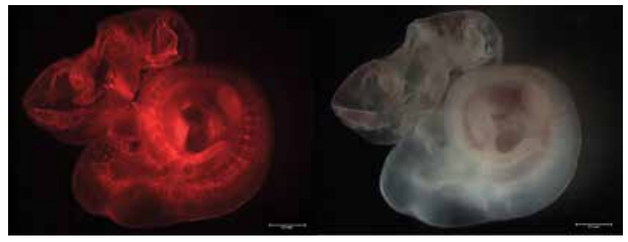
LAB MEMBERS

Assistant professor: Michihiro Kobayashi MD, PhD
Post-doctoral fellow: Haizi Cheng
Research assistants: Chika Nishida MD, Augusto Latorre



A lineage tracing mouse model
Cdh5CreERT2: Rosa-Tomato embryos. Tamoxifen was injected into day 9 pregnant mother and cdh5+ endothelial cells were labeled with Tomato+ on the following day.

Dung-Fang Lee, PhD
Assistant Professor

Familial cancer syndromes in a dish

After leukemia, osteosarcoma is the second leading cause of cancer mortality among children. Genetic alterations (e.g., *p53* mutation and *RB1* deletion) are strongly associated with osteosarcoma development. Patients with Li-Fraumeni syndrome (LFS), a genetically inherited autosomal dominant cancer disorder caused by germline mutations in the *p53* tumor suppressor gene, have increased incidence of osteosarcoma development, which provides a perfect model system to study osteosarcoma.

Modeling human genetic disease has recently become feasible with induced pluripotent stem cell (iPSC) methodologies developed by Dr. Shinya Yamanaka in 2006. Characterized by their ability to self-renew indefinitely and differentiate into all cell lineages of an organism like embryonic stem (ES) cells, iPSCs provide a powerful and unlimited source of cells to generate differentiated cells that can be used to elucidate disease pathogenesis for drug discovery and development, toxicology screening, personalized healthcare, and eventually cell transplantation-based therapies.

Our research is dedicated to understanding cancer pathological mechanisms by applying patient-specific iPSCs and/or engineered ESCs. We have established the first human Li-Fraumeni syndrome (LFS) disease model by using LFS patient-specific iPSCs to delineate the pathological mechanisms caused by mutant p53 in osteosarcoma (Lee, et al, Cell 2015; Gingold, et al, Trends Cancer 2016). LFS iPSC-derived osteoblasts recapitulate osteosarcoma features, including defective osteoblastic differentiation and tumorigenic ability, suggesting that our established LFS disease model is a "disease in a dish" platform for elucidating p53 mutation mediated disease pathogenesis. Since these iPSCs were generated from non-transformed fibroblasts, any recapitulated features of osteosarcoma must be due to the single gene alteration. The patient-specific iPSC model therefore provides a powerful system to elucidate unique gene function in tumor etiology. We continue applying patient-specific iPSCs and TALEN/CRISPR genetically engineered hESCs to illuminate cancer pathological mechanisms.

RESEARCH PROJECTS

- Systems-level analyses and characterization of mutant p53 in LFS-associated osteosarcoma.
- Systematic analyses of genome alterations during LFS-associated osteosarcoma development.
- Model familial cancer syndrome with predisposition to osteosarcoma by patient-specific iPSC approaches.

KEY PUBLICATIONS

Huensuk Kim, Seungyeul Yoo, Ruoji Zhou, An Xu, Jeffrey M. Bernitz, Ye Yuan, Andreia M Gomes, Michael G Daniel, Jie Su, Elizabeth G. Demicco, Jun Zhu, Kateri A. Moore, Dung-Fang Lee, Ihor R Lemischka, Christoph Schaniel. Oncogenic role of SFRP2 in p53-mutant osteosarcoma development via autocrine and paracrine mechanism. *Proc Natl Acad Sci U S A*. 2018 Nov 20:115(47):E11128-E11137.

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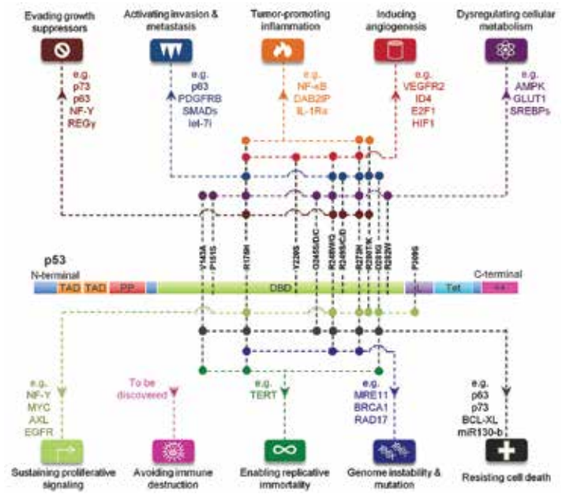
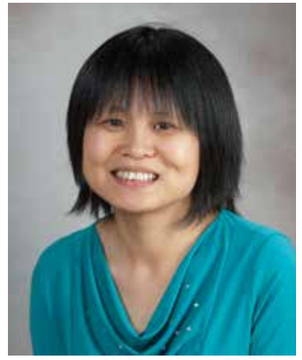
Brittany E. Jewell1, An Xu, Dandan Zhu, Mo-Fan Huang, Linchao Lu, Mo Liu, Erica L. Underwood, Jun Hyoung Park, Huihui Fan, Julian A. Gingold, Ruoji Zhou, Jian Tu, Zijun Huo, Ying Liu, Weidong Jin, Yi-Hung Chen, Yitan Xu, Shu-Hsia Chen, Nino Rainusso, Nathaniel K. Berg, Danielle A. Bazer Christopher Vellano, Philip Jones, Holger K. Eltzschig, Zhongming Zhao, Benny Abraham Kaipparettu, Ruiying Zhao, Lisa L. Wang, Dung-Fang Lee. Patient-derived iPSCs Link Elevated Mitochondrial Respiratory Complex I Function to Osteosarcoma in Rothmund-Thomson Syndrome. *PLoS Genet*. 2021 (in press)

LAB MEMBERS

Post-doctoral fellows: An Xu, Mo Liu, Dandan Zhu
Graduate student: Mo-Fan Huang
Research assistant: Ying Liu



Mutant p53 gain-of-function driver cancer through cancer hallmarks. Different mutations on p53 protein arm p53 with new weapons (downstream targets indicted in the figure) to drive cancer development and progression. Each color-coded node indicates gain-of-function of specific mutation of TP53, which further drives cancer through various cancer hallmarks.



Ying Liu, MD, PhD
Assistant Professor

Human pluripotent stem cells in cell-based therapy for CNS diseases

Our research focuses on dissecting the neural developmental pathways and the corresponding pathogenesis in CNS injury and neurodegenerative diseases. Our long-term goal is to identify therapeutic targets for the treatment of CNS diseases.

Human induced pluripotent stem cells (iPSCs) provide autologous materials for patients, which theoretically omit the need for immune suppression. We have optimized the more clinically relevant, integration-free hiPSC generation protocol and performed directed differentiation of patient-specific iPSCs into neural stem cells, neuronal and glial progenitors, as well as mature cell types for disease modeling, transplantation studies, neural regeneration and repair, and drug screening and testing. The highly efficient CRISPR gene editing tool adapted in the lab allows for quick creation of neural lineage reporters and multigene activation for lineage induction. These neural lineage specific cells are applied to in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

- Generation of patient-specific, integration-free iPSCs.
- Identification of optimal neural lineage progenitors for cell-based therapy in spinal cord injury.
- Down syndrome disease modeling using patient derived iPSCs and neural populations
- Molecular changes in gene expression regulatory networks in glioblastoma.

KEY PUBLICATIONS

Liu, Y.*, Zheng, Y., Li, S., Xue, H., Schmitt, K., Hergenroeder, G.W., Wu, J., Zhang, Y., Kim, D.H., Cao, Q*. (2017) Human neural progenitors derived from integration-free iPSCs for SCI therapy. *Stem Cell Res*. 2017 Jan 5;19:55-64. doi: 10.1016/j.scr.2017.01.004. [Epub ahead of print] (*corresponding authors) PMID:28073086

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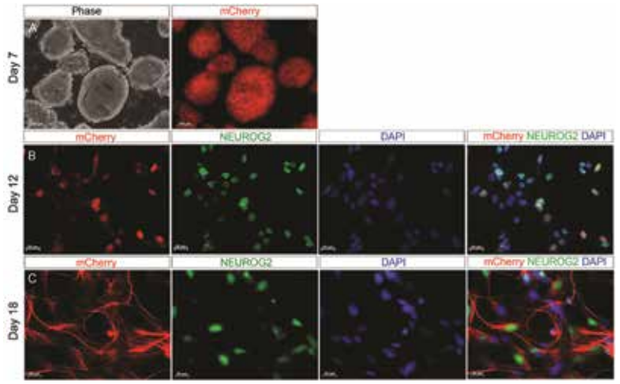
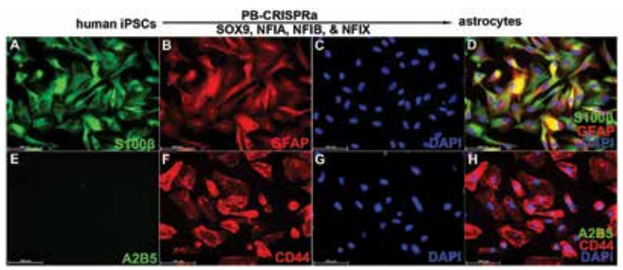
LAB MEMBERS

Research scientist: Shenglan Li
Research associate: Haipeng Xue



A Neurogenin 2 knockin human iPSC reporter cell line made using the CRISPR/Cas9 system. NEUROG2-mCherry human iPSC clones are induced as embryoid bodies (EBs) which glow red under the fluorescence microscope (A). NEUROG2 antibody staining (green) confirms that mCherry (red, native signal) expression faithfully reflects the endogenous NEUROG2 expression along the differentiation pathway (B, C).



Rapid generation of astrocytes from human iPSCs by endogenous activation of astrocyte lineage specific transcription factors with the piggyBac-CRISPR activation system. Human iPSCs cell line was transfected with all-in-one vectors expressing guide RNAs that activate SOX9-NFIA-NFIB-NFIX transcription factors. Fourteen days post transfection, nearly all cells expressed astrocyte markers S100B (A), GFAP (B) and CD44 (F), while did not express glial progenitor marker A2B5 (E). Nuclei are revealed by DAPI (C, G). (D) and (H) are overlapped images.

Nami McCarty, PhD
Professor
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

Investigating and targeting signaling pathways involved in protein homeostasis in cancers and neurodegenerative diseases

Protein homeostasis is orchestrated by coordinated protein synthesis, folding, transport, and degradation. Inappropriate protein assembly or modification promotes protein misfolding, which can lead to not only disruptions to protein homeostasis but also to normal cellular functions. Chaperones are regulators of protein folding processes and include the heat shock proteins. Misfolded proteins that escape these control mechanisms must be targeted for degradation, either through the UPS or by autophagic processes.

One crucial mechanism that marks the target protein for degradation in both the UPS and autophagy pathways is ubiquitination. UPS-mediated protein degradation is mediated by the recognition of the protein substrates marked through polyubiquitination. E1-E2-E3 ubiquitin activators, conjugases, and ligases work together to conjugate polyubiquitin chains to the substrate targeted for degradation. Polyubiquitinated proteins, including misfolded proteins, are recognized by the ATP-dependent proteasome complex and subsequently degraded. In the autophagy-mediated degradation pathway, the polyubiquitin chain is recognized by proteins such as SQSTM1/p62 and adaptor proteins that recruit substrates to autophagosomes for degradation.

When misfolded proteins are not efficiently degraded by proteasome complexes, they form toxic aggregates, and their removal becomes critical for cell survival. The accumulation of misfolded proteins and protein aggregates is a central pathological feature of many neurodegenerative diseases, such as Alzheimer, Parkinson, and Huntington. Balanced proteostasis is also important for cancer cell survival. In cancer cells that have uncontrolled protein synthesis, misfolded protein accumulation suppresses growth. Therefore, understanding the regulatory mechanisms of proteostasis control is critical for understanding not only normal cell growth but also malignant cell survival.

We previously discovered a member of tripartite motif (TRIM) family, TRIM44, which is highly upregulated in quiescent multiple myeloma (MM) cells isolated from the osteoblastic niches of the bone marrow. Since TRIM44 was first cloned in 2001, only a few studies have reported on TRIM44 functions. We found that TRIM44 deubiquitinates HIF-1α to stabilize it, which in turn promotes quiescent MM cell survival. Upregulated TRIM44 promotes bone destruction in a similar manner to that observed in symptomatic MM patients. Silencing TRIM44 reverses bone destruction.

We also discovered novel TRIM44 functions in proteostasis control. TRIM44 associates with ubiquitinated aggregates via its CC domains and plays critical functions in misfolded protein clearance. Upregulated TRIM44 expression in aggregate-prone models showed a remarkably reduced number of protein aggregates with decreased overall aggregate volume. In a cell model that localized misfolded proteins to aggresomes, TRIM44 colocalized with aggresome components, revealing that TRIM44 is part of an aggresome complex. In addition, TRIMM44, a deubiquitination enzyme, activates autophagy via promoting p62 oligomerization and plays critical roles in aggregate removal. Our data support a novel function of TRIM44 in aggregate binding and removal and are the first to show that TRIM44 plays an essential function in protein homeostatic control and possibly plays important functions in cancers and neurodegenerative diseases.

