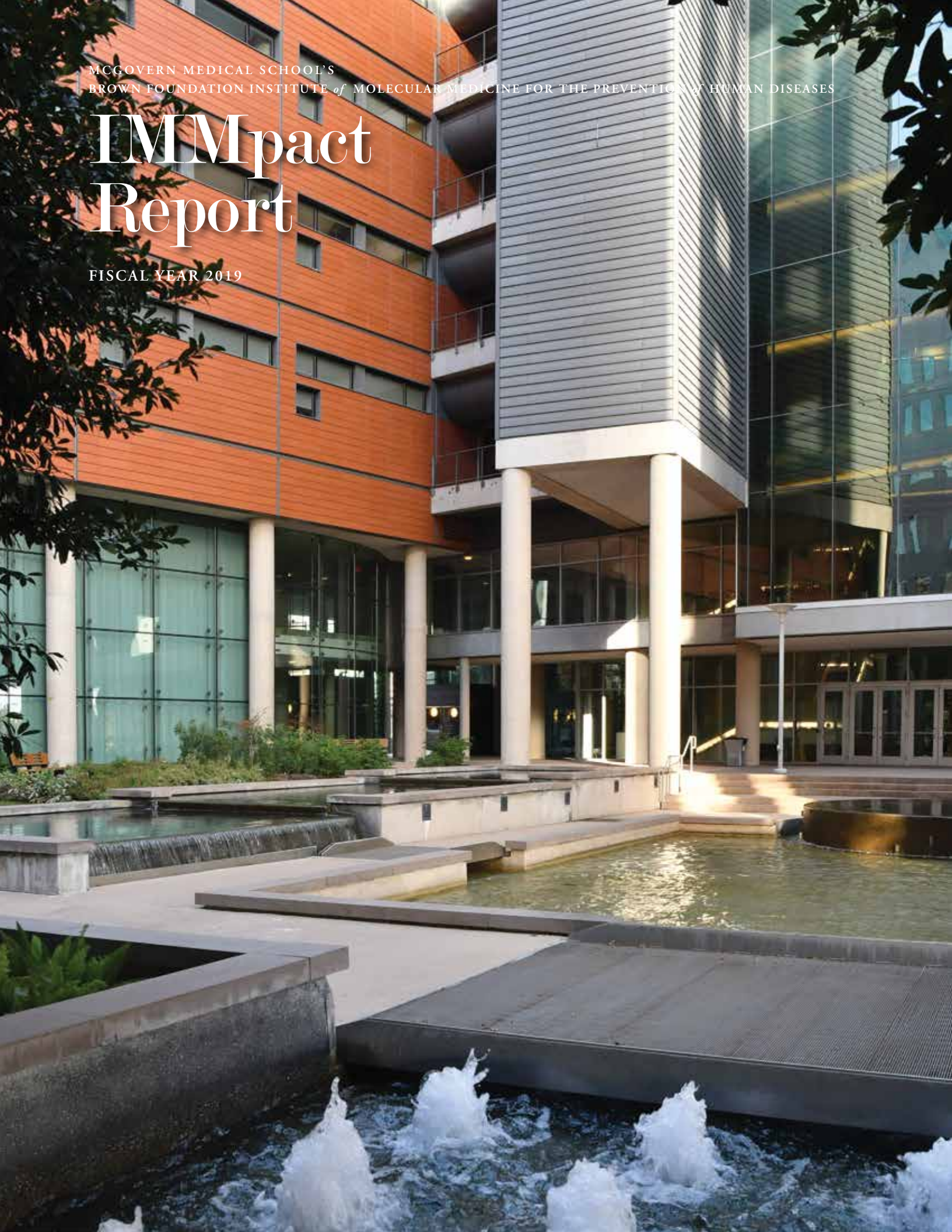


MCGOVERN MEDICAL SCHOOL'S
BROWN FOUNDATION INSTITUTE *of* MOLECULAR MEDICINE FOR THE PREVENTION *of* HUMAN DISEASES

IMM Report

FISCAL YEAR 2019



ABOUT THE COVER

A butterfly garden is a new addition to the courtyard of McGovern Medical School's Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases.

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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 IMM^{act} 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. To this end, we have teams of outstanding basic and translational scientists who collaborate closely with our clinical colleagues. Inside the report you will find in-depth articles on some of our faculty and donors plus an account from each IMM faculty member describing their research programs.

The environment for scientific research funding continues to be extremely challenging, especially from the NIH. Despite this, IMM faculty have excelled again. Over the financial year just ended, our new grants and contracts were up again over the preceding year, which in turn had seen a considerable increase over the prior year. Indeed, we have now substantially increased our extramural grant funding for each of the last seven years! It is a testament to the remarkable quality and creativity of our scientists that the IMM remains so successful in attracting research funds from what is an ever-diminishing national pool. That said, 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.

In addition to advancing science and medicine, we therefore wish to develop our relationships with all in our community who value the aspiration of our mission to translate molecular discoveries into new therapies for human disease. In this regard, we are 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. If you would like to investigate how you can also be involved, I would be pleased to talk with you personally. Alternatively, I would be delighted to see you at our annual IMM symposium. Last year, 145 guests listened to two talks in the Beth Robertson Auditorium and attended a reception in the James T. Willerson, M.D. Discovery Hall.

This year the symposium will be held April 29, 2020, and will feature two talks on using antibodies to fight cancer. We chose this novel and exciting topic in part to showcase a major new \$6M grant from the Cancer Prevention and Research Institute of Texas (CPRIT) that was awarded to Dr. Zhiqiang An and colleagues in the Texas Therapeutics Institute at IMM. The CPRIT grant is designed to help cancer researchers from all around Texas take advantage of unique expertise at IMM to develop highly specific therapeutics to target 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, you will need to come to the symposium. It will be a very illuminating and entertaining evening. As in previous years the symposium is an excellent opportunity to hear exciting research stories directly from our faculty, to discuss its implications for the future of medicine and health care, and to have all your questions answered. Full details are in this report; please mark the date in your calendar because it is a great opportunity to visit the IMM.

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 (SCR_{B3})



- SCR_{B3} 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.



The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases

IMMpact Symposium

Fayez S. Sarofim Research Building
1825 Pressler Street

Wednesday, April 29, 2020
4:00 - 6:30 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*

UNLOCKING THE MYSTERIES OF EATING DISORDERS

There is no secret that eating a proper diet is a key component to living a healthy lifestyle. Trendy superfoods and the next-best diet are a constant reminder that eating healthy should be a part of every individual's day-to-day goal.

For those suffering with eating disorders, however, healthy eating may not always be an option. That is why the lab of associate professor Qingchun Tong, PhD, focuses on finding effective treatments to alleviate the symptoms of eating disorders.

"I've always been interested in this research, because I see this as a huge problem in our society," Dr. Tong says. "It affects almost every family."

Dr. Tong's research spans both extremes of the spectrum of feeding behavior. One side looks at over-eating leading to obesity, while the other end focuses on the refusal to eat causing anorexia and malnourishment. However, in addition to special diets or exercise, Dr. Tong is specifically interested in helping patients whose symptoms may be starting in the brain.

"We're trying to understand the brain pathways that can regulate feeding behavior and identify key neurons and neuropathways in the brain that are defective," Dr. Tong says. "Our idea is that defective feeding behaviors in obesity and anorexia are co-regulated by neurons that

also regulate emotional states."

Scientists currently do not have sufficient knowledge of the biology of eating disorders, with medication as the only effective treatment for the more than 30 percent of Americans who are affected by obesity. On the other side, though the incidence rate for patients with anorexia is much smaller, the fatality rate is higher due to no known effective treatments for the disease.

"The hope is that our study can reveal key mechanisms underlying development so that we can discover effective targets for future cures or prevention," Dr. Tong explains. "We hope that we can identify the brain neurons to first, understand the biology, and second design a suitable drug that can prevent or cure both obesity and anorexia."

Using animal models in a typical feeding center, Dr. Tong and his lab are able to manipulate neural activities to see whether they experience emotional changes. The lab is also able to manipulate the neural activity in the aggression center, which can cause the development of feeding abnormalities.

In his research, Dr. Tong and his team have discovered a neural pathway from the brain feeding center to the brain aggression center, which controls emotional changes, meaning that pathway could be important in regulating eating disorders. Since the neurons can change activity in oppo-

site ways, they can be used to control overconsumption or refusal to eat. By regulating each of the biological processes, Dr. Tong has confirmed that feeding and emotional regulation can be controlled by the same pathway.

"My research was originally in obesity," Dr. Tong says. "But when I discovered that neurons can go both ways, I became interested in anorexia research, because I think it's the same neural program that controls both. It was the logical next step."

Dr. Tong's lab is using advanced technologies, such as optogenetics and newly available neural tracing technologies, to study brain neuronal activities that direct regulated feeding behavior. One of the goals of his research is to find a means of training those neural activities to control eating disorders through behavioral treatments instead of through medication.

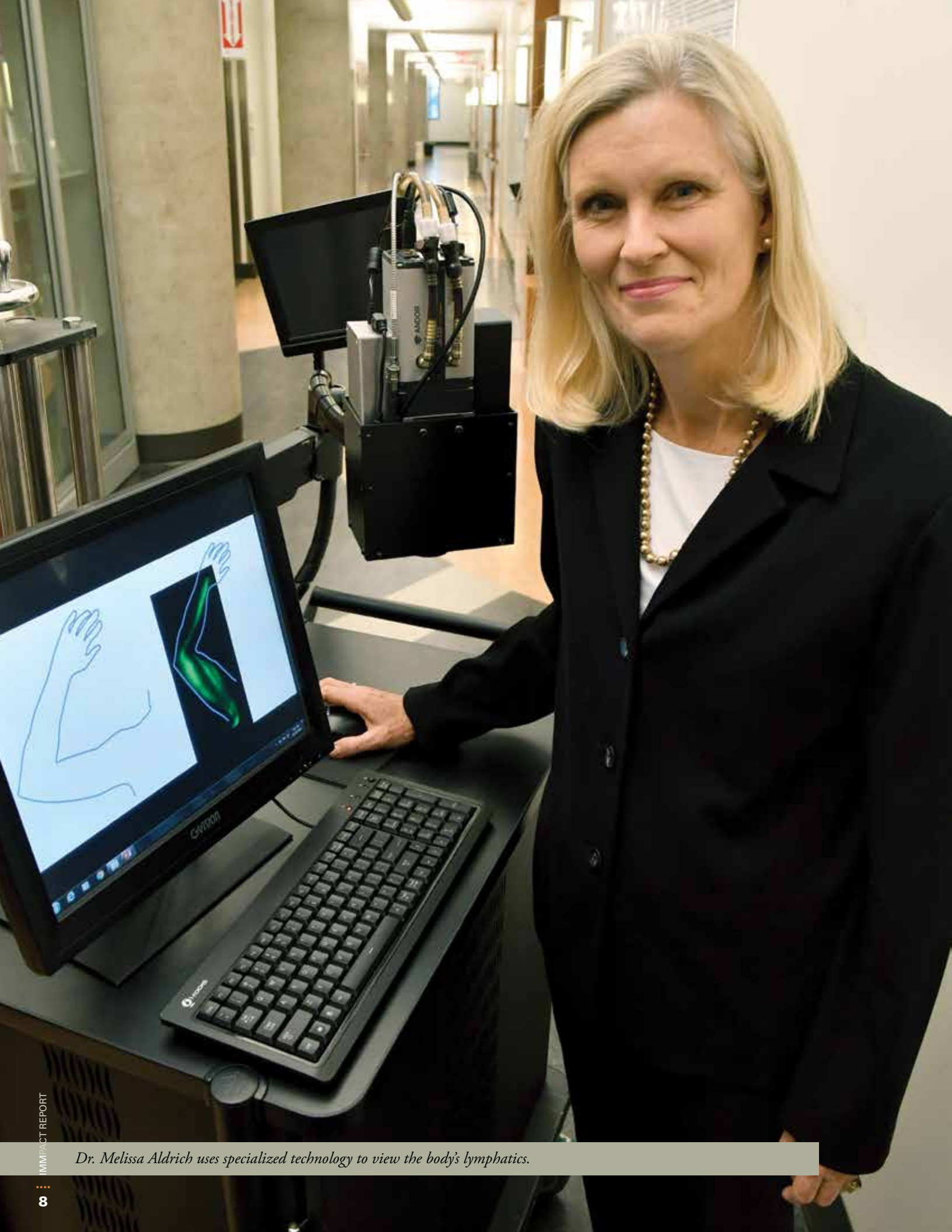
"Some behavioral treatments already exist," Dr. Tong says. "You can go the gym and participate in certain programs of exercise. So if you can use behavioral intervention in those key neurons, you can reduce the symptoms of both obesity and anorexia."

Dr. Tong completed his undergraduate and masters programs in China before earning his PhD from SUNY Downstate Medical Center in 2003. He joined the staff at McGovern Medical School in 2009.

“Our idea is that defective feeding behaviors in obesity and anorexia are co-regulated by neurons that also regulate emotional states.” — Dr. Qingchun Tong



Dr. Qingchun Tong is on a mission to find treatments for both sides of the eating disorder spectrum.



Dr. Melissa Aldrich uses specialized technology to view the body's lymphatics.

SHINING A LIGHT ON A NEGLECTED SYSTEM

We all are familiar with the body's skeletal system, muscle system, and blood system. But what about the lymphatic system? Considered a neglected system, it only came into scientific focus about a decade ago when researchers realized that this network of vessels and immune organs filters 2 to 3 gallons of lymph through our bodies each day.

A clear fluid comprised of immune cells, large proteins, and cell waste fluid, lymph can pose a serious problem for cancer patients presenting as lymphedema – excess fluid pooling in limbs and in areas where lymph nodes have been removed.

About 10 million people live with lymphatic disease in the United States, and Melissa Aldrich, PhD, an assistant professor in the Center for Molecular Imaging, predicts that number will increase as cancer survivorship rates grow.

Dr. Aldrich, who investigates immune factors that influence lymphatic dysfunction and translates lymphatic imaging technology to the clinical setting, is leading a five-year National Institutes of Health-funded study of breast cancer patients who are at high risk of developing lymphedema.

“No one knows the exact cause of lymphedema – surgery, radiation, and cancer are blamed – and it can be permanent,” Dr. Aldrich explains. “What we are trying to do is to detect developing lymphedema at the early stages – the earlier you catch it and treat it, the earlier

you can halt it and even turn it around.”

The study started in 2016, and so far, 60 breast cancer patients from The University of Texas MD Anderson Cancer Center have been enrolled. The goal is to include 75-100 patients. Simona Shaitelman, MD, associate professor of radiation oncology at MD Anderson, is the co-principal investigator on the grant.

The key to the research is the innovative imaging technology, which was invented by Eva Sevick, PhD, professor and director of the Center for Molecular Imaging and Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research, and John Rasmussen, PhD, assistant professor in the Center for Molecular Imaging.

“Up until now, visualizing what was going on inside the lymphatic system was like looking at a black box and guessing. I am fortunate to work with engineers who have developed the best system in the world that can see if the lymphatics are working,” Dr. Aldrich says.

“With the tool, starting before cancer treatment, we look through patients' skin to see whether they are developing lymphedema. Many times we catch it well before the standard old-fashioned way, which is just waiting until limb volume is 5-10 percent above normal.”

Patients in the study also have their blood tested for immune abnormalities before cancer surgery and at 6, 12, and 18 months following radiation treatment.

Dr. Aldrich says her research has a two-pronged approach.

“Our first goal is catching the lymphedema early, and the second is finding the cause,” Dr. Aldrich says. “Lymphedema is more than just a disease of disruption of the lymphatic channels due to surgery and radiation – if it were that, then everyone who had surgery would get it. It's an autoimmune-like disorder, and there are cellular and molecular factors that have yet to be uncovered that could be targets for treating lymphedema with a pill, or other therapeutics.”

Dr. Aldrich earned her PhD in immunology from the MD Anderson UTHealth Graduate School and joined the IMM faculty in 2007.

“I hadn't even heard of lymphedema before joining Dr. Sevick's lab – and this was after years of working in cancer research,” Dr. Aldrich added.

In order to spread the word about lymphedema, Dr. Aldrich recently served as chair of a committee with the advocacy organization LE&RN, Lymphatic Education & Research Network.

“Our committee developed standards for centers of excellence for the diagnosis and treatment of lymphatic diseases. This will help patients understand their options and make informed decisions,” Dr. Aldrich said.

For Dr. Aldrich, her work is all about the patients.

“A pill for lymphedema is my main goal, at the top of my bucket list. A pill, or an easy treatment.”



Dr. Ali Azhdarinia's research could lead to safer and more effective cancer treatments in tumor-specific drug delivery.

A VISION FOR THE FUTURE OF CANCER TREATMENT

Perhaps one of the scariest words throughout the world is cancer. Whether it is a family member or friend who has been diagnosed, or an awareness program at work or school, chances are cancer will affect a person's life in one way or another. In fact, the American Cancer Society estimates that nearly one in every three Americans will develop cancer in their lifetime.

Research all over the world has been poured into finding a cure for cancer. However, in the lab of associate professor Ali Azhdarinia, PhD, the goal is to apply new technologies to enhance treatments that already exist.

"Specifically, my lab focuses on using imaging techniques to improve how we see and treat disease," Dr. Azhdarinia says.

In current cancer treatments for tumors that have spread across the body, extremely toxic chemotherapy is injected into the body, which goes to the tumor and wipes it out. With this approach however, you cannot control where the drug goes, so while wiping out a dangerous tumor, you are also affecting healthy, normal tissue.

Similar problems can arise due to chemotherapies not being potent enough to completely kill off a cell, or if a tumor adapts and becomes resistant to a certain

chemotherapy.

"We are developing new strategies to take imaging compounds, and then we use them not only to deliver an isotope to see what's going on, but also to deliver a cytotoxic agent that will kill the cell," Dr. Azhdarinia says.

Using concepts that have been clinically adopted in nuclear medicine for nearly half a century, the Azhdarinia lab has attached chemotherapy treatments with radioactive compounds to see exactly where the drug is going and how effective it is at destroying the tumor.

"If you have a toolkit of three or four drugs that may help the patient, you want to figure out which one is the best for each individual," Dr. Azhdarinia says. "We are working on methods that will allow us to noninvasively see how each drug interacts with the tumor so we can predict which will be most effective."

Azhdarinia's lab has been working on tumor-specific drug delivery since 2010 and made a breakthrough in 2014 after receiving an NIH grant that supported research for a fluorescent compound that uses the same chemistry platform that is used for delivering chemotherapy. This project consists of a peptide that is attached to a fluorescent dye

and specifically seeks out tumor cells to allow surgeons to more efficiently remove cancerous cells without cutting healthy tissue.

"Collectively, our drug design strategy can help surgical and nonsurgical populations," Dr. Azhdarinia explains. "We have the same concept for both, but now instead of just putting a fluorescent dye to allow us to see the tumor; we can substitute toxic compounds to kill the tumor."

Dr. Azhdarinia's interest in his field developed while at The University of Texas Graduate School of Biomedical Sciences, where he obtained his PhD in the area of pharmacology and nuclear medicine. He recalls that at the beginning of his research career, cancer treatment was not nearly as individualized as it is today.

"Now treatments are tailored to the biology of the cancer and are going in so many different ways," Dr. Azhdarinia says.

"So, our strategy was: why not take imaging, since you can see uptake and measure it, and apply it to targeted therapies so we can monitor how much reaches the tumor and see how it responds? Potentially, you can achieve the ultimate goal of seeing how much drug needs to get to that tumor in order to get the response you want."

“We are working on methods that will allow us to noninvasively see how each drug interacts with the tumor so we can predict which will be most effective.” — Dr. Ali Azhdarinia

TRANSLATING RESEARCH IN THE LAB

A balanced life. A balanced diet. A balanced checkbook. These are goals for many of us. But did you know that this sense of balance is actually based in our cells?

Cellular homeostasis is key to keeping our entire body in balance. When cells die, fold, or grow, that can spell trouble, signaling cancer or another troubling disease. About 38% of us will be diagnosed with cancer; 1 in 10 Americans over the age of 65 has Alzheimer's; and 1 million Americans are believed to have Parkinson's.

But what if these diseases, which all start at the cellular level, could be stopped there?

Working at the intracellular level, Nami McCarty, PhD, associate professor of Stem Cell and Regenerative Medicine at the IMM, is discovering how cells make that fatal switch from balanced to unbalanced.

"Healthy cells have to make proteins in the correct balance, homeostasis. If the cell becomes out of balance, it dies," explains Dr. McCarty, the Annie and Bob Graham Distinguished Chair in Stem Cell Biology.

Detecting how cells become out of balance, Dr. McCarty and her lab have discovered a

protein that is implicated in cancer progression, acting as fuel for the cell. This same protein, Dr. McCarty says she believes, is a factor in Alzheimer's and Parkinson's.

"This protein is like the trash mechanism for the cell," she explains. "The cells can't get rid of the trash; the cell gets overloaded and dies."

Dr. McCarty's lab discovered a protein that regulated inactivity in blood cancer, multiple myeloma.

"Multiple myeloma is a good model for drug development, and I have been able to publish a lot and been well-funded in the cancer space," she says. "I would like to now add in Alzheimer's and expand to the neurological research."

Having made these findings in cancer, Dr. McCarty says her research applies not only to neurological diseases but also to autoimmune diseases.

"Ultimately, we will create drug targets, which will provide therapies to these devastating diseases," she says. "That would be our goal – to halt the progression of bone marrow cancer, Alzheimer's, Parkinson's, and Huntington's."

Dr. McCarty earned her PhD in medicine chemistry

and molecular pharmacology at Purdue University in 2000, and pursued postdoctoral research at the Dana-Farber Cancer Institute and Harvard Medicine School, which involved investigating mechanisms of immune homeostasis and central tolerance. She received a Research Scholar Development Award, K22, from the National Institute of Allergy and Infectious Diseases in 2006, based on a gene signature she discovered.

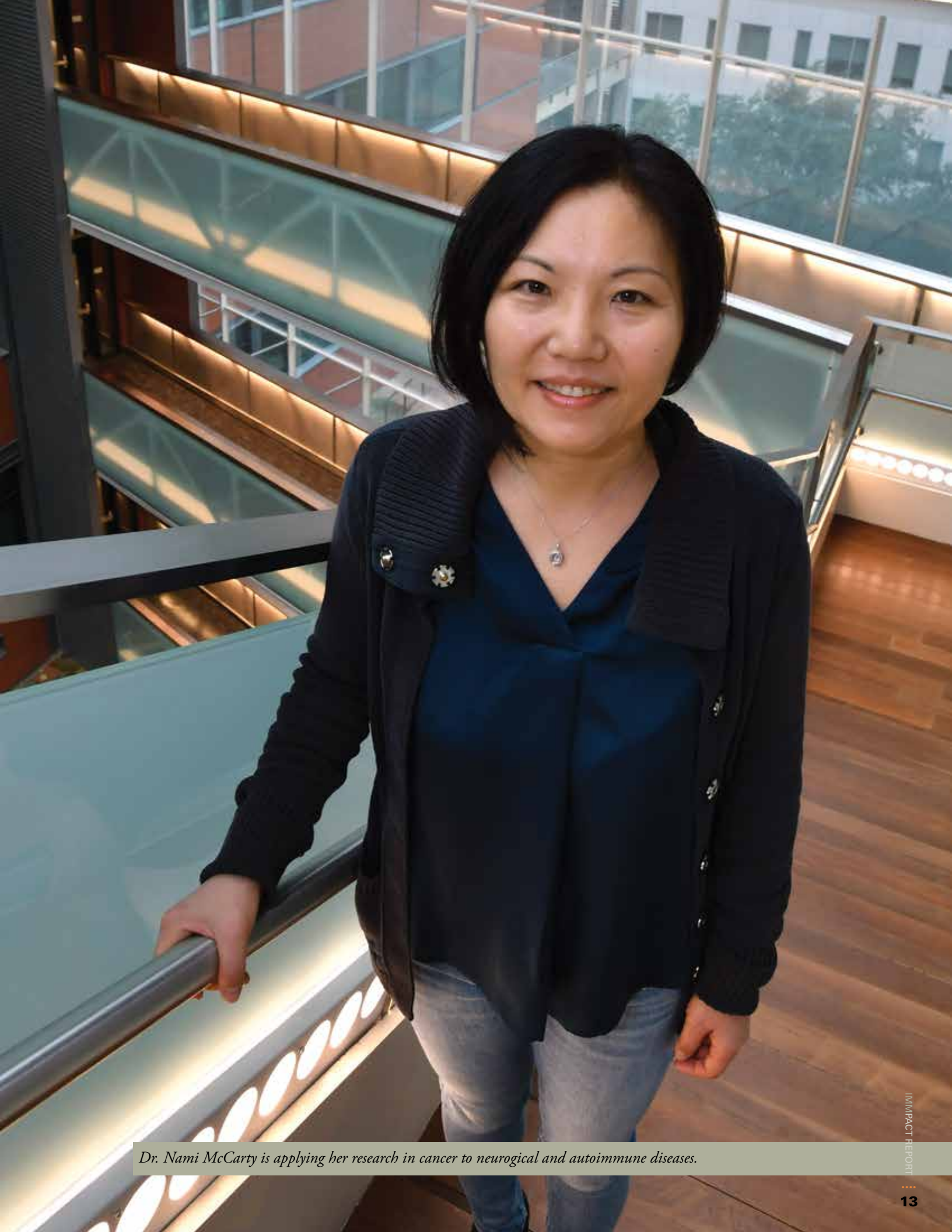
That was the same year she was recruited to the Institute of Molecular Medicine and was one of the first faculty to occupy the 229,000-square-foot Faye S. Sarofim Research Building.

"I had the whole floor to myself back then," she says. "Dr. Irma Gigli and Dr. Tom Caskey, who was the director at that time, interviewed me for my position."

Upon joining the IMM, Dr. McCarty switched her research focus from immunology to cancer. And with her intent to broaden her studies to include the neurosciences, she again is looking for a big change.

"I can do this," she says. "What I am passionate about is to make something of my research."

“That would be our goal – to halt the progression of bone marrow cancer, Alzheimer's, Parkinson's, and Huntington's.” — Dr. Nami McCarty



Dr. Nami McCarty is applying her research in cancer to neurological and autoimmune diseases.



Members of the Bovay Foundation include Edward R. Naumes, from left, Michael L. Patrick, Peggy Larkin Kelly, Frances Escriva, Carl F. Jaedicke, and C. Ronald Dorchester.

THE BOVAY FOUNDATION – BUILDING BRIDGES

As a child, Harry E. Bovay Jr. enjoyed a poem that hung in his father's office. Called "The Bridge Builder" by Will Allen Dromgoole, it describes an old traveler who builds a bridge across a wide chasm after crossing safely – not for himself but for the youth who may travel after him.

That concept of building for others remains as Bovay's legacy in the Harry E. Bovay Jr. Foundation.

Bovay lived in the Houston area for most of his life following graduation from Cornell University in 1936 as a civil engineer. He established Bovay Engineers and, following his retirement in 1984, established the Mid-South Telecommunications Company, working there until a few months before his death in 2011 at the age of 96.

In 1991, he formed the Harry E. Bovay Jr. Foundation to support education and community development in rural areas, the Boy Scouts, and other charities, including research efforts at the IMM.

Bovay was one of the original donors to the IMM's \$200 million New Frontier Campaign, which established the IMM as the research institute it is today. His gift of \$1.5 million created the Harry E. Bovay Jr. Distinguished University Chair in Metabolic Diseases, and his generosity

helped to create the IMM's Research Center for Metabolic Diseases.

Mikhail Kolonin, PhD, director of the IMM's Center for Metabolic and Degenerative Diseases, is the holder of the Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Research and principal investigator on the Bovay Lectureship, an endowed lecture series. He joined the IMM faculty in 2007, earning his PhD from Wayne State University and completing a postdoctoral fellowship at The University of Texas MD Anderson Cancer Center.

Dr. Kolonin and his team have focused their research on the role fat plays in cancer progression and aging. The group's work has shown that fat cells promote the progression of prostate and breast cancers and makes them more resistant to chemotherapy.

"According to published statistics, 15 to 25 percent of cancer-related deaths could have been avoided by preventing obesity. It is possible that with weight-loss surgery, or other prevention measures, we can also reduce cancer progression, the risk of cancer recurrence, or even use it as a cancer treatment," Dr. Kolonin says.

His group is investigating experimental therapies targeting fat tissue as a potential complementary approach to cancer treatment.

Funded by the Bovay Foundation, the team also is looking at the science of healthy aging.

"When we age, telomeres (the protective tips of the chromosome) shorten and, as a result, stem cells can no longer divide to renew cells in fat tissue," Dr. Kolonin says.

His team now has evidence that in obesity, stem cell depletion is accelerated, which underlies premature diabetes development. The group is now looking at how feeding affects stem cell exhaustion in fat, as well as in other tissues, and how this knowledge can be applied in preserving skeletal muscle fitness.

"We have a lot to learn and many hypotheses to test, but the stars are aligned within our team to make important new discoveries that will improve health," Dr. Kolonin says.

In December 2019, keeping with the Bovay mantra "build on what you gave," Dr. Kolonin and his team were the recipients of another gift from the Bovay Foundation to support their important and innovative work.

"We are largely dependent upon the Bovay Foundation's generosity," Dr. Kolonin says. "We have NIH support, but reviewers of NIH want preliminary data, they want the nitty gritty details to get started, and that is why this support is so vital."

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; AJ Marian, MD, Professor; Raffaella Lombardi, MD, PhD, Adjunct Assistant 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 identification of new disease-causing genes and have advanced the genetic causes of heart failure. We are actively recruiting additional probands and family members.

II. Genomics 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 by RRBS, and chromatin remodeling by ChIP-Seq of specific histones and proteins. The integrated findings are used for preventive and therapeutic targeting.

III. 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. Several active programs are current underway.

IV. 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
James T. Willerson Distinguished
Chair in Cardiovascular Research*



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 entails an integrated approach that includes human molecular genetic studies through high throughput whole exome and genome sequencing to identify the causal genes and mutations, followed by genomic studies, including transcriptomics and epigenetics to define molecular remodeling of chromatin in the presence of causal mutations. The aim is to

link the causal mutations to genomic remodeling and to the pathogenic pathways. The responsible molecular mechanisms are identified through molecular mechanistic studies in genetically modified animal models and cultured cells. The mechanistic discoveries are then utilized to intervene in model organisms, utilizing genetic and pharmacological approaches that target the pathogenic pathways, in order to prevent the evolving phenotype and reverse or attenuate the established phenotype. These findings in the model organisms are extended to human studies through pilot randomized placebo-controlled double-blind clinical trials. The findings, if favorable, are pursued through collaborative large-scale clinical trials.

