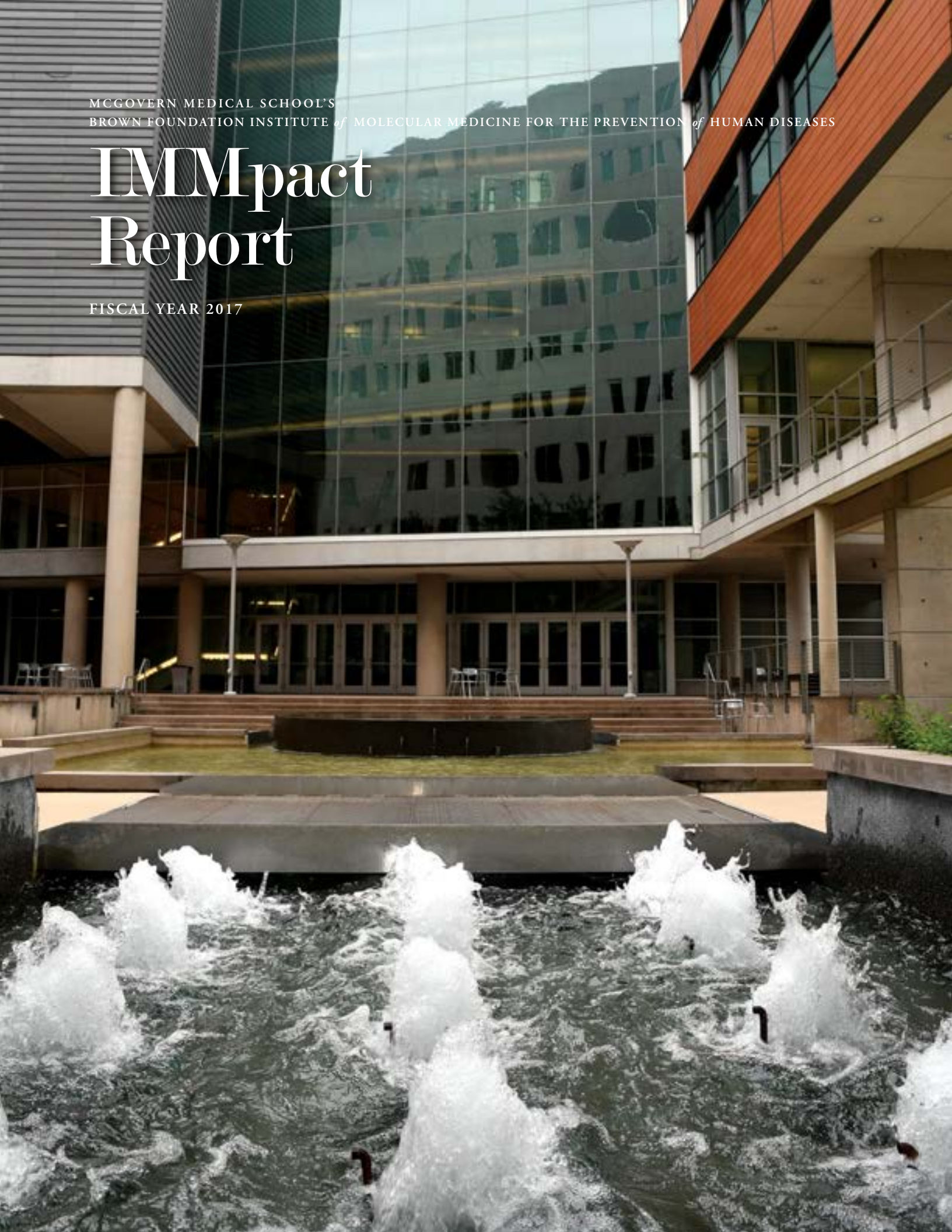

MCGOVERN MEDICAL SCHOOL'S
BROWN FOUNDATION INSTITUTE *of* MOLECULAR MEDICINE FOR THE PREVENTION *of* HUMAN DISEASES

IMMpact Report

FISCAL YEAR 2017

About the cover

It's a beautiful day at McGovern Medical School's Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases.

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McGovern Medical School.
All correspondence should be addressed to:
Office of Communications
6431 Fannin, B.340
Houston, TX 77030
E-mail: m.darla.brown@uth.tmc.edu

Editor
Darla Brown, Director, Office of Communications

Contributors:
Darla Brown
Rob Cahill
Jonathan Garris
IMM Faculty

Design:
Roy Prichard

Photography:
Dwight Andrews
Maricruz