KEY PUBLICATIONS

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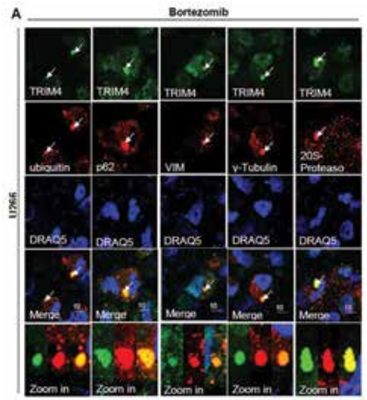
LAB MEMBERS

Post-doctoral fellows: Lin Lyu, PhD, Yuquin Wang, PhD
Research assistant: Raksha Rao, PhD



TRIM44 is associated with ubiquitin proteins
(A) U266 cells were treated with Bortezomib (5 nM) for 24 hr, and visualized with an antibody to ubiquitin, p62, vimentin, γ-Tubulin, and 20S-Proteasome. Scale bars: 10 µm
(B) U266 cells were treated with DMSO or tunicamycin (1µg/mL) for 24 hr or Thasigargin (2.5µM/mL) for 6h , and immunostained with antibodies against to TRIM44 and ubiquitin. Scale bars: 10 µm.
(C) A549 cells were transfected with expression plasmids for GFP-CFTR-ΔF508 or GFP-250 as indicated. GFP-CFTR-ΔF508 transfected cells were treated with MG132 (5 µM) for 6 or 24 hr, and visualized with an antibody to TRIM44. The colocalization of TRIM44 with GFP-250 aggresomes was assessed at 24 hr after GFP-250 transfection.

Pamela Wenzel, PhD
Associate Professor
Director of Immunology Program, MD Anderson

Effects of flow on stem cell potential and immune function

Our lab studies how biomechanical force generated by the flow of blood in the circulatory system impacts cell fate and behavior. One of our primary research projects addresses how frictional force caused by blood flow promotes emergence of blood stem cells during embryo development. We are interested in how we might use this information in the laboratory to expand improved sources of these stem cells for treatment of hematologic disorders and cancers, such as bone marrow failure syndromes and leukemias.

Complex signaling occurs in response to flow that potentiates stem cell potential, including activation of integrins, mechanosensitive ion channels, and primary cilia (Fig. 1). In our prior published work, we have shown that fluid frictional force in biomimetic microfluidics that matches the intensity of blood flow present in the developing embryo can stimulate calcium sparks within the cytoplasm, thus triggering the cell to produce prostaglandin E2. Elevated prostaglandin synthesis is key to forming hematopoietic stem cells that later will supply the body with blood and immune cells into adulthood. We have additionally shown that the force generated by this flow activates classic developmental signaling, including Notch and Wnt. Both of these signal transduction pathways are known regulators of blood development and must be tightly modulated in order to direct differentiation of certain immune cell lineages, including T lymphocytes. Lastly, in work spanning various model systems, evidence has begun to emerge that implicate focal adhesion kinase and the Src family kinases in regulation of transcription factors such as Yap and Taz downstream of fluid force. We are currently pursuing both collaborative and independent studies aimed at better understanding the mechanosensors and intracellular signaling that are central to dictating how blood stem cells respond to biomechanical cues to ensure proper self-renewal and differentiation.

Another related area of research in our lab includes the study of how flow alters bioenergetics and, specifically, how the powerhouses of the cell – the mitochondria - adapt to meet the changing metabolic needs of stem cells. We are finding that these organelles change shape and move differently within the cell depending upon the biophysical cues in the environment. This is particularly relevant during fate commitment of hematopoietic stem cells in the embryo, but could also be important in the adult. Mitochondria are critical in both hematopoietic stem cells and mesenchymal stem cells of the adult bone marrow, the latter of which are known to be capable of promoting repair of damaged tissues by mitochondrial transfer to injured cells when administered as a cellular therapeutic. Ongoing studies are directed at determining how mitochondria contribute to ensuring that hematopoietic stem cells are properly specified in the embryo and how we might modify mitochondrial behavior to enhance stem cell activity in bone marrow transplantation.

RESEARCH PROJECTS

- Effects of flow on hematopoietic stem cell fate and the bone marrow niche
- Biomechanical force in modulation of mitochondrial dynamics

KEY PUBLICATIONS

Diaz, M.F., Horton, P.D., Kumar, A., Livingston, M., Mohammadalipour, A., Xue, H., Skibber, M.A., Ewere, A., Toledano Furman, N.E., Aroom, K.R., Zhang, S., Gill, B.S., Cox, C.S., & Wenzel, P.L. Injury intensifies T cell mediated graft-versus-host disease in a humanized model of traumatic brain injury. *Scientific Reports*. 10(1):10729. doi: 10.1038/s41598-020-67723-x, 2020.

Mohammadalipour, A., Dumbali, S.P., & Wenzel, P.L. Mitochondrial transfer and regulators of mesenchymal stromal cell function and therapeutic efficacy. *Frontiers in Cell and Developmental Biology*. 8:603292. doi: 10.3389/fcell.2020.603292, 2020.

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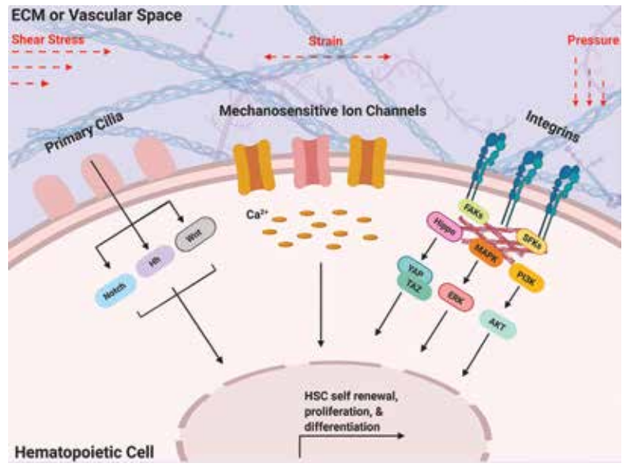
LAB MEMBERS

Graduate student: Paulina Horton
Post-doctoral fellow: Sandeep Dumbali
Senior research associate: Miguel Diaz



Integrins, mechanosensitive ion channels, and primary cilia sense mechanical features of the hematopoietic niche. Activation of mechanotransduction pathways alter gene expression and cell behavior critical for homeostasis and response to stress.

Jiaqian Wu, PhD
Associate Professor

Gene transcription and regulation of stem cell differentiation and neural injuries

An associate professor with tenure in the Vivian L. Smith Department of Neurosurgery and Center for Stem Cell and Regenerative Medicine, I led the NIH Mammalian Gene Collection effort and cloned thousands of mammalian genes which are publicly available through GE Dharmacon now. I also was closely involved in the ENCODE project and she employed interdisciplinary approaches to study gene expression, transcription factor regulation, and regulatory networks of stem cell self-renewal and differentiation. In her independent laboratory, I have carried out unprecedented transcriptome profiling of eight highly purified neuron, glia, and vascular cells from brain by RNA-Seq. Our lab identified a large number of novel long non-coding RNAs and identified the role of lncRNA in oligodendrocyte precursor cell (OPC) formation for the first time using functional and genetic experiments. One of the neurological diseases of focus is spinal cord injury (SCI). The lab has already published studies for acute and chronic SCI phases in mouse and rat contusive injury models. We provided valuable data source and a powerful analysis framework for functional investigations of coding and long non-coding RNAs in CNS cell types and SCI. Our work has been recognized with prestigious honors and awards, including the National Institutes of Health Ruth L. Kirschstein National Research Service Award for Individual Postdoctoral Fellows, and the International Society for Stem Cell Research (ISSCR) Annual Meeting Travel Award, the National Institute of Health Pathway to Independence (PI) Award (K99/R00), R01 and the Senator Lloyd and B.A. Bentsen Investigator Award. A reviewer for NIH, New York State Department of Health-Spinal Cord Injury Research Board, MRC, ANR, and many journals, I have presented invited talks and lectures at national and international conferences and institutions. I have developed a patent, authored two books, and wrote many articles that have appeared in *Nature, PNAS, the Journal of Neuroscience, Cell Reports, Genome Research,* and *Nature Neuroscience* among others.

RESEARCH PROJECTS

- Combines stem cell biology and systems-based approaches involving genomics, bioinformatics, and functional assays to investigate gene expression and regulatory mechanisms during stem cell differentiation; pinpoint key transcription factors and regulatory RNAs, and modulate key regulators to steer the direction of stem cell differentiation and improve efficiency/safety
- Characterize molecular signatures and identify therapeutic targets for spinal cord injury and neurological diseases

KEY PUBLICATIONS

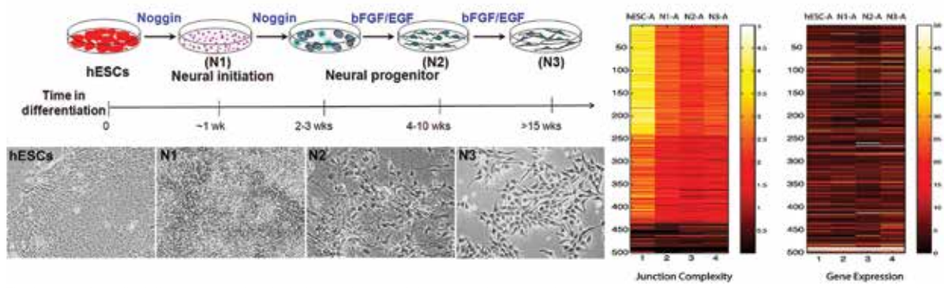
Wei, H*., Wu, X*., You, Y*., Cuevas-Diaz Duran, R., Zheng, Y., Narayanan, L.K., Hai, B., Li X., Tallapragada, N., Prajapati, T.J, Kim, D.H., Deneen, B., Cao, Q., Wu, J. Q. Systematic analysis of purified astrocytes after SCI unveils Zeb2os function during astrogliosis. *Cell Reports*. 2021 Feb 2;34(5):108721. doi: 10.1016/j.celrep.2021.108721. PMID: 33535036.

Wei, H*., Dong, X*., You, Y., Hai, B., Cuevas-Diaz Duran, RC., Wu, X., Kharas, N., Wu, J. Q. OLIG2 regulates lncRNAs and its own expression during oligodendrocyte lineage formation. *BMC Biol*. 2021 Jun 25;19(1):132. https://doi.org/10.1186/s12915-021-01057-6. PMID: 34172044.

Escartin, C*., Galea, E*., Lakatos, A., O'Callaghan, JP., Petzold, GC., ..., Wu, J. Q., ..., Zheng, B., Zimmer, ER., Zorec, R., Sofroniew, MV., Verkhratsky, A. Reactive astrocyte nomenclature, definitions, and future directions. *Nature Neuroscience*. 2021. doi: 10.1038/s41593-020-00783-4. PMID: 33589835.

LAB MEMBERS

Post-doctoral fellows: Haichao Wei, PhD; Xizi Wu, MD, MS
Research associate:Andrew Rolfe
Resident physician: Michael Monterey, MD
Undergraduate students: Tanuj Prajapati, Neha Tallapragada



The lab uses interdisciplinary approaches including molecular biology, genetics, genomics, proteomics, and bioinformatics to study gene expression and transcriptional regulation in stem cells and the nervous system.

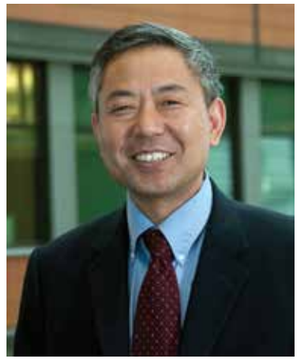
Translational cancer research aims to identify novel drug targets followed by the discovery and development of drug candidates as potential cancer therapeutics. The goal is to translate discoveries made in basic cancer research to potential drugs that could be tested in human patients. It relies on a plethora of information and data on cancer origination, progression, metastasis, drug-resistance, and disease relapse to uncover the driving mechanisms of tumor growth and invasion. Technologies such as next generation sequencing of DNA and RNA in cancer and non-cancer cells of tumor tissues, CRISPR screening, proteomics, imaging, patient-derived tumor models, drug candidate discovery, and bioinformatics are utilized to reveal drug targets and validate potential drug candidates.

The current research in the Center for Translational Cancer Research emphasizes several areas, including the application of cutting-edge bioinformatic and experimental technologies to identify and validate novel drug targets in several major types of solid tumors, the discovery of specific molecules against the targets with a focus on antibody/protein-drug conjugates, the development of targeted contrast agents for disease visualization, and the study of proteome alterations to elucidate disease mechanism and discover biomarkers.