RESEARCH PROJECTS

- Identification of causal genes for heart failure and sudden cardiac death.
- Identification and characterization of epigenetic and transcriptomic changes, including non-coding RNAs and histone modifications in hereditary cardiomyopathies.
- Identification and characterization of the molecular pathways that link the genetic mutations to the clinical phenotype in patients with cardiomyopathies, including delineation of the mechanical signaling pathways regulated at the intercalated discs.
- HALT-HCM (Hypertrophic Regression with N-Acetylcysteine in Hypertrophic Cardiomyopathy) clinical trial (ClinicalTrials.org NCT01537926).
- Maverick & Explorer studies: Industry – sponsored clinical trials to test efficacy of an ATPase modulator on improve symptoms and exercise tolerance in patients with obstructive (Maverick) and non-obstructive (Explorer) hypertrophic cardiomyopathy.

KEY PUBLICATIONS

Exercise Restores Dysregulated Gene Expression in a Mouse Model of Arrhythmogenic Cardiomyopathy. Cheedipudi, S.M.; Hu, J.; Fan, S.; Yuan, P.; Karmouch, J.; Czernuszewicz, G.; Robertson, M.J.; Coarfa, C.; Hon, g K.; Yao, Y.; Moore, H.C.; Wehrens, X.; Gurha, P.; Marian AJ. *Cardiovasc Res.* 2019 Jul 26. PMID: 31350552

Genomic Reorganization of Lamin-Associated Domains in Cardiac Myocytes is Associated with Differential Gene Expression and DNA

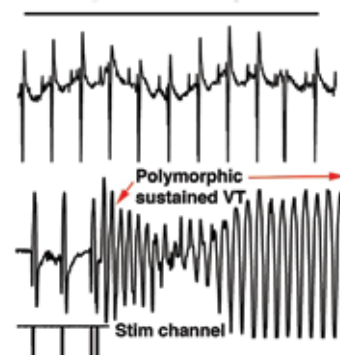
Methylation in Human Dilated Cardiomyopathy. Marreddy-Cheedipudi, S.; Matkovich, S.J.; Coarfa, C.; Hu, X.; Robertson, M.J.; Sweet, M.; Taylor, M.; Mestroni, L.; Cleveland, J.; Willerson, J.T.; Gurha, P.; Marian, AJ. *Circ Res.* 2019 Apr 12;124(8):1198-1213, PMID: 30739589

DNA Damage Response/TP53 Pathway is Activated and Contributes to the Pathogenesis of Dilated Cardiomyopathy Caused by Lamin A/C Mutations. Chen, S.N.; Lombardi, R.; Karmouch, J.; Tsai, J-Y.; Czernuszewicz, G.; Taylor, M.R.G.; Mestroni, L.; Coarfa, C.; Gurha, P.; Marian, AJ. *Circ Res.* 2019 Mar 15;124(6):856-873. PMID: 30696354

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LAB MEMBERS

Post-doctoral fellows: Gaëlle Auguste, PhD.; Sirisha C Marreddy; Leila Rouhigharabaei, PhD
Research assistant: Ping Yuan, Siyang Fan
Research associate: Grace Czernuszewicz, MS
Research and clinical nurse: Yanli Tan, RN



Inducible polymorphic ventricular tachycardia in a mouse model of arrhythmogenic cardiomyopathy.



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 human DCM/heart failure (HF). Recently, we uncovered the role of DNA methylation and Lamin Associated Domain in Human HF and identified an epigenetic regulator KDM5, and a novel cardiac myocyte enriched long intergenic non-coding RNAs (lincRNA) in the phenotypic manifestation of HF. The role of KDM5 and CM enriched lincRNAs in 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 towards human HF.

RESEARCH PROJECTS

- Role of lincRNAs 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

Gurha, P. Noncoding RNAs in cardiovascular diseases. *Curr Opin Cardiol*. 2019 May; 34(3):241-245.

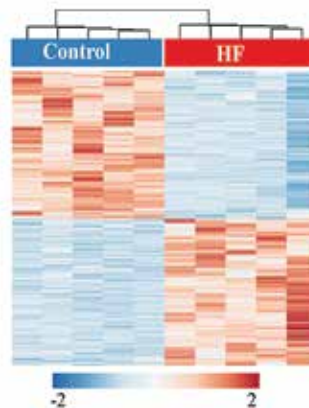
Marreddy Cheedipudi, S.; Matkovich, S.J.; Coarfa, C.; Hu, X.; Robertson, M.J.; Sweet, M.E.; Taylor, M.; Mestroni, L.; Cleveland, J.C.; Willerson, J.T.; Gurha, P#; Marian, AJ#. Genomic Reorganization of Lamin-Associated Domains in Cardiac Myocytes is Associated with Differential Gene Expression and DNA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

Gurha, P.*#; Chen, X.*; Lombardi, R.; Willerson, J.T.; 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).

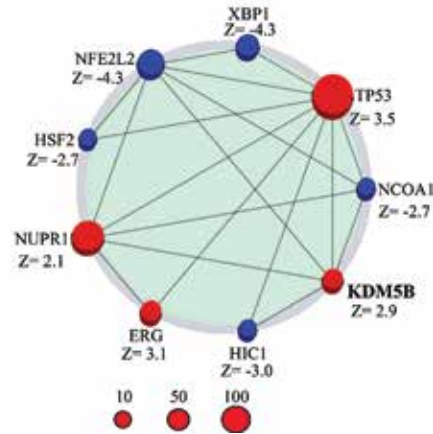
LAB MEMBERS

Research assistant: Jordi Coste Pradas

Differentially expressed transcripts from human heart



Upstream regulators induced and suppressed in human heart failure



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 effective 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. The challenge of identifying these genetic variants is made more complex by the fact that there is a lot of genetic variation affecting our immune responses. In order to be able 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 disease.

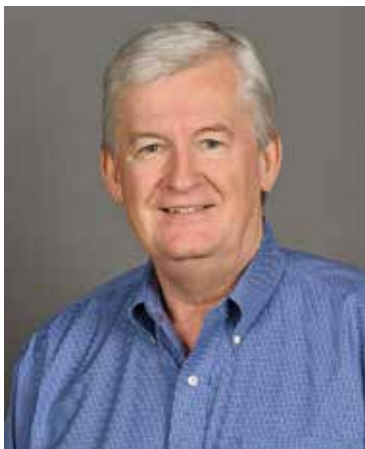
Progress in the laboratories of our investigators continues to yield exciting and important insights. Our human population geneticists, working



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 newest faculty member, Dr. Ashish Kapoor, is an emerging leader in this field. We have shown that kidney injury associated with increased blood pressure results from the emergence of auto-antibodies that damage tissues. This unexpected finding from Dr. Doris’ lab points to a role of immune system genetic variation in creating disease risk. Dr. Ba-bie Teng continues to advance understanding of susceptibility to atherosclerosis and the interplay between new drug targets, such as PCSK9, and lipoprotein uptake by cells. As our understanding of the complexity of information storage and retrieval in the genome expands, 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.

All of us have had, or will have, one of our close relationships in life disrupted by common cardiovascular disease. In the Center for Human Genetics, we have the opportunity to work for change, pushing forward the knowledge from which current medicine draws 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 how 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 dichotomy of hypertensive renal disease risk seen in humans is also present in these rats. Some lines get progressive renal injury, 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 also have 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.

Pharmacological suppression of T and B cell function limit renal disease in hypertension.

We have used a drug to prevent adaptive immune responses in this model. This work confirms our attribution of disease to the adaptive immune system and suggests that antibody-producing B cells may be the main disease driver.

Genetic deletion of antibodies eliminates hypertensive renal disease.

To prove that antibodies causes hypertensive renal injury in our model organism, we deleted the immunoglobulin gene. These animals cannot generate antibodies. They have high levels of blood pressure but have no renal injury.

Loss of antibodies reveals a bacterial input to

the hypertensive immune system.

When hypertensive rats unable to produce antibodies are raised without antibody replacement, they experience blood infection (sepsis), even though they live in an environment free from bacterial pathogens. Blood culture indicates that the infecting bacteria are non-pathogenic bacteria that live in the gut. The genetic differences in immune function respond to this bacterial antigenic input and may cause antibodies to form that are harmful to the kidney. When antibiotics are given to hypertensive rats prone to injury, renal injury was markedly reduced.

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

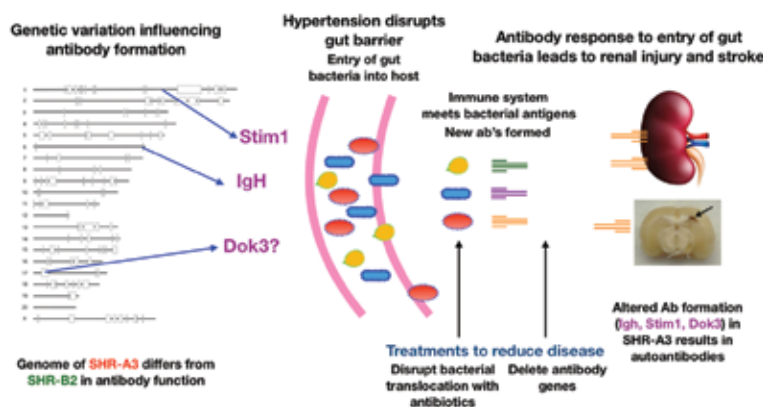
Dhande, I.S.; S.C. Kneedler; A.S. Joshi; Y. Zhu; M.J. Hicks; S.E. Wenderfer; M.C. Braun; P. A. Doris. Germ-line genetic variation in the immunoglobulin heavy chain creates stroke susceptibility in the spontaneously hypertensive rat. *Physiological Genomics*, 2019 PMID: 31608789.

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*. 2019. In Press

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*. 2019. In Press

LAB MEMBERS

Post-doctoral fellow: Isha S. Dhande, PhD
 Research assistants: Yaming Zhu, MD; Aniket Joshi, BS



Our discoveries in hypertensive renal disease genetics include the detection of genetic variation in genes contributing to antibody formation (Left panel). We also discovered that gut bacteria are able to activate an immune response and that we can block this immune response and reduce disease. We can block it with antibiotics, or we can block it by deleting the gene from which antibodies are formed. This indicates that there are pathogenic antibodies that are creating disease (center panel). We want to know the targets of these antibodies so that we can understand how they create disease. This knowledge may allow us to block their harmful effects while still allowing normal protections that antibodies provide (right panel).



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. Novel 'omics' technologies allow us to characterize and quantify the sets of biological molecules that make up cells, tissues, and organisms on a population scale. These powerful technologies have opened new avenues toward biomarker discovery for risk prediction and risk stratification, enabling informed preventive and therapeutic interventions to slow, or reverse, brain aging.

Our research program investigates the molecular basis of diseases of the aging brain, such as stroke and dementia. In collaboration with researchers in the United States and Europe, we apply genome sequencing technologies to identify genes and gene variants that influence risk for stroke, Alzheimer's disease, brain MRI abnormalities, and their cardiovascular risk factors. These complex traits are determined by DNA sequence variations occurring in many genes that have small effect sizes and act over long periods of time. The total number of genetic variants that an individual has inherited, which increases their risk of developing a particular disease, can be measured as their 'polygenic risk score' (PRS). Using genetic data, we estimate these scores to predict whether it is likely that someone will develop a specific disease during their lifetime. For example, obesity is a major risk factor for stroke and dementia. We used a computational algorithm to generate a PRS to predict body mass index (BMI) based on an individual's genotype data. We then showed that individuals with a PRS in the top 10% of the population have a substantially elevated risk of obesity, bariatric surgery, cardiovascular diseases, and death. We also showed that differences in weight among individuals with different PRS emerged as early as childhood. Although

challenges remain, our study exemplifies the potential of PRS for clinical applications.

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 assayed the metabolome, the collection of all metabolites in the blood of 3,904 men and women at midlife, and identified two novel circulating molecules that conferred a greater risk of future ischemic stroke. These discoveries may yield new insights into disease mechanisms and lead to the development of new therapeutics.

RESEARCH PROJECTS

- Discovering DNA sequence variants influencing subcortical brain structures on MRI, which are integral to motion, consciousness, emotions, and learning.
- 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

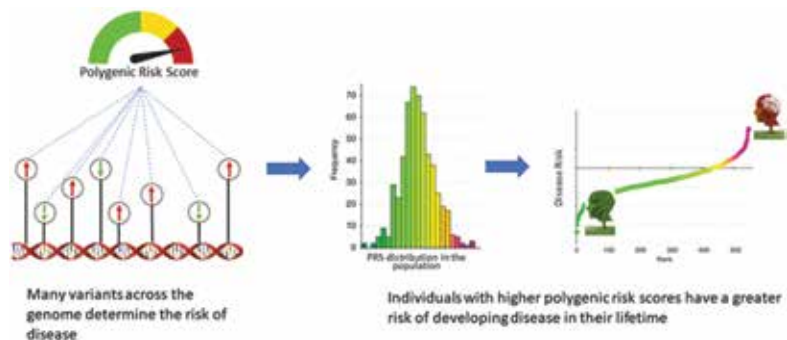
Satizabal, C.L.; Adams, H.H.H.; Hibar, D.P.; Fornage, M.; Wardlaw, J.M.; Schmidt, R.; DeCarli, C.; De Jager, P.L.; Villringer, A.; DeBette, S.; Gudnason, V.; Medland, S.E.; Shulman, J.M.; Thompson, P.M.; Seshadri, S.; Ikram, M.A. Genetic architecture of subcortical brain structures in 38,851 individuals. *Nat Genet.* 2019 (in press)

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Khera, A.V.; Chaffin, M.; Wade, K.H.; Zahid, S.; Brancale, J.; Xia, R.; Distefano, M.; Senol-Cosar, O.; Haas, M.E.; Bick, A.; Aragam, K.G.; Lander, E.S.; Smith, G.D.; Mason-Suares, H.; Fornage, M.; Lebo, M.; Timpson, N.J.; Kaplan, L.M.; Kathiresan, S. Polygenic Prediction of Weight and Obesity Trajectories from Birth to Adulthood. *Cell.* 2019; 177:587-596.

LAB MEMBERS

Graduate students: Yunju Yang, PhD program; Nitesh Enduru, MPH program
 Research assistants: Lassana Samarakoon, MPH, Biostatistician; Emy Thomas, MS, Biostatistician; Ping Wang, PhD
 Research associate: Rui Xia, PhD, Biostatistician



Polygenic (genetic) risk profiling can identify groups of individuals who could benefit from the knowledge of their probabilistic susceptibility to disease.



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 heartbeat. 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 GWAS signals to identify the underlying causal variants, genes and their mechanisms.
- Evaluation of constitutive and heart-restricted

Nos1ap null mice to understand its role in cardiac electrophysiology.

- Functional genomic approaches to understand cardiac gene expression regulation.

KEY PUBLICATIONS

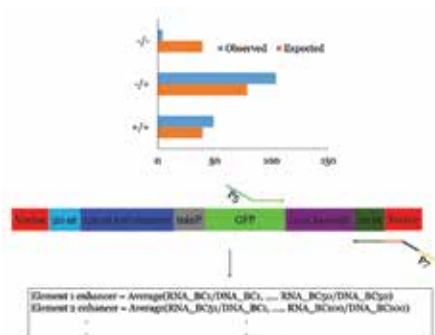
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Kapoor, A.; Auer, D.R.; Lee, D.; Chatterjee, S.; Chakravarti, A. Testing the Ret and Sema3d genetic interaction in mouse enteric nervous system development. *Human Molecular Genetics* 26:1811-20, 2017.

LAB MEMBERS

Research assistant: Alexa Smith, BS



Human genetic and *in vitro* gene targeting studies have identified NOS1AP as a candidate gene regulating QT interval variation and cellular electrophysiology, respectively. With the aim to further characterize *Nos1ap* function *in vivo*, we have generated mice lacking *Nos1ap*, however constitutive loss of *Nos1ap* in mouse models leads to embryonic lethality. The top panel shows a chart of number of wild type (+/+; 2 normal gene copies), heterozygote mutant (-/+;

1 mutant and 1 normal gene) and homozygote mutant (-/-; 2 mutant gene copies) mice observed and expected at weaning from a heterozygote intercross (-/+ × +/-); we are now evaluating heart-restricted *Nos1ap* loss in our mouse models. QT interval GWAS in humans have identified a large number of non-coding sequence variants associated with the trait. As a first step toward molecular characterization of these association signals, we are performing a sequencing-based high throughput enhancer screen in cardiomyocyte cell lines, schematic shown in the bottom panel, where ~1000 variant-centered test elements are evaluated by calculating the RNA/DNA read counts from the unique barcodes attached to each test element (50 per element).



Ba-Bie Teng, PhD, FAHA

Professor

The Jerry and Maury Rubenstein Foundation

Distinguished Professorship in Heart Disease Research

Pathogenesis of atherosclerosis and immunity and the development of genetic and cell therapies for the treatment of atherosclerotic vascular diseases

Atherosclerosis is an inflammatory disease in the aorta that increases in severity as we age. The disease includes imbalance lipid metabolism that leads to hyperlipidemia and maladaptive immune responses that affect the arterial vasculature. Our research focuses on understanding the development of atherosclerosis and to elucidate the cross-regulation between atherosclerosis and immunity. We have generated a mouse model that mimics humans with hyperlipidemia by deleting both LDL receptor (LDLR) and RNA editing enzyme (Apobec1) genes (*LDb=Ldlr/-Apobec1/-*). These mice develop atherosclerosis as they age. Feeding on a Western high-fat diet accelerates their atherosclerosis development. Moreover, male mice develop atherosclerosis faster and more severely than females.

PCSK9 (proprotein convertase subtilisin/kexin type 9) is a newly identified causative gene for hyperlipidemia. Patients with elevated PCSK9 levels have increased plasma cholesterol and premature coronary artery disease. We delete PCSK9 gene from *LDb* mice (*LTP=Ldlr/-Apobec1/-Pcsk9/-*), showing decreased atherosclerosis and apolipoprotein B levels with improved function of endothelial cell. PCSK9 modulates autophagy signaling pathway by inhibiting the degradation of molecules and organelles via autophagosome/lysosome. PCSK9 modulates SREBP-1c lipogenesis, producing more atherogenic LDL containing elevated levels of cholesteryl ester and phospholipids, resulting in increased atherosclerosis. Furthermore, the presence of PCSK9 down regulates the protection role of TGF-β in endothelial cells. Thus, PCSK9 contributes to atherosclerosis through its multiple effects on autophagy, hepatic lipid metabolism and cellular immune function in endothelial cells.

The participation of various helper T cells in the development of atherosclerosis is complex and controversial. We show that *LDb* atherogenic mice exhibit increased plasma interleukin-17 (IL-17), which is associated with increased numbers of T helper 17 cells (Th17).

By deleting PCSK9 from *LDb* mice, these triple knockout *LTP* mice show opposite effect with decreased IL-17 and reduced Th17 cells. We hypothesize that PCSK9 might regulate the function of Th17 cells, which are critical for the pathogenesis of atherosclerosis.

Taken together, our laboratory is using current technologies, including RNA-Seq, ATAC-Seq, RPPA and CRISPR/Cas9 to define the cellular and molecular mechanisms by which proatherogenic factors modulate disease development. Our discovery will provide insight into the understanding of physiological and pathological of disease process. It will provide a basis to develop efficient therapeutic approaches to combat the progression of diseases.

RESEARCH PROJECTS

- The role of PCSK9 in autophagy, inflammation, and atherosclerosis.
- Using CRISPR/Cas9 technique to generate IL-17 RC triple knockout mice to study its effect on atherosclerosis.
- Using genetic tools and proteomics to identify genes associated with atherogenesis and to develop genetic therapy for the treatment of atherosclerotic vascular diseases.

KEY PUBLICATIONS

Proatherogenic conditions promote autoimmune Th17 responses *in vivo*: Hoyong Lim, Young Uk Kim, Hua Sun, Joseph M. Reynolds, Shino Hanabuchi, Ba-Bie Teng, and Yeonseok Chung. *Immunity*. 40, 153-165 (2014). PMID: 24412615.

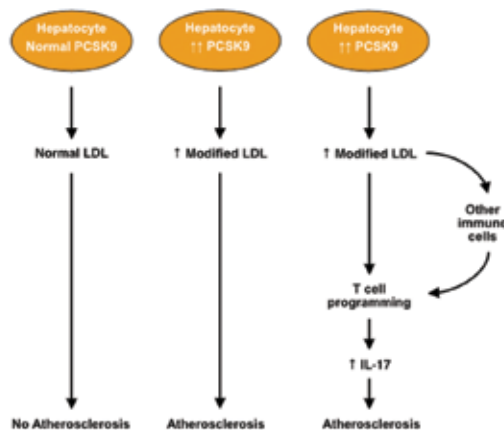
PCSK9 Deficiency Reduces Atherosclerosis, Apolipoprotein B Secretion and Endothelial Dysfunction: Hua Sun, Ronald M. Krauss, Jeffrey T. Chang, and Ba-Bie Teng. *J Lipid Res* 59: 207-223 (2018). PMID: 29180444*. (This article was selected as the Cover for J. Lipid Research as shown below).

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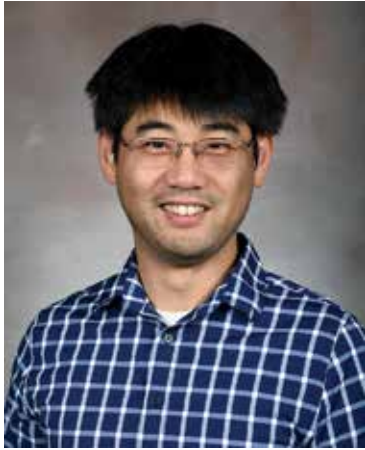
LAB MEMBERS

Research assistant: Xin Li

Mentorship: Dr. Patrick Kee, Department of Internal Medicine, McGovern Medical School



This diagram displays the role of PCSK9 in atherosclerosis and immune response mediated via IL-17 cells and other immune cells, including Th1, Treg, and Tfh cells. Under normal lipidemic condition with low PCSK9 levels, the hepatocytes produce and secrete normal LDLs. These LDLs do not influence the development of atherosclerosis. Under hyperlipidemia condition with increased PCSK9 levels, the hepatocytes produce increased amounts of modified LDLs, which are cholesterol ester and phospholipid enriched. These modified LDLs contribute to the development of atherosclerosis by possibly altering T cells programming shifted toward IL-17 producing T cells to increase IL-17 production or via induce cytokines production to modulating immune cells, including Th1, Treg, or Tfh T cells to influence IL17 production. Increased IL17 contributes to the development of atherosclerosis.



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 focuses on addressing these questions in biological contexts that are relevant to human health. Our immediate goals are to develop novel tools to systematically 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. We are further expanding this line of research in the context of stress response, where global scale changes in translational regula-

tion 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. In order to evaluate how general this observation is, we are now expanding our analysis to other tissue types and species.

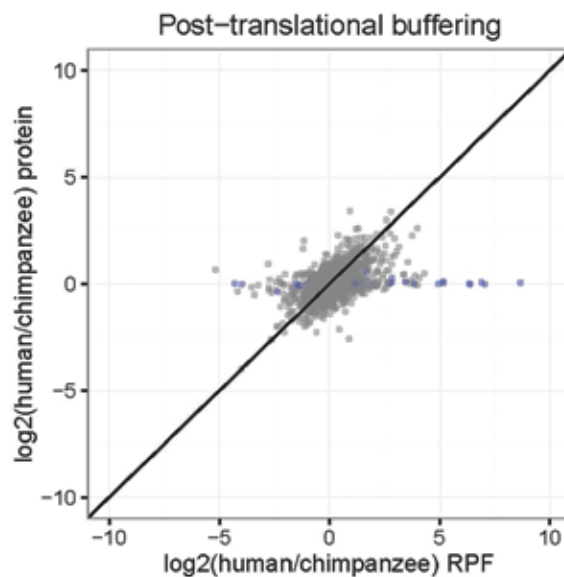
KEY PUBLICATIONS

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

LAB MEMBERS

Post-doctoral fellow: Sandeep Bansal



Post-translationally buffered genes are identified between human and chimpanzee by comparing the inter-species differences at the protein level to the inter-species differences at the level of translation. Each data point on the plot represents a gene, the position along each axis indicates fold expression differences between human and chimpanzee. The blue data points represent genes under significant post-translational buffering identified at a family-wise error rate of 1%.



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. Muller-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 other 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

Mazzilli, J.L.; Snook, J.D.; Simmons, K.; Domozhirev, A.Y.; Garcia, C.A.; Wetsel, R.A.; Zsigmond, E.M.; Westenskow, P.D. A Preclinical Safety Study of Human Embryonic Stem

Cell-Derived Retinal Pigment Epithelial Cells for Macular Degeneration. *J Ocul Pharmacol Ther.* 2019; Oct 9. doi: 10.1089/jop.2019.0039 (PMID: 3159663).

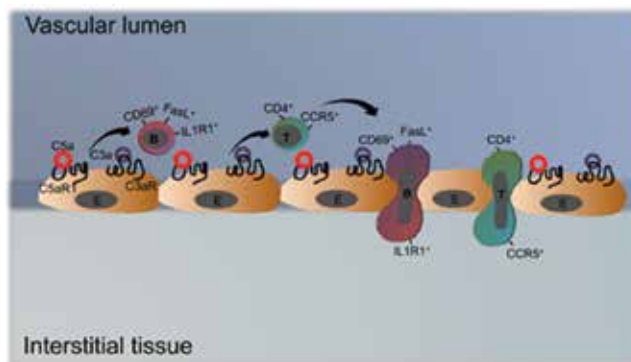
Mueller-Ortiz, S.L.; Calame, D.G.; Shenoi, N.; Li, Y.-D.; Wetsel, R.A. The complement anaphylatoxins, C5a and C3a, suppress IFN- β production in response to *Listeria monocytogenes* by inhibition of the cyclic dinucleotide-activated cytosolic surveillance pathway. *J Immunol.* 2017; 198: 3237-3244 (PMID: 28275134).

Mazzilli, J.L.; Domozhirev, A.Y.; Mueller-Ortiz, S.L.; Garcia, C.A.; Wetsel, R.A.; Zsigmond, E.M. 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).

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LAB MEMBERS

Senior research scientist: Stacey Mueller-Ortiz, PhD
 Research scientist: Ken Simmons, PhD
 Post-doctoral fellow: John L. Mazzilli, MD
 Instructor: Pooja Shivshankar, PhD



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,
 MD Anderson UTHealth Graduate School
 Professor and Vice Chairman, 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

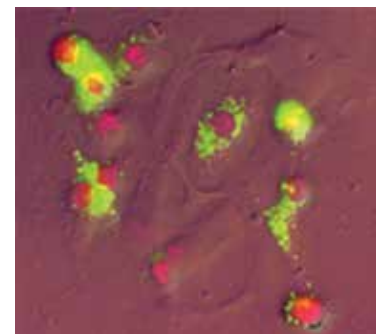
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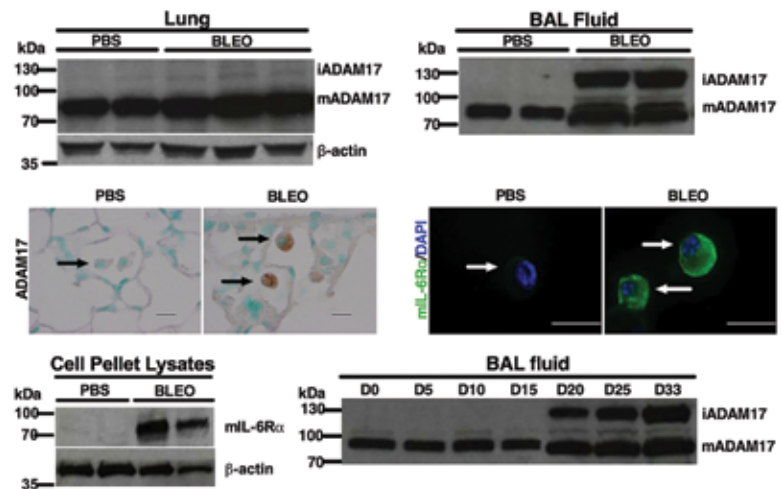
Karmouty-Quintana, H.; Philip, K.; Chen, N.Y.; Weng, T.; Molina, J. G.; Luo, F.; Davies, J.; Acero, L.; Le, Bao; Bunge, I.; Volcik, K.; Le, T.; Johnston, R. A.; Xia, Y.; Eltzschig, H. K.; and Blackburn, M. R. (2015) Deletion of ADORA2B from myeloid cells dampens lung fibrosis and pulmonary hypertension.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

Associate Professor, Center for Immunology and Autoimmune Diseases and 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 dollars. In addition, patients suffering from CRS often are diagnosed with asthma. 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.

CRS is clinically classified into 2 groups defined by the absence or presence of nasal polyps (see image 1). This clinical classification has been supported generally by immunologic profiles of the inflamed sinus tissue. CRS without nasal polyps is characterized by predominance of neutrophils and elevated T helper cell type 1 (Th1) cytokines, while CRS with nasal polyps (CRSwNP) has high presence of eosinophils, mast cells, and basophils and expression of type 2 cytokines such as IL-4, IL-5, and IL-13. However, recent study by our labs using cluster analysis of genetic information has identified endotypes within these clinical phenotypes, allowing for possible personalized treatment.

Allergic fungal rhinosinusitis (AFRS) is a clinical subtype of CRSwNP that is associated with an accumulation of thick entrapped mucus laden with fungal hyphae and eosinophils between the nasal polyps and within sinus cavities. This trapped mucus can cause expansion of sinus cavities and ultimately erosion of bone separating the sinuses from the intracranial and

orbital cavities which can result in intracranial complications and blindness, respectively.

Epithelial cells
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. 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, 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. We currently believe allergic fungal rhinosinusitis may result from a defect in local anti-fungal immune response. 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. Current studies are focused on the pathways that regulate antimicrobial peptides with antifungal activity as it relates to CRS.