These efforts connect us with collaborators, such as physicians, pathologists, biologists, bioinformaticians, and bioengineers, across UTHealth, institutions within the Texas Medical Center, and across Texas to enhance basic, translational and clinical research. At the IMM, we have state-of-the-art mass spectrometers that provides in-depth proteomic analysis of cells, tissues or biological fluids, with the goals to discover novel targets and biomarkers to inform the development of therapeutic treatment and early detection of diseases. We combine critical data from cancer genetics, genomics, and proteomics to identify drug targets, create targeted antibodies and peptides, and synthesize drug conjugates that are then evaluated in tumor models. We also have expertise in the development and application of novel antibody-based agents that have imaging implications in cancer as well as infectious diseases. Furthermore, the Center specializes in the development of multifunctional peptides that combine radioactive and fluorescent contrast to enable tumor identification before, during, and after surgery, thus introducing a precision surgery approach. Recently, the center received major funding from the Cancer Prevention and Research Institute of Texas (CPRIT) to establish a core facility to carry out pharmacokinetics and toxicology studies of large molecule therapeutics candidates, which will fill a major gap in drug discovery for cancer researchers in Texas.

In addition to the CPRIT-funded Preclinical Development Core for Large Molecule Therapeutics, the Center houses several other core facilities, including the Nanochemistry Service Center, 3D-printing Service Center and Clinical and Translational Proteomics Service Center, to support many research labs through service and collaborative efforts.

Qingyun "Jim" Liu, Ph.D.
Center Director & Professor
Janice Davis Gordon Distinguished Professor for Bowel Cancer Research



Qingyun (Jim) Liu, PhD
Professor
Janice Davis Gordon Chair for Bowel Cancer Research

Investigation of normal and cancer stem cells for the discovery of cancer therapeutics

Adult stem cells are specialized cells that can self-renew and give rise to all the other types of differentiated cells in the tissue where the stem cells reside. They are essential for the maintenance of tissues with high turnover rate, such as the gut and skin, and for tissue repair after injury. However, these cells are also believed to be the cells-of-origin for many types of cancer as they are programmed to divide indefinitely. Furthermore, tumor tissues are also heterogeneous in which only a subpopulation of cells can self-renew and provide daughter cells that make up the bulk of the tumor. These self-renewing cancer cells, designated cancer stem cells or tumor-initiating cells, often bear great similarity to normal stem cells in molecular profile and regulatory systems. Understanding of the mechanisms that govern the control of the self-renewal and differentiation of normal and cancer stem cells will provide crucial knowledge to the discovery and development of novel therapeutics for regenerative medicine and cancer treatment.

Our research is focused on delineating the function and mechanisms of a group of cell surface receptors called LGR4, LGR5, and LGR6 (LGR4-6) that play critical roles in the survival of normal stem cells and tumor cells. Previously, we discovered that LGR4-6 function as receptors of a group of stem cell factors called R-spondins (RSPOs) that are essential for the survival and growth of stem cells. We are now focused on understanding how RSPOs and LGRs work together to regulate the growth and migration of normal and cancer cells. We found that LGR4 and LGR5 work through a different mechanism to control the survival and expansion of intestinal stem cells, which challenges a major current paradigm that LGR4 and LGR5 works in an identical way in cell signaling. Meanwhile, we showed that drug conjugates of ant-LGR5 antibodies showed excellent anti-tumor efficacy in preclinical models of colon cancer. Recently, we have discovered a novel approach that can target all three LGR receptors for the treatment of cancers of the digestive system. We are currently optimizing this approach by protein engineering followed by drug conjugation to increase potency and efficacy in tumor models.

RESEARCH PROJECTS

- Delineation of signaling mechanisms of stem cell receptors.
- Determination of the function and mechanism of the receptors in the control of normal and cancer cell growth.
- Investigation of the roles of aberrant expression of the RSPOs in the control of tumor metastasis of lung and colon cancer
- Identification of lead molecules targeting the RSPO-LGR system as novel anticancer therapeutics.
- Optimization of antibody-drug conjugates targeting the RSPO-LGR system for the treatment of colorectal and other cancers with high LGR expression.
- Determination of the function of a common mutation of RNF43 found in colon, stomach and uterine cancer.

KEY PUBLICATIONS

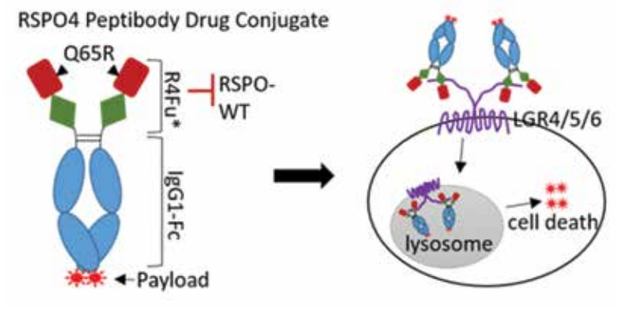
Tu, J., Park, S., Yu, W., Zhang, S., Wu, L., Carmon, K., Liu, Q.J. The most common RNF43 mutant G659Vfs*41 is fully functional in inhibiting Wnt signaling and unlikely to play a role in tumorigenesis. *Scientific reports.* 9(1):18557 (2019).

Park, S., Wu, L., Tu, J., Yu, W., Toh, Y., Carmon, K.S., and Liu, Q.J. Unlike LGR4, LGR5 does not sequester E3 ligases to potentiate Wnt/β-catenin signaling. *Science Signaling,* 13:eaaz4051, 2020.

Cui, J., Toh, Y., Park, S., Yu, W., Tu., J., Wu, L., Li, L., Jacob, J., Pan, S., Carmon, K.S., Liu, Q.J. Drug Conjugates of Antagonistic R-Spondin 4 Mutant for Simultaneous Targeting of Leucine-Rich Repeat-Containing G Protein-Coupled Receptors 4/5/6 for Cancer Treatment. *J. Med. Chem.* 64:12572-12581, 2021.

LAB MEMBERS

Sr. research associates: Wangsheng Alice Yu, Ling Wu, and Jianghua Tu
Research scientist: Yukimatsu Toh



A schematic model illustrating how drug conjugates of R-spondin antagonist can bind to LGR4/5/6 to deliver drugs into and kill cancer cells. Left side depicts the fusion of an RSPO4 furin domain mutant fused to the Fc domain of human IgG1 antibody and the right sides shows that cytotoxic drugs attached to the fusion protein enter cells through LGR4/5/6 to eradicate tumors.

Ali Azhdarinia, MS, PhD
Associate Professor
John S. Dunn Research Scholar III

Cancer theranostics

My laboratory is at the interface of chemistry and biology and is focused on developing molecules for the visualization and treatment of disease. Using novel chemistry platforms, we have the ability to produce molecules with multiple labels and thus, multiple applications. For example, the addition of radioactive and fluorescent labels onto tumor-seeking agents has allowed us to develop new approaches to specifically identify cancer by whole-body and intraoperative imaging, respectively. This could potentially provide surgeons with real-time intraoperative images that will distinguish cancer from normal tissue, minimize removal of healthy tissues, and identify small tumors, which would otherwise be missed by the naked eye. In cases where cancer has spread and surgery is not possible, we aim to use our chemistry platform to specifically deliver toxins to tumors and visualize the effects to personalize treatment protocols. Importantly, our fundamental expertise in chemistry, imaging, and drug characterization has allowed us to establish diverse collaborations to study the *in vivo* properties of novel disease-targeted peptides and antibodies, evaluate the potential benefits of modulating biomarker trafficking in cancer cells, and assess the effectiveness of emerging antibody-based cancer treatments. Common to each project is our focus on translation of discoveries and technologies into the clinic to improve human health.

RESEARCH PROJECTS

- Development of contrast agents for real-time surgical guidance.
- Receptor-targeted delivery of chemotherapy agents for treatment of cancer.

KEY PUBLICATIONS

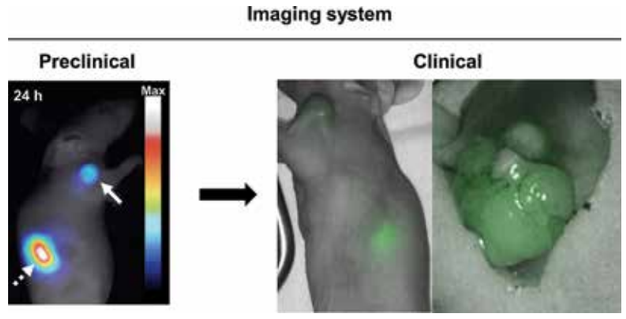
Hernandez Vargas, S., Kossatz, S., Voss, J., Ghosh, S.C., Tran Cao, H.S., Simien, J., Reiner, T., Dhingra, S., Fisher, W.E., Azhdarinia, A.* Specific targeting of somatostatin receptor subtype-2 for fluorescence-guided surgery. *Clin Cancer Res.* 25(14):4332-4342, 2019. PMID: 31015345.

Hernandez Vargas, S., Lin, C., AghaAmiri, S., Voss, J., Ikoma, N., Tran Cao, H., Ghosh, S., Uselmann, A., and Azhdarinia, A.* A proof-of-concept methodology to validate the *in situ* visualization of residual disease using cancer-targeted molecular agents in fluorescence-guided surgery. *SPIE BiOS.* Vol. 11222. 2020.

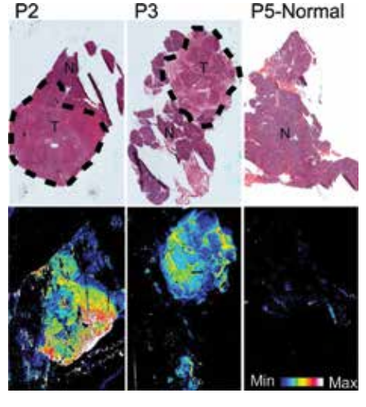
Subramanian, S., Daquinag, A., AghaAmiri, S., Ghosh, S.C., Azhdarinia, A., Kolonin, M.G. Characterization of peptides targeting metastatic tumor cells as probes for cancer detection and vehicles for therapy delivery. *Cancer Res.* 2021. doi: 10.1158/0008-5472. CAN-21-1015. Online ahead of print. PMID: 34607842.

LAB MEMBERS

Research scientist: Sukhen Ghosh
Post-doctoral fellow: Solmaz AghaAmiri
Graduate student: Servando Hernandez Vargas



A fluorescent contrast agent that targets neuroendocrine tumors clearly identified cancerous tissue (solid arrow) using a preclinical imaging system (left; dashed arrow shows kidney). Imaging was then performed using a clinical imager that is part of a surgical robotic system and showed similar detection capabilites. These findings suggest that the selected combination of imaging agent and device can be effectively used in a surgical oncology setting in patients



Pancreatic neuroendocrine tumors were surgically resected from patients and used to prepare frozen sections for tissue analysis. Top: Hematoxylin and Eosin (H&E) staining was used to identify the boundary between tumor (T) and normal (N) tissue. Bottom: Fluorescence images from *ex vivo*-stained tissues showed that the tumor-seeking contrast agent, Ga-MMC(IR800)-TOC, bindings preferentially to tumor cells with low uptake in normal tissue. P5 represents normal tissue from a patient and showed no binding, further demonstrating the cancer-specific binding of the agent.

Kendra Carmon, MS, PhD
Assistant Professor

Therapeutic strategies for targeting colorectal tumors and cancer stem cells

Drug resistance, metastasis, and relapse continue to be leading causes of colorectal cancer-related deaths, demonstrating the need for new therapeutic approaches. Cancer stem cells (CSCs) or tumor-initiating cells are a subpopulation of tumor cells that behave like normal stem cells and have been shown to mediate drug resistance, metastasis, and relapse; making them a major impediment for the effective treatment of colorectal cancer. Therefore, recent strategies have been focused on the development of novel therapies that can ultimately target and destroy CSCs.

LGR5 (Leucine-rich repeat-containing, G protein-coupled Receptor 5), is highly upregulated in colorectal tumors and found on the cell surface of colorectal CSCs. LGR5 also has been shown to be significantly elevated in several other major tumor types, including liver, gastric, and ovarian cancers. The colorectal CSCs, which express LGR5, are capable of driving tumor growth. Interestingly, LGR5-postive CSCs have been shown to have the ability to transition to a more drug resistant LGR5-negative cancer cell type as a means to evade therapy and metastasize. Once LGR5-negative cancer cells initiate metastasis, they can then transition back to LGR5-positive CSCs to increase metastatic growth. As a means to eliminate CSCs, we generated LGR5-targeted antibody-drug conjugates (ADCs). ADCs, referred to as target-guided missiles, are innovative therapeutics that destroy tumors, while sparing normal healthy tissues. They are comprised of a highly specific antibody attached to a cytotoxic chemical "warhead" that is only released once the ADC binds and enters tumor cells. We previously showed that LGR5-targeted ADCs were highly effective in eliminating LGR5-positive colorectal tumors without major adverse effects. However, after treatment was terminated, a fraction of tumors eventually relapsed. These findings suggested that targeting LGR5-positive CSCs alone may not be sufficient to eliminate colorectal tumors because of their ability to escape treatment by converting to an LGR5-negative state. Instead, to successfully eliminate colorectal cancer (CRC) it may require a dual- or multi-targeted approach.

One of the current research interests of my lab focuses on investigating the roles of LGR5 in colorectal tumor growth, metastasis, and drug resistance. Secondly, we are working to discover a more effective treatment for colorectal cancer by taking novel approaches to modify and improve our LGR5-targeting ADCs and evaluating them in combination with other targeted therapies. We are also generating bispecific antibodies, which are aimed at binding two different cancer targets at the same time. Thirdly, our lab is identifying and characterizing new cancer targets for antibody and ADC development. One of these new targets is a cell receptor called GPR56, which is highly expressed in colorectal cancer and correlates with poor patient survival. We found that GPR56 can promote tumor growth and drug resistance, and we are investigating the cellular mechanisms that drive its function. Our group has acquired colorectal tumor samples from patients and established 3D cultures called patient-derived organoids or PDOs. We use the PDOs to study the function of our different cancer targets and evaluate the efficacy of our ADCs before testing in animal models. Our work will lead to elucidating the function and mechanism of different receptors in colorectal cancer and generate innovative therapeutics for the improved treatment and eradication of colorectal cancer.

RESEARCH PROJECTS

- Investigation of LGR5 function in cancer stem cells, metastasis, and drug resistance
- Identification of novel therapeutic targets and development of antibody-drug conjugates and combination therapies to target colorectal tumors and cancer stem cells
- Elucidating the role and signaling pathways of GPR56 in colorectal cancer

KEY PUBLICATIONS

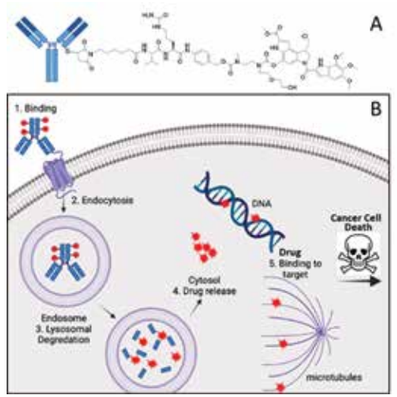
Chatterjee, T., Zhang, S., Posey, T.A., Jacob, J., Wu, L., Yu, W., Francisco, L.E., Liu, Q.J., Carmon, K.S.* Anti-GPR56 monoclonal antibody potentiates GPR56-mediated Src-Fak signaling to modulate cell adhesion. *J Biol Chem.* 296:100261, 2021.

Zhang, S., Chatterjee, T., Godoy, C., Wu, L., Liu, Q.J., Carmon, K.S.* GPR56 drives colorectal tumor growth and promotes drug resistance through upregulation of MDR1 expression via a RhoA-mediated mechanism. *Mol Cancer Res.* 17(11):2196-2207, 2019.

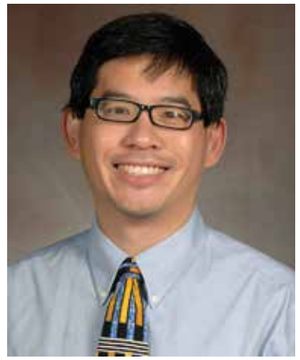
Azhdarinia A., Voss J., Ghosh S.C., Simien J.A., Hernandez Vargas S., Cui J., Yu W.A., Liu Q., Carmon K.S.* Evaluation of Anti-LGR5 Antibodies by ImmunoPET for Imaging Colorectal Tumors and Development of Antibody-Drug Conjugates. *Mol. Pharm.* 15(6):2448-2454, 2018.

LAB MEMBERS

Post-doctoral fellow: Liezl Francisco Brown
Students: Joan Jacob, Tressie Posey, Ashlyn Parkhurst
Research associates: Sheng Zhang, Zhengdong Liang



(A) Antibody-Drug Conjugate (ADC) structure. (B) Schematic showing the mechanism by which an ADC destroys a cancer cell.



Jeffrey Chang, PhD
Associate Professor
CPRIT Scholar in Cancer Research

Deciphering the signaling programs underlying cancer metastasis

Our lab is focused on understanding the signaling programs underlying cancer progression and developing therapeutic strategies to prevent or treat metastasis. We wish to understand the events that lead tumor cells to become metastatic, whether through acquired mutations or epigenetic mechanisms. Our ultimate goal is to translate these findings into the clinic through the development of genomic biomarkers and repositioning of drugs. To do this, we use a range of approaches encompassing genomics, cell biology, and biochemistry; and use models including cell culture, mouse models, and clinical samples.

Our research program encompasses two broad and complementary areas of emphasis:

1. Breast cancer metastasis. It is estimated that up to 90% of cancer deaths are due to metastasis, in part because metastatic cells do not respond to traditional therapies. To address this problem, we have used computational approaches to characterize the metastatic state and to reposition drugs to target cells that exhibit phenotypes that promote metastasis. Through these studies, we have found that metastasis is driven in part by cells that acquire a stem-like state through deregulation of cholesterol metabolism through altered expression of the ABCA1 cholesterol efflux channel. We are currently identifying therapeutic strategies to inhibit this pathway to reprogram breast cancer stem cells so that they become more amenable to therapies.

2. Artificial intelligence for genomic analysis. Many of our projects require the integration with bioinformatics to mine public data sets, develop hypotheses, or analyze results. To amplify our ability to do bioinformatics, we have developed an artificial intelligence, BETSY, that can automatically plan and execute these tasks, presenting us with finished results. It is a backwards-chaining expert system that leverages a knowledge base containing descriptions of common bioinformatics algorithms.

RESEARCH PROJECTS

- The role of cholesterol trafficking in cancer stem cell differentiation, the epithelial-to-mesenchymal transition, and cancer metastasis.
- Heterogeneity and progression of metastatic cancers.
- Intelligent computational pipelines for bioinformatic analysis.

KEY PUBLICATIONS

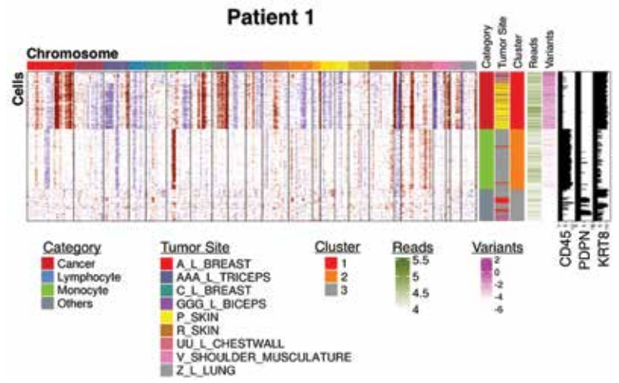
Liu X, Gosline SJC, Pflieger LT, Wallet P, Iyer A, Guinney J, Bild AH, and Chang JT: Knowledge-based classification of fine-grained immune cell types in single-cell RNA-Seq data with ImmClassifier. *bioRxiv* doi: 10.1101/2020.03.23.002758, 2020.

Zhao W., Prijic S., Urban B., Tisza M.J., Li L., Tan Z., Chen X., Mani S.A., and Chang J.T.: Candidate anti-metastasis drugs suppress the metastatic capacity of breast cancer cells by reducing membrane fluidity. *Cancer Research* 76(7):2037-49, 2016.

Chen X and Chang J.T.: Planning bioinformatics workflows using an expert system. *Bioinformatics* 33(8), 2017.

LAB MEMBERS

Instructor: Weina Zhao, PhD
Research scientist: Xuan Liu, PhD
Research assistant II: Jiayi Liu, MB



Profiles of cells from Patient 1. The large heatmap shows the predicted copy number profiles of cells (rows) from metastatic tumors from patient 1. Chromosomes are organized across the length of the heatmap. The colors indicate predicted copy number alterations. Colored bars to the right of the heatmap show various annotations for the cells. The Category is the cell type predicted to be cancer or other types, based on a range of criteria. The Tumor Site is the tumor that the cell came from. Cluster is based on an unbiased clustering of the copy number prediction. The Reads is the log2 of the total number of reads for the cell, and Variants is the log2 of variants per read of the cell. The black bars on the far right indicate the log2 of the counts per million of markers for immune cells (CD45), fibroblasts (PDPN), and luminal epithelial cells (KRT8).

Sheng Pan, PhD
Associate Professor / Director, the Clinical and Translational Proteomics Service Center
Rochelle and Max Levit Chair in the Neurosciences

Deciphering proteome alterations associated with diseases

Proteins are essential functional biomolecules that are involved in all aspects of cellular physiologic activities and have been important targets for drug development and early detection of diseases. Proteomics, especially quantitative proteomics, have been a vital tool in basic, translational and clinical research, providing a unique avenue to investigate disease-associated molecular alterations at a functional level. Proteome alterations that are associated with diseases may include changes in protein expression, sequence, post-translational modifications (PTMs), and protein interactions with proteins and other biomolecules, which may all lead to a malfunction of cellular processes. In our lab, mass spectrometry based proteomic technologies are applied to study cancer, neurodegeneration, and other diseases. These studies are carried out with various goals, such as aiming to better understand the molecular mechanisms underlying tumorigenesis, to investigate changes in PTM status associated with diseases, to identify protein biomarkers or therapeutic targets, or to interrogate microbiome dysbiosis. The samples involved in our studies include a variety of research and clinical specimens, including tumor tissues, blood, and other bodily fluids, as well as isolated cells from various clinical specimens. Currently, our main disease focuses are pancreatic cancer and other GI-tract malignances. In addition, through collaborative efforts, our lab also supports proteomic study of neurodegeneration, chronic inflammations, and infectious diseases, as well as therapeutic drug development. Mass spectrometry, bioinformatics, systems biology, and chemical biology are important components in our study.

RESEARCH PROJECTS

- Mechanistic and biomarker studies of pancreatic ductal adenocarcinoma (PDAC) and its precursors, including pancreatic intraepithelial neoplasia (PanIN) and pancreatic cyst neoplasms (PCNs).
- Investigation of protein glycation and advanced glycation end products (AGEs) in malignances, aging, diabetes, and chronic inflammation.
- Metaproteomic study of microbiome implicated in GI-tract malignancies and other diseases.
- Investigation of the proteome and glycoproteome alterations associated with cancer, Alzheimer's disease, and Lewy Body Dementia (LBD).
- Innovation of proteomic technologies, including singe cell proteomics and glycoproteomics, for basic, translational, and clinical applications.

KEY PUBLICATIONS

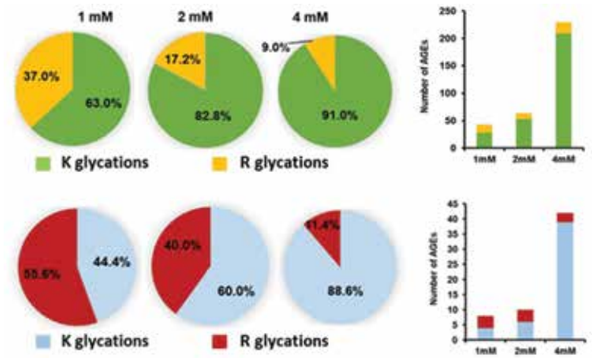
Pan S, Brand RE, Lai LA, Dawson DW, Donahue TR, Kim S, Khalaf NI, Othman MO, Fisher WE, Bronner MP, Simeone DM, Brentnall TA, Chen R, "Proteome heterogeneity and malignancy detection in pancreatic cyst fluids," *Clinical and Translational Medicine,* 2021, Aug;11(8):e506.

Senavirathna L, Ma C, Chen R, Pan S, "Proteomic investigation of glyceraldehyde-derived intracellular AGEs and their potential influence on pancreatic ductal cells," *Cells.* 2021 Apr 24;10(5):1005.

Wang H, Li X, Lai LA, Brentnall TA, Dawson DW, Kelly KA, Chen R, Pan S, "X-aptamers targeting Thy-1 membrane glycoprotein in pancreatic ductal adenocarcinoma," *Biochimie.* 2021 Feb;181:25.

LAB MEMBERS

Post-doctoral fellow: Lakmini Senavirathna, PhD
Research scientist: Cheng Ma, PhD
Research coordinator: Li Li



Proteome wide proteomic identification of intracellularly formed proteins AGEs on K and R residues in cellular proteins of HPDE cells (upper) and PANC-1 cells (lower).

David Volk, PhD
Associate Professor

Targeting cancer with X-aptamers and nanoparticles

The focus of my lab is to develop targeting agents and smart particles that attack cancer or infectious organisms, such as tuberculosis. Current treatments are often ineffective or create harsh side effects for patients. We use modified DNA joined to drug-like or protein-like attachments (X-aptamers). X-aptamers can be used alone or as complex particles containing anti-cancer agents to act as a one-two punch. Such particles can also be loaded into larger silicon particles for a sustained release of the disease fighting particles.

Aptamer Development - In recent years, we have developed DNA aptamers targeting breast and ovarian cancer. Such DNA can greatly reduce cancer in a dose-dependent manner. However, DNA aptamers are even more effective when used in combination therapy together with chemotherapeutic agents such as siRNA or drugs like Paclitaxel. We have shown that our aptamer targeted approach reduces tumor size and, more importantly, the spread of metastatic cancer. Furthermore, we have also shown our method is safe in preclinical testing. Our recent aptamer-related research has shown the following.

ESTA1 multistage particles directed anticancer siRNA to the bone marrow, reducing breast cancer metastasis and leading to increased survival rates.

Our Annexin A2 (Mangala et al., 2016) aptamer directed delivery of siRNA improves vascular maturation to enhance anti-tumor effects in ovarian cancer.

Our AXL aptamer (Kanlikilicer et al., 2017; Amero et al. 2021) can reduce cancer alone and enhances anti-tumor effects in combinatorial therapy.

Developed aptamers (Liu et al. 2018) targeting the endothelium of lymphoma in bone marrow.