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

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Dietz, C.J.; Sun, H.; Yao, W.C.; Citardi, M.J.; Corry, D.B.; Luong, A.U. *Aspergillus fumigatus* induction of IL-33 expression in chronic rhinosinusitis is PAR2-dependent. *Laryngoscope*. 2019 Oct;129(10):2230-2235.

LAB MEMBERS

Hua Sun, PhD, Yi-Dong Li



Nasal polyps

Nasal polyps seen by nasal endoscopy within nasal cavity of CRSwNP patient.



Eva M. Zsigmond, PhD
Associate Professor
Director, Transgenic and Stem Cells Core Facility

Transgenic and stem cells core facility

RESEARCH SERVICES

- CRISPR/Cas9-mediated genome editing.
- Microinjection of DNA, BAC or YAC clones for the production of transgenic animal models.
- Microinjection of ES cells for the production of knock-out and knock-in mice.
- Re-derivation of mice and rats from fertilized eggs.
- Cryopreservation of fertilized mouse and rat eggs.
- Gene targeting, selection, expansion, cryopreservation of mouse ES cells.
- Derivation of novel mouse ES cells and other cell lines.
- Availability of germline-competent mouse ES cells and MEF feeder layer cells.

Accomplishments:

- Consistently high transgenic rates (average 23%).
- 100% success rate of germline transmission in the production of knock-out mice when using ES cells derived at the facility.
- 100% success rate in re-derivation of mice.
- Derivation of over 20 mouse and human cell lines, including human ES cells approved for NIH-funded research.

The Transgenic and Stem Cells Core Facility provides a unique service to the UTHealth scientific community by generating animal models of specific human diseases in order to develop novel treatments. The laboratory was established in 1998 and since that time, it has generated more than 850 new transgenic, knock-out and knock-in animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions.

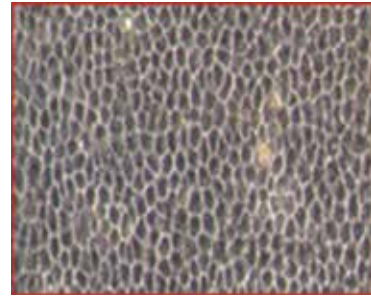
In addition to the production, cryopreservation and re-derivation of genetically-engineered mice and rats, the services of the facility also include gene targeting, derivation of new cell lines, and technical support in different aspects of animal microsurgery, cell culture, and stem cells research.

The embryonic stem (ES) cell lines derived in the Core Laboratory are highly effective for studies involving cellular differentiation. In a current research project, our laboratory is using human ES cell-derived retinal pigment epithelial (RPE) cells as a therapeutic strategy for the treatment of age-related macular degeneration (ARMD). In the United States, ARMD is a leading cause of blindness. The aim of our study is to use RPE cells derived from human ES cells in a clinical trial of sub-retinal transplantation into patients with ARMD for the reversal of the visual loss associated with the disease. We have derived functional human RPE cells in our laboratory and have tested the efficacy and safety of these cells in animal models. In preparation of clinical trials, we are examining the long-term viability of the transplanted cells in murine animal models of ARMD, and we will generate transplantable human RPE cells in a GMP-certified facility.

Our laboratory also has generated human ES cells with stable deletion of an X-chromosome. This cell line represents a pluripotent stem cell model to study the disease mechanisms of Turner's syndrome, one of the most common genetic abnormalities seen in female embryos.

RESEARCH PROJECT

Stem Cell Therapy for Age-Related Macular Degeneration



CR-4 human ES cell-derived retinal pigment epithelial cells

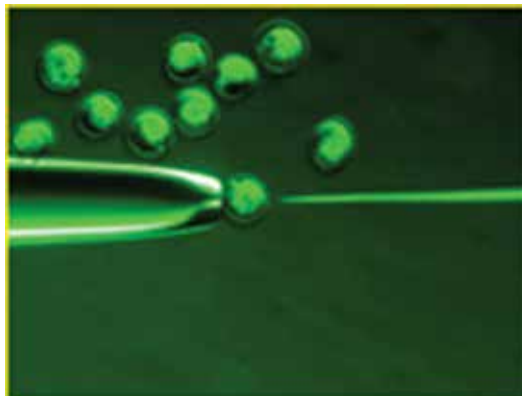
KEY PUBLICATIONS

Mazzilli, J.L.; Snook, J.D.; Simmons, K.; Domozhrov, A.Y.; Garcia, C.A.; Wetsel, R.A.; Zsigmond, E. M.; and Westenskow, P.D. A Preclinical Safety Study of Human ES Cell-Derived RPE Cells for the Treatment of Macular Degeneration *J. Ocul. Pharm Ther.* Doi.10.1089/jop. 2019.

Domozhrov, A.Y.; Mazzilli, J.L.; Wetsel, R.A.; and Zsigmond, E. M.; Generation of CDML012-A-1: A Pluripotent Human ES Cell Model of Turner's Syndrome. *Stem Cell Res.* 39:101508, 2019.

LAB MEMBERS

Senior research associate: Aleksey Domozhrov
Post-doctoral fellow: John Mazzilli, MD



CRISPR/Cas9-mediated gene editing for the production of novel animal models.



The eight laboratories of the Center for Metabolic and Degenerative Diseases investigate aging-associated diseases, including type-2 diabetes, muscle wasting, vascular insufficiencies, neurodegeneration, and cancer. Mechanisms of aging, stress, and obesity-associated changes in brain activity, energy metabolism, vascular function, cell signaling, protein homeostasis, and cell fate determination that lead to physiological abnormalities are being interrogated in animal models and studies on clinical specimens. The specific questions being addressed by the center's faculty include the following:

- How does replicative senescence of adipocyte progenitors underlie diabetes development?
- How do adipocyte-derived fatty acids contribute to diabetes and cancer progression?
- Can cells of adipose tissue be targeted for therapeutic purposes?
- How is angiogenesis, fibrosis, and inflammation implicated in metabolic dysfunction?
- How do stress hormones regulate energy utilization in diabetes?
- What vascular genes can be targeted to treat muscle disease and diabetes?

- How does the brain and circadian clock control the body's energy balance?
- How does the circadian clock protect against liver disease and 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 cellular homeostasis cause neurodegeneration?
- How does stress promote Alzheimer's disease and post-traumatic stress disorder?

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

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



Mikhail Kolonin, PhD

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

Adipocyte progenitor cells: Dysfunction in disease and aging

My laboratory investigates mechanisms underlying aging-related diseases, including type-2 diabetes, muscle degeneration, and cancer. We are focusing on the role of fat (adipose) tissue in the context of these pathologies. While white adipocytes store lipids and 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 diseases, and cancer. In contrast, active brown fat can prevent the onset of metabolic disease. Both white and brown adipocytes are continuously replaced as we age, and their pools in fat tissue are maintained by adipose stem cells (ASCs). In obesity, increased numbers of white fat ASCs are generated. Our laboratory has discovered that tumors recruit these ASCs that fuel cancer progression. Currently, no drugs targeting cells in fat tissue for obesity or cancer indications are available. Taking advantage of our expertise in targeted therapeutics, we have developed the first experimental drug (D-WAT) targeting ASCs. Our publications demonstrate that D-WAT prevents obesity and suppresses tumor growth in mice. We are now using this experimental drug to investigate the mechanism through which ASC promote cancer progression

to chemotherapy resistance and metastasis and assess them as a drug target. We are also applying ablation of ASC as a New Therapeutic Approach to Duchenne muscular dystrophy treatment. In collaboration with bariatric surgeons, we recently showed that D-WAT targets human ASCs. Our reports indicate that D-WAT treatment spares brown fat ASCs, leads to generation of brown adipocytes and enables a short-term metabolic benefit. However, our recent data indicate the importance of maintaining functional ASCs for healthy aging. As we age, fat cell numbers decrease and the deficient fat tissue fails to effectively absorb lipids, which start spilling into other organs. This can cause inflammation and metabolic disorders accounting for cancer and organ failure in the elderly. Our experiments in animal models suggest that adipocytes run out because ASCs lose replicative potential with age due to telomere shortening and become 'exhausted,' which is accelerated by obesity. Understanding the mechanisms and function of fatty acid transport through CD36 in the context of type-2 diabetes and cancer is our most recent pursuit. Another recent research direction is focused on the role of inflammatory signaling and fat tissue remodeling in metabolic response to anti-diabetes drugs.

health, and targeting in disease.

- Aging-associated exhaustion of adipocyte progenitors and its role in metabolic disease
- Cancer-associated adipocytes as the source of lipids driving cancer progression.

KEY PUBLICATIONS

The role of adipose stroma in prostate cancer aggressiveness. Kolonin M.G.; DiGiovanni J. *Transl Androl Urol*. 2019

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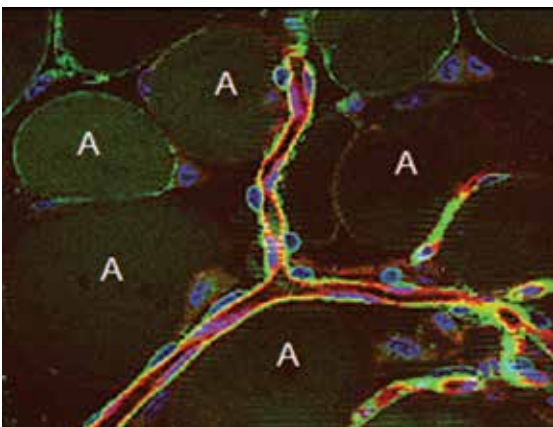
Adipose stromal cell targeting suppresses prostate cancer epithelial-mesenchymal transition and chemoresistance. Su, F.; Ahn, S.; Saha, A.; DiGiovanni, J.; Kolonin, M.G. *Oncogene*. 2019

LAB MEMBERS

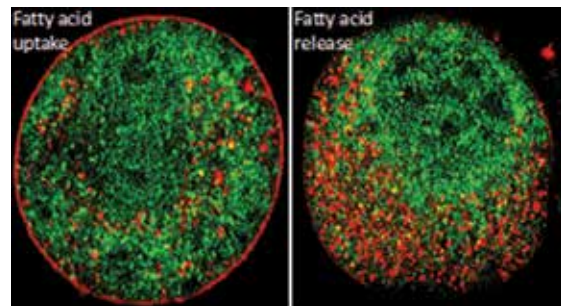
Senior research scientists: Alex Daquinag, Zhanguo Gao
Research scientist: Fei Su
Graduate student: Shradha Subramanian
Research assistant: Cale Fussell

RESEARCH PROJECTS

- Adipocyte progenitors: lineages, function in



A micrograph showing localization of adipose stromal cells (green) around blood vessels (red) in fat tissue. A: adipocytes. Nuclei are blue.



Changes in subcellular localization of fatty acid transporter CD36 (red) in adipocytes treated with fatty acids (left) or induced to undergo lipolysis (right).



Rebecca Berdeaux, PhD

Associate Professor

Regulation of muscle metabolism and regeneration by cellular signaling pathways

Skeletal muscle comprises approximately 40% of body mass in healthy individuals. In addition to the major function of skeletal muscle to contract and perform work such as lifting objects and escaping predators, skeletal muscle is important for utilization and storage of energy, maintenance of spine stability and mobility, and generation of heat to help maintain body temperature. In fact, skeletal muscle is responsible for ~85% of insulin-induced uptake of sugar from the blood after a meal. Muscle insulin resistance is a major contributing factor to high blood sugar levels in people with type 2 diabetes. During aging, muscle mass declines, causing both disruptions in mobility as well as metabolism. In aging individuals, loss of muscle stem cell activity is thought to be partly responsible for the loss of muscle mass.

Our lab studies hormone-activated pathways in mature skeletal muscle and in muscle stem cells in hopes of identifying novel therapeutic targets to improve muscle metabolism and muscle stem cell function. The overall goal is to ameliorate insulin resistance and promote healthy aging by maintaining healthy muscle metabolism and promoting the capacity of muscle stem cells to be activated. We found that intracellular pathways activated by hormones, like adrenaline, stimulate muscle stem cell activity, and we aim to identify how that occurs at the molecular level. One of the proteins induced by hormone activity in muscle is an enzyme called SIK1. One of our major aims is to understand how SIK1 contributes to impaired muscle energy utilization in type 2 diabetes and whether SIK1 might be a useful drug target to treat insulin resistance and type 2 diabetes.

RESEARCH PROJECTS

- Identification of the roles of SIK1 in muscle glucose metabolism in obesity.
- Study of how cAMP signaling regulates skeletal muscle stem cell activity in aging.
- Understand how different hormonal pathways collaborate to regulate energy utilization and storage in skeletal muscle in times of feast and famine.

KEY PUBLICATIONS

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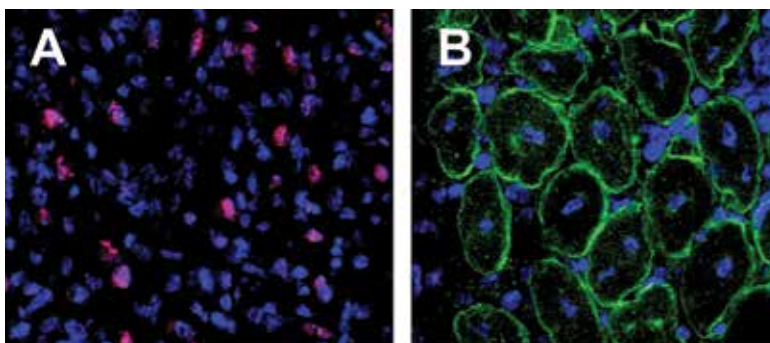
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LAB MEMBERS

Research scientist: Dmitry Akhmedov
 Research assistants: Alexander Ambrocik, Mahmoud Farhan
 Medical student: Victor Gonzalez



Characterization of regenerating skeletal muscle. A) Three days after muscle injury, muscle stem cells become activated to grow and divide. The red stain recognizes the proliferating cells within the damaged muscle. Blue shows all cells in the tissue section. B) Two days later, new muscles are formed and regrow. In this experimental model, we expressed a receptor (stained in green) in the newly formed muscle cells. This receptor allows us to stimulate the same hormonal pathways with a drug that does not act on any other receptors normally expressed in the muscle. In this way, we can mimic hormone-activated pathways and study the effects of those pathways on muscle regeneration and regrowth of new muscle fibers.



Kristin Eckel-Mahan, PhD

Assistant Professor

Circadian clocks in health and disease

The goals of my lab center on the role of the circadian clock in health and disease. The circadian clock in an exquisite internal time-keeping system present in all cells of our body that drives 24-hr. rhythms in physiology and tissue-specific function. Examples of our daily rhythms include the sleep/wake cycle, food intake, internal body temperature, and hormone secretion. This internal clock adapts to and is aligned with the 24-hr. rotation of the earth on its axis. Recent evidence from large epidemiological studies (which is well supported by carefully controlled experiments in other organisms) 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. When the circadian clock is disrupted genetically or environmentally, several deleterious outcomes result, including accelerated aging, cancer, and metabolic disease. We are trying to understand why circadian disruption produces these effects.

While the central clock of the brain is predominantly controlled by light, circadian oscillations in peripheral organs are heavily controlled by other *zeitgebers* (“time-givers”) such as food. Poor quality nutrients as well as food intake at the wrong time can impair circadian communications across the body, increasing the risk of metabolic diseases, such as obesity and diabetes. Our current experiments include those designed to reveal which *zeitgebers* are most important for tissue-specific clock function and the mechanisms by which tissue-specific clocks protect against metabolic disease.

Our lab and others have shown that nutrient timing and quality have a strong influence on peripheral circadian clocks, such as the clocks of the liver, muscle, and adipose tissue. Overconsumption of an unhealthy, high-fat diet can cause substantial disruption of the circadian clock in normally highly insulin sensitive tissues

that become insulin resistant under these feeding conditions. Thus, we hypothesize that a high-fat diet induces weight gain in part by “misaligning” the circadian rhythms of normally highly insulin-sensitive peripheral tissues, vs. the brain clock. Clock misalignment is thought to be involved with several metabolic disorders, including type 2 diabetes. In addition, we have discovered a new circadian mechanism of pre-adipocyte proliferation in adipose tissue, which we hypothesize controls both the size and health of adipose tissue stores over the lifetime of an organism.

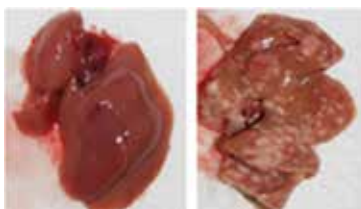
In addition to metabolic diseases, such as obesity and diabetes, circadian disruption has also been linked to cancer. We have identified that a nuclear receptor with circadian activity gets altered in the context of specific liver cancers. Attempts to restore the circadian function in these cells causes cell death and impairs tumor growth. We are using this information to determine whether these subsets of liver cancers might be responsive to particular circadian manipulations not previously thought of as being applicable to hepatic tumorigenesis.

RESEARCH PROJECTS

- Mechanisms by which circadian disruption leads to metabolic disease.
- Mechanisms linking circadian disruption to liver cancer.
- Understanding the role of the circadian clock in human adipose tissue.

KEY PUBLICATIONS

“HNF4 α -deficient fatty liver provides a permissive environment for sex-independent



Loss of circadian HNF4 α activity in the liver (which can also occur under conditions of chronic high-fat feeding) combined with prolonged high-fat diet results in hepatocellular carcinoma in male and female mice. (left liver= Hnf4a knockout mice fed low-fat diet; right= Hnf4a knockout mice fed low-fat diet)

hepatocellular carcinoma” Fekry, B.; Ribas Latre, A.; Baumgartner, C.; Mohamed, A.M.T.; Kolonin, M.G.; Sladek, F.M.; Younes, M.; and Eckel-Mahan, K.L. *Cancer Research* Oct. 2019, (Epub ahead of print).

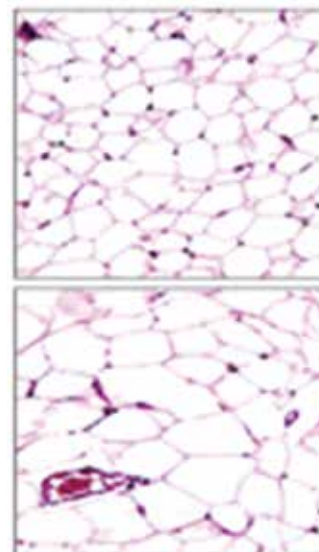
“Incompatibility of the circadian protein BMAL1 and HNF4 α in hepatocellular carcinoma” Baharan Fekry; Aleix Ribas-Latre; Corrine Baumgartner; Jonathan R. Deans; Christopher Kwok; Pooja Patel; Loning Fu; Rebecca Berdeaux; Kai Sun; Mikhail G. Kolonin; Sidney H. Wang; Seung-Hee Yoo; Frances M. Sladek; and Kristin Eckel-Mahan. *Nature Communications* 2018; 9: 4349.

“Rosiglitazone Reverses High Fat Diet-Induced Changes in BMAL1 Function in Muscle, Fat, and Liver Tissue in Mice” Ribas Latre, A.; Fekry, B.; Kwok, C.; Bamgarner, C.; Shivshankar, S.; Sun, K.; Chen, Z.; and Eckel-Mahan, K. *International Journal of Obesity* 2018 May 24.

LAB MEMBERS

Post-doctoral fellows: Baharan Fekry, Aleix Ribas Latre, and Rafael Bravo Santos

Graduate student: Rachel Van Drunen



Abnormally large adipocytes in the visceral fat of mice harboring a genetic disruption of the circadian clock (top= normal adipocytes; bottom=adipocytes of Clock knockout mice).



Nicholas Justice, PhD
Assistant Professor

Stress signaling in psychiatric and degenerative disease

High stress causes anxiety- and depression-related diseases that are among the most common suffered by our population. In addition, the consequences of chronic stress, including elevations in the stress hormone, cortisol, can negatively impact other seemingly unrelated conditions, from metabolic diseases like diabetes, to age-dependent degeneration of neurons that occurs in Alzheimer's disease. One specific project is now focused on understanding how stress pathways interact with motor control circuitry. We discovered new circuits by which stress responsive neurons connect to circuits that control movement. When these circuits malfunction, diseases such as Parkinson's disease, occur, dramatically altering our ability to move. However, the same circuits are involved in determining why we make the choices we make and are intimately a part of the circuitry that controls decision-making, which is a problem in diseases from addiction to obesity. We are interested in how the stresses in our lives affect the way we move, as well as why we make the decisions that we do. A second project is attempting to describe a new type of cell that we identified and how it is involved when we encounter stress or threatening situations. We are also constantly developing new genetic tools that will improve our ability to answer these questions; the field of neuroscience is currently experiencing a renaissance due to the ability to manipulate neurons and neural circuits as we try to understand how they control our thoughts and behaviors.

We are actively investigating the biological mechanisms by which stress changes the way our brains function to alter the bodies' physiology and negatively affect our health. The identification of specific stress-related mechanisms that drive disease will not only inform us as to the importance of controlling stress in our own lives, it will perhaps identify new drug targets that can both alleviate feelings of stress, and also block the negative effects of stress on disease progression. Our

long-term goal is to prevent, improve, or eliminate metabolic and degenerative diseases in patients. The Justice lab is working every day to understand how the neural and hormonal circuits that respond to stress impact disease, and how we can use that knowledge to meet the goal of preventing, slowing, or curing disease.

I obtained a B.A. from the University of California at Berkeley and a Ph.D. in Neuroscience at the University of California, San Francisco (UCSF), and studied in the lab of Yuh Nung Jan. I moved to the Salk Institute in San Diego for post-doctoral training and studied under Wylie Vale, my first Houstonian mentor. At the Salk Institute, I learned the molecular mechanisms by which the body responds to stress. I moved to Houston as an instructor at Baylor College of Medicine studying the influence of stress in Alzheimer's Disease with Hui Zheng at the Huffington Center on Aging, before accepting a faculty position at the IMM.

RESEARCH PROJECTS

- Defining the anatomy of neuronal circuits that respond to stress.
- Investigating why we respond to stress the way that we do.
- Searching for genetic and environmental factors that cause stress resilience and stress sensitivity.
- Defining a neural circuit that controls and coordinates stress hormone release.
- Searching for biomarkers that predict PTSD in recently traumatized patients
- Understanding stress and stress-related diseases influence Alzheimer's Disease progression.

KEY PUBLICATIONS

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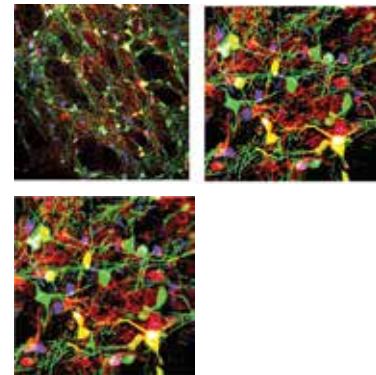
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amygdalar CRF neurons synapse in the external globus pallidus. *Brain Struct. Funct*

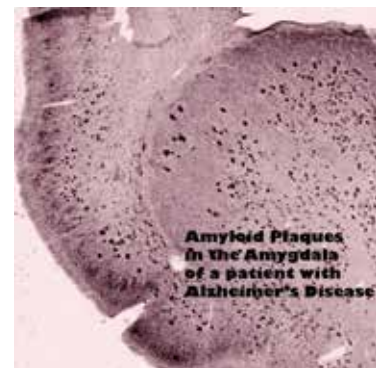
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LAB MEMBERS

Post-doctoral fellow: Shivakumar Rajamanickam, PhD
Research technician: Jonathan Tao



"Stress Responsive neurons." Neurons in the basal ganglia express receptors for the stress peptide CRF (green), which causes a shift in the function of circuits that control our movement. One example is when a prey animal switches from hiding to running away from a predator.



Human brain tissue from an Alzheimer's patient shows that amyloid plaques form in the amygdala, the brain area that controls our emotions.



Vihang Narkar, PhD

Associate Professor

George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research

Gene regulation in metabolic-vascular syndromes

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 also have 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* and *Nature Communications*.

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

Vila, I.K.; Park, M.K.; Setijono, S.R.; Yao, Y.; Kim, H.; Badin, P.M.; Choi, S.; Narkar, V.; Choi, S.W.; Chung, J.; Moro, C.; Song, S.J.; Song, M.S. A muscle-specific UBE2O/AMPK α 2 axis promotes insulin resistance and metabolic syndrome in obesity. *JCI Insight*. 4(13). pii: 128269, 2019.

Likhite, N.; Yadav, V.; Milliman, E.J.; Sopariwala, D.H.; Lorca, S.; Narayana, N.P.; Sheth, M.; Reineke, E.L.; Giguère, V.; Narkar, V. Loss of Estrogen-Related Receptor Alpha Facilitates Angiogenesis in Endothelial Cells. *Mol Cell Biol*. 39(5). pii: e00411-18, 2019.

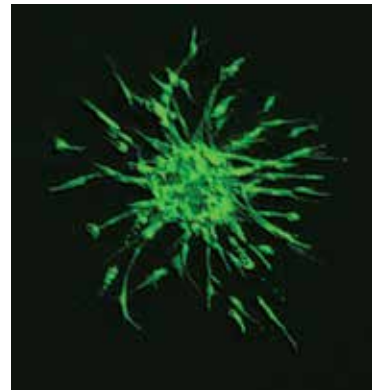
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LAB MEMBERS

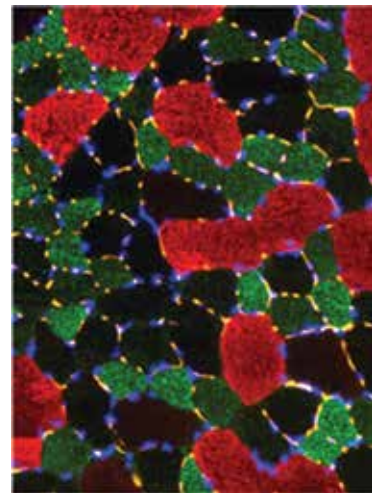
Post-doctoral fellows: Danesh Sopariwala, Neah Likhite

Research assistants: Meghna Seth, Nitya Narayana, Lisa Lin

Visiting fellows: Vikas Yadav, Hygor Araujo



Vascular cells in culture. Microscopic image of vascular cells grown in culture, where they spontaneously form tube-like structures called sprouts. This phenomenon is called 'angiogenesis', which is the process by which new blood vessels are formed.



Skeletal muscle cross-section view. Immunohistology image of a skeletal muscle cross-section showing different types of myofibrils (green, red, dark) decorated with blood vessels (yellow).



Kai Sun, MD, PhD

Assistant Professor

Targeting adipose tissue remodeling for treatment of obesity and diabetes

Research in our laboratory examines the essential contributions of adipocyte-derived factors to the dynamics of adipose tissue remodeling during obesity development and pinpoints them as critical factors with clinical significance in human obesity and insulin resistance.

In the past years, we discovered that obese fat pads are frequently hypoxic and HIF1 α induction is the initial step which ultimately leads to local fibrosis and inflammation in adipose tissue. More importantly, we further demonstrated that VEGF-A induced angiogenesis in white adipose tissue could be dichotomous and metabolic context dependent: at the early stage of obesity development, angiogenesis is metabolically beneficial by improving vascularization and inducing a “browning” phenotype in white adipocytes. In contrast, in pathologically expanded adipose tissue, antiangiogenic action leads to improvements in metabolism by ablating dysfunctional adipocytes.

We further explored the fine-tuned regulation of adipose tissue remodeling at other levels in obese and diabetic animal models. Indeed, we found fibrosis is the hallmark in the metabolically dysfunctional adipose tissue and MT1-MMP (MMP14) plays a critical role in regulation of the levels of extracellular matrix (ECM). Of note, our recent research suggests that the regulation of ECM flexibility by MT1-MMP is also metabolic context dependent: On the one hand, at early stages of obesity, MT1-MMP cleaves collagenous proteins and stimulates angiogenesis in combination with VEGF-A and leptin, thus relieving the pathological conditions caused by hypoxia. On the other hand, in the context of pre-existing unhealthy adipose tissue, it digests collagen 6 α 3 and produces endotrophin which accelerates fibrosis and inflammation, ultimately leading a highly unfavorable microenvironment to sustain metabolic flexibility.

Most recently, we purified lipid droplet proteins from the brown fat tissues and analyzed them by mass spectrometry. Excitingly, we found many novel proteins that translocate onto lipid

droplets in response to sympathetic activation. Carboxyl esterase 3 (Ces3) is one of them which possesses lipolytic activity. We found that Ces3 is involved in thermogenesis during cold exposure by regulating the thermogenic molecule UCP-1. We are now investigate the mechanism(s) by which Ces3 up-regulates UCP-1.

RESEARCH PROJECTS

- Hypoxia-induced pathological changes in adipose tissue.
- Sympathetic innervation during adipose tissue healthy expansion.
- Reversibility of adipose tissue fibrosis by novel anti-fibrotic therapies.
- Dynamics of lipid droplets and the metabolic outcomes.

KEY PUBLICATIONS

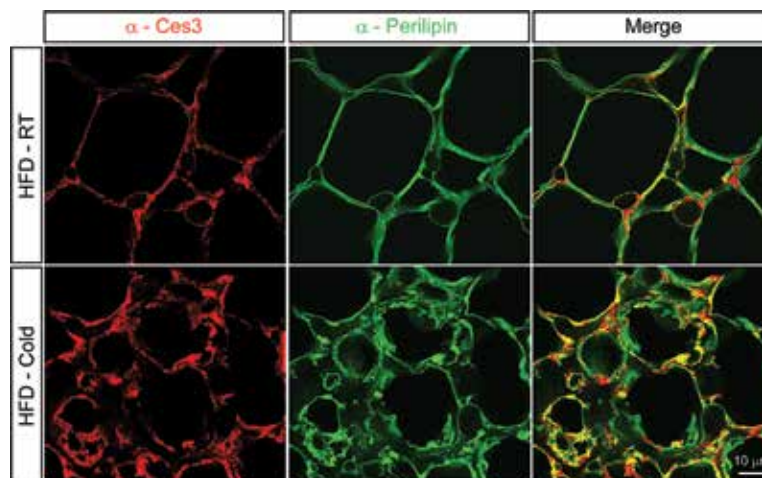
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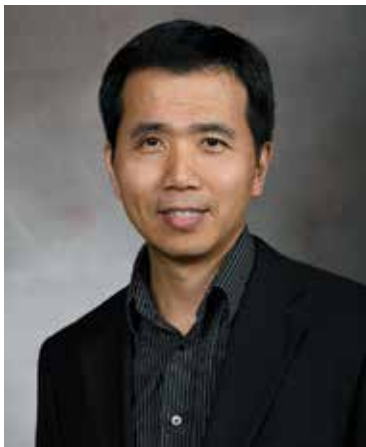
Li, X.; Mao, Z.; Yang, L.; Sun, K. Co-staining Blood Vessels and Nerve Fibers in Adipose Tissue. *J Vis Exp.* 2019 Feb 13;(144).