X-aptamers can be used to develop biomarkers in schizophrenia (Walss-Bass et al, 2019)

We have recently (Costello et al, 2021) developed improved versions of our vimentin-binding aptamer which targets ovarian cancer.

We recently (Leonard et al. 2017) showed that our ESTA1 and CD44 aptamers deliver mesoporous silicon particles to macrophages infected with *M. tuberculosis*, thereby enhancing the immune system and reducing the *M. tuberculosis* (Tb) burden.

RESEARCH PROJECTS

- Development of smart particles to attack breast and ovarian cancers.
- Developing new X-aptamers targeting other diseases.

KEY PUBLICATIONS

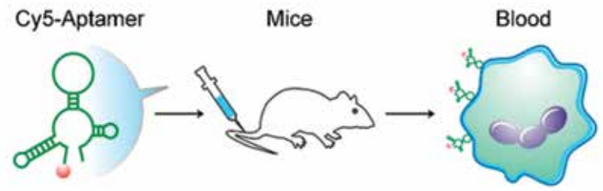
X-Aptamer Technology Identifies C4A and ApoB in Blood as Potential Markers for Schizophrenia. Walss-Bass, C, Lokesh, GLR, Dyukova, E, Gorenstein, DG, Roberts, DL, Velligan D, Volk, DE. *Molecular Neuropsychiatry,* (2019) 5:52–59.

Tracking biodistribution of myeloid-derived cells in murine models of breast cancer. *Genes* (2019) 10:297. Li, J, Mai, J, Hinkle, L, Lin, D, Zhang, J, Liu, X, Ramirez, MR, Zu, Y, Lokesh, G, Volk, DE, Shen, H.

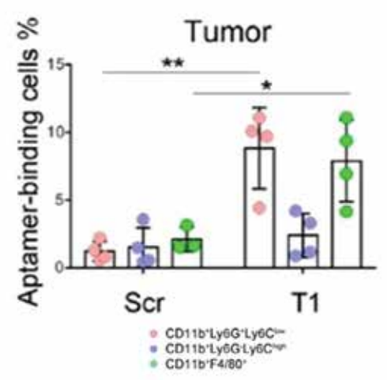
Functional Blockade of E-selectin in Tumor-associated Vessels Enhances Anti-Tumor Effect of Doxorubicin in Breast Cancer. Morita, Y., Leslie, M., Kameyama, H., Lokesh, G.L.R., Ichimura, N., Davis, R., Natalie Hills, N., Hasan, N., Zhang, R., Kondo, Y., Gorenstein, D.G., Volk, D.E., Cheveroneva, I., Hallgeir, R., and Tanaka, T. Cancers, (2020) 12:725.

Conversion of RNA Aptamer into Modified DNA Aptamers Provides for Prolonged Stability and Enhanced Antitumor Activity. Paola Amero, P., Lokesh, G.L.R., Chaudhari, R.R., Cardenas-Zuniga, R.C., Schubert, T., Yasmin M Attia, Y.M., Montalvo-Gonzalez, E., Elsayed, A.M., Ivan, C., Zhihui Wang, Z., Cristini, V., de Franciscis, V., Zhang, S., Volk, D.E., Mitra, R., Rodriguez-Aguayo, C. , Sood, A.K., Lopez-Berestein, G. *J Am Chem Soc* (2021) 143:7655-7670.

Selection and Characterization of Vimentin-Binding Aptamer Motifs for Ovarian Cancer. Costello, A.M., Elizondo-Riojas, M.-A., Li, X., David E Volk, D.E., Pillai, A.K., Wang, H. *Molecules,* (2021) 26:6525.



Selecting aptamers targeting myeloid-derived cells in breast cancer.



Enhanced delivery to tumors relative to scrambled oligo.

Hongyu Wang, MD, PhD
Assistant Professor

Integrate multidisciplinary approaches for cancer biomarker discovery

Aptamer-Mediated Biomarker Discovery: Aptamer-mediated biomarker discovery and targeted therapy are attractive approaches for cancer treatment. Aptamers are single-stranded oligonucleotides with high affinity and specificity to the target molecules. DNA aptamers have many significant advantages over monoclonal antibodies in terms of feasibility, low cost, non-immunogenicity, and facile modification for various applications. We created a systems biology approach that combines a bead-based modified aptamer library with flow cytometry sorting and mass spectrometry to identify proteomic biomarkers. Patient's plasma was incubated with beads-based aptamer library and sorted for aptamer-protein complex by flow cytometry based (Figure 1). Using this approach, we selected a panel of prognostic biomarkers for hepatocellular carcinoma (HCC) patients under Lipiodol-based transarterial chemoembolization (TACE) treatment.

Artificial Intelligence (AI) Image Analysis: Unlike most solid cancers, the diagnosis of HCC is based on multiphasic CT or MRI without histological confirmation in patients with cirrhosis. AI has the capacity of converting images into mineable data by high-throughput extraction of quantitative features. A seamlessly integrated AI component within the imaging workflow would increase efficiency, reduce errors, and improve diagnostic performance with minimal manual input by interpreting radiologists. Most of the current deep learning approaches focus on image segmentation at a single time point rather than a series of images at the different diagnosing stages of the disease. We will develop a Long Short-Term Memory (LSTM) network based time-series model combined with 3D neural networks (3DCNN-LSTM) and domain adaptation to learn the disease transformations from cirrhosis to HCC and make disease trajectory predictions.

Integrated Multi-Aspects Biomarker Analysis: By studying differentially expressed mRNAs using data downloaded from TCGA and validating those mRNA encoded proteins in tissue and blood samples, we have identified a group of genes and proteins that are significantly differentially expressed between HCC and healthy control groups. We seek to shift current clinical surveillance and early diagnosis of HCC into a new platform (AiCat) to integrate multidisciplinary approaches into one setting, including artificial intelligence (AI) image analysis, proteomics, and genomics biomarkers to improve early diagnosis and outcome prediction for liver cirrhosis patients who are at high risk of developing HCC.

RESEARCH PROJECTS

- Identify proteomic biomarkers for outcome prediction of lipiodol TACE treatment
- Artificial intelligence improves liver cancer surveillance and early detection
- Genetic and proteomic biomarker discovery for hepatocellular carcinoma

KEY PUBLICATIONS

Wang H., Li, X., Volk, D.E., Lokesh, R. L.G., Elizondo-Riojas M-A., Nick, A.M., Sood, A.K., Rosenblatt, K.P., Gorenstein, D.G. Morph-X-Select, a Morphologically-Based Aptamer Tissue Selection for Personalized Ovarian Cancer Biomarker Discovery. *Biotechniques.* 2016 Nov 1;61(5):249-259. PubMed PMID: 27839510.

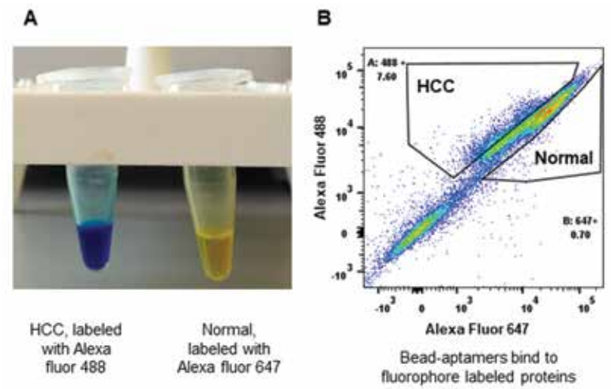
Wang H, Lam CH, Li X, West DL, Yang X. Selection of PD1/PD-L1 X-Aptamers. *Biochimie.* 2018 Feb; 145:125-130. doi: 10.1016/j.biochi.2017.09.006. Epub 2017 Sep 11. PMID: 28912094; PMCID: PMC5794648.

Wang H, Li X, Lai LA, Brentnall TA, Dawson DW, Kelly KA, Chen R, Pan S. X-aptamers targeting Thy-1 membrane glycoprotein in pancreatic ductal adenocarcinoma. *Biochimie.* 2020 Nov 23; 181:25-33. doi: 10.1016/j.biochi.2020.11.018. Epub ahead of print. PMID: 33242496.

Costello AM, Li X, Volk DE, Pillai AK, Wang H. Selection and characterization of Vimentin-binding aptamer motifs for treatment of ovarian cancer. *Molecules* 2021, 26, 6525. https://doi.org/10.3390/molecules26216525.

LAB MEMBERS

Research associate: Xin Li



Protein biomarker discovery using bead-based X-aptamer library. (A) Patient and health donor plasma were labeled with different fluorophores. (B) Proteins bound to bead-X-aptamer were sorted by flow cytometer.

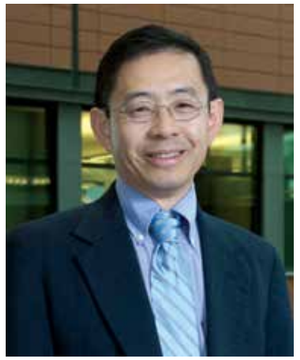
The Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston. TTI-IMM was created for the discovery, development, and commercialization of therapeutic agents and diagnostic tools. Research conducted at the center focuses on the establishment of proof-of-principle for therapeutics and the identification and validation of drug targets.

TTI-IMM investigators have brought in significant funding from biopharmaceutical companies, such as Merck and Johnson & Johnson, and from government organizations, including the National Institutes of Health, the Cancer Prevention and Research Institute of Texas, and the Department of Defense. TTI investigators have made significant scientific discoveries in the areas of cancer biology, fungal natural products, and antibody drug development.

Current research activities at TTI-IMM include: 1) signaling mechanisms of receptors and enzymes that have critical roles in human diseases; 2) discovery of biologics and natural products that modulate the activity of these targets as potential lead molecules for drug discovery; and 3) characterization of antibodies from animals and humans in response to viral infections and experimental vaccines.

In addition to basic and translational research programs, TTI has built a major drug discovery platform for therapeutic monoclonal antibody lead discovery optimization and development. Over the last 12 years, TTI established a network of collaborators from institutions across Texas and the nation. TTI has more than 30 active drug discovery projects targeting cancer, metabolic diseases, neurodegenerative diseases, spinal cord injury, fibrosis, acute drug induced liver injury, and viral infections. Ten TTI inventions have been licensed to biotech companies for drug development. Five antibody based therapeutics discovered by TTI scientists are currently in human clinical trials. In response to the COVID-19 pandemic, TTI scientists quickly discovered neutralizing antibodies targeting the SARS-CoV-2 virus. These antibodies are in development as potential therapies for the treatment of COVID-19. Licensing deals resulted in significant upfront payments, potential milestone payments, and royalties. The Texas Therapeutics Institute is recognized as the drug discovery engine of McGovern Medical School and UTHealth.

Zhiqiang An, PhD
Professor & Center Director
Robert A. Welch Distinguished University Chair in Chemistry



Zhiqiang An, PhD
Professor and Co-Director of the Texas Therapeutics Institute
Robert A. Welch Distinguished University Chair in Chemistry

Discovery and development of therapeutic antibodies

Our group focuses on the discovery and development of therapeutic antibodies against human diseases. Currently, we have two major research areas.

RESEARCH PROJECTS

- Antibody Response to Viral ilfections and Vaccination: Identification of highly immunogenic vaccines that induce neutralizing antibodies against a broad range of clinical isolates is one approach to develop effective viral vaccines. We have ongoing projects to aid the design of HCMV dengue by profiling antibody response to the experimental vaccines in rhesus and humans, and to develop SARS-CoV-2 targeting antibodies for the potential treatment of COVID-19.
- Cancer Therapeutic Monoclonal Antibody Drug Discovery: Our group has built a comprehensive antibody drug discovery platform with a focus on antibody lead optimization technologies, such as antibody phage display, deep sequencing of antibody encoding genes from individual antibody expressing B cells, affinity maturation, and humanization. Currently, we have multiple in-house and collaborative antibody drug discovery projects targeting various cancer types.

KEY PUBLICATIONS

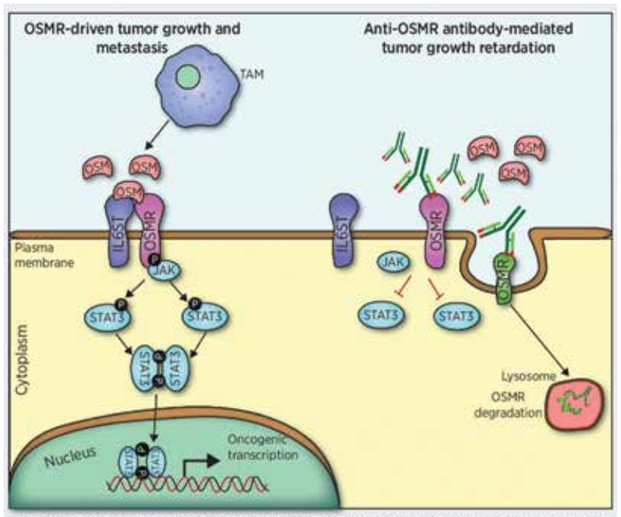
Anjali Geethadevi, Ajay Nair, Deepak Parashar, Zhiqiang Ku, Wei Xiong, Hui Deng, Yongsheng Li, Jasmine George, Donna M. McAllister, Yunguang Sun, Ishaque P. Kadamberi, Prachi Gupta, Michael B. Dwinell, William H. Bradley, Janet S. Rader, Hallgeir Rui, Robert F. Schwabe, Ningyan Zhang, Sunila Pradeep, Zhiqiang An and Pradeep Chaluvally-Raghavan 2021. Oncostatin M Receptor-targeted antibodies suppress STAT3 signaling and inhibit ovarian cancer growth. *Cancer Research* DOI: 10.1158/0008-5472. CAN-21-0483.

Zhiqiang Ku, Xuping Xie, Paul Hinton, Xinli Liu, Xiaohua Ye, Antonio Muruato, Dean Ng, Sujit Biswas, Jing Zou, Yang Liu, Deepal Pandya, Vineet Menachery, Sachi Rahman, Yu-An Cao, Hui Deng, Wei Xiong, Kevin Carlin, Junquan Liu, Hang Su, Elizabeth Haanes, Bruce Keyt, Ningyan Zhang, Stephen Carroll, Pei-Yong Shi, and Zhiqiang An. 2021. Nasal delivery of an IgM against SARS-CoV-2 offers potent protection and coverage of variants. *Nature* 595:718–723 https://doi.org/ 10.1038/s41586-021-03673-2.

Leike Li, Daniel C. Freed, Yaping Liu, Fengsheng Li, Diane F. Barrett, Wei Xiong, Richard E. Rupp, Dai Wang, Ningyan Zhang, Tong-Ming Fu, Zhiqiang An. 2021. A conditionally replication-defective cytomegalovirus vaccine elicits potent and diverse functional monoclonal antibodies in humans. *NPJ Vaccines* doi.org/10.1038/s41541-021-00342-3..

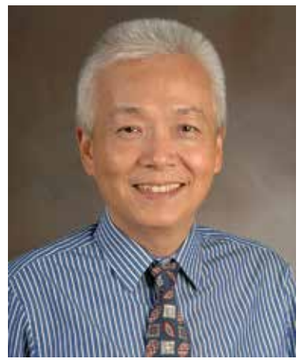
LAB MEMBERS

Post-doctoral fellows: Zhiqiang Ku, Junquan (Jake) Liu, Jwala Sivaccumar, Lingxiao Tan, Xiaohua Ye, Peng Zhao, Zhuan Zhang
Graduate students: Joshua W. Morse, Mason Ruiz
Research technician: Hannah Boyd



Anti-OSMR antibody can mediate disruption of OSM-induced OSMR–IL6ST dimerization and oncogenic signaling, thus documenting the preclinical therapeutic efficacy of human OSMR antagonist antibodies for immunotherapy in ovarian cancer.

Could a Nasal Spray of Designer Antibodies Help to Beat COVID-19?



Xiaodong Cheng, PhD
Professor
Walter and Mary Mischer Distinguished Professor in Molecular Medicine

cAMP - mediated cell signaling and drug discovery

Our laboratory studies intracellular signaling associated with second messenger cAMP, a major stress signal implicated in the development of human diseases. We apply multidisciplinary approaches, coupling biochemistry, biophysics, and cell biology with pharmacology and chemical biology, to understand the structure and function of a family of cAMP sensors: exchange proteins directly activated by cAMP (EPAC). Our goals are to unravel the signaling intricacies of EPAC proteins and to design pathway-specific modulators for these important signaling molecules so that their functions can be exploited and controlled pharmaceutically for the treatment of human diseases. We have developed first-in-class EPAC selective inhibitors and EPAC knockout mouse models to study the physiological functions and diseases relevance of this family of important signaling molecules. Recently, we have identified a potential use of EPAC inhibitors in the prevention and treatment of proliferative retinopathy. Currently, we are developing second-generation isoform specific EPAC inhibitors and agonists and in exploring their potential uses in various human diseases including cardiovascular diseases and chronic pain.

RESEARCH PROJECTS

- Structural and functional analyses of the exchange proteins directly activated by cAMP (EPAC).
- Examine the roles of EPAC proteins in major human diseases, such chronic pain and proliferative vascular diseases using EPAC knockout mouse models and pharmacological inhibitors.
- Preclinical development of novel drug candidates targeting EPAC1 for the treatment of diabetic retinopathy.

KEY PUBLICATIONS

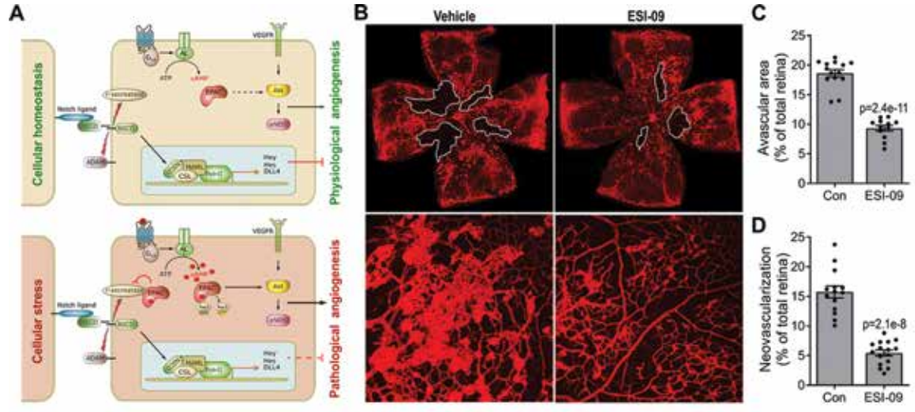
Liu, H., Mei, F. C., Yang, W., Wang, H., Wong, E., Toth, E., Luo, P., Li, Y.-M., Zhang, W. and Cheng, X. Epac1 inhibition ameliorates vaso-proliferative retinopathy through coordinated activation of Notch and suppression of VEGF signaling. *Science Advances*. 6: eaay3566, 2020.

Robichaux, W. G., Mei, F. C., Wang, H., Yang, W., Sun, H., Zhou, Z., Milewicz, D. M., Teng, B. B. and Cheng, X. EPAC1 promotes foam cell formation and atherosclerosis development by upregulating the oxidized LDL scavenger receptor LOX-1 through a PKC dependent pathway in macrophages. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 40:e322-e335. doi: 10.1161/ATVBAHA.119.314238, 2020. [ATVB Editor's Pick].

White, M. A., Lin, W. and Cheng, X. Discovery of COVID-19 inhibitors targeting the SARS-CoV2 Nsp13 helicase. *J Phys Chem Lett.* 11:9144-9151. doi: 10.1021/acs.jpclett.0c02421, 2020.

LAB MEMBERS

Research assistant professor: Fang Mei
Instructor: Wenli Yang
Instructor: William Robichaux
Research associate: Wei Lin
Graduate student: Ningzhou Gu



Epac1 in pathological angiogenesis and as a therapeutic target for retinopathy. (A) Epac1 promotes pathological angiogenesis through sensitization of VEGF signaling and suppression of Notch activation via γ-secretase inhibition. (B) Pharmacological inhibition of Epac prevents neovascularization associated with oxygen-induced retinopathy (OIR). Representative retinal vasculature (upper panel) and high magnification images (lower panel) at P17 in OIR mice treated with Epac inhibitor ESI-09 or vehicle (Con). White lines outline the area of vaso-obliteration. (C, D) Graphs represent avascular and neovascularization area at P17.

Xin (Alex) Ge, PhD
Associate Professor
Kay and Ben Fortson Distinguished Chair in Neurodegenerative

Bio-pharmaceutical discovery and engineering

Our research is in the area of protein engineering, focusing on bio-pharmaceuticals such as monoclonal antibodies (mAbs), T cell receptors, and therapeutic enzymes. We develop novel techniques to facilitate these biologics discovery, optimization, and production, and further evaluates their therapeutic efficacy *in vitro* and *in vivo*.

RESEARCH PROJECTS

- Protease-Inhibiting Therapeutic mAbs (Funded by NIGMS) Proteases are important signaling molecules and represent one of the largest families of pharmaceutical targets. My laboratory has been committed to the development of enabling methodologies for the generation of therapeutic mAbs as safe and effective protease inhibitors. Over the past decade, we established a series of novel technologies, including camelid-inspired convex paratope human antibody libraries (PNAS 2016), and inhibition-based rather than binding-based selection/screening methods (PNAS 2019). Combining these approaches, we discovered, characterized, and optimized panels of potent and specific mAbs inhibiting numerous proteases of biomedical importance. Furthermore, our protease inhibitory mAbs have shown significant therapeutic efficacy in animal models of cancers, neuropathic pains, obesity, and stroke.
- Efficient Transportation Across Blood-Brain Barrier (Funded by NINDS) The blood-brain barrier (BBB) poses a great challenge for developing effective therapies for neurological disorders, such as brain cancer and neurodegenerative diseases. We design protease-activated bi-specific antibody prodrugs as a highly efficient BBB delivery approach for treating neurological diseases.
- Monoclonal Antibody-Based Therapeutics for Diabetic Neuropathy (Funded by DoD) One of the most common complications of diabetes is nerve damage-associated diabetic peripheral neuropathy (DPN), which affects up to 50% of diabetic patients. This research develops, optimizes, and evaluates highly specific mAb therapeutics directly targeting the mechanisms of DPN pathogenesis, and thus with great values in the management of diabetic neuropathy.
- Broad Neutralizing mAbs of Snake Venom Metalloproteases (Funded by NIAID) Snake envenomation is a serious global health concern, and in N.A., the majority of venomous snakes belong to family Viperidae (e.g. rattlesnakes and copperheads), which bites can lead to fatal hemorrhage and coagulopathy caused by snake venom metalloproteinases (svMPs). This research develops humanized broad neutralizing mAbs by targeting svMPs reaction cleft, and further tests their efficacy *in vitro* and *in vivo*.

KEY PUBLICATIONS

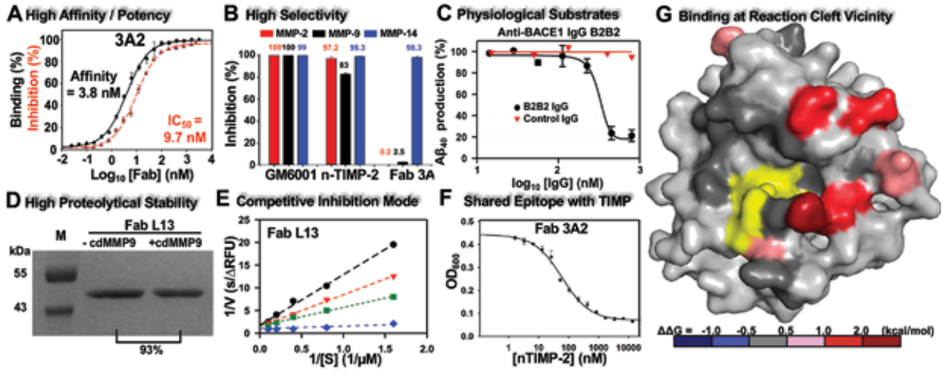
Lopez T, Mustafa Z, Chen C, Lee KB, Ramirez A, Benitez C, Luo X, Ji R-R, Ge X. 2019. Functional Selection of Protease Inhibitory Antibodies. *Proceedings of the National Academy of Sciences of the United States of America* 116 [33]:16314-16319.

Nam DH, Lee KB, Kruchowy E, Pham H, Ge X. 2020. Protease Inhibition Mechanism of Camelid-Like Synthetic Human Antibodies. *Biochemistry* 59[40]:3802-3812.

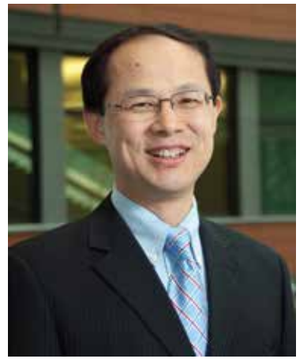
Ge X, Nam DH, Lopez T. 2021. Inhibitory Antibodies Against MMP-14. United States Patent 10,975,166

LAB MEMBERS

Post-doctoral fellows: Hyunjun Choe, Kibaek Lee, Zening Wang, Xin Wei



Biochemical characterizations of protease inhibitory mAbs. (A) Nanomolar affinity and potency; (B) Exclusive selectivity; (C) Inhibition with physiological substrates; (D) High proteolytic stability; (E) Competitive mode of inhibition; (F) Epitope overlaps with endogenous inhibitors; (G) Epitope determined by alanine scanning.



Wenliang Li, PhD
Associate Professor

Studying and targeting cancer metastasis and drug resistance

My research programs are (1) to obtain critical new knowledge of cancer metastasis and drug resistance of human cancer cells, (2) to identify new biomarkers and drug targets for the development of better therapeutics for human cancers.

Cancer metastasis, the spread of tumor to other parts of patient's body, is responsible for over 90% of cancer death. However, cancer metastasis is still poorly understood, and the current approaches to prevent or treat human metastatic cancers are mostly unsuccessful. Therefore, there is a huge unmet medical need to better understand cancer metastasis and to develop new therapies against cancer metastasis. Through genomics, RNAi, and cDNA functional screens, Our lab has identified several crucial but previously unknown regulators for cancer metastasis. Some of the novel regulators control epithelial-mesenchymal transition (EMT), while some others are essential for survival and proliferation of highly metastatic cancer cells (i.e. essential genes). EMT, a developmental process, is believed to play a key role in cancer metastasis, drug resistance, organ fibrosis, and stem cell phenotypes. Essential genes for metastatic cancer cells may be the key to understand colonization, the rate-limiting step of cancer metastasis. Signaling pathways and molecular mechanisms of these novel regulators are under investigation with molecular, cellular, biochemical, genomic, proteomic approaches, and mouse models. These studies are yielding critical new insights for cancer metastasis and facilitating the development of new therapeutics and biomarkers.