LAB MEMBERS

Post-doctoral fellows: Xin Li, MD, PhD; Li Yang, PhD; Hyunho Lee, PhD



Ces3 Translocates onto Lipid Droplets: Co-immunostaining with α -Ces3 (red) and α -Perilipin 1 (green) antibodies on white adipose tissue of high fat-diet fed mice upon cold exposure. While most of Ces3 molecules locate in ER, significant amounts target lipid droplets upon cold exposure. The yellow color indicates co-localization of Ces3 with lipid droplet protein marker perilipin 1 in response to cold stimulation (Yang et al. *Diabetes*, 2019).



Qingchun Tong, PhD
Associate Professor
Cullen Chair in Molecular Medicine

Brain control of feeding, body weight, and glucose 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 our goals, we employ various animal models in combination with the state-of-the-art techniques, including electrophysiology, optogenetics, chemogenetics, and *in vivo* live imaging. Cre-lox P mouse genetics is used to achieve neuron-specific manipulations in the brain. Also various adenoassociated viral vectors (AAV) harboring genes that exhibit Cre-dependent expression or inactivation will be stereotaxically delivered to specific brain regions of Cre-expressing neurons, achieving neuron-expression or inactivation of foreign tool genes. 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 CRISPR/Cas9 technology to achieve neuron-specific gene deletion in adult mice. These advanced techniques ensure our studies are effective and our 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, psychiatric patients, 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.

KEY PUBLICATIONS

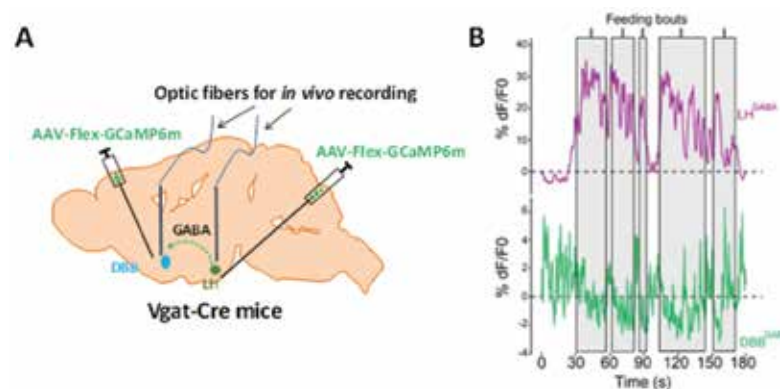
Cassidy, R.M.; Lu, Y.; Jere, M.; Tian, J.B.; Xu, Y.; Mangieri, L.R.; Felix-Okoroji, B.; Selever, J.; Xu, Y.; Arenkiel, B.R.; Tong, Q. A lateral hypothalamus to basal forebrain neurocircuit promotes feeding by suppressing responses to anxiogenic environmental cues. *Sci Adv.* 2019 Mar 6;5(3):eaav1640. doi: 10.1126/sciadv.aav1640. *eCollection* 2019 Mar. PMID: 30854429.

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Xu, Y.; Lu, Y.; Cassidy, R.M.; Mangieri, L.R.; Zhu, Y.; Huang, X.; Jiang, Z.; Justice, N.J.; Xu, Y.; Arenkiel, B.R.; and Tong, Q. Identification of a Neurocircuit Underlying Regulation of Feeding by Stress-related Emotional Responses. *Nat Commun.* 2019 Aug 1;10(1):3446. doi: 10.1038/s41467-019-11399-z. PMID: 31371721.

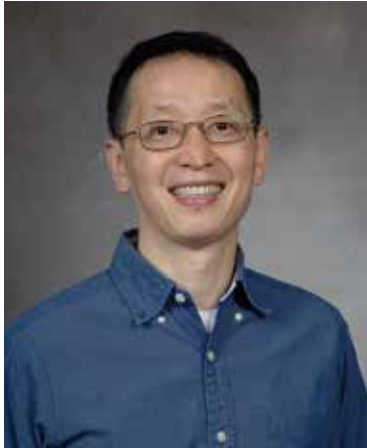
LAB MEMBERS

Instructor: Yuanzhong Xu, MD, PhD
Postdoctoral fellow: Zhiying Jiang, PhD
Graduate students: Ryan Cassidy, Canjun Zhu (visiting), Jessie Morrill, Jing Cai, Hongli Li (visiting)



A) Diagram showing injections of GCaMP6 virus to both lateral hypothalamus (LH) and DBB (Diagonal Band of Broca) of Vgat-Cre mice and implantation of optic fibers to both LH and DBB for *in vivo* recording of neuron activity in both locations simultaneously.

B) Simultaneous recording of LHVgat-GCaMP6m and DBBVgat-GCaMP6m population activity during feeding bouts (shaded areas), showing opposite phase of neuron activities between the two groups of neurons during feeding bouts due to GABAergic connections between them.



Sheng Zhang, PhD

Associate Professor

Becker Family Foundation Professor in Diabetes Research

Molecular mechanisms of neurodegenerative diseases

As we enjoy unprecedented longer life expectancy, we are also becoming more vulnerable to aging-related neuronal degenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and others. By now, these incapacitating brain diseases still do not have effective preventive and therapeutic avenues, but they inflict unbearably high emotional and financial tolls to patients and their families, becoming a pressing threat to society. Our lab studies how neurons normally utilize certain molecular machineries, inside cells to stay healthy during normal ageing that no longer operate properly in affected brains. Findings from these studies should help find cures for these devastating diseases.

Our senses, reasoning, and responses are achieved through neurons and their functional connections inside the body. However, unlike some cells, such as those from skin and blood that are constantly dividing and being replenished, neurons face one particularly unique challenge: once they are born and mature into interconnected functional units, they mostly lose their ability to reproduce and no longer get replaced. To maintain longevity, these long-lived neurons harbor robust self-clearance machineries to stay healthy and ward off internal crisis and external insults for decades to come.

The self-maintenance machines inside cells include *chaperones* that help proteins to stay in shape, as well as proteasomes, *autophagy* (meaning "self-eating" in Greek) and lysosomal systems, which are cellular clearance and protective machineries that clean up and recycle worn-out or toxic cellular materials. In neurodegenerative diseases, these protective machineries often become inefficient or nonfunctional, leading to deposition of toxic proteins and RNAs in the brain, causing eventual neuronal loss. Commonly known as aggregates, tangles and plaques, such abnormal deposits are a common pathological hallmark of almost all brain degenerative diseases.

Using genetic, biochemical, and cell biology tools in both model organism *Drosophila* and

mammalian systems, we study how these self-maintenance machines operate inside the cell to recognize and efficiently clear away toxins, while spare and protect normal cellular constituents. Our goal is to find ways to command these innate protection machineries to fight against neurodegenerative diseases. In particular, we focus on the following directions:

(1) Chaperones and autophagy pathways on neuronal survival

Chaperone Hsp110 is one of the most abundant proteins in the brain. It helps proteins to fold into proper shapes to achieve their normal activities and is also a major component of *disaggregase*, a potent molecular machine capable of dismantling large and tightly packed protein deposits.

During autophagy, cells produce autophagosomes as garbage bags to collect unwanted or harmful cellular components for their eventual disposal and recycling. *Selective autophagy* is a subtype of autophagy that shows specificity for its clearance targets. Interestingly, we found that Huntingtin, the gene responsible for a fatal degenerative Huntington's disease (HD), plays an important role in selective autophagy.

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

Specialized cellular organelles, such as autophagosomes, lysosome-related organelles, and synaptic vesicles control many aspects of neuronal functions. Their disruptions are linked to a spectrum of disorders including AD, PD, HD, and schizophrenia.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

David-Morrison, G.; Xu, Z.; Rui, Y.N.; Charng, W.L.; Jaiswal, M.; Yamamoto, S.; Xiong, B.; Zhang, K.; Sandoval, H.; Duraine, L.; Zhang, S.;* Bellen, H.J.* (2016) "WAC Regulates mTOR Activity by Acting as an Adaptor for the TTT and Pontin/Reptin Complexes." *Dev Cell*. 36(2):139-51. (*corresponding authors)

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Mao, D.X.; Lin, G.; Senturk, M.; Zuo, Z.Y.; Tepe, B.; Zhang, S.; Arenkiel, B.R.; Sardiello, M.; and Bellen, H.J. (2019): "Vap proteins are required for autophagic and lysosomal degradation". *Autophagy* 15(7): 1214-1233.

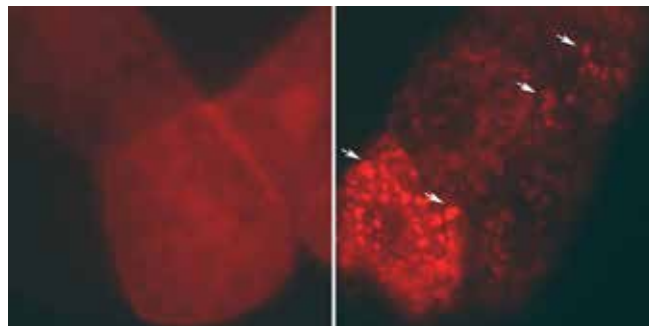
LAB MEMBERS

Instructor: Shiyu Xu, PhD

Post-doctoral fellow: Gang Li, PhD

Graduate Students: Yue Yu, Amanda Solbach (rotating GSBS student)

Research assistants: Xin Ye, PhD; Mrs. Lili Ye



Induction of autophagosomes in autophagy.

Numerous autophagosomes (red puncta in right panel), not found in control cells (left panel), are present in cells expressing autophagy-inducer human ULK1 gene.



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, antibody engineering, 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 lymphatic system using

photonics technologies for better diagnosis and directed treatments. Conditions such as vascular anomalies, congenital heart disease, peripheral vascular disease, 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, CSF 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.

*Eva Sevick-Muraca, PhD
Center Director & Professor
Nancy and Rich Kinder Distinguished Chair in
Cardiovascular Disease Research
Director, Center in the NCI Network for
Translational 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

Understanding how the lymphatic watersheds mediate immune health and chronic disease

As higher vertebrates evolved from the sea into land dwellers, terrestrial antigen exposure increased and the adaptive immune system evolved from a centralized lymphatic system to one dependent upon regional draining lymph nodes. The decentralized lymphatic system is organized into watersheds that drain into lymph node basins before emptying into the hemovascular circulatory system. In the regional draining lymph nodes, antigens are presented to activate immune cells that then leave the lymph node and disseminate through the body via the blood vasculature. This organization enables regional processing of immune responses to multiple antigens without overwhelming the immune system and breaking central tolerance, or tolerance to self. Yet despite the watershed organization of lymphatics, drugs that are intended to alter immune responses by targeting key signaling molecules within the lymphatics are administered or dosed systemically. Whether dosed to stimulate the immune system as in cancer checkpoint blockade immunotherapies, or to attenuate immune responses against self as in autoimmune therapies, these pharmaceutical strategies frequently lead to suboptimal results and, perhaps not surprisingly, adverse immune responses that break central tolerance.

In addition, all tissues drain to at least one lymphatic watershed not only to ensure immunosurveillance but also to collect cellular waste products and excess fluid for return to the hemovascular circulatory system. Lymphatic insufficiency can result in the build-up of waste products and unresolved inflammation. For example, in the lower extremities of aging populations, we have found that lymphatic insufficiencies accompany peripheral vascular disease and precede ulcer formation. In the brain, the cerebrospinal fluid (CSF) generated in the brain drains into the cervical lymphatic watershed, and in animal models of Alzheimer's disease, is impaired and presumably leads to Aβ aggregation and plaque formation.

In our research program, we employ near-infrared fluorescence imaging of the

lymphatic vasculature and its function in order to understand chronic conditions that involve the lymphatics and to more effectively deliver therapeutics that can modulate immunity. Specifically, we conduct translational imaging of infants, children, and adults on the Texas Medical Center with chronic conditions and investigate the corresponding animal models of these conditions. Our studies are designed to develop new biological insights that could lead to better prevention and treatment of these conditions. We also engineer new methods of lymphatic imaging to provide better diagnostics of chronic conditions.

RESEARCH PROJECTS

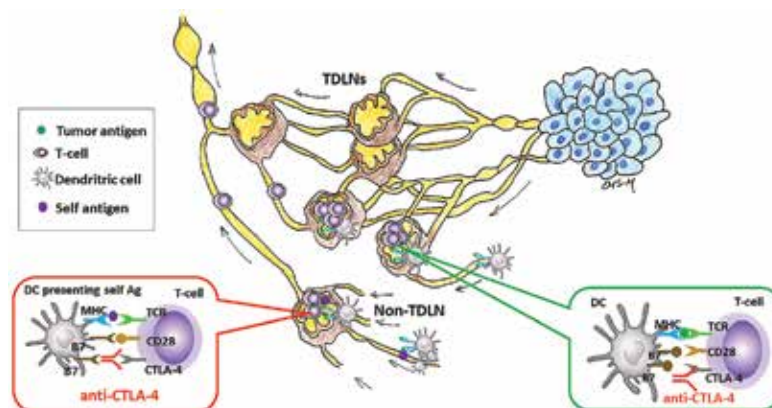
- Lymphatic delivery of immunotherapies for cancer and autoimmune disorders.
- Evaluating the role of CSF outflow in brain health and Alzheimer's.
- Assessing the role of lymphatics in metabolic disorders.
- Refining measurements of lymphatic anatomy and function.

KEY PUBLICATIONS

Aldrich, M.A.; Velasquez, F.C.; Kwon, S.; Azhdarinia, A.; Pinkston, K.; Harvey, B.R.; Chan, W.; Rasmussen, J.C.; Ross, R.F.; and E.M. Sevick-Muraca, "Lymphatic delivery of etanercept via nanotopography improves response to collagen-induced arthritis," *Arthritis Research And Treatment*, 19(1): 116, 2017 PMID: 28566090.

Kwon, S.; Morena-Gonzalez, I.; Taylor-Prese, 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*, 9(26):8332-8343, 2019.



Site of action for checkpoint blockade immunotherapy is in the regional tumor draining lymph nodes (TDLNs). There anti-CTLA-4 takes the "brakes" of T cell activation against tumor antigen (green ball). However, in non-TDLN, anti-CTLA-4 takes the brakes of T cell activation against self antigens leading to immune related adverse events. Adapted from Kwon, S et al., *Theranostics*, 2019.



Melissa B. Aldrich, MBA, PhD

Assistant Professor

Imaging in immunology

Cancer survivors face the possibility of developing a devastating side effect of cancer treatment: lymphedema, which manifests as a permanently swollen arm, leg, neck, or trunk. Lymphedema requires constant compression garment wear, meticulous skin care, and specialized massage. Lymphedema patients suffer discomfort, depression, cellulitis bouts, and there is no cure—only palliative treatment. Studies have shown that, if caught early in development, lymphedema treatment can reverse the disease. Near-infrared fluorescence lymphatic imaging (NIRFLI) imaging delivers high-resolution, low-cost images of lymphatic vessel architecture and pumping. In disease states such as lymphedema, NIRFLI imaging can provide information for early diagnosis and evaluation of treatment efficacy. I lead a five-year prospective and longitudinal study using NIRFLI surveillance of breast cancer patients to identify early lymphedema development and biomarkers that could suggest pharmacological treatment. I recently presented evidence at an international conference showing that lymphedema is reversible at early stages. I also participate as a team member imaging treatment responses to head and neck lymphedema, which affects ~90% of head and neck cancer patients. The treatment used, pneumatic compression therapy, removes stagnant lymph in lymphedema patients, but needs NIRFLI to visually “prove” to medical insurers that the therapy actually works. I am very active in the lymphedema 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 for LE&RN’s Centers of Excellence designation, which will enable patients to locate institutions with lymphatic expertise.

Delivery of pharmacological therapeutics directly to the site of disease activity could reduce the amount of pharmaceutical required,

and minimize off-target toxicities. In a rat model of rheumatoid arthritis, NIRFLI revealed that delivery of a tumor necrosis factor-alpha (TNF-alpha) blocker directly through lymphatic vessels to lymph nodes resulted in significantly reduced disease activity, as evidenced by improved lymphatic pumping.

NIRFLI studies of patients with lipedema, a fat disorder that affects ~11% of women, revealed that leg lymphatic vessels are dilated and slow-pumping, suggesting the disease is an inflammatory disorder. Compression garment wear to promote leg lymph movement and anti-inflammatory dietary practices have improved outcomes for these patients. I am a member of the Center for Molecular Imaging (CMI) team that participates with a national coalition of researchers to investigate lipedema.

Chylothorax occasionally affects neonatal heart surgery patients. My colleagues and I here at CMI and Memorial Hermann Hospital have used NIRFLI to help visualize the source of pleural effusion in babies with chylothorax. We also have imaged numerous pediatric patients with lymphovenous anomalies to help physicians direct optimal care.

RESEARCH PROJECTS

- Longitudinal study of breast cancer-related lymphedema.
- Imaging of lymphatics in lipedema.
- Imaging of neonatal chylothorax and pediatric lymphovenous anomalies.

KEY PUBLICATIONS

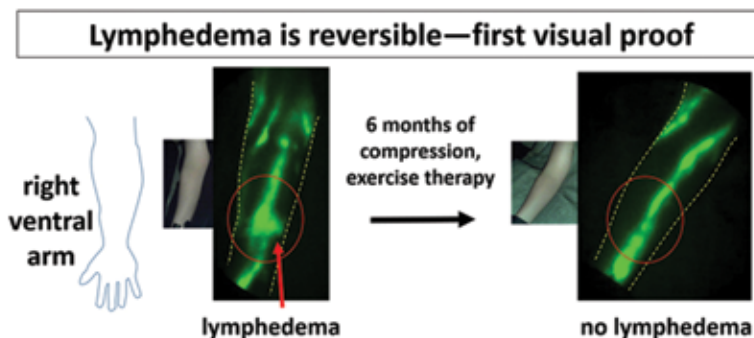
Schaverien, M.V.; Aldrich, M.B. New and emerging treatments for lymphedema. *Semin Plast Surg* 32:48-52, 2018.

Aldrich, M.B.; Velasquez, F.C.; Kwon, S.; Azhdarinia, A.; Pinkston, K.; Harvey, B.; Chan, W.; Rasmussen, J.C.; Ross, R.F.; Fife, C.E.; Sevick-Muraca, E.M. Lymphatic delivery of etanercept via nanotopography improves response to collagen-induced arthritis. *Arthritis Res Ther* 19:116, 2017.

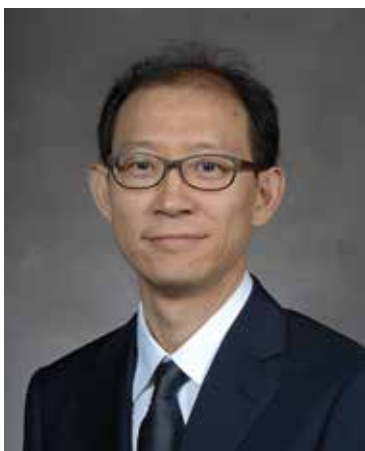
Aldrich, M.B.; Gross, D.; Morrow, J.R.; Fife, C.E.; Rasmussen, J.C. Effect of pneumatic compression therapy on lymph movement in lymphedema-affected extremities, as assessed by near-infrared fluorescence lymphatic imaging. *J Innov Optical Health Sciences*, Vol. 10, No. 02, 1650049 doi: 10.1142/S1793545816500498, March 2017.

LAB MEMBERS

Medical student: Kay Pham



Near-infrared fluorescence lymphatic imaging (NIRFLI) of arm lymphatics in a patient with breast cancer-related lymphedema. Green fluorescent dye is visualized through the skin, pumping through vessels (in a healthy arm) or pooling as backflow (in lymphedema). Medical insurers need objective evidence that currently prescribed physical therapy for lymphedema actually works. These images are the first visual proof of reversal of lymphedema in response to therapy.



Sun Kuk Kwon, PhD

Assistant Professor

Carolyn Frost Keenan Professor in Cardiovascular Disease Research

Lymphatic function in health and disease

I lead the development and application of small animal imaging techniques to address biological questions. My main research interest focuses on investigating the microcirculatory movement of fluid and macromolecules, particularly in the lymphatic system. Recently, we developed non-invasive, dynamic near-infrared fluorescence (NIRF) imaging methods for imaging and quantifying lymphatic function in health and disease. Using this novel technique, we showed abnormal lymphatic function and drainage patterns in animal models of lymph node metastasis, hypertension, and inflammation.

Recently, CMI demonstrated that direct infusion of an immune-attenuating biologic into the lymphatics can result in improved local and systemic responses when compared to conventional routes of administration in an arthritis rat model. Recently, I used the novel lymphatic infusion device for immunotherapy to maximize drug exposure to tumor-draining lymph nodes and to reduce toxicity by localizing immune stimulation to the regional lymphatics for systemic anti-tumor immunity. I demonstrated that when the repeated dose of anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) was successfully delivered via the lymphatic system, tumor shrinkage occurred when compared to the untreated cohort.

Recent evidence demonstrates that cerebral spinal fluid (CSF) and brain interstitial fluid (ISF) are exchanged through “glymphatics” that ultimately drain into the peripheral lymphatic vasculature within the head and neck area. Recently, I showed that the peripheral lymphatic system of transgenic mouse models of Alzheimer’s disease (AD) is impaired and may impact glymphatic function at early onset of amyloid beta (Aβ) plaque accumulation in collaboration with Drs. Claudia Soto and Ines Moreno-Gonzalez in the Mitchell Center for Alzheimer’s disease at McGovern Medical School. This is the first time to show that peripheral lymphatics outflow from the head and neck can be used as a diagnostic target for predicting onset, progression, and response to

AD pharmacological intervention.

Other directions of my scientific interests revolve around multi-modality molecular imaging. The Center for Molecular Imaging is developing and translating imaging agents. I am currently conducting molecular imaging of cancer and lymph node metastasis, inflammation, and myocardial infarct in mice.

RESEARCH PROJECTS

- Non-invasive dynamic lymphatic imaging to show lymphatic remodeling in a mouse model of melanoma and restoration of normal lymphatic vessel integrity after cancer therapy.
- Characterizing impaired cerebrospinal fluid (CSF) drainage into peripheral lymph nodes in Alzheimer disease animal models in collaboration with Dr. Claudio Soto at the medical school.
- Developing PET/CT methodology for quantifying infarct size in gene therapy trials of myocardial infarct in mice in collaboration with Dr. Jung Suh at Rice University.
- Assessing the normal lymphatic drainage in the lower extremities as well as in the cervical region after intradermal and intraoral administration of ICG, under conditions of supine position and head down tilt (HDT), the latter of which mimics aspects of microgravity.
- Using non-invasive lymphatic imaging to show

efficacy of drug delivery with SOFUSA™ into the lymph nodes in mice and successfully translated to humans supported by Kimberly Clark Coop (Currently Sorrento Therapeutics).

KEY PUBLICATIONS

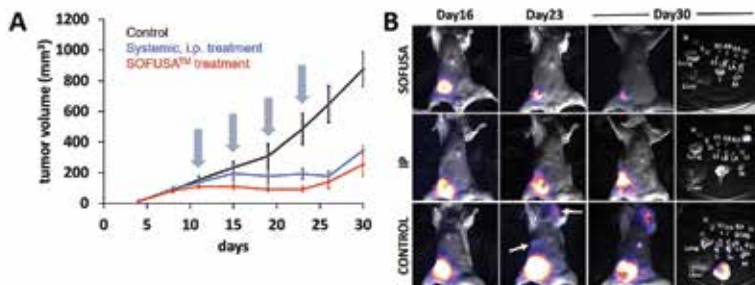
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Sunkuk Kwon, Christopher F. Janssen, Fred Christian, Velasquez, and Eva M. Sevick-Muraca, “Fluorescence imaging of lymphatic outflow of cerebrospinal fluid in mice,” *Journal of Immunological Methods*, 449; 37-43. 2017.

Sunkuk Kwon, Fred Christian, Velasquez, and Eva M. Sevick-Muraca, “Near-infrared fluorescence lymphatic imaging in vascular endothelial growth factor-C overexpressing murine melanoma,” *Biomedical Optics Express*, 9; 4631-4637. 2018.

LAB MEMBERS

Research assistants: C.J. Vaelasquez, Amanda Pinal (summer pre- Baccalaureate student)



A. Growth rates of orthotopic 4T1-Luc in Balb/c mice treated with control antibody (black) or with CTLA-4 administered i.p., (blue) or with SOFUSA™ delivered CTLA-4 (red). Grey arrows indicate treatment days. The mean tumor volumes ± SE (bars) are shown at the times that tumor measurements were made. B. Bioluminescence imaging of orthotopic 4T1-Luc in Balb/c mice 16, 23, and 30 days after tumor inoculation and tissues after euthanasia. CTLA-4 was treated i.p or via SOFUSA™ at 11, 15, 19, and 23 days post implantation. H, heart. RS/LS, right/left submandibular LN, RB/LB, right/left brachial LN. RA/LA, right/left axillary LN. RI/LI, right/left inguinal LN. T, tumor. M, muscle. S, stratum. Sc, Scapula.



John Rasmussen, PhD

Assistant Professor

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. It has been known for many years that 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 active venous leg ulcers and demonstrated abnormal lymphatics in all the legs with advanced disease. However, what was most surprising was that we also observed lymphatic abnormalities in all the contralateral legs, including those with no external sign of venous disease. In following studies of subjects with early venous disease, we have observed a degradation of lymphatic anatomy and decreased lymphatic pumping, which corresponds with increased classification of venous disease. A better understanding of the role of the lymphatics in early vascular disease may enable the development of more effica-

cious therapeutic approaches.

We are also using NIRF imaging to assess the recovery, or lack thereof, of the lymphatics after cancer treatment. We are particularly interested in the head and neck cancer population as it has been reported that 75% of head and neck cancer survivors will develop lymphedema. Our imaging has shown the development of abnormal lymphatics in this population, with the extent of abnormal lymphatics generally increasing with time (months) after the end of radiation treatment. We recently used NIRF imaging to assess the lymphatic response to a new, advanced pneumatic compression device developed specifically for subjects with head and neck lymphedema and reported a reduction in the extent of abnormal lymphatics with as little as two weeks of intervention in 75% of subjects. Ongoing studies also seek to assess treatment efficacies of improved pneumatic compression devices in subjects with breast cancer-related lymphedema, as well as the relationship between the lymphatics and other disorders, including lipedema.

We continue the development of this technology, including assessing novel imaging and drug delivery technologies, improving device sensitivity, automating different aspects of the hardware, and developing analytical tools to facilitate lymphatic image processing and analysis with the ultimate goal of answering new biological and clinical questions not addressed by other technologies.

RESEARCH PROJECTS

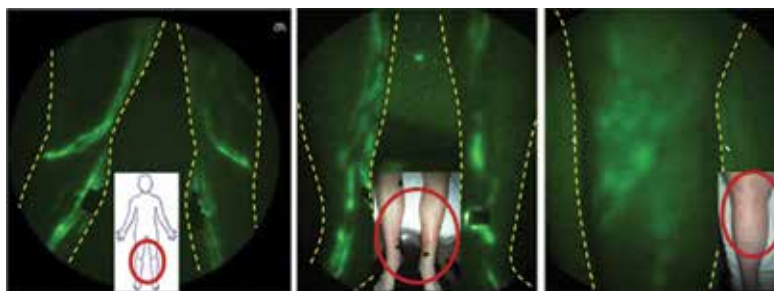
- Understanding the role of lymphatics in the development of peripheral venous and arterial disease.
- Assessing the development of cancer-related lymphedema and its response to intervention.
- Translation of lymphatic imaging to the pediatric population.

KEY PUBLICATIONS

Gutierrez, C.; Karni, R.J.; Naqvi, S.; Aldrich, M.B.; Zhu, B.; Morrow, J.R.; Sevick-Muraca, E.M.; and Rasmussen, J.C., "Head and neck lymphedema: treatment response to single- and multiple sessions of advanced pneumatic compression therapy," *Otolaryngology-Head and Neck Surgery*, 160(4):622-626, 2019.

Zhu, B.; Kwon, S.; Rasmussen, J.C.; Litorja, M.; and Sevick-Muraca, E.M., "Comparison of NIR versus SWIR fluorescence image device performance using working standards calibrated with SI units," *IEEE Transactions on Medical Imaging*, epub 8/27/2019.

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*, 9(26):8332-8343, 2019.



Normal lymphatic vessels in a healthy volunteer (left, adapted from Rasmussen, et al., *J. of Vas. Surg.: Ven. and Lymph. Dis.*, 4(1), 2016) and abnormal lymphatics in a patient with (center) C3 venous disease with segmented lymphatics and (right) C4 venous disease with indistinct lymphatic vessels.



Banghe Zhu, PhD

Assistant Professor

Cap-based transcranial optical tomography for whole brain functional mapping and comparison of NIR versus SWIR fluorescence image device performance using working standards calibrated with SI units

Cap-based Transcranial Optical Tomography for whole brain functional mapping: Although Blood Oxygenation Level Dependent (BOLD) functional MRI (fMRI) is widely used to examine brain function in adults, the need for general anesthesia limits its practical utility in infants and small children. Functional Near-Infrared Spectroscopy - Diffuse Optical Tomography (fNIRS-DOT) imaging promises to be an alternative brain network imaging technique. Yet current versions of continuous wave fNIRS-DOT systems are limited in the number of source-detector pairs, detector sensitivity as well as the field-of-view that restrict measurements to the cortical surface. Currently, we are developing a novel transcranial near infrared optical imaging paradigm, called Cap-based Transcranial Optical Tomography (CTOT) to image whole brain hemodynamic activity. With an increased amount of high-quality CTOT data, we are able to obtain a high-resolution functional map of the cerebral cortex in an awake child and the static CTOT volumes are strongly correlated with the averaged BOLD fMRI volume.