Another research topics in the lab is to investigate the mechanisms of cancer cell plasticity and drug resistance. In particular, we study how prostate cancers become resistance to new generation of androgen receptor pathway inhibitors, and how non-small cell lung cancers become resistant to EGFR inhibitors. The common theme is to better understand and to target a process called neuroendocrine differentiation (NED), which is increasingly accepted as a critical process in cellular plasticity and drug resistance in many cancers. NED is still poorly understood and currently there are no effective treatments to prevent or overcome drug resistance related to NED. I investigate the underlying mechanisms of NED, cellular plasticity and drug resistance, especially the roles and mechanisms of action of several novel epigenetic regulators.

Finally, in collaborations with Drs. Ningyan Zhang and Zhiqiang An at TTI, we are identifying novel therapeutic antibodies. We are also exploring new combinatory strategies to enhance efficacy of immune therapy, such as combining our kinase inhibitors with immune checkpoint blockade, e.g. anti-PD-1 and anti-PD-L1 antibodies.

RESEARCH PROJECTS

- Targeting critical regulators of cancer metastasis.
- Defining new pathways and mechanisms of epithelial-mesenchymal transition.
- Investigating lineage plasticity and acquired resistance to cancer therapeutics.
- Identifying novel cancer therapeutic antibodies.
- Exploring new combinatory strategies to enhance immune therapy.

KEY PUBLICATIONS

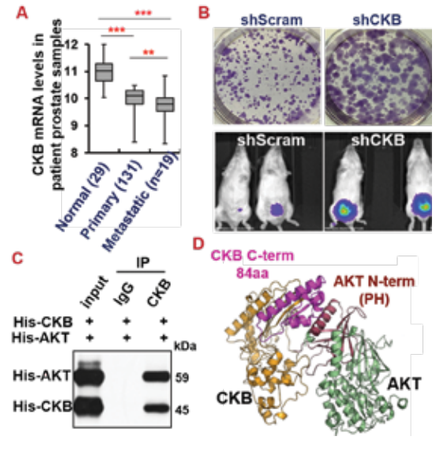
Zhang Y, Zheng D, Zhou T, Song H, Hulsurkar M, Su N, Liu Y, Wang Z, Shao L, Ittmann M, Gleave M, Han H, Xu F, Liao W, Wang H, Li W*. Androgen deprivation promotes neuroendocrine differentiation and angiogenesis through CREB-EZH2-TSP1 pathway in prostate cancers. *Nature Communications,* 2018 Oct 4; 9(1):4080. *corresponding author

Li, L, Su, N., Zhou, T., Zheng, D., Wang, Z., Chen, H., Yuan, S., Li, W*. Mixed lineage kinase ZAK promotes epithelial-mesenchymal transition in cancer. *Cell Death & Disease* 2018 Feb 2; 9(2):143. *corresponding author

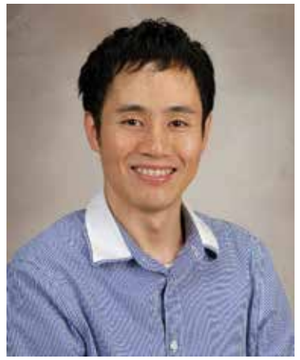
Wang Z, Hulsurkar M, Zhuo L, Xu J, Yang H, Naderinezhad S, Wang L, Zhang G, Ai N, Li L, Chang JT, Zhang S, Fazli L, Creighton CJ, Bai F, Ittmann MM, Gleave GE, Li W*. CKB inhibits epithelial-mesenchymal transition and prostate cancer progression by sequestering and inhibiting AKT activation. *Neoplasia* 2021 Oct 23;23(11):1147-1165. *corresponding author

LAB MEMBERS

GSBS graduate students: Samira Naderinezhad, Boxuan Yang



CKB inhibits EMT and prostate cancer progression by sequestering and inhibiting AKT activation. (A) Creatine kinase B expression is downregulated as prostate cancers progress in patients. (B) CKB silencing promotes colony formation and prostate tumor growth in mouse xenografts. (C) CKB physically interacts with oncogene AKT. (D) Modeling on CKB and AKT crystal structures indicates that CKB C-terminal 84aa interacts with AKT N-terminal PH domain, sequestrates AKT and blocks its activation, which was confirmed experimentally.



Kyoji Tsuchikama, PhD
Assistant Professor

Linker and conjugation technologies for generating novel antibody-drug conjugates (ADCs) toward innovative cancer therapeutics

Antibody-Drug Conjugates (ADCs) represent a rapidly growing class of anticancer therapeutics. As demonstrated with 12 FDA-approved ADCs and more than 100 promising ADCs in clinical trials, successful clinical outcomes using ADCs have inspired scientists and clinicians to further advance this new molecular format for effective treatment of cancers. ADCs deliver anticancer drugs (payloads) selectively to blood cancer cells or solid tumors while avoiding healthy tissues, enabling the use of highly active payloads that are too toxic to be used alone. The ADC chemical linker connecting the antibody and the payload molecule is a critical component for enabling tumor-specific drug delivery. Thus, the use of properly designed ADC linkers is a key for successful implementation of ADC-based chemotherapy.

My research group is focused on the development of novel chemical ADC linkers by taking advantage of the power of organic chemistry, medicinal chemistry, and chemical biology. We have developed a glutamic acid-valine-citrulline tripeptide linker as a new-generation ADC linker with high translatability from bench to clinic. Our tripeptide linker is highly stable in circulation but immediately degraded once a given ADC gets into the target cell, maximizing ADC stability and therapeutic efficacy. Using this technology, we recently developed ADCs equipped with two distinct payloads (termed "dual-drug ADCs", Figure 1A). Our dual-drug ADC showed improved treatment efficacy in xenograft mouse models representing intratumor HER2 heterogeneity and elevated drug resistance, which are clinical issues often seen in breast cancer patient samples. Notably, our dual-drug ADC exerts greater treatment effect and survival benefit than does co-administration of two single-drug variants (Figure 1B). Our findings suggest that simultaneous delivery of two payloads with distinct drug properties is a promising approach to combating breast cancer heterogeneity and drug resistance. Our next goal is to advance this novel ADC to in-depth preclinical studies and following first-in-human studies within several years.

With our cutting-edge conjugation technology platform in hand, we are currently pursuing next-generation ADCs for treating refractory cancers, including inflammatory breast cancer, glioblastoma multiforme (GBM), pancreatic cancers, and other solid tumors with drug resistance and/or high intratumor heterogeneity. We are also evaluating novel antibody conjugates that can elicit strong anti-tumor immune response with minimal systemic toxicity. Patients with resistant and heterogeneous cancers often suffer from recurrence of malignancy and exacerbated quality of life because of ineffective chemotherapy. Our lab's long-term goal is to create novel therapeutic options for overcoming such clinical issues. We envision that our novel ADC linker technology platform will help the whole biomedical research community achieve this overarching goal.

RESEARCH PROJECTS

- Design, synthesis, and evaluation of novel chemical linkers for constructing multi-loading ADCs
- Structural optimization of ADC linkers for high plasma stability, rapid drug release, and enhanced permeability to the brain
- Modulation of the ADC function by chemical modification for organ-specific delivery
- Evaluation of ADCs in refractory cancer models

KEY PUBLICATIONS

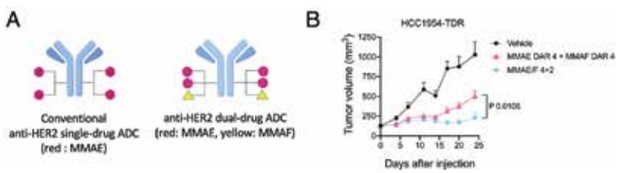
Anami, Y., Yamazaki, C. M., Xiong, W., Gui, X., Zhang, N., An, Z., Tsuchikama, K.* (2018) Glutamic acid–valine–citrulline linkers ensure stability and efficacy of antibody–drug conjugates in mice, *Nature Communications,* 9:2512.

Yamaguchi, A., Anami, Y., Ha, S. H. H., Roeder, T., Xiong, W., Lee, J., Ueno, N. T., Zhang, N., An, Z., and Tsuchikama, K.* (2021) Chemical generation of small molecule-based bispecific antibody-drug conjugates for broadening the target scope. *Bioorganic & Medicinal Chemistry* 32:116013, 2021. DOI: 10.1016/j.bmc.2021.116013.

Yamazaki, C. M., Yamaguchi, A., Otani, Y., Anami, Y., Xiong, W., Lee, J., Ueno, N. T., Zhang, N., An, Z., and Tsuchikama, K.* (2021) Antibody-drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance. *Nature Communications* 12:3528, 2021.

LAB MEMBERS

Instructor: Yasuaki Anami, PhD
Senior research scientist: Chisato Tsuchikama, PhD
Post-doctoral fellows: Aiko Yamaguchi, PhD, Yin Yuen Ha (Summer), PhD



Dual-drug ADCs for combating breast cancer heterogeneity. (A) Schematic structure of anti-HER2 dual-drug ADC equipped with monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF). (B) Compared to co-administration of two conventional single-drug ADCs (magenta), our dual-drug ADC (cyan) provides improved therapeutic effect in a mouse model of HER2 heterogeneous breast tumor with elevated drug resistance (HCC-1954-TDR cell line). All mice were treated with a single dose of each ADC at 1 mg/kg (n = 11 for vehicle; n = 12 for other groups).

Ningyan Zhang, PhD
Professor

Cancer resistance mechanisms to therapeutic antibodies and modulation of anticancer immunity

Monoclonal antibody therapies have revolutionized cancer treatment and been successfully used for treatment of many types of cancer in the clinic. However, similar to many targeted cancer therapies, both innate and acquired resistance are widely reported for monoclonal antibodies. Understanding the mechanism of cancer resistance to therapeutic antibodies is of paramount importance for improvement of efficacy of the antibody therapies to benefit more patients.

Cancer immune evasion is being recognized as one of hallmarks of cancer. Our research has demonstrated the prevalence of proteolytic impairment of antibody IgG in the tumor microenvironment. Trastuzumab and pertuzumab (anti-HER2 antibody) with a single hinge cleavage showed a loss of immune effector function against cancer cells *in vitro* and reduced antitumor efficacy *in vivo*. Based on our findings and reports by others, we hypothesize that antibodies recognizing tumor associated antigens (TAA) in the tumor microenvironment are susceptible to proteolytic impairment through a hinge cleavage by matrix metalloproteinases (MMPs). Such proteolytic hinge cleavage of antibodies not only weakens antibody anticancer immunity but also leads to an immune suppressive tumor microenvironment. Our current research programs are centered on better understanding of tumor evasion of antibody immunity and developing therapeutic strategies to modulate anticancer immunity for improvement of cancer treatment. We employ a wide array of experimental approaches including *in vitro* 2D and 3D cell co-cultures, mouse tumor models, and studies with clinical samples from cancer patients to determine factors influencing proteolytic impairment and to identify mechanisms of cancer immune evasion triggered by proteolytic impairment of antibody hinge. State-of-the-art technologies are used in our studies, such as high content fluorescence imaging, mass spectrometry, fluorescence activated cell sorting (FACS), and single cell cloning of antibodies. We have established a monoclonal antibody platform technology to discover and select novel anticancer antibodies for functional evaluation and preclinical development. The long-term goal of my research is to understand mechanisms of cancer evasion of antibody therapies and to identify key molecular targets for development of effective anticancer immunotherapies.

RESEARCH PROJECTS

- Understand mechanisms of cancer immune suppression.
- Develop platform technologies for discovery of therapeutic antibodies.

KEY PUBLICATIONS

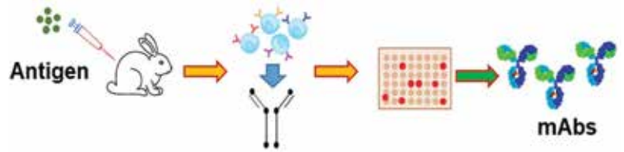
Guojin Wu; Yixiang Xu; Robbie D Schultz; Heyu Chen; Jingjing Xie; Mi Deng; Xiaoye Liu; Xun Gui; Samuel John; Zhigang Lu; Ningyan Zhang; Zhiqiang An; Chengcheng Zhang (2021) LILRB3 supports development of acute myeloid leukemia and regulates NF-κB signaling by recruiting TRAF2 and cFLIP. *Nature Cancer,* doi.org/10.1038/s43018-021-00262

Ila Mishra, Clemens Duerrschmid, Zhiqiang Ku, Wei Xie, Jennifer Hoffmann, Ningyan Zhang, Zhiqiang An, and Atul R. Chopra (2021). Asprosin Neutralizing Antibodies as a Treatment for Metabolic Syndrome. *eLife.* doi.org/10.7554/eLife.63784.