Comparison of NIR versus SWIR fluorescence image device performance using working standards calibrated with SI units: Recently, fluorescence imaging using shortwave infrared light (SWIR, 1,000-2,000 nm) has been proposed as having advantage over conventional near-infrared fluorescence (NIRF) imaging due to the reduced tissue scattering, negligible autofluorescence, comparable tissue absorption, and the discovery that indocyanine green (ICG), used clinically as a NIRF contrast agent, also has fluorescence emission in SWIR regime. Images of ICG in small animals acquired by commercial Si-based and InGaAs-based imaging cameras have been qualitatively compared, however the lack of working standards to quantify performance of these imaging systems limits quantitative comparison. Without quantification using a traceable *in vitro* test, clinical adoption of rapidly evolving advances in both NIRF and SWIR imaging devices will become limited. Currently, we are developing an ICG based fluo-

rescent solid working standard calibrated with SI units ($mW \cdot cm^{-2} \cdot sr^{-1}$) for quantification of measurement sensitivity of Si, GaAs-intensified Si, and InGaAs based camera systems, their signal-to-noise ratio (SNR), and contrast in non-clinical tests. In addition, we are performing small animal and large animal imaging with ICG for qualitative comparison of the same SWIR fluorescence and NIRF imaging systems. Our results suggest that SWIR fluorescence imaging of ICG may have superior resolution in small animal imaging compared to NIRF imaging, but lack of measurement sensitivity, SNR, contrast, as well as water absorption limits deep penetration in large animals.

RESEARCH PROJECTS

- Develop CTOT imaging system.
- Develop working standards for device performance comparison.
- Perform peripheral vascular disease clinical studies.
- Perform head & neck surgery clinical studies.

KEY PUBLICATIONS

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 Medi-*

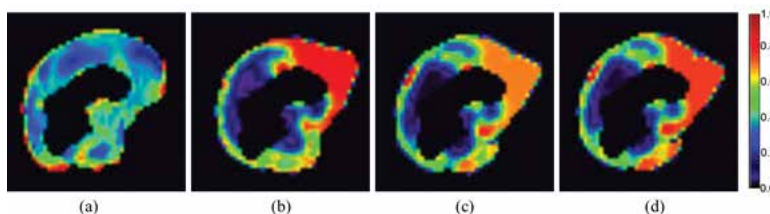
cal Imaging, DOI: 10.1109/TMI.2019.2937760 (2019).

Guenther, C. M.; Brun, M.; Bennett, A.; Ho, M. L.; Chen, W.; Zhu, B.; Lam, M.; Yamagami, M.; Kwon, S.; Evans, A. C.; Voss, J.; Sevick-Muraca, E. M.; Agbandje-McKenna, M.; and Suh, J., "Protease-activatable adeno-associated virus vector for gene delivery to damaged heart tissue," *Molecular Therapy*, 27, 611-622, (2019).

Alvarez-Urena, P.; Zhu, B.; Sonnet, C.; Henslee, G.; West, J.; Sevick, E. M.; Davis, A.; Olmsted, D. E., "Development of a Cell-based Gene Therapy Approach to Selectively Turn off Bone Formation". *J. Cell. Biochem.* 9999: 1-8, 2017.

Zhu, B.; Robinson, H.; Zhang, S.; Wu, G.; and Sevick-Muraca, E. M., "Longitudinal far red gene-reporter imaging of cancer metastasis in preclinical models: a tool for accelerating drug discovery", *Biomedical Optics Express*, 6:3346-3351 (2015).

Zhu, B.; Rasmussen, J.C.; and Sevick-Muraca, E. M., "Non-invasive fluorescence imaging under ambient light conditions using a modulated ICCD and laser diode," *Biomedical Optics Express*. 5(2):562-572 (2014).



(a) Sagittal slice of the one minus the normalized intensity processed BOLD image (1-nBOLD), (b) Sagittal slice of the normalized intensity CTOT-HbO image (CTOT-nHbO), (c) Sagittal slice of the normalized intensity CTOT-HbR image (CTOT-nHbR), and (d) Sagittal slice of the normalized intensity CTOT-HbT image (CTOT-nHbT). NIH heat map was used (see right color bar). All images had non-brain structures and the ventricles masked prior to cross correlation. For all images the top of image is superior, and right is anterior.

Precision medicine aims to personalize patient care with tailored medical decisions, treatments, and practices. It relies on the context of a patient's genome, proteome, health history, demographics, and other molecular or cellular information. Thus, technologies, such as "omics" approaches, molecular diagnostics, imaging, targeted delivery, and bioinformatics, are implicated to facilitate precision medicine.



The current research in the Center for Precision Biomedicine emphasizes several areas, including application of analytical and translational technologies to investigate molecular events underlying malignancy and other diseases, development of molecules for selective targeting of tissues or toxins, development of targeted contrast agents for disease visualization, and study of proteome alterations to elucidate disease mechanism and discover biomarkers to facilitate early detection and therapeutic treatment, as well as technology innovation in proteomics, imaging agents, and nanomedicine.

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, providing 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. These studies, with the aid of bioinformatics and systems biology, span from characterization of protein profiles and post-translational modifications to interrogation of protein network alterations, as well as targeted analysis of protein biomarkers in cancer, neurological disease and other diseases

for large cohort study. We also have an active probe development program that includes the development of new aptamers and multi-functional nanoparticle therapeutics for targeting pathological tissues, such as cancer. In addition, we have expertise in the development and application of novel biologics, namely antibody-based agents that have imaging and therapeutic 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. We also have large-scale, multi-color, high-resolution state-of-the-art 3D printers for both fast prototypes and finished production level models of new surgical tools and instruments or patient-specific organ models.

Our center houses several 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.

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



Ali Azhdarinia, MS, PhD

Associate Professor
John S. Dunn Research Scholar III

Molecular imaging probe development

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 in areas beyond cancer such as imaging of "good" fat tissue, characterization of novel nanomaterials for biomedical use, and assessing 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

(* denotes corresponding author)
 Ghosh, S.C.; Rodríguez, M.; Carmon, K.S.; Voss, J.; Wilganowski, N.L.; Schonbrunn, A.; Azhdarinia, A.* A Modular Dual Labeling Scaffold That Retains Agonistic Properties for Somatostatin Receptor Targeting. *J. Nucl Med.* 58(11):1858-1864, 2017. PMID:28572490.

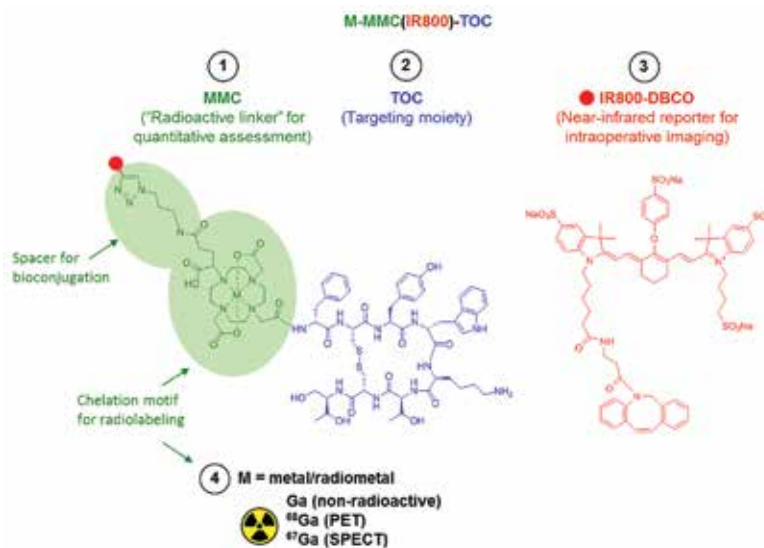
Hernandez Vargas, S.; Ghosh, S.C.; Azhdarinia, A.* New Developments in Dual-labeled Molecular Imaging Agents. *J Nucl Med.* 60(4):459-465, 2019. PMID: 30733318.

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

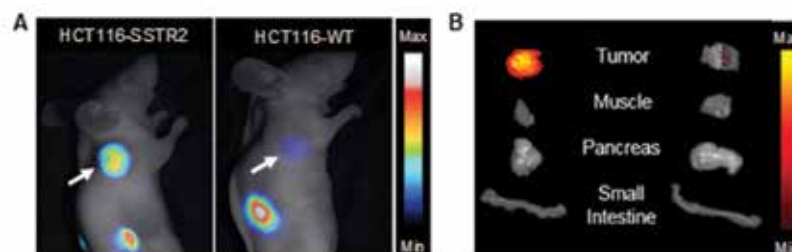
targeting of somatostatin receptor subtype-2 for fluorescence-guided surgery. *Clin Cancer Res.* 25(14):4332-4342, 2019. PMID: 31015345.

LAB MEMBERS

Postdoctoral fellows: Solmaz AghaAmiri
 Graduate students: Servando Hernandez Vargas
 Research scientist: Sukhen Ghosh



Chemical structure of a hybrid somatostatin analog containing radioactive and fluorescent labels for multimodality imaging.



In vivo and *ex vivo* imaging with the multimodality contrast agent, MMC(IR800)-TOC. (A) *In vivo* near-infrared fluorescence imaging in mice with (HCT116-SSTR2) and without (HCT116-WT) showed specific uptake in tumors that express somatostatin receptors (arrows indicate tumor). (B) *Ex vivo* imaging confirmed *in vivo* findings.



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 reposition drugs to target cells that exhibit phenotypes that promote metastasis. Through this study, 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 requires 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 backward-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

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.

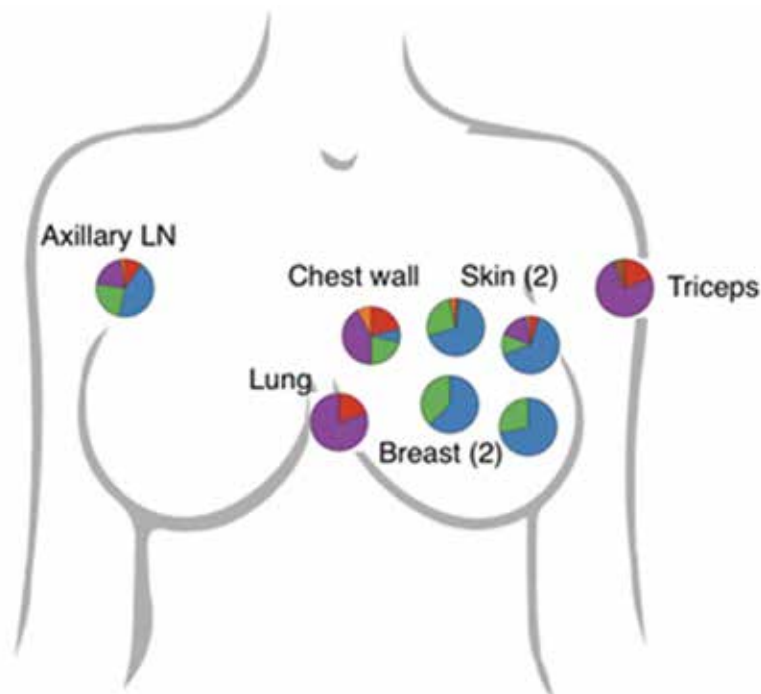
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* Co-Corresponding Authors

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LAB MEMBERS

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



We are using genomics to profile metastatic tumors to determine patterns of dissemination and phenotypic changes that occur in cells as metastases develop. This shows the diversity of genetic subclones from a patient with metastatic disease originating from the left breast. While the genotypes of the breast tumors are relatively homogeneous, they diverge as the disease spreads outward.



Barrett R. Harvey, PhD

Assistant Professor
John S. Dunn Research Scholar I

Development of therapeutics and diagnostics targeting cancer and infectious disease

Engineered to recognize and specifically target disease, antibodies are powerful tools for both basic, diagnostic, and translational research applications. As basic research tools, antibodies designed by our lab have been instrumental in helping to improve our understanding of bacterial physiology, and the factors that govern gram positive infection. Taking advantage of antibody specificity and technological achievements in antibody engineering, our lab also develops antibody based diagnostic agents with focused efforts in imaging cancer and bacterial infection. Combined with modern imaging equipment, these agents have direct clinical applications to help guide physicians with clinical diagnosis or in the removal of disease.

Finally, with emphasis on targeting clinically relevant signaling pathways for novel therapeutic approaches in cancer treatment, our laboratory is addressing the role of members of the Transforming Growth Factor Beta (TGFβ) family which are associated with aggressive cancer phenotypes. Nodal, a TGFβ family member, is overexpressed in breast cancer, with high levels of expression correlating with cancer cell stemness and causing increased cancer aggressiveness and drug resistance. We have developed a novel antibody agent, which specifically targets the Nodal pathway and are using relevant translational animal models of breast cancer to assess its efficacy. Our goal is to deliver an innovative drug to target Nodal signaling, while building a strong dataset on its synergistic performance in combination with current clinical cancer therapy.

RESEARCH PROJECTS

- Molecular targeting of nodal in aggressive breast cancer.
- Virulence factor regulation governing bacterial infection.

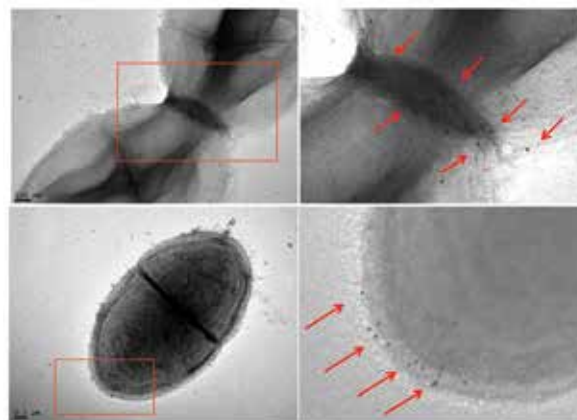
KEY PUBLICATIONS

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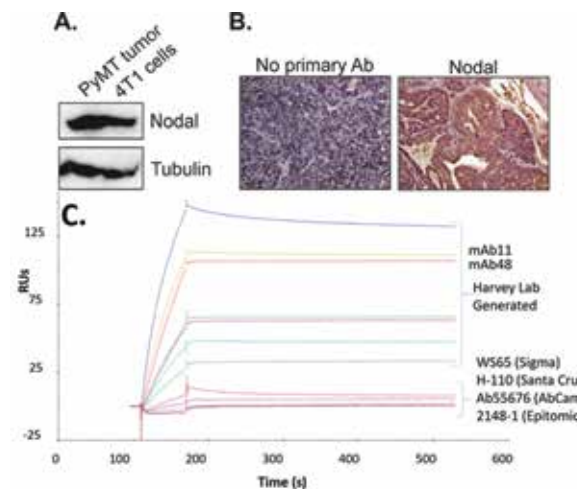
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Bacterial chaining, thought to encourage colonization and infection is regulated by AtlA, an enzyme important in cell wall cleavage. Beads (designated by red arrows) denote AtlA localization at poles and septum of dividing bacterial chains.



A. Western blot analysis of mouse breast cancer PyMT, and 4T1 tumor cells. B. Immunohistochemical analysis of PyMT tumor labeled with anti-Nodal antibody for primary detection. C. Surface plasmon resonance analysis demonstrating poor Nodal binding among commercial antibodies compared to in house generated monoclonals.



Sheng Pan, PhD

Associate Professor / Director, the Clinical and Translational Proteomics Service Center

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, has 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 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 cancer associated 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 malignancies. In addition, through collaborative efforts, our lab also supports proteomic study of neurological diseases, chronic inflammations, degenerative diseases, infectious diseases, and therapeutic drug development. Mass spectrometry, bioinformatics, systems biology, and chemical biology are important components in our study.

RESEARCH PROJECTS

- Discovery of protein biomarkers and therapeutic targets in pancreatic ductal adenocarcinoma (PDAC), especially those associated with cancer precursors, such as pancreatic intraepithelial neoplasia (PanIN) and pancreatic mucinous neoplasms (IPMN, MCN).
- Investigation of protein glycosylation, glycation and other PTMs in malignancies, neurological diseases, diabetes and chronic inflammation.
- Innovation of proteomic technologies for basic, translational and clinical applications.

KEY PUBLICATIONS

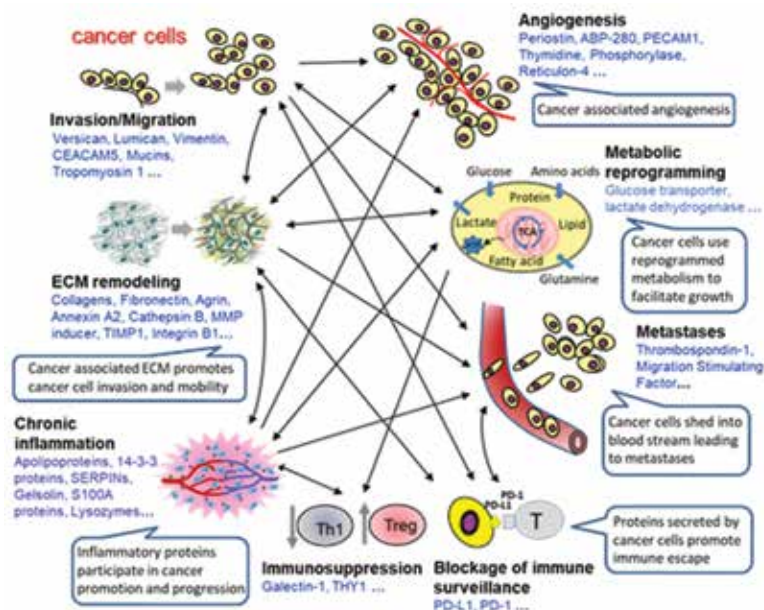
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LAB MEMBERS

Post-doctoral fellow: Hong Peng, PhD
 Research coordinator: Li Li
 Research associate: Xin Li
 Undergraduate student: Alisha Kashyap



Differential proteins identified in PDAC tissues are broadly involved in many aspects of tumorigenesis to facilitate cancer progression.



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 anti-cancer 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) can reduce cancer alone and enhances anti-tumor effects in combinatorial therapy.
- Developed aptamers (Fig 3, 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)

Other projects target infectious agents such as Dengue 2 virus, *C. difficile* infections and tuberculosis. 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.

Software Development - Another focus of the lab is to provide bioinformatics support and to develop novel software for the analysis of next-generation sequencing (NGS) data. We therefore developed Aptaligner (Lu et al. 2014), a completely automated program with easy-to-use graphical user interfaces. We are currently working on software for the analysis of DNA sequence changes during recombination events in *B. burgdorferi* infections, the cause of Lyme disease. Such antigenic variation is thought to cause long-term Lyme disease infection and post-infection deficits.

RESEARCH PROJECTS

- Development of smart particles to attack breast and ovarian cancers.
- Developing new X-aptamers targeting other diseases.
- Software to analyze how Lyme disease escapes host immune systems.

KEY PUBLICATIONS

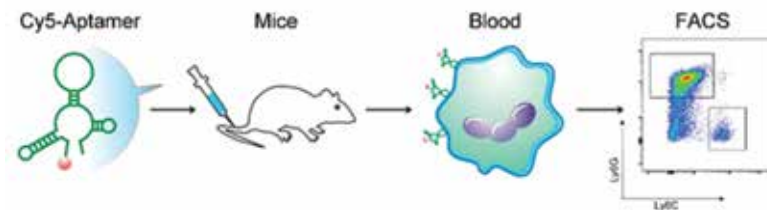
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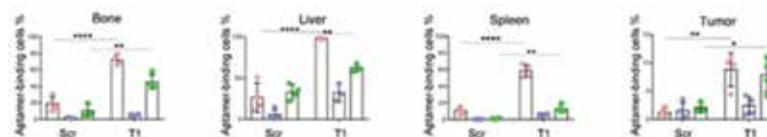
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LAB MEMBERS

Research associate: Xin Li, MS



Selecting aptamers targeting myeloid-derived cells in breast cancer.



Enhanced delivery to tumors.



Hongyu Wang, MD, PhD
Assistant Professor

Cancer biomarker discovery and targeted therapy

Biomarker discovery and targeted therapy are important parts of precision medicine. Aptamer mediated biomarker discovery and targeted therapy are attractive approaches for precision cancer treatment. Aptamers are single-stranded oligonucleotides with high affinity and specificity to the target molecules or cells. 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 systematic biology approach that combines a bead-based modified aptamer library with flow cytometry sorting and mass spectrometry to identify proteomics biomarkers. Patients' plasma were incubated with beads-X-aptamer library and sorted by flow cytometry based on fluorescence intensity (Figure1). Using this approach, we selected a panel of prognostic biomarkers for hepatocellular carcinoma (HCC) patients under Lipiodol-based transarterial chemoembolization (TACE) treatment. In combination with quantitative imaging analysis, we will integrate blood biomarkers with quantitative imaging features to establish a non-invasive platform for precision treatment

and outcome prediction for HCC patients. As aptamers can serve as an important category of molecular targeting ligand, aptamer mediated targeted therapy and targeted imaging offer a unique opportunity for selective delivery of therapeutic siRNA and drugs, or imaging agents. Several modified aptamers have been successfully identified in our lab for further targeted studies, such as Annexin A2, CD44, PD1, PD-L1, Vimentin, and Thy1. Those selected aptamers have great application potential in targeted drug delivery or targeted imaging. By conjugating the specific aptamer with nanoparticles that are loaded with drug or siRNA, we demonstrated specific delivery and targeting to ovarian cancer after systemic administration in vivo.

RESEARCH PROJECTS

- Proteomics biomarker discovery for hepatocellular carcinoma.
- Targeted cancer therapy with aptamer mediated nanoparticle-drug delivery.
- Aptamer-mediated targeted imaging.

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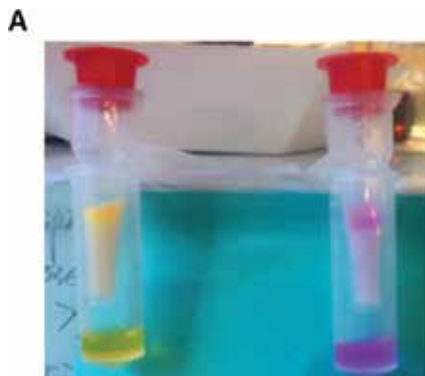
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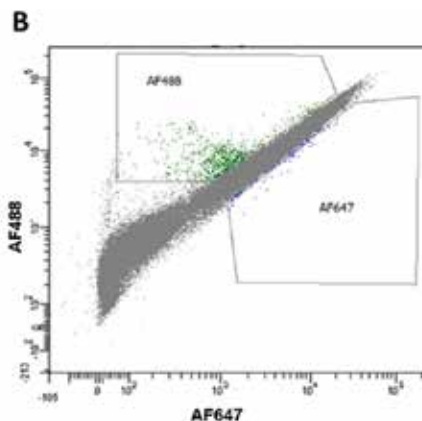
LAB MEMBERS

Research associate: Xin Li
Medical student: Andrea Costello



A
Proteome A, labeled with Alexa flour 488

Proteome B, labeled with Alexa flour 647



B
XA beads with fluorescent labeled proteome

X-aptamer based proteome selection. Patient plasma and health donor plasma cells were labeled with Alexa Fluor 488 or Alexa Fluor 647, respectively (A), mixed and then incubated with X-aptamer beads library (10^8 different X-aptamers). Proteome bound to bead-X-aptamer were sorted based on fluorescence intensity at each channel by a two-color BD FACS ARIA II flow cytometer (B).

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 healthy 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 of these are 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 turnover, aging, injury, or disease. A second class of stem cells of significant therapeutic 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. Faculty within the Center are seeking to develop efficient and robust methodologies to convert iPSCs into various cells/tissues of therapeutic interest including neural, blood, lung, muscle, and cartilage – as well as how to best deliver and maintain such cells/tissues for therapeutic benefit.

For patients presenting with genetically inherited disease, Center faculty are utilizing recently developed gene editing technologies to correct the disease-causing mutations in either tissue-resident stem cells or iPSCs. The goal of these studies is development of therapies that

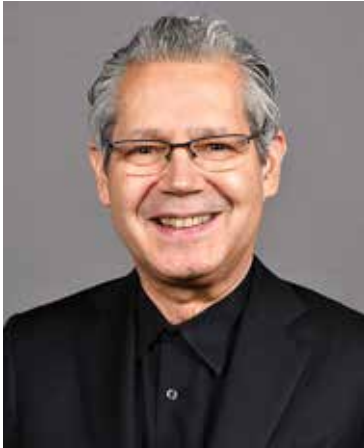
include correcting the mutations in a patient's own stem cells, then delivering either the corrected stem cells or cells/tissues derived from them back into the same patient.

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 such as lymphoma.

In the pages following you will find several examples of Center faculty exploring the potential therapeutic value of stem cells for repairing tissues such as spinal cord, brain, muscle, cartilage, 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, University of Texas Rising STARS Program, Mission Connect, 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 Center Director
The C. Harold and Lorine G. Wallace Distinguished
University Chair*



Brian R. Davis, PhD

Professor

Director of the Center for Stem Cell and Regenerative Medicine
C. Harold and Lorine G. Wallace Distinguished University Chair

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

RESEARCH PROJECTS

- Correction of airway basal stem cells and iPS cells from Cystic Fibrosis patients.
- Derivation of airway basal stem cell from Cystic Fibrosis patient-specific iPS cells.
- Correction of blood stem cells from Wiskott-Aldrich Syndrome patients.

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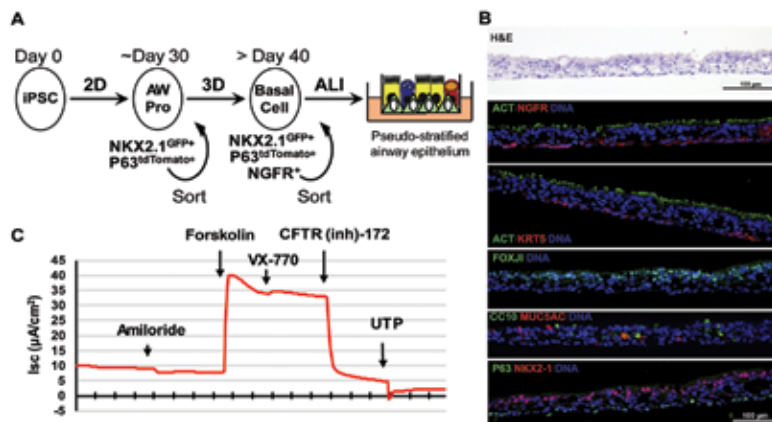
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LAB MEMBERS

Post-doctoral fellows: Dr. John M. Avila, Dr. Cristina Barilla, Dr. Shingo Suzuki
Graduate students: Varada Anirudhan
Research staff: Dr. Ana M. Crane, Dr. Nadine Matthias, Haipeng Xue

Dr. Davis' laboratory has as its primary objective the sequence-specific genetic correction of mutations in the chromosomal DNA of induced pluripotent stem (iPS) cells and/or tissue-specific stem 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. We have 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 introduced lung-specific fluorescent reporters into iPS cells and utilized to specifically isolate early lung progenitors and then airway basal stem cells for purposes of molecular and functional characterization. One of our objectives is to employ CF patient-specific iPS cell-derived lung epithelium for testing sensitivity to specific CF drugs -- in order to facilitate a personalized therapeutic approach. We are also presently utilizing the fore-mentioned gene correction methodologies to correct the CF mutations in tissue-specific stem cells directly obtained from CF patients. The second 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. Again, we are seeking to correct the disease-causing mutations in patient-specific blood stem cells. In both the CF and WAS projects, 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.



Deriving airway epithelium from induced pluripotent stem cells.
A. Schematic of differentiation protocol
B. Derived airway epithelium
C. Derived airway epithelium exhibits CFTR function



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 of 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 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 lineages 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

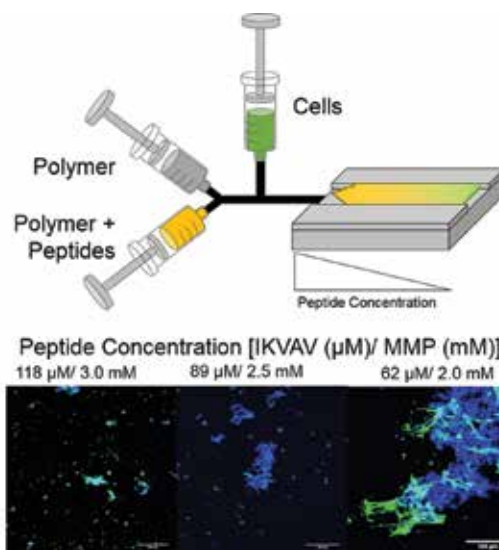
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LAB MEMBERS

Post-doctoral fellows: T. Hiran Perera, Xi Lu



Schematic of hydrogel with a continuous gradient in two peptide concentrations (IKVAV and MMP) and response of human induced pluripotent stem cell derived neural stem cell cytoskeletal organization (green) encapsulated in the hydrogel for 1 week undergoing neural differentiation. Nuclear staining is blue. Scale bars= 100µm.



Qi Lin Cao, MD
Professor

Stem cells for neurological diseases

Transplantation of neural stem cells (NSCs) is proved a promised therapeutic approach to promote functional recovery after neurological diseases, including spinal cord injury (SCI) and stroke. However, there is no consensus as to which NSC resource is optimal for SCI. Human central nervous system stem cells isolated from fetal cadaver brain tissue and neural progenitor cells derived from human embryonic stem cells (hESCs)-derived have been approved for clinical trials for SCI patients. However, these cells are associated with ethical controversy and graft rejection. Cells derived from hESCs have additional risk of teratoma formation. Human induced pluripotent stem cells (hiPSCs) are recently developed remarkable pluripotent, ESC-like cells reprogrammed from adult somatic cells by over-expression of four developmental/pluripotency transcription factors. Compared with ESCs, hiPSCs offer significant additional advantages in terms of availability of source material without ethical concerns of embryo use, and especially the ability to generate isografts without the need of immunosuppression. We have developed protocol to differentiate and purify NSC, neuronal precursor cells or glial precursor cells from hiPSCs. Our results show that hiPSC-derived NSCs can proliferate over long time *in vitro* and be induced to differentiate into functional neurons, astrocytes and oligodendrocytes. Importantly, hiPSC-derived NSCs can survive and differentiate into both neurons and glia after transplantation into the contused spinal cord and promote functional recovery. These studies suggest that transplantation of hiPSC-derived NSC is an effective therapy to preserve and restore neurological functions. Currently, we are testing the therapeutic efficacy and long-term safety of NSCs, neuronal or glial precursor cells to identify the optimal cell graft for SCI and stroke. Recently, we are testing whether we can directly reprogram the astroglial cells in the injured spinal cord or stroke brain into neurons. Astroglial scar are the major inhibitor for axonal regeneration. *In situ* reprogramming active astrocytes into neuronal

precursor cells will decrease astrocyte inhibition to promote axonal regeneration. The newly reprogrammed neuronal precursor cells could replace the lost neurons after SCI or stroke. These two mechanisms may work synergistically to promote great functional recovery after SCI or stroke. Our long-term goal is to develop novel stem cell-based therapies to treat human SCI or stroke.

RESEARCH PROJECTS

- *In vivo* reprogramming of reactive astrocyte and chemogenetic approach for SCI repair.
- Treating neuropathic pain by *in vivo* reprogramming of astrocytes after SCI.
- Combinatorial approaches to promote axonal regeneration and functional recovery after SCI
- Human iPSC-derived neural stem or precursor cells for spinal cord injury and stroke.

KEY PUBLICATIONS

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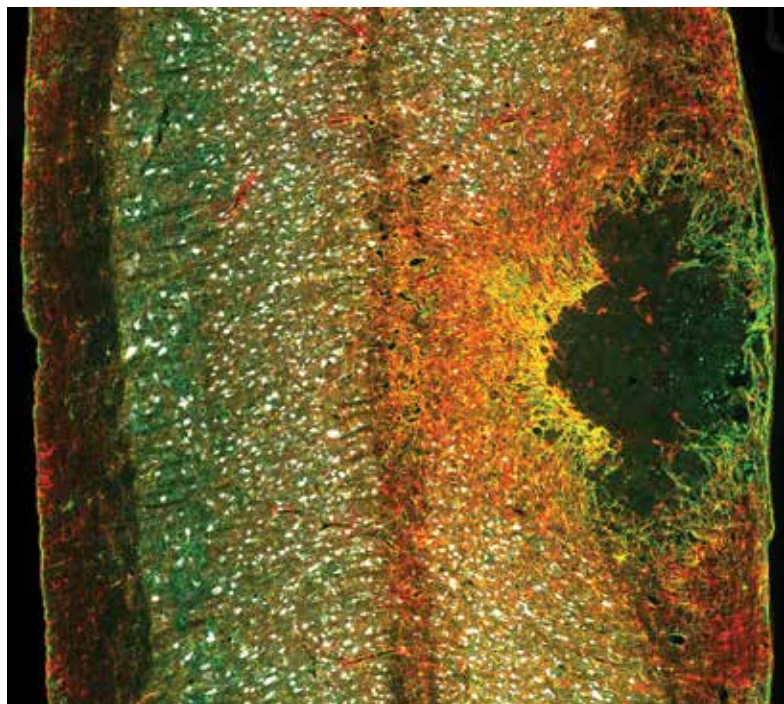
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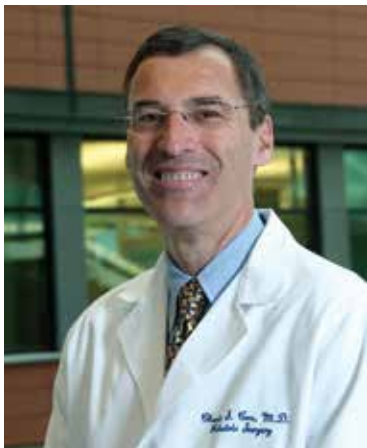
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LAB MEMBERS

Post-doctoral fellow: Yiyang Zheng
Graduate student: Chrystine Gallegos
Research associate: Haipeng Xue
Undergraduate student: Matthew Carey



Astroglial scar formation after traumatic spinal cord injury in double-transgenic mice of GFAP-cre/Ai9.



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

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LAB MEMBERS

Steven Kosmach, MSN, RN, CCRC-TBI clinical

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Akshita 'Jade' Kumar, MD, TBI clinical and cell therapy

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Scott Olson, PhD, assistant professor

Katherine Ruppert, PhD, Sr research associate

Karthik Prabhakara, Sr research assistant

Cecilia Martin, PhD, research associate

Supinder Bedi, PhD, assistant professor

Amit Srivastava, PhD, assistant professor

Naama Toledo-Furman, PhD, flow cytometry/innate immunity

Hasen Xue, MD, research associate

Fabio Triolo, PhD, GMP center director

Sufira Kiran, GMP, QA director

Romina Gipson-Love, quality improvement

coordinator

Deepa Bhattarai, Sr research associate

Matteo Costantini, research assistant

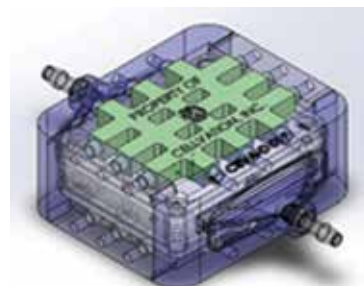
Kevin Aroom, scientific programmer

Tushar Sharma, scientific programmer

Max Skibber, research assistant

Christina Willingham, program manager

Stephanie Baca, program manager



Development of a novel bioreactor for stem cell production.



Radbod Darabi MD, PhD

Associate Professor

Using stem cells to treat muscle disorders

Skeletal muscle is the largest tissue in the body and therefore, is very prone to various disorders. Most common types of muscle disorders include gradual muscle deterioration during aging (sarcopenia) or after severe systemic diseases (such as heart or kidney failure, or in patients with cancer). Muscle loss injuries are also very common after accidents or during combat injuries. In addition, genetic disorders of the muscle such as muscular dystrophies are also a major health problem with no definitive cure. All of these disorders will eventually lead to progressive muscle weakness and loss of its function. Unfortunately, in severe cases, these disorders are fatal due to the involvement of heart or breathing muscles.

Here at our center, we are interested to use pluripotent stem cells (PSCs) for the treatment of these common disorders. During the last few years, our lab has developed novel methods for myogenic differentiation of induced pluripotent stem cells (iPSCs) as cell therapy in different skeletal muscle disorders, such as Duchenne muscular dystrophy (DMD) and muscle loss injuries.

Here at IMM, by using cutting-edge gene targeting/editing technologies (such as CRISPR/Cas9) our lab has successfully generated knock-in human stem cell lines for early myogenic genes such as PAX7 and MYF5. This allows us to study the emergence of early myogenic progenitors from human PSCs, a crucial step to identify and isolate myogenic progenitors useful for stem cell therapy in muscle disorders. These cells have been tested for the engraftment potential in the mice models for Duchenne muscular dystrophy, which so far have demonstrated superior engraftment and regeneration potential in the treated muscles. Study of the muscle function recovery after stem cell therapy is another exciting goal in the lab, which is currently under investigation.

Other major goals of our lab include using high throughput screening (HTS) to identify important pathways for myogenesis, evaluation of the engraftment potential of human

PSCs in mice models for muscle disorders, as well as identification of novel regulators of myogenic program using genome-wide sgRNA library screen. Our research team also works on derivation of iPSCs from patients with new types of muscular dystrophies such as LGMD2Z, gene correction of patient-derived iPSCs, as well as enhancing cell delivery and engraftment in the skeletal muscle. Our research program is currently funded by two NIH grant awards from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) to support these exciting and novel projects.

RESEARCH PROJECTS

- Evaluation of the engraftment and functional recovery potential of hPSCs in the mice models for Duchenne muscular dystrophy (DMD) and muscle mass loss injury.
- Gene correction of patient-derived iPSCs using CRISPR/Cas9 system.
- In vivo gene activation to ameliorate muscle degeneration.

KEY PUBLICATIONS

Wu, J.; Hunt, S.D.; Matthias, N.; Servián-Morilla, E.; Lo, J.; Jafar-Nejad, H.; Paradas, C.; Darabi, R. Generation of an induced pluripotent stem cell line (CSCRMi001-A) from a patient with a new type of limb-girdle muscular dystrophy (LGMD) due to a missense mutation in POGlut1 (Rumi). *Stem Cell Research*, Sep. 24: 102-105. 2017

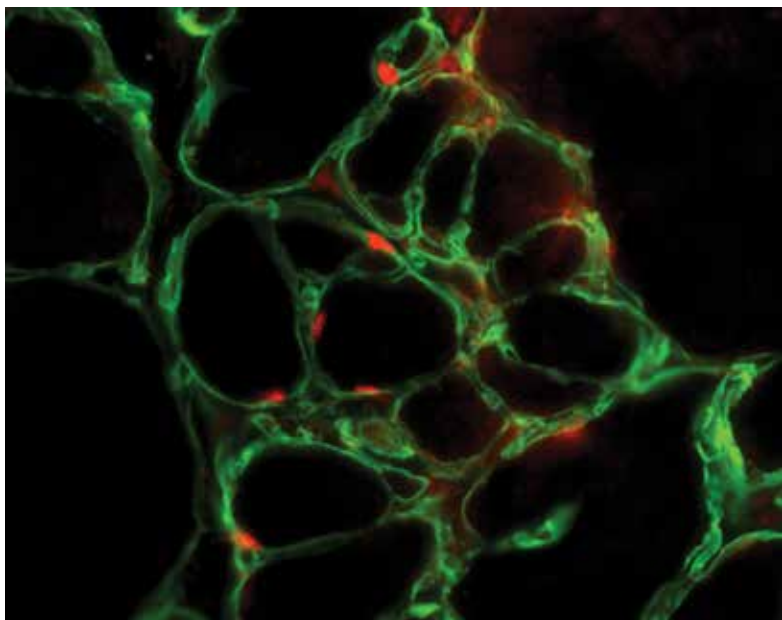
Matthias, N.; Hunt, S.D.; Wu, J.; Lo, J.; Smith Callahan, L.A.; Li, Y.; Huard, J.; Darabi, R. Volumetric muscle loss injury repair using in situ fibrin gel cast seeded with muscle-derived stem cells (MDSCs). *Stem Cell Research*. Mar. 27: 65-73. 2018

Wu, J.; Matthias, N.; Lo, J.; Ortiz-Vitali, J.L.; Shieh, A.W.; Wang, S.H.; Darabi, R. A Myogenic double-reporter human pluripotent stem cell line allows prospective isolation of skeletal muscle progenitors. *Cell Reports*. Nov. 25/7: 1966-1981. 2018

LAB MEMBERS

Instructor: Jianbo Wu

Research associate: Nadine Matthias



Muscle stem cells (satellite cells) stained in red in a muscle tissue cross section. Green stain marks myofibers' cross sections.



Pramod Dash, PhD

Professor

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 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.

A large number of studies have reported decreased energy utilization and impaired mitochondrial function after moderate-severe TBI. Unlike other organs, the brain does not store energy and relies on a continuous supply of glucose to generate the ATP it requires for normal function. When mitochondrial dysfunction occurs, the brain cannot generate sufficient ATP. In addition, impaired mitochondrial respiration can give rise to increased levels of free radicals that can damage cell components, exacerbate tissue damage, and worsen outcome. While studies have demonstrated a role for mitochondrial dysfunction in more severe forms of TBI, limited information exists

on the status of mitochondrial respiration after repeated mild TBI. To address this knowledge gap, we utilized our recently developed tissue respiration assay to measure mitochondrial function in mice subjected to repeated mild TBI. Our results indicate that repeated mild brain injury, but not a single mild injury, significantly increases proton leak in the hippocampus. Maintenance of a proton gradient is critical for mitochondrial function and leakage of protons across the mitochondrial membrane and has been linked to insufficient ATP generation and increased free radical production. Consistent with this, we have found that treatments which reverse proton leakage after repeated mild TBI increase ATP-linked mitochondrial respiration. These beneficial effects were found to be associated with reduced white matter damage and improved cognitive outcome.

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

Maynard, M.E.; Underwood, E.L.; Redell, J.B.; Zhao, J.; Kobori, N.; Hood, K.N.; Moore, A.N.; and Dash, P.K. Carnosic acid improves outcome after repetitive mild traumatic brain injury. *J. Neurotrauma*. 36:2147-2152, 2019.

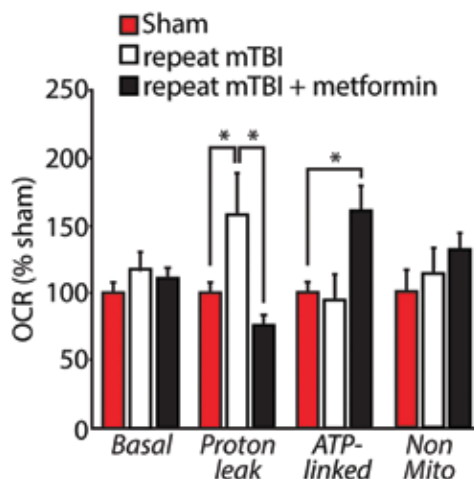
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COLLABORATORS

Dr. James McCarthy: Chairman of Emergency Medicine; Medical Director, Emergency Center at Memorial Hermann Hospital-TMC
 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
 Dr. Cameron Jeter: Assistant Professor of Diagnostic and Biomedical Sciences



Summary data of mitochondrial respiration from hippocampal tissue punches showing the various aspects of mitochondrial function. Repeated mild TBI caused an increase in proton leak that is reversed by the anti-diabetes drug metformin. This reduction in proton leak was accompanied by an increase in ATP-linked respiration.



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. The 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, human pluripotent stem cell-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 disease studies by other research groups. Currently, we are working on culture conditions that can direct the early human lung cells towards either airway or distal alveolar cells. The availability of each of such enriched cell population provides a valid platform for studying lung diseases originating in both airways and alveolar, such as severe influenza viral infection and lung cancer.

RESEARCH PROJECTS

- Use patient hiPSC differentiated lung epithelial cells to test rare mutations in TLR3 as causative for severe influenza infection induced by H1N1 virus.
- Understanding the basic mechanisms of lung cell fate determination using molecular, genetic and epigenetic approaches.

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.

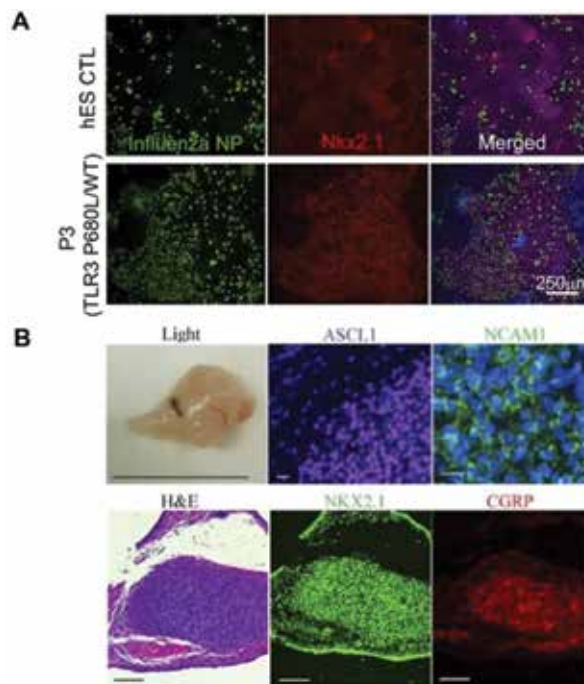
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pulmonary neuroendocrine cells and SCLC-like tumors from human embryonic stem cells. *J Exp Med*. 2019 Mar 4;216(3):674-687.

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.

LAB MEMBERS

Post-doctoral fellows: Nicolas Focioli-Conti, Nadine Matthias



A. Lung epithelial cells (NKX2.1+) derived from patient hiPSC (P3) carrying TLR3 gene mutation (bottom panels) are more susceptible to H1N1 viral infection (influenza NP) compared with those from healthy control (top panels); B. human pluripotent stem cell-derived pulmonary neuroendocrine progenitors formed small cell lung cancer-like tumors in immuno-deficiency mice, upon the suppression of two tumor suppressor genes RB and P53. The tumor cells express key lung and neuroendocrine markers (NKX2.1, ASCL1, NCAM1 and CGRP).



Dong Kim, MD

Professor and Chair, Vivian L. Smith Department of Neurosurgery
 Director, Mischer Neuroscience Institute
 Memorial Hermann-TMC
 Clive and Nancy Runnels Chair in Neurosurgery

Advancing the field of neuroscience

neural progenitors derived from integration-free iPSCs for SCI therapy. 2016; *Stem Cell Res.* 2017 Jan 5;19:55-64.

thoracic spinal cord injury. *Neurosurgery.* 24 May 2017.

LAB MEMBERS

Research Assistant Professor: Yanning Rui; Zhen Xu
 Assitant Professor: Yuanqing Yan
 Genetic Counselor: Krista Qualmann
 Clinical Trials Nurse: Rebecca Martinez

I am professor and chair of the Department of Neurosurgery at McGovern Medical School. As director of the Mischer Neuroscience Institute (MNI), I also lead the clinical neuroscience efforts for the Memorial Hermann Healthcare System. Currently, this group includes over 100 faculty and residents/fellows.

My research has focused on the origin, development, and treatment of brain aneurysms. Our group recently identified the first gene defect proven to cause intracranial aneurysms in familial patients. We also work to develop neural stem cells for implantation into the brain and spinal cord.

I was named to the US News and World Report's Top 1% Doctors, and America's Top Surgeons. I am the recipient of grants from the National Institutes of Health and the American Stroke Association.

A graduate of Stanford and the University of California, San Francisco (UCSF) School of Medicine, I completed general surgery training at Harvard, then neurosurgery at UCSF. Prior to coming to Texas, I held positions at Harvard Medical School, Brigham and Women's Hospital, the Dana-Farber Cancer Institute, Cornell University Medical College, The New York Hospital, and Memorial Sloan Kettering Cancer Center.

RESEARCH PROJECTS

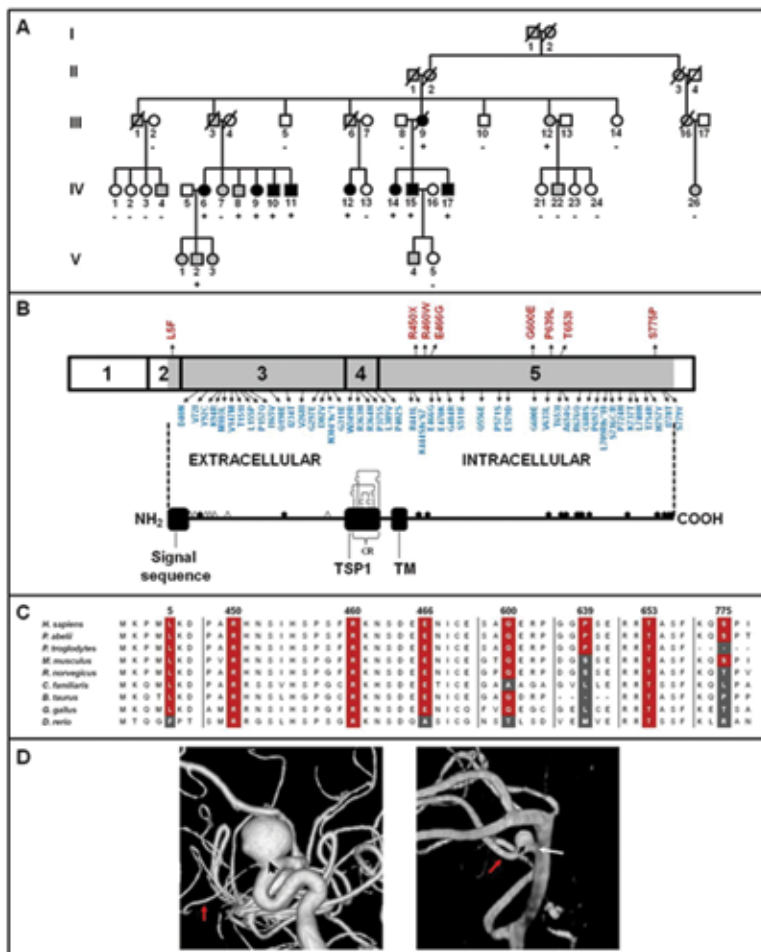
- Stem cell therapy for spinal cord injury.
- Genetic aneurysm research.
- Clinical trials.

KEY PUBLICATIONS

Santiago-Sim, T.; Fang, X.; Hennessy, M.; Naibach, S.; DePalma, S.; et al. THSD1 (Thrombospondin Type 1 Domain Containing Protein 1) Mutation in the Pathogenesis of Intracranial Aneurysm and Subarachnoid Hemorrhage. *Stroke.* 2016 Dec;47(12):3005-3013. Epub 2016 Nov 15.

Ying Liu; Yiyan Zheng; Shenglan Li; Haipeng Xue; Georgene W. Hergenroeder; Jiaqian Wu; Yuyan Zhang; Dong H. Kim; Qilin Cao. Human

Levi, A.D.; Okonkwo, D.; Park, P.; Jenkins, A.; Kurpad, S.; Parr, A.; Ganju, A.; Arabi, B.; Kim, D.; Casha, S.; Fehlings, M.; Anderson, K.D.; Gage, A.; Hsieh, J.; Huhn, S.; Curt, A.; Guzman, R. Emerging safety of intramedullary transplantation of human neural stem cells in chronic cervical and



Identification of the THSD1 R450X Mutation in Large Family with IA and the Spectrum of THSD1 Rare Variants.



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. We have recently found that innate-like B-1 lymphocytes and the first HSCs are produced simultaneously from HECs. We are elucidating 1) what molecular signals determine the divergent point between innate-like B-1a biased and multi-potent HSCs, 2) how embryo-derived B-1 progenitors contribute to postnatal peritoneal B-1 cell pool, 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. It has been postulated for decades that B-1 cells are derived from fetal progenitor cells, not from adult bone marrow HSCs, based on the results of transplantation assays. Our aim is to identify the main source of HSC-independent B-1 progenitor cells and evaluate its real contribution to postnatal B-1 cell pool, utilizing various lineage tracing mouse models.

B-1 biased progenitors and precursor of HSCs are produced from hemogenic endothelial cells simultaneously in the embryo. However, it is still unknown what molecules determine the cell fate of hemogenic endothelial cells into these two types of progenitors. By utilizing transplantation assays, lineage tracing mouse models, and single-cell RNA-sequencing, we are elucidating the biological and molecular mechanisms that are responsible for this cell

fate decision and maturation.

Knowledge obtained from these 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.
- Elucidating cell-intrinsic and cell-extrinsic mechanisms for maintaining B-1a cell self-renewal ability.
- Understanding the divergent point of HSC and B-1 biased progenitor differentiation from the hemogenic endothelial cells in the mouse embryo.
- Identify important molecules for HSC maturation in the mouse embryo utilizing single-cell RNA-sequencing.

KEY PUBLICATIONS

Kobayashi, M.; Tarnawsky, S.P.; Wei, H.; Mishra, A.; Azevedo Portilho, N.; Wenzel, P.; Davis, B.; Wu, J.; Hadland, B.; Yoshimoto, M. Hemogenic Endothelial Cells Can Transition to Hematopoietic Stem Cells through a B-1 Lymphocyte-Biased State during Maturation in the Mouse Embryo. *Stem Cell Reports*. 13 (1): 21-30, 2019.

Lin, Y.; Kobayashi, M.; Azevedo Portilho, N.; Mishra, A.; Gao, H.; Liu, Y.; Wenzel, P.; Davis, B.; Yoder, M.C.; Yoshimoto, M. Long-Term Engraftment of ESC-Derived B-1 Progenitor Cells Supports HSC-Independent Lymphopoiesis. *Stem Cell Reports*. 12(3):572-583, 2019.

Yoshimoto, M. B-1 progenitor acute lymphoid leukemia. *Blood*. 133(24):2557-2558, 2019.

LAB MEMBERS

Assistant professor: Michihiro Kobayashi MD, PhD

Research assistants: Chika Nishida MD, Samuel A. Cornelius, Noemi Valiente



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 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

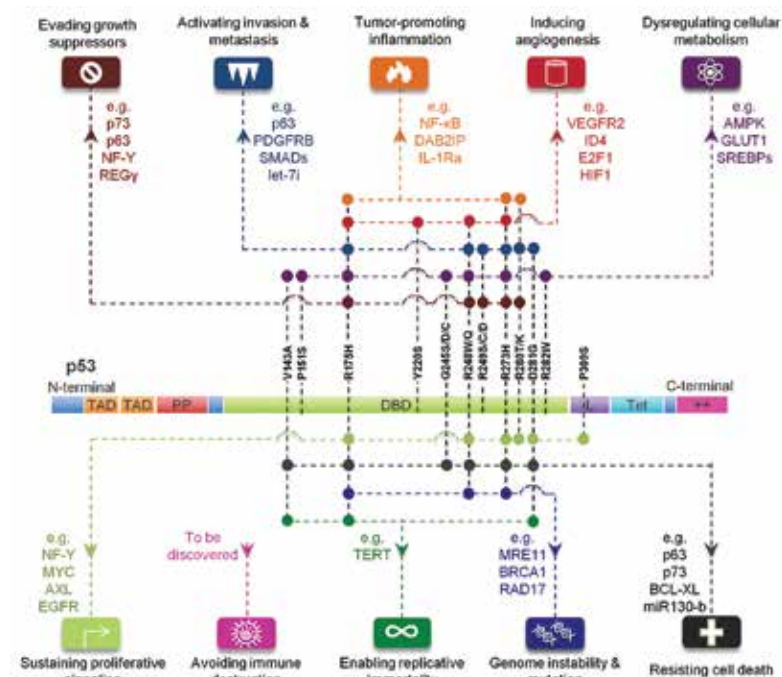
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Huensuk Kim; Seungyeul Yoo; Ruoji Zhou; An Xu; Jeffrey M. Bernitz; Ye Yuan; Andreia M Gomes; Michael G Daniel; Jie Su; Elizabeth G. Demicco; Jun Zhu; Kateri A. Moore; Dung-Fang Lee; Ihor R Lemischka; Christoph Schaniel. Oncogenic role of SFRP2 in p53-mutant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

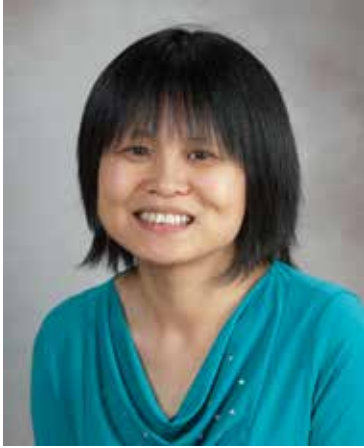
Jie Su; Dandan Zhu; Zijun Huo; Julian A. Gingold; Yen-Sin Ang; Jian Tu; Ruoji Zhou; Yu Lin; Haidan Luo; Huiling Yang; Ruiying Zhao; Christoph Schaniel; Kateri A. Moore; Ihor R. Lemischka; Dung-Fang Lee. Genomic integrity safeguards self-renewal in embryonic stem cells. *Cell Rep*. 2019 Aug 6;28(6):1400-1409.e4

LAB MEMBERS

Post-doctoral fellows: An Xu, Mo Liu, Dandan Zhu
Students: Brittany E Jewell
Technicians: 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

We have been pursuing basic and translational research in the following two areas: (i) stem cell biology and regenerative medicine, and (ii) pathogenesis of neurodegenerative disease and CNS injury. We focus on dissecting the neural developmental pathways and the corresponding pathogenesis in spinal cord injury. 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)

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PMCID:PMC5485764

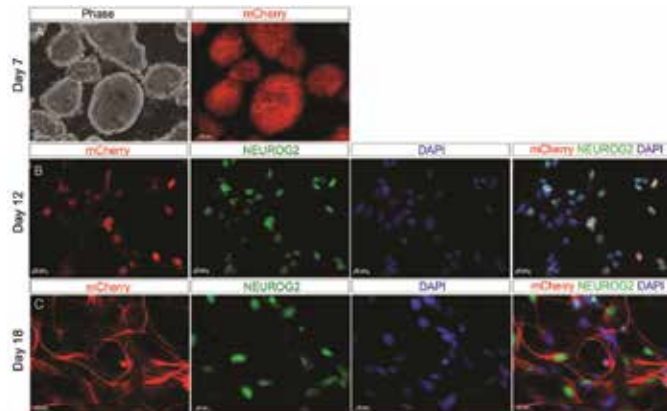
Long, B.; Li, S.; Xue, H.; Sun, L.; Kim, D. H.; Liu, Y. (2017). Effects of propofol treatment in neural progenitors derived from human-induced pluripotent stem cells. *Neural Plasticity.* Oct

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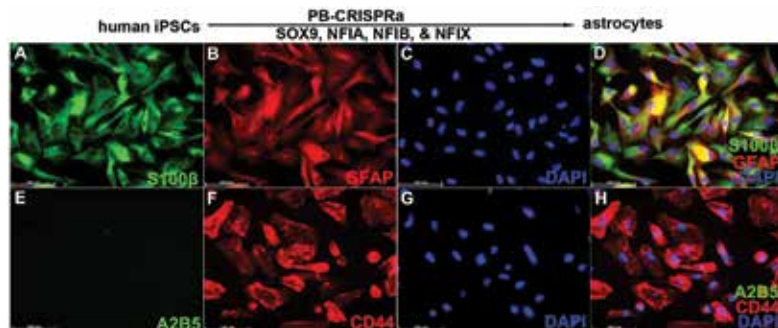
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LAB MEMBERS

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

Associate Professor

Annie and Bob Graham Distinguished Chair in Stem Cell Biology

Deciphering mechanisms of human cancer cell survival within the bone microenvironment

The behavior of cancer cells is not only dependent on their genomic abnormalities, but also requires complex relationships between malignant cells and their local bone marrow niche, which provides an environment for multiple myeloma cell growth as well as protection from chemotherapy-induced apoptosis. The bone marrow niches provide a “hiding place” for dormant clones, which are often resistant to chemotherapeutic agents.

The major goals of my research program are to decipher molecular pathways that confer selective growth and survival advantages to malignant B cells and delineating their interaction with bone marrow microenvironment. One of those factors is paired box 5 (PAX5), a determinant of normal B cell lineage development. We discovered that PAX5 silencing in mantle cell lymphoma leads to increased tumor formation in xenograft mice, indicating that PAX5 is a potential tumor suppressor. Moreover, PAX5 silencing led to increased cancer cell survival in the bone marrow.

We have conducted high throughput drug screening using libraries comprised of 3,991 compounds of NCI oncology, custom clinical, and prestwick libraries. We discovered that select compounds target the survival pathways of PAX5 silenced cells. Given that PAX5 silenced cells are highly drug resistant, discovery of compounds that target drug resistance populations in cancer cells will have direct translational applications.