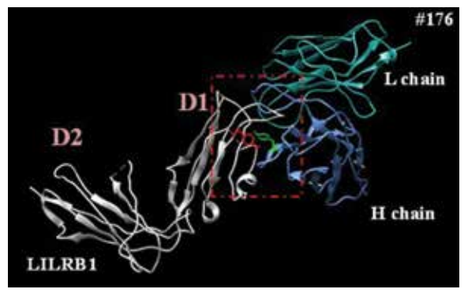
Jingnan An, Yi Du, Xuejun Fan, Y Wang, C Ivan, X Zhang, Anil K. Sood, Zhiqiang An, Ningyan Zhang (2019) EGFL6 Promotes Breast Cancer by Simultaneously Enhancing Cancer Cell Metastasis and Stimulating Tumor Angiogenesis. *Oncogene,* doi.org/10.1038/s41388-018-0565-9.

LAB MEMBERS

Senior research associate: Hui Deng, MS
Senior research scientist: Xuejun Fan, MD, PhD
Research scientist: Leike Simon Li, PhD
Research associate: Xin Li, MS
Senior research scientist: Wei Xiong, PhD



Schematic diagram for generation and screening of monoclonal antibodies (mAbs) using our established technology platform.



Structural identification of binding epitopes of a functional anti-LILRB1 monoclonal antibody for development of cancer therapeutics. From H. Chen et al. (2020) *Journal for Immunotherapy of Cancer*, doi.org/10.1136/jitc-2019-000515.

IMM Service Centers

The IMM is focused on studying and preventing disease at the genetic, cellular, and molecular levels using DNA and protein technologies and animal models. Our service center goal is to provide the latest technology and the highest quality services to our colleagues and customers while operating in a cost-effective manner. IMM's Service Centers are staffed by top research experts in the technologies offered.

To accomplish IMM's strategic goal of providing high quality and effective support services for our research capacity, we have initiated a systematic process to further improve our infrastructure and to provide to our faculty and customers access to cutting-edge technology. The establishment of key service centers at UTHealth-IMM is a critical component of this commitment.

Antibody Engineering and Expression Service Center

Antibody therapeutics represents a major breakthrough in combating human diseases, including cancer. Even though the pharmaceutical and biotechnology industries are in the center stage of drug discovery and development, academic researchers are increasingly engaged in discovering new antibody drug candidates. However, advancement of some of the promising antibodies in the early stage of discovery from academic research laboratories is often hindered by the lack of access to the expertise and infrastructure required for antibody engineering and other related key technologies. Our antibody engineering and expression service center offers the services to fill the gap of the much-needed expertise in early discovery of monoclonal antibodies and lead optimization for the research and drug discovery communities. The objective of the service center is to provide technical support and services to antibody identification, molecular cloning, antibody expression, and purification. Results generated from the service center will strengthen the collaborators' ability to attract external funding to continue development of the optimized therapeutic antibodies with the ultimate goal of translating basic research to novel therapies.

Clinical and Translational Proteomics Service Center

Proteins are the essential functional biomolecules that participate in a vast array of physiological cellular activities and are implicated in all aspects of disease mechanisms. Disease associated proteome alterations may reflect on changes in protein expression, structure, localization, polymorphism, as well as post-translational modifications (PTMs) status. Proteomics can deliver dynamic information of a protein profile in a complex system and thereby provide a vibrant picture of cellular function under biological conditions. Furthermore, quantitative proteomics can identify steady or perturbation-induced proteome alterations associated with a disease status or biological state and is highly relevant to translational and clinical applications.

Our center provides state-of-the-art proteomics services to support basic, translational, and clinical research. The main services include protein profiling, label-free or label-based quantitative analysis, therapeutic protein characterization, and essential PTM analysis. We have the capability to analyze a broad range of research or clinical specimens, from purified proteins to complex mixtures, including cell and tissue extracts, plasma/serum, and other biofluids or biological samples.

We also provide more advanced support through collaborative efforts, such as biomarker discovery and verification, glycoproteomics/glycomics analysis and microbiome profiling.

The center contains state-of-the-art instrumentation and well-trained personnel to provide an integrated proteomics service, including sample preparation, mass spectrometric analysis, and bioinformatics data processing.

Flow Cytometry Service Center

Flow cytometry is a single-cell analysis technology used for cell counting and fluorescent marker detection. It allows high-speed identification, and even isolation, of specific subsets within mixtures of cells. The fluorescence can be measured to determine cellular properties like relative size, complexity, cell type, and response to specific stimuli, such as drugs and genetic manipulations.

These specialized multicolor cell analysis instruments allow researchers to evaluate a large number of samples in a short time frame and gather information on very rare populations of cells and additionally isolate cell populations to be sorted. The current instrumentation allows simultaneous acquisition of more than 10 fluorescent signals from thousands of individual cells per second.

The Flow Cytometry Service Center offers FACS acquisition and analysis, cell sorting, user training, and consultation for experimental design, interpretation, and troubleshooting.

Our instruments are available on a fee-for-service charge to all research investigators from UTHealth and external organizations.

Transgenic and Stem Cell Service Center

Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cells service center, which was established in 1998. It has generated over 800 new transgenic and knock-out mouse animal models for all research investigators from UTHealth and external organizations on a fee-for-service basis.

The stem cell lines that have been derived in the laboratory are highly effective for the generation of knock-out/ knock-in mice and for cell differentiation studies. In addition to the production, cryopreservation, and re-derivation of genetically-engineered mice and rats, the services of the facility also include gene targeting, CRISPR/Cas9 genome editing, derivation of new cell lines, and intellectual/technical support in different aspects of microsurgery, cell culture, and stem cell research.

Nano 3D Printing Service Center

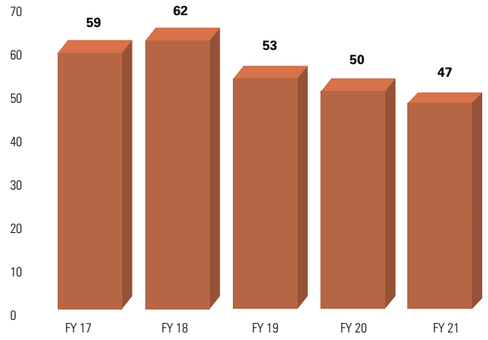
Nano 3D Printing Service Center provides state-of-the-art 3D printing services. We provide 3D printed models of human and laboratory animal organs, novel surgical tools, and custom-made laboratory supplies, in prototype or final production models.

We have both traditional FDM (Fortus 450mc) thermoplastic as well as multi-color, resin-based, high-resolution Stratasys J750 (14 micron) 3D printers with large print beds.

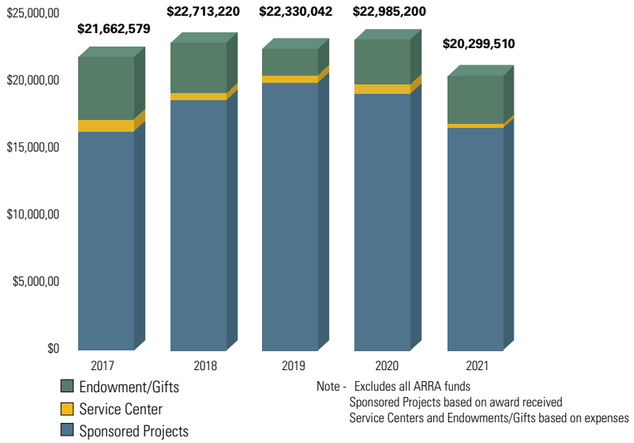
A wide range of materials with varying Shore A values (hardness) is available. STL files, SolidWorks, or medical imaging files can be used to produce the 3D models.

We are located on the 3rd floor of the Fayez S. Sarofim Research Building.

Number of Faculty

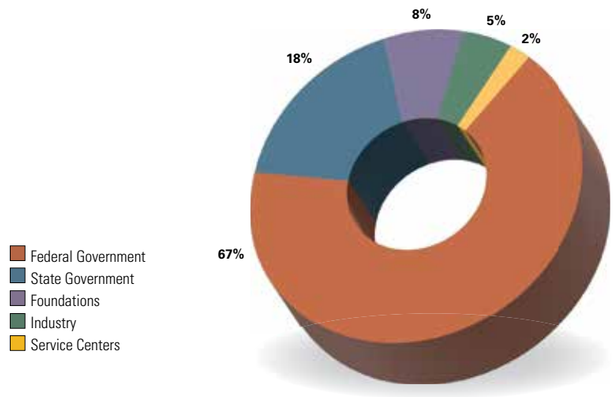


Total Funds Supporting Research

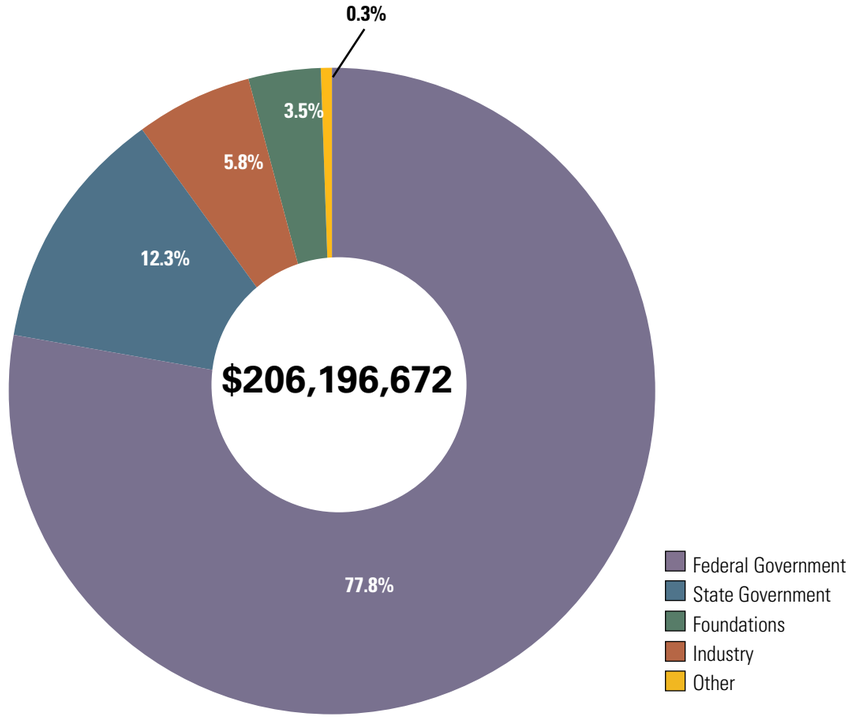


Note - Excludes all ARRA funds
Sponsored Projects based on award received
Service Centers and Endowments/Gifts based on expenses

Total Expenses Supporting Research



IMM Extramural Funding Inception to Date



IMM Commercial Outcomes Inception to Date

U.S. Patents Issued
57

License & Option Agreements Executed
82

Startup Companies Formed
21

Income Generated from Intellectual Property
$19,935,995

Institute of Molecular Medicine Endowments

Annie and Bob Graham Distinguished Chair in Stem Cell Biology
Becker Family Foundation Professorship in Diabetes Research
C. Harold and Lorine G. Wallace Distinguished University Chair
Chair in Biomedical Engineering
Cullen Chair in Molecular Medicine
D. Dudley and Judy White Oldham Research Fund
Dr. Edward Randall, Jr. Memorial Fund
George and Cynthia Mitchell Distinguished Chair in Neurosciences
George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research
Hans J. Muller-Eberhard, MD, PhD, and Irma Gigli, MD, Distinguished Chair in Immunology
Harry E. Bovay Lecture Series in Molecular Medicine
Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research
IMM General Endowment
James T. Willerson Distinguished Chair in Cardiovascular Research in Tribute from the Ewing Halsell Foundation
Janice Davis Gordon Chair for Bowel Cancer Research
Jerold B. Katz Distinguished Professorship in Stem Cell Research
John S. Dunn Research Scholar Fund I
John S. Dunn Research Scholar Fund II
John S. Dunn Research Scholar Fund III
John S. Dunn Research Scholar IV
Kay and Ben Fortson Distinguished Chair in Neurodegenerative Disease Research
Kozmetsky Family Chair in Human Genetics
Linda and Ronny Finger Foundation Distinguished Chair in Neuroimmunologic Disorders
Marjorie B. Poyner and Herbert F. Poyner, Jr. Endowment for Medical Research in the Institute of Molecular Medicine for Prevention of Human Diseases
Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research
Müller-Eberhard Memorial Lecture Series
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research
Nina and Michael Zilkha Distinguished Chair in Neurodegenerative Disease Research
Pierce Runnells Memorial Research Fund
Robert A. Welch Distinguished University Chair in Chemistry
Rochelle and Max Levit Chair in the Neurosciences
Rodney J. Sands New Initiatives Stem Cell Research Endowment Fund
Shavonnah Roberts Schreiber Women's Health Endowment
The Carolyn Frost Keenan Professorship in Cardiovascular Disease Research
The Jerry and Maury Rubenstein Foundation Distinguished Professorship in Heart Disease Research
The Laurence and Johanna Favrot Distinguished Professorship in Cardiology
The Welch Foundation Endowment in Chemistry and Related Sciences
Walter and Mary Mischer Distinguished Professorship in Molecular Medicine
William S. Kilroy, Sr. Distinguished University Chair in Pulmonary Disease

Thank you to our donors,
who through the establishment of these endowments, enable the IMM to recruit and retain top scientists from around the world.