We are also conducting research delineating roles of the quiescent multiple myeloma (MM) and their interaction with the bone marrow microenvironment. MM is a plasma cell malignancy that proliferates primarily in bone marrow and causes osteolytic lesions. Since quiescent cells can escape the chemotherapeutic treatment and potentially lead to drug resistance and increased tumor formation, it is important to understand the molecular mechanisms of their survival in bone marrow. Characterization of quiescent cells and their interaction with microenvironment is underway.

RESEARCH PROJECTS

- Survival mechanisms of dormant multiple myeloma cells and their microenvironment in the bone marrow: We conducted microarray analyses to identify genes expressed in quiescent multiple myeloma cells from the different niches of the bone marrow. We will continue to characterize functions of these genes in the multiple myeloma interaction with bone marrow microenvironment to delineate how dormant multiple myeloma cells evade chemotherapies.
- Development of small molecule inhibitors to target drug-resistant lymphomas: We have conducted high throughput chemical screening to identify the compounds that selectively target mantle cell lymphoma cells that develop drug resistance. We will further develop and test these compounds in animal models for pre-clinical studies and plan to test their efficacies in the patients.
- 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. We focuses on delineating functions of protein homeostasis control in cancer progression.

KEY PUBLICATIONS

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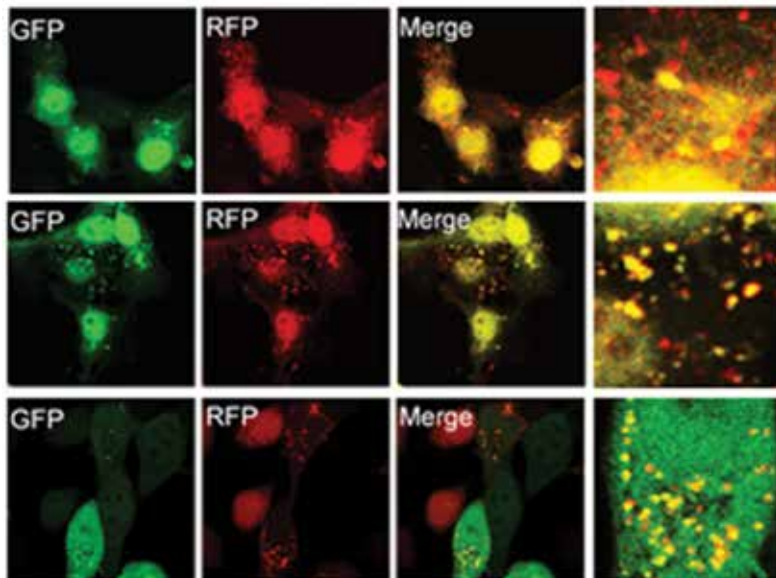
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LAB MEMBERS

Post-doctoral fellow: Lyn Liu, PhD

Research assistants: Jiacui Chen



TRIM44 increases autophagy flux processes. TRIM44 expression was modified in cancer cells and assayed for the appearance of autophagosomes using a confocal microscopy.



Naoki Nakayama, PhD

Associate Professor

Annie & Bob Graham Distinguished Chair in Stem Cell Biology

Pluripotent stem cell biology and Synovial joint morphogenesis

Synovial joint is composed of articular cartilage (meniscal cartilage), ligament and synovial membrane and is formed during embryogenesis from a multipotential joint progenitor. Injured joint cartilage is not spontaneously repaired in humans, leading to osteoarthritis. There has been considerable interest in the clinical application of stem cells to the repair of damaged cartilage; however, current cell therapies using expanded articular chondrocytes and adult stem cells, such as mesenchymal stromal cells face the problems of low yield of cells and their tendency to yield unstable cartilage that is later degraded or mineralized. A transient cartilage, destined to form bone, is typically found in the growth plate. In contrast, joint cartilage is a permanent cartilage generated from joint progenitor cells distinct from those for generating the growth plate cartilage. Therefore, we hypothesize that the embryonic joint progenitor would be the best cell-type for the regeneration of joint cartilage, which may also allow ligament regeneration. Pluripotent stem cells (PSCs), whether derived from an embryo, or induced from adult cells, are expected to differentiate into any cell-types of the human body through processes that mimic embryogenesis, making human (h) PSCs a promising source of embryonic cells for regenerative medicine. Therefore, we have been investigating the developmental process from human and mouse (m) PSCs to joint progenitor cells.

What is the molecular basis of human stem/progenitor cells to form permanent cartilage? We have previously developed and purified from hPSCs, progeny representing the three embryonic origins of chondrocytes, and demonstrated that they are able to expand and differentiate into corresponding chondrocyte precursors (chondroprogenitors). All such chondroprogenitors are capable of giving rise to hyaline cartilage in culture. However, most of them are mineralized and turned into bone when transplanted into immunocompromised mice, resembling growth-plate cartilage and adult stem cell-derived cartilage. In order to

establish methods to generate permanent cartilage that stays as unmineralized cartilage for a long time after transplantation, we aim to achieve the following two goals; 1) generating the embryonic joint progenitor from hPSCs, and 2) demonstrating that they allow the culture-made cartilage to be stably maintained even after transplantation, and that they show superior capacity of articular cartilage repair to currently available cells. We previously discovered a way to selectively generate, and to a limited extent, expand joint progenitor-like cells from hPSCs. To purify and further characterize the joint progenitor-like cells, we have generated hiPSC lines that carry fluorescence marker genes in the joint progenitor marker gene loci. Furthermore, we have recently discovered that cartilage made with the joint progenitor-like cells is in fact maintained as unmineralized cartilage in mice. In addition, we have determined that controlling cAMP signaling leads to cartilage displaying very limited bone forming capacity after transplantation (i.e. pseudo-permanent cartilage) even from the chondroprogenitors. Therefore, we are currently focusing both on the characterization of the joint progenitor-like cells to elucidate molecular basis of their permanent cartilage-forming capacity, and on the elucidation of signaling mechanisms required for the formation of permanent cartilage. We expect that such mechanistic studies may lead to a proper method to make clinically relevant adult stem cells more suitable for joint cartilage regeneration.

Large quantity of articular cartilage-forming cells; long-term expansion of PSC-derived human chondroprogenitors. Rapid loss of chondrogenic activity of adult stem cells during expansion culture is still a problem to be solved for application of them to cartilage regenerative therapy. We previously established culture conditions that maintained and expanded the hPSC-derived chondroprogenitors for an extended period of time, without loss of their cartilage-forming capacity. Such stable expansion of chondrogenic activity is currently hard to achieve with adult stem cells. We are interested in elucidating the mechanistic basis of such capacity, which may be applied to improve the expansion culture method for adult stem cells in the future.

RESEARCH PROJECTS

- Further defining the process of chondrogenesis from the hPSC-derived chondroprogenitors and joint progenitors to give rise to articular chondrocytes.
- Comparative omics analyses to elucidate the molecular basis of permanent, hyaline cartilage formation from the hPSC-derived joint progenitor-like cells.
- Joint articular cartilage repair with hPSC-derived chondroprogenitors and joint progenitor-like cells in immunocompromised animal models.

KEY PUBLICATIONS

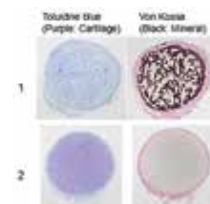
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LAB MEMBERS

Research assistant: Berke E. Sahbazoglu
Senior research associate and animal specialist: Nadine Matthias



Transient cartilage from standard chondroprogenitors (1), and permanent cartilage from joint progenitor-like cells (2) recovered from NSG mice after 8 weeks.



Pamela Wenzel, PhD

Associate Professor

Effects of flow on stem cell potential and cancer metastasis

Our lab studies how biomechanical force generated by the flow of blood in the circulatory system and lymph in the lymphatics impacts cell fate and behavior.

One of our 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, and we employ various tools to evaluate stem cell function, including microfluidics, pharmacology, mouse genetics, and transplantation assays.

Fluid flow and hydrostatic pressure have also been implicated in tumor biology, but it remains unclear what role lymphatic or vascular forces may play in regulating how aggressively cancer cells spread throughout the body. Using microchips designed to model the lymphatic vasculature, we modulate the frictional force experienced by cancer cells and evaluate the impact of fluid force on invasive potential and activation of oncogenic pathways that contribute to the systemic spread of cancer from the primary tumor. By application of bioengineering approaches to study the tumor microenvironment, we hope to identify new treatment options for patients affected by cancer.

RESEARCH PROJECTS

- Effects of flow on hematopoietic stem cell fate.
- Lymphatic flow as a regulator of the spread of cancer.

KEY PUBLICATIONS

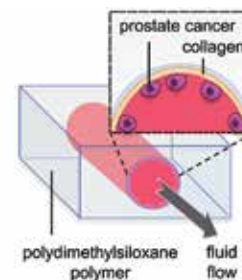
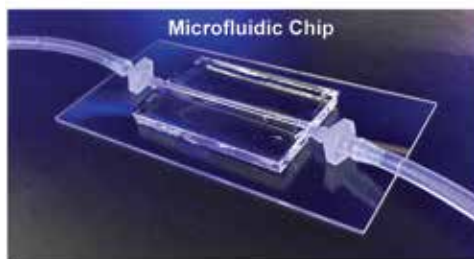
Lee, H.J.; Diaz, M.F.; Price, K.M.; Ozuna, J.A.; Zhang, S.; Sevick-Muraca, E.M.; Hagan, J.P.; and Wenzel, P.L. Fluid shear stress activates YAP1 to promote cancer cell motility. *Nature Communications*. 8:14122, 2017.

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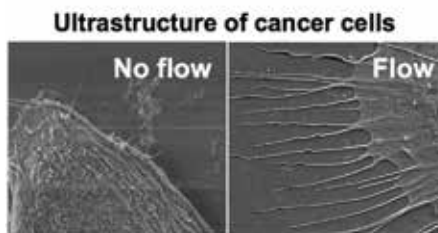
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LAB MEMBERS

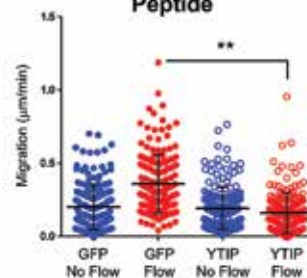
Graduate student: Paulina Horton
 Post-doctoral fellows: Amina Mohammadalipour, Sandeep Dumbali
 Senior research associate: Miguel Diaz



We engineer biomimetic microchips that permit real-time visualization of cancer cell migration and monitoring of gene activity under conditions that a cancer cell may experience during metastasis through the lymphatic vasculature. A microchannel embedded in the center of the microfluidics device is treated with collagen matrix, followed by introduction of cancer cells. Culture medium is pushed through the microchannel to mimic lymphatic flow.



Motility Reduction by YTIP Peptide



Flow elevates cancer cell motility, a process important for movement of cells from the primary tumor site to secondary metastatic sites in the body. The shape of the cell changes to accommodate increased movement, but the increased migration can be blocked by treatment with a small peptide YTIP that interrupts YAP1 function, a proto-oncogene, in the cancer cells. Asterisks represent a significant reduction in cancer cell motility by treatment with YTIP under fluid flow.



Jiaqian Wu, PhD
Associate Professor

Gene transcription and regulation of stem cell differentiation and neural injuries

Dr. Jiaqian Wu is an associate professor with tenure in the Vivian L. Smith Department of Neurosurgery and Center for Stem Cell and Regenerative Medicine. Dr. Wu led the NIH Mammalian Gene Collection effort and cloned thousands of mammalian genes which are publicly available through GE Dharmacon now. Dr. Wu was also 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, Dr. Wu has carried out unprecedented transcriptome profiling of eight highly purified neuron, glia, and vascular cells from brain by RNA-Seq. The Wu lab identified a large number of novel long non-coding RNAs, and they 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 that Dr. Wu is focusing on is spinal cord injury (SCI). The Wu lab has already published studies for acute and chronic SCI phases in mouse and rat contusive injury models. The Wu lab 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. Dr. Wu's 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, Dr. Wu has presented talks and lectures at national and international conferences and institutions. She has developed a patent, authored two books, and wrote many articles that have appeared in *Nature*, *PNAS*, the *Journal of Neuroscience*, *Plos Genetics*, *Genome Research*, and *Scientific Reports*, among others.

RESEARCH PROJECTS

- Wu laboratory 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 regula-

tory 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

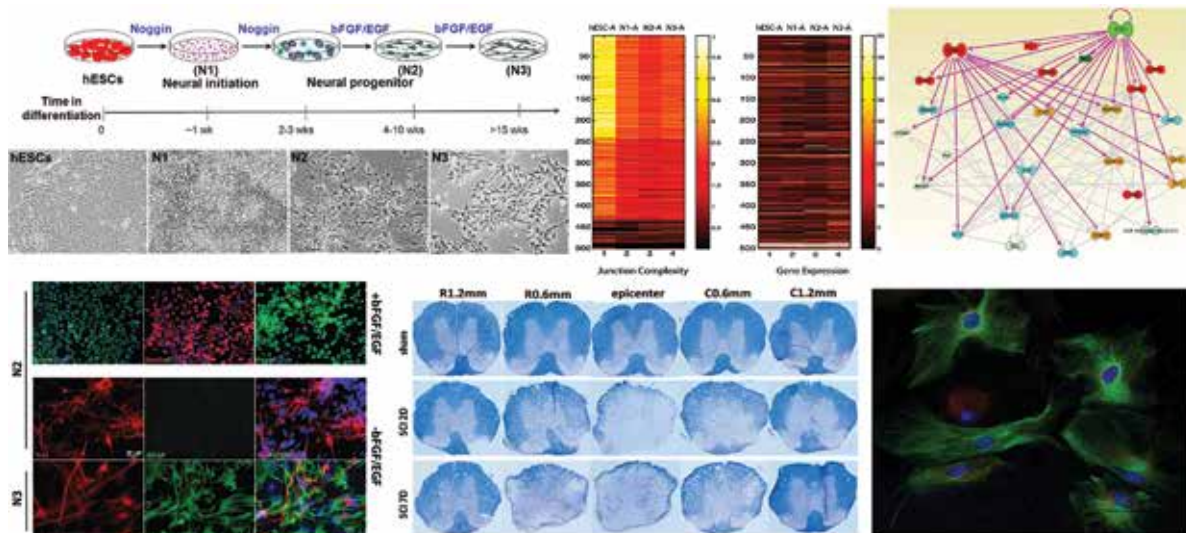
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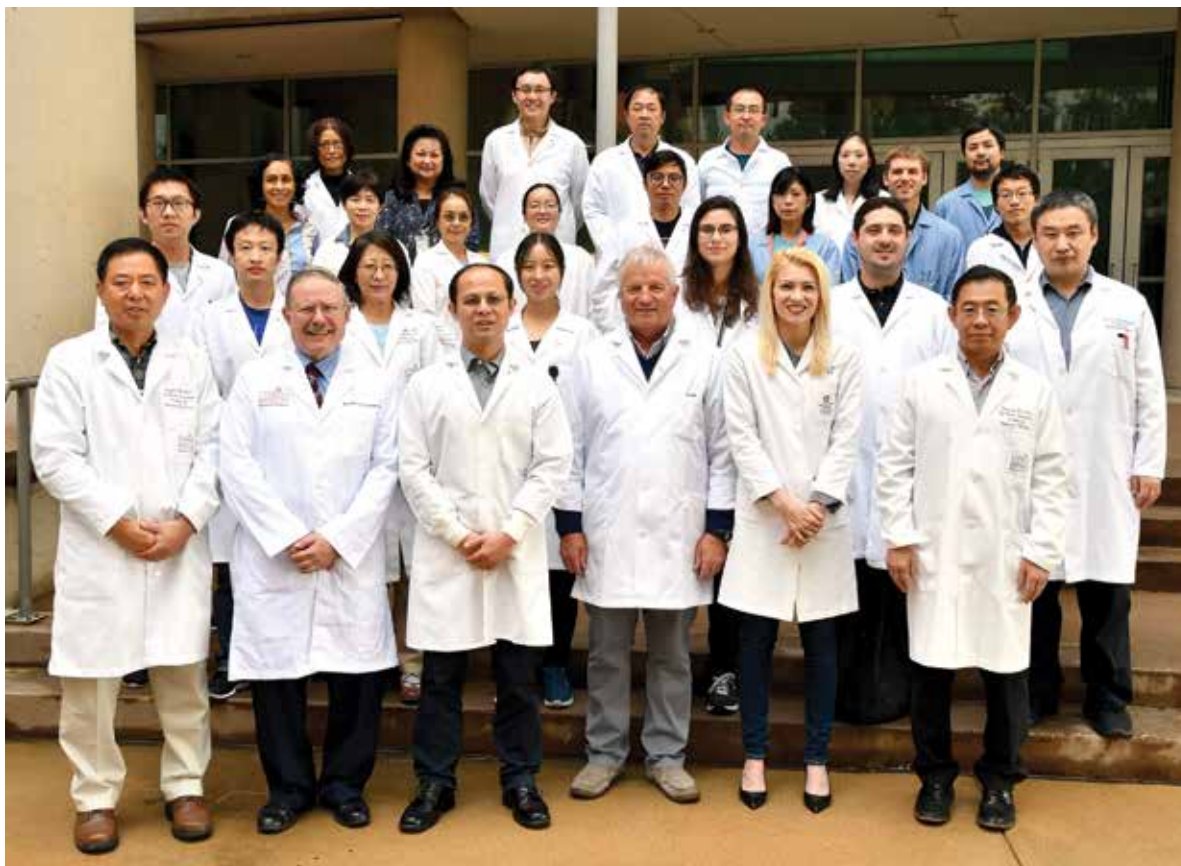
Long non-coding RNAs: important regulators in CNS and disorders. *Neuropathology and Applied Neurobiology*. 2019 Jan 13, doi: 10.1111/nan.12541. PMID: 30636336.

LAB MEMBERS

Post-doctoral fellows: Haichao Wei., PhD; Xizi Wu, MD, MS; Xu Li, PhD
Undergraduate student: Tanuj Prajapati



Wu 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.



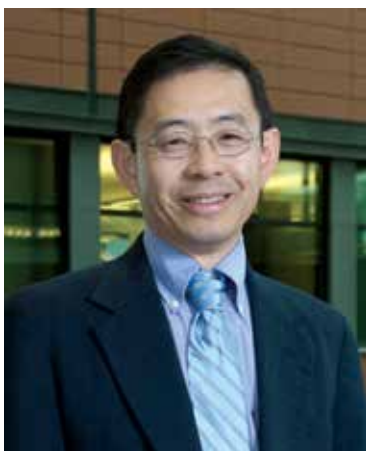
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston for the discovery, development, and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

TTI-IMM investigators have brought in significant funding from the pharmaceutical and the biotechnology industry, such as Merck, the National Institutes of Health, the Cancer Prevention and Research Institute of Texas, and the Department of Defense, and have made significant scientific discoveries in the areas of cancer biology, fungal natural products, and cancer 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, natural products, and synthetic small molecules 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 the basic and translational research programs, TTI has built a major drug discovery platform focusing on the therapeutic monoclonal antibody lead discovery optimization and development. During the last nine years, TTI has established a network of collaborators from institutions across Texas and the nation with 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. Five TTI inventions have been licensed to biotech companies for drug development. These 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 three major research areas.

RESEARCH PROJECTS

- Cancer antibody drug resistance mechanisms. Immune suppression is recognized as a hallmark of cancer, and this notion is largely based on studies on cellular immunity. Our recent studies have demonstrated a new mechanism of cancer suppression of immunity by impairment of antibody effector function mediated by proteolytic enzymes in the tumor microenvironment.
- Antibodies response to viral infections and vaccination. Design of highly immunogenic vaccines that induce neutralizing antibodies against a broad range of clinical isolates is one of the approaches in developing effective viral vaccine. We have an on-going project to aid the design of HCMV and dengue vaccines by profiling antibody response to the experimental vaccines in rhesus and humans.
- 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 collaborative antibody drug discovery projects targeting various cancer types.

KEY PUBLICATIONS

Xiaohua Ye; Xun Gui; Daniel C. Freed; Zhiqiang Ku; Leike Li; Yuanzhi Chen; Wei Xiong; Xuejun Fan; Xi He; Jian Liu; Hua Zhu; Dai Wang; Ningyan Zhang; Tong-Ming Fu; Zhiqiang An. 2019. Identification of APMAP as a novel modulator of HCMV infection. *PLoS Pathogens* 15(7): e1007914. <https://doi.org/10.1371/journal.ppat.1007914>.

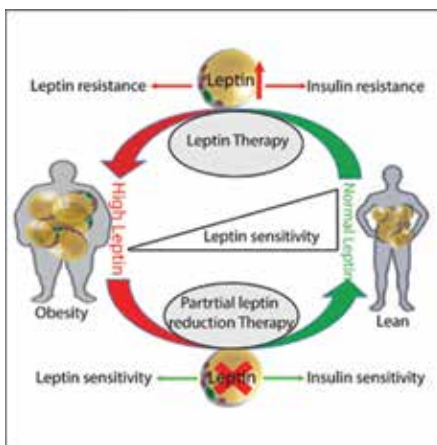
Xun Gui; Mi Deng; Hao Song; Yuanzhi Chen; Jingjing Xie; Zunling Li; Licai He; Fangfang Huang; Yixiang Xu; Yasuaki Anami; Hai Yu; Chenyi Yu; Leike Li; Zihao Yuan; Xiaoying Xu; Qihui Wang; Yan Chai; Tao Huang; Yi Shi; Kyoji Tsuchikama; X. Charlene Liao; Ningshao Xia; George F. Gao; Ningyan Zhang; Cheng Cheng Zhang; and Zhiqiang An. 2019. Disrupting LILRB4/APOE interaction by an efficacious humanized antibody reverses T cell suppression and blocks AML development. *Cancer Immunology Research* doi: 10.1158/2326-6066.CIR-19-0036.

Shangang Zhao; Yi Zhu; Robbie D. Schultz; Na Li; Zhenyan He; Zhuzhen Zhang; Alexandre Caron; Qingzhang Zhu; Kai Sun; Wei Xiong;

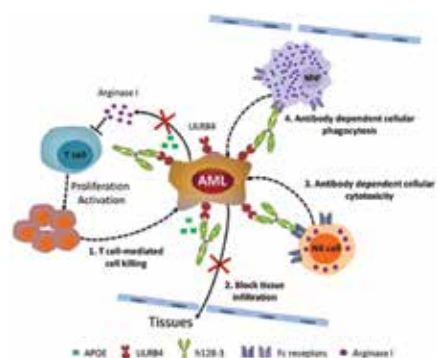
Hui Deng; Jia Sun; Yingfeng Deng; Min Kim; Charlotte E. Lee; Ruth Gordillo; Tiemin Liu; Angela K. Odle; Gwen V. Childs; Ningyan Zhang; Christine M. Kusminski; Joel K. Elmquist; Kevin W Williams; Zhiqiang An; and Philipp E. Scherer. 2019. Partial Leptin Reduction as an Effective Novel Weight Loss Strategy. *Cell Metabolism* doi: org/10.1016/j.cmet.2019.08.005.

LAB MEMBERS

Post-doctoral fellows: Zhiqiang Ku, Leike (Simon) Li; Xiaohua Ye; Peng Zhao; Lingxiao Tan; Junquan (Jake) Liu; Zihao Yuan
Graduate student: Hang Su
Research coordinator: Georgina T. Salazar



Partial leptin reduction as an effective novel weight loss strategy. In the context of obesity, partial leptin reduction restores hypothalamic leptin sensitivity and leads to reduced food intake, increased energy expenditure, and improved insulin sensitivity. Thus, strategies aimed at partially reducing circulating leptin may represent a promising approach for the treatment of obesity and diabetes (Zhao et al., 2019. *Cell Metabolism* doi: org/10.1016/j.cmet.2019.08.005).



Multiple mechanisms of a LILRB4 neutralizing antibody contribute to anti-AML activity. Mechanistic studies revealed four concordant modes of action for the anti-AML activity of a LILRB4 neutralizing antibody: 1) reversal of T cell suppression; 2) inhibition of monocytic AML cell tissue infiltration; 3) antibody-dependent cellular cytotoxicity (ADCC); and 4) antibody dependent cellular phagocytosis (ADCP). Therefore, targeting LILRB4 with antibody represents a new therapeutic strategy for treating monocytic AML (Gui et al., 2019. *Cancer Immunology Research* doi: 10.1158/2326-6066.CIR-19-0036).



Gerald F. Bills, PhD

Professor

Kay and Ben Fortson Distinguished Chair in Neurodegenerative Disease Research

Genome mining, biosynthesis, and discovery of microbial metabolites for infectious diseases and cancer therapies

Microorganisms have produced many of our most important drugs. Their hyper-biodiversity and genetic capacity for synthesis of organic molecules continue to yield breakthrough molecules for invention in human disease. Multidisciplinary microbial biomedical research in the Texas Therapeutics Institute and the Institute of Molecular Medicine brings together members of our lab and collaborators from diverse backgrounds, including pharmaceutical sciences, organic chemistry, biochemistry, molecular biology, and microbiology. Our research involves testing microbial natural products for therapeutic applications, making natural products through fermentation to support medicinal chemistry synthesis, and elucidating biosynthetic pathways of bioactive natural products. We seek to test various hypotheses that natural product-producing microorganisms harbor biosynthetic gene clusters and novel biosynthetic mechanisms that can be harnessed to generate new bioactive chemistry useful in intervention in infectious diseases and cancers. In parallel, we use pathway genetics and genomic manipulation in the producing organisms to aid in supplying large quantities of these natural products to support synthesis of new derivatives.

Our lab employs genomics to interpret and predict genetically encoded chemical diversity of microorganisms using filamentous fungi as model organisms, especially for biosynthetic families relevant for pharmaceutical intervention in human diseases. For example, we have characterized biosynthetic pathways responsible for the family of echinocandin antifungal drugs, including pneumocandin B0, the starting molecule for the antifungal drug CANGIDAS. We have re-programmed pneumocandin biosynthesis to produce new strains with improved product purity and new analogues with increased potency. In parallel, we use pathway genetics and genomic manipulation in the producing organisms to aid in supplying large quantities of these natural products to support synthesis of new derivatives and overproduce drug-precursor

molecules.

We also carry out fundamental discovery of new bioactive natural products that inhibit growth of human pathogens, including *Cryptococcus neoformans*, a yeast causing *Cryptococcus meningitis* and cryptococcosis. Extracts of fermented fungi are evaluated for useful biological effects using an ensemble of assays directed at finding molecules that affect human pathogens. After preliminary chromatography, such as flash or column chromatography, active fractions of the extracts are identified through our bioassays against the target pathogens. More refined chromatographic techniques, e.g., preparative HPLC and bioautography, guide us to the activity-causing natural products. These extracts are available through collaborations with other academic and industrial laboratories.

RESEARCH PROJECTS

- Biosynthesis of natural products and pathway engineering for improved antifungals.
- Development of methods for reprogramming transcription of biosynthetic genes of fungi to discover or overproduce natural products useful for treating human diseases.

- Discovery of new antifungals and other therapeutic agents.

KEY PUBLICATIONS

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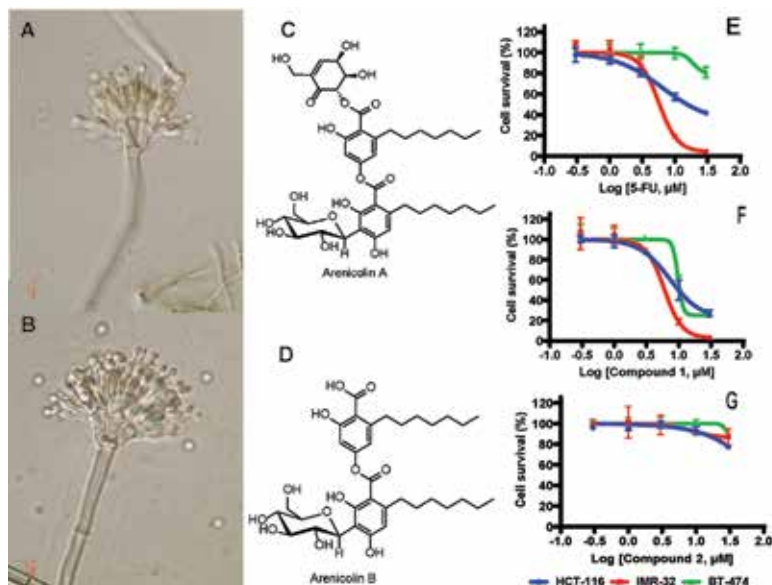
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Yue, Q.; Y. Li; L. Chen; X. Zhang; X. Liu; Z. An; and G.F. Bills. 2018. Genomics-driven discovery of a novel self-resistance mechanism in the echinocandin-producing fungus *Pezizula radicola*. *Environmental Microbiology* 20:3154-3167.

LAB MEMBERS

Post-doctoral fellows: Dr. Nan Lan, Dr. Bruno Perlatti

Research assistant: Mr. Travis Roeder



Discovery of the Arenicolins, novel C-glycosylated depsides from the fungus *Penicillium arenicola*. A-B. *Penicillium arenicola* strain NRRL 8095 from soil from British Columbia, Canada. C. Arenicolin A, note the unusual acylated 2-hydroxymethyl-4,5,6-trihydroxycyclohexenone responsible for selective cytotoxicity for human cell lines. D. Arenicolin B. E-G. In vitro toxicity assay on cancer cell lines HCT-116 (colorectal carcinoma), IMR-32 (neuroblastoma) and BT-474 (ductal carcinoma) for: E. 5-fluorouracil (positive control); F. Arenicolin A; G. Arenicolin B.



Kendra S. Carmon, MS, PhD

Assistant Professor

Therapeutic strategies for targeting colorectal tumors and cancer stem cells

Emerging evidence has shown that within several different malignant tumors types there exists a subpopulation of cancer cells that behave like normal stem cells. These cells are referred to as cancer stem cells (CSCs) or tumor-initiating cells since they have the capacity to fuel tumor growth. CSCs have been implicated in drug resistance, metastasis, and relapse, making them a major impediment for the effective treatment of cancer. Therefore, it is essential to develop novel therapies that can ultimately target and destroy CSCs.

Recent studies have established that LGR5 (Leucine-rich repeat-containing, G protein-coupled Receptor 5), a receptor expressed on normal adult stem cells, is highly upregulated in primary colorectal tumors. Furthermore, colon CSCs which express LGR5 are capable of driving tumor growth and metastasis. In addition, LGR5 expression has been shown to be significantly elevated in several other major tumor types, including liver, gastric, and ovarian cancers. My previous work led to the discovery that LGR5 functions as a receptor of secreted growth factors, called R-spondins, to promote cancer cell adhesion and regulate cell signaling pathways involved in stem cell survival and tumor growth. These findings suggest that LGR5 plays an important role in cancer and could serve as a novel target for the development of innovative therapies which can eliminate CSCs.

My current research is focused on investigating the function and cell signaling mechanisms of LGR5 in colon CSCs using colon cancer cell lines and patient-derived tumor models. This work will lead to identifying the role of LGR5 in the control of tumor growth, metastasis, and drug resistance. Furthermore, we are developing innovative therapeutics called antibody-drug conjugates (ADCs) that target and destroy colon tumors and CSCs, similar to guided missiles. ADCs are comprised of a highly specific monoclonal antibody attached to a cytotoxic chemical "warhead" that is only released once the ADC binds and enters target tumor cells. We have successfully generated LGR5-targeted

ADCs that incorporate the cytotoxin monomethyl-auroistatin E (MMAE) and showed they could destroy colon cancer cells and tumors in mice. Currently, we are taking novel approaches to modify and improve our LGR5-targeting ADCs in order to effectively treat a larger number of tumors. Our lab is also identifying and characterizing new cancer targets for ADC development. In collaboration with Dr. Ali Azhdarinia's group, we are using PET/CT imaging to select optimal therapeutic antibodies and evaluate them as diagnostics to stratify tumors which would respond best to LGR5-targeted ADC therapy. Our work will lead to the elucidation of the function and mechanism of LGR5 in CSCs and generate innovative therapeutic leads to target CSCs for the treatment and eradication of colon cancer.

RESEARCH PROJECTS

- Development of antibody-drug conjugates to target colon tumors and cancer stem cells.
- Investigation of the LGR5 function in cancer stem cells, metastasis, and drug resistance.
- Identification of novel therapeutic targets and associated signaling pathways in colon cancer.

KEY PUBLICATIONS

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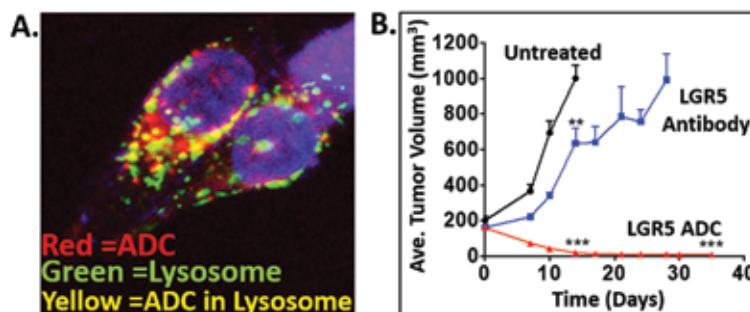
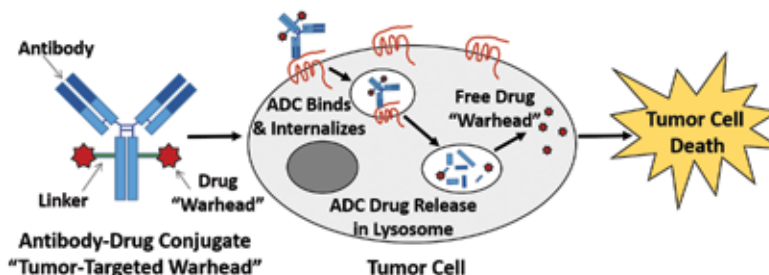
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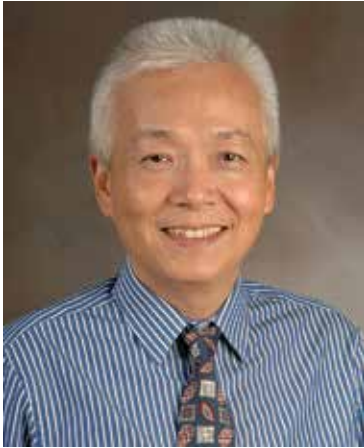
LAB MEMBERS

Research associate: Sheng Zhang

Students: Tressie Posey, Treena Chatterjee



Schematic of an antibody-drug conjugate (ADC) and its mechanism of action in tumor cells. Experiments show (A) LGR5-targeted ADCs internalize within a colorectal tumor cell for drug release and (B) ADC treatment eliminates tumors in mice.



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 disease relevance of this family of important signaling molecules.

Recently, we have identified a potential use of EPAC inhibitors in the prevention and treatment of fatal rickettsioses. Currently, we are actively engaged in developing second generation isoform specific EPAC inhibitors and agonists and in exploring their potential uses in various human diseases including chronic pain and cardiovascular diseases.

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 EPAC for the treatment of microbial infections caused by tick-borne bacteria Rickettsia.

KEY PUBLICATIONS

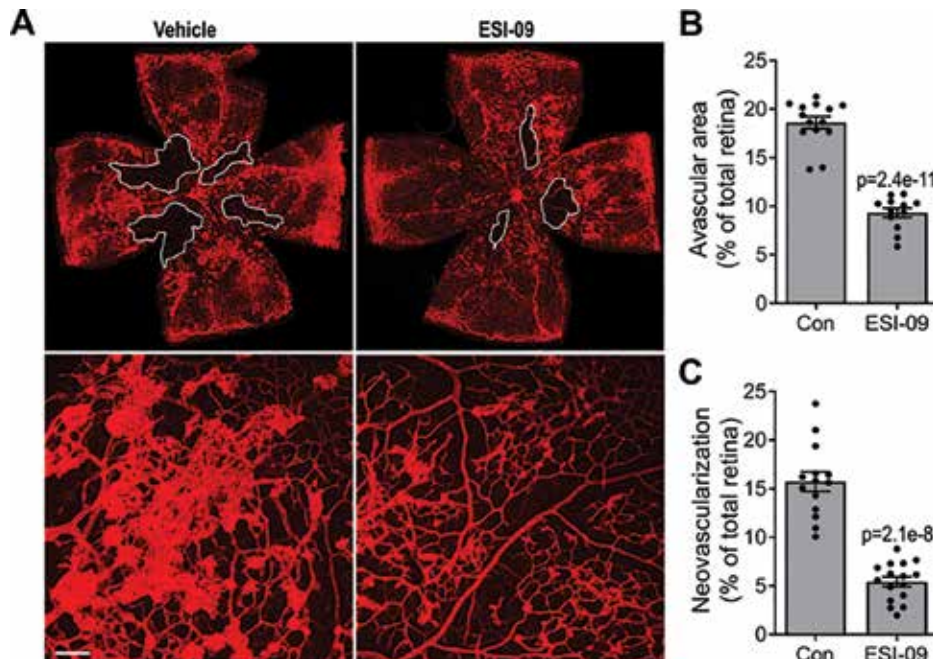
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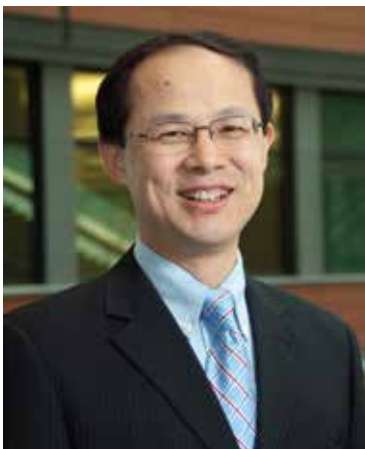
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LAB MEMBERS

Research assistant professor: Fang Mei
 Research scientist: Wenli Yang
 Instructor: William Robichaux
 Research associate: Wei Lin



Epac1 in pathological angiogenesis and as a therapeutic target for retinopathy. (A) Pharmacological inhibition of Epac prevents OIR-induced neovascularization. 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. (B, C) Graphs represent avascular and neovascularization area at P17.



Wenliang Li, PhD

Associate Professor

Studying and targeting cancer metastasis and drug resistance

Dr. Wenliang Li's research programs are (1) to obtain critical new knowledge of cancer metastasis and drug resistance of human cancer cells, and (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 a 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, Dr. Li's 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 topic in Dr. Li's lab is to investigate the mechanisms of cancer cell plasticity and drug resistance. In particular, Dr. Li studies how prostate cancers become resistant to new generations of androgen receptor pathway inhibitors (ARPIs) and how non-small cell lung cancers (NSCLC) become resistant to EGFR inhibitors. The common theme in this topic 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 of these two major, solid cancer types. Upon the acquisition of resistance to ARPIs, some AR-positive prostate adenocarcinoma cancers become AR-low/negative aggressive neuroendocrine prostate cancers. Similarly, after becoming resistant to EGFR inhibitors, some NSCLC demonstrates phenotypes of small cell lung cancer, which is neuroendocrine in nature and very aggressive. NED is still poorly understood, and currently there are no effective treatments, to prevent or overcome drug resistance related to NED. Dr. Li investigates the underlying mechanisms of NED, cellular plasticity and drug resistance, especially the roles and mechanisms of action of several novel epigenetic regulators.

RESEARCH PROJECTS

- Mechanisms of action for novel regulators of cancer metastasis.
- New pathways and mechanisms of epithelial-mesenchymal transition.
- Lineage plasticity and acquired resistance to cancer therapeutics.
- Epigenetic mechanisms of beta adrenergic signaling in tumor progression and angiogenesis.

KEY PUBLICATIONS

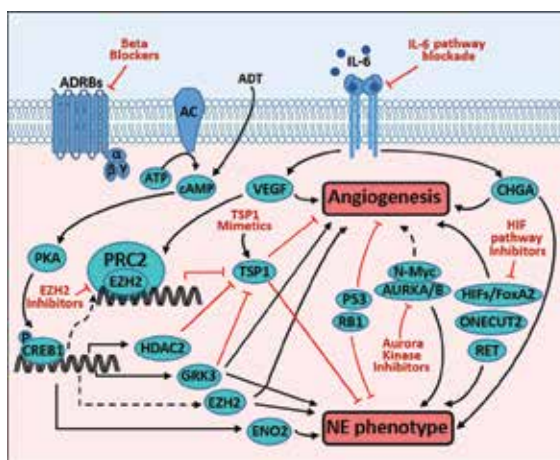
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LAB MEMBERS

Post-doctoral fellows: Zheng Wang, Lijuan Zhuo.
Research assistant II: Han Yang



Targeting molecular links for angiogenesis and neuroendocrine differentiation, two prominent phenotypes in advanced prostate cancers. Activated CREB1 directly induces transcription of several genes involved in neuroendocrine differentiation (NED) or angiogenesis. GRK3 and HDAC2 promotes angiogenesis, at least in part through downregulating TSP1, an anti-angiogenesis factor. CREB1 activation also enhances the PRC2 function of EZH2, which is critical for NED and angiogenesis induced by androgen derivation therapy. Several other players/pathways also contribute to both phenotypes. Novel strategies targeting these pathways may be effective to treat neuroendocrine prostate cancers that are lethal with no effective treatment.



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 have now uncovered how RSPOs and LGRs work together to regulate cell growth and migration. In particular, we uncovered that RSPO3-LGR4 has a major role in the aggressiveness of lung adenocarcinomas and colorectal cancer. Most recently, we showed that drug conjugates of anti-LGR5 antibodies showed excellent anti-tumor efficacy in preclinical models of colon cancer. We have also identified and characterized a series of anti-LGR4 antibodies and generated their drug conjugates. The modified antibodies displayed robust anti-tumor activity in animal models of colorectal and ovarian cancer. Our current efforts are focused on further optimizing

these drug leads targeting the RSPO-LGR system as potential treatment for colorectal cancer and other types of malignancies. Recently, we have discovered a novel approach that can target all three LGR receptors for the treatment of cancers of the digestive system. We also discovered that LGR5 interacts with the Wnt signaling complex directly, as shown in the picture.

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 cancer types.

KEY PUBLICATIONS

Carmon, K.S.; Gong, X.; Lin, Q.; Thomas, A.; and

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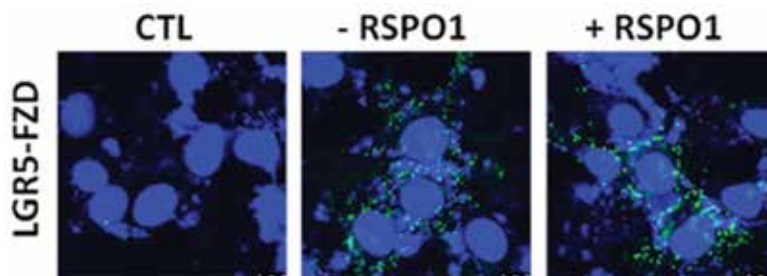
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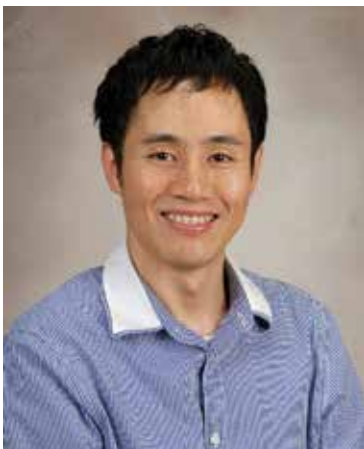
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LAB MEMBERS

Post-doctoral fellows: Soohyun Park, Jianghua Tu
Sr. research associates: Wangsheng Alice Yu, Ling Wu



LGR5 directly interacts with the Wnt receptor Frizzled (FZD) in the presence and absence of R-spondin1 (RSPO1) as shown the proximity ligation assay. Two molecules can be linked (ligated) to give a signal only if they are located next to each other. Each green dot represents an amplified fluorescence signal as a result of the ligation of LGR5 with FZD. Blue represents staining of the nuclei of the cells. CTL = control experiment (no FZD). - and + RSPO1 = the absence and presence of RSPO1, respectively.



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 5 FDA-approved ADCs (Adcetris[®], Kadcyca[®], Besponsa[®], Mylotarg[®], and Polivy[®]) and more than 80 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 and 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 needs to selectively deliver and release payloads only at the tumor sites. 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. Unlike the conventional valine-citrulline linker that is known to undergo degradation in mouse circulation, our tripeptide linker is stable in both human and mouse plasma, maximizing ADC circulation stability and therapeutic efficacy. Recently, we developed dual-loading ADC linkers, enabling simple and easy installation

of two distinct drug molecules onto a single antibody (Figure 1). This dual-loading linker enables construction of a variety of ADCs with high homogeneity and defined drug-to-antibody ratios. We have demonstrated that our dual-drug ADCs exert greater therapeutic effect in mouse models of human breast cancer than can be achieved by conventional single-drug variants. Notably, one of the dual-drug ADCs was more efficacious in a clinically relevant, heterogeneous breast tumor model than a combination of two single-drug ADCs (manuscript in preparation). Taken together, these technologies could add flexibility to ADC molecular design and maximize ADC therapeutic efficacy, which may help increase success rates in preclinical studies.

With our technology platform in hand, we are currently pursuing next-generation ADCs for combating the cancer drug resistance and heterogeneity issues. These are unsolved issues in cancer chemotherapy leading to discontinuation of medication and recurrence of malignancy. Another research direction in my lab is to improve antibody and ADC delivery efficiency to the brain and brain tumors. We envision that our novel ADC linkers will help not only ourselves but also other researchers establish various approaches for overcoming such unsolved issues in cancer management.

RESEARCH PROJECTS

- Design, synthesis, and evaluation of novel branched 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
- Quorum sensing-guided drug delivery.

KEY PUBLICATIONS

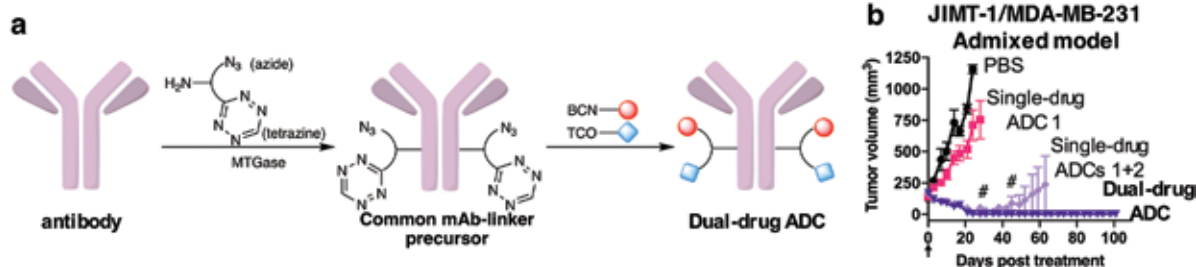
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Anami, Y.; Tsuchikama, K. * (2019) Transglutaminase-Mediated Conjugations, *Methods in Molecular Biology*, 2078:71-82.

LAB MEMBERS

Post-doctoral fellows: Yasuaki Anami, PhD, Chisato Tsuchikama, PhD, Aiko Yamaguchi, PhD
Research assistants: Travis Roeder



Novel ADC linker technologies developed by our lab. (a) Construction of dual-drug ADCs with high homogeneity using branched linkers. (b) In vivo comparison of ADCs equipped with single or dual drugs in the JIMT-1/MDA-MB-231 admixed xenograft mouse model (n = 5). All tumor-bearing mice were treated with a single dose of each ADC (3 mg/kg) at Day 0 (indicated with a black arrow).



Ningyan Zhang, PhD

Associate Professor

Cancer resistance mechanisms to therapeutic antibodies and modulation of anticancer immunity

Monoclonal antibodies are becoming a major drug modality for cancer treatment and have shown clinical success for treatment of various types of cancer. Tumor targeting monoclonal antibodies, such as trastuzumab against HER2 and bevacizumab targeting tumor angiogenesis factor VEGF, have been successfully used for treatment of many types of cancer. However, both innate and acquired resistance to these therapeutic antibodies are widely reported. Understanding the mechanism of cancer resistance to therapeutic antibodies is of paramount importance for improvement of these cancer targeted therapies to benefit more cancer patients. Our current research programs are centered on better understanding of tumor evasion of antibody immunity and develop therapeutic strategies to modulate anticancer immunity for improvement of cancer treatment.

Cancer immune evasion is recognized as a hallmark 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 recent 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. To test our hypothesis, 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 and cellular immunity 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

Leike Li; Weixu Meng; Melanie Horton; Daniel R. DiStefano; Elizabeth A. Thoryk; Qihui Wang; Georgina T. Salazar; Jennifer M. Pfaff; Trevor Barnes; Benjamin J. Doranz; Andrew J. Bett; Danilo R. Casimiro; Kalpit Vora; Zhiqiang An; Ningyan Zhang. * (2019) Potent neutralizing antibodies

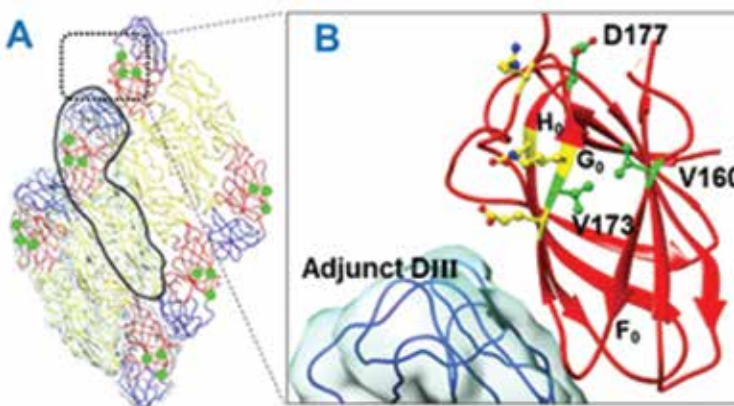
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LAB MEMBERS

Research associate/scientists: Hui Deng, MS; Xuejun Fan, MD, PhD; Wei Xiong, PhD
 Post-doctoral fellows: See the list under Dr. Zhiqiang An's group



Antibody d462 epitope mapped on envelope (80E) protein structure of dengue virus (PDB, 4CBF). The black line (A, on the left) shows the boundary of a single asymmetric unit. The residues that interact with d462 are shown as green spheres. Enlarged view (B, on the right) shows the epitope region (V160, V173, D177) on FO, GO β -strands, and the GOHO loop on E protein domain I. Atoms colored red for O, blue for N. From L. Li et al. (2019) *PLoS Pathogens*.

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, and 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 the innovative 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 technique used to analyze the characteristics of individual cells suspended in fluid. Typically, a variety of cellular components are fluorescently labelled and then passed in front of lasers of varying wavelengths. 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 up to 19 fluorescent signals from thousands of individual cells per second. The Flow Cytometry Service Center offers FACS acquisition and analysis, cell sorting, and consultation for experimental design, interpretation, and troubleshooting. Our instruments are available on a fee-for-service charge to all research investigators from UTHHealth and external organizations.

TRANSGENIC AND STEM CELL SERVICE CENTER

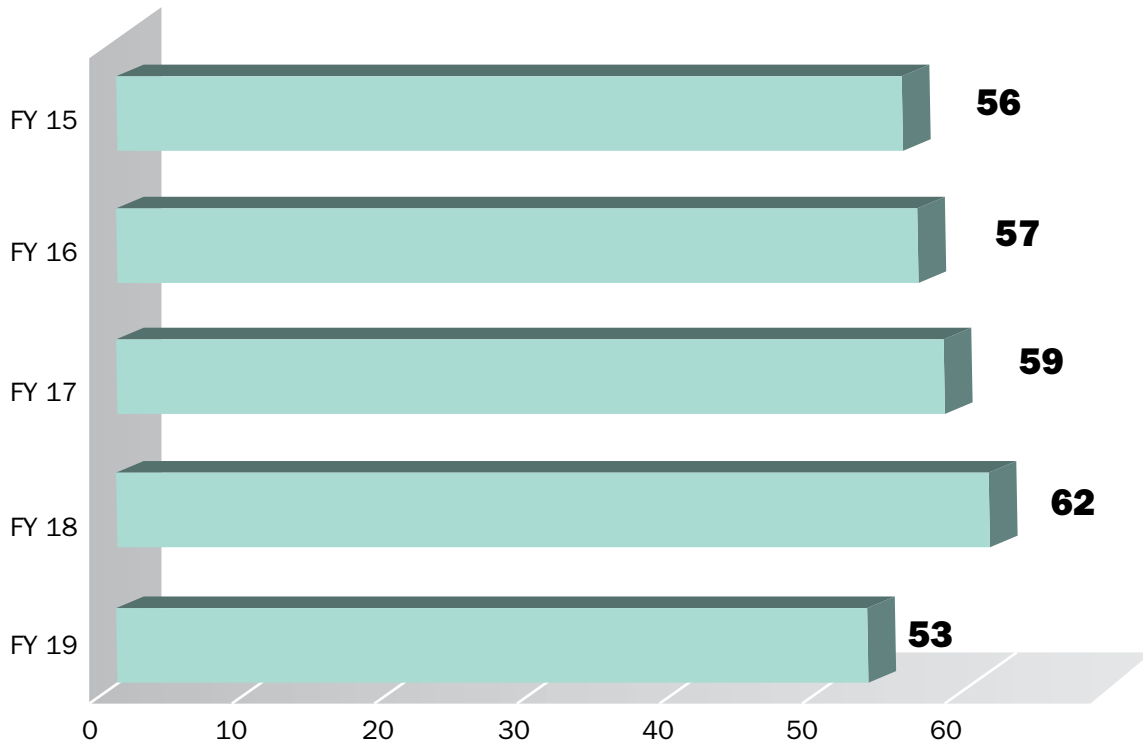
Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cells service center which was established in 1998. It has generated over 800 new transgenic and knock-out mouse animal models for all research investigators from UTHHealth and external organizations on a fee-for-service basis.

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 cells research.

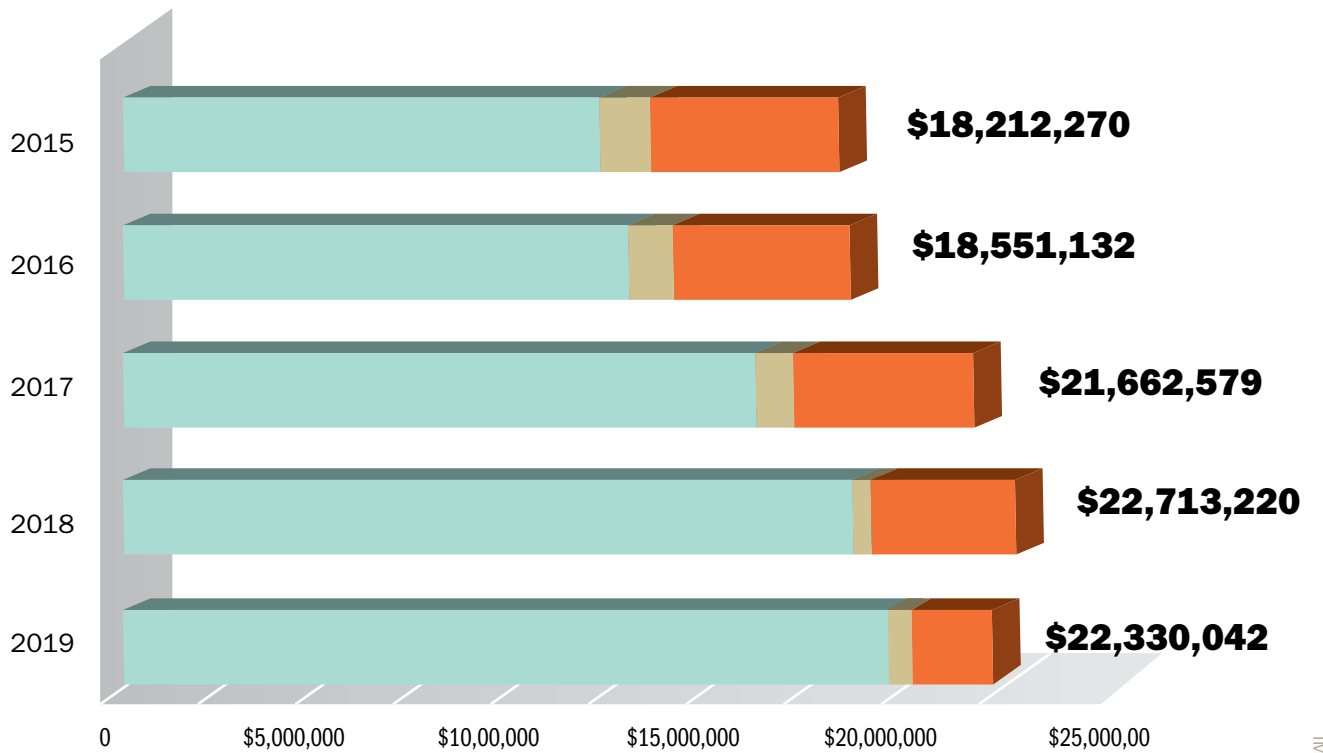
NANO 3D PRINTING SERVICE CENTER

Nano 3D Printing Service Center provides state-of-the-art 3D printing services. We provide 3D printed models of human organs and novel surgical tools, in prototype or final production models. We have both traditional FDM (Fortus 450mc) thermoplastic and 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) are available. STL files, SolidWorks, or medical imaging files can produce 3D Models. We are located on the third floor of the Faye S. Sarofim Research Building.

NUMBER OF FACULTY



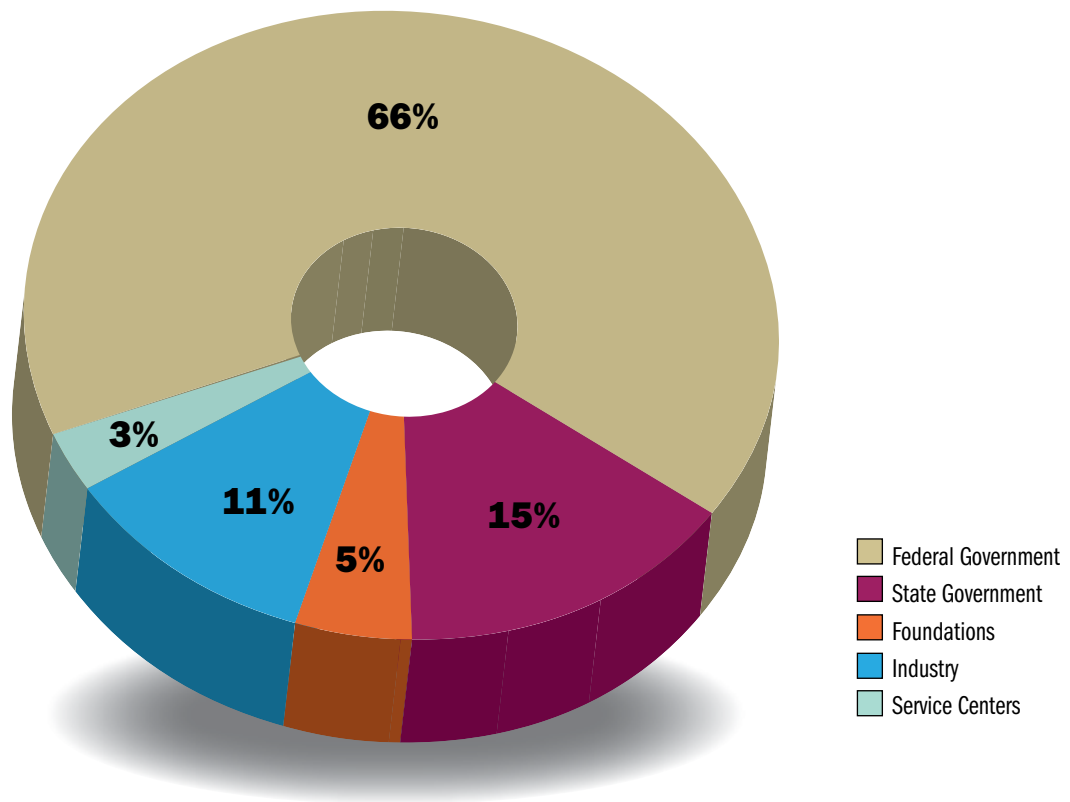
TOTAL FUNDS SUPPORTING RESEARCH



- Endowment/Gifts
- Service Center
- Sponsored Projects

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

TOTAL EXPENSES SUPPORTING RESEARCH



GIFT REPORT

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 TO RECRUIT AND RETAIN TOP SCIENTISTS FROM AROUND THE WORLD.**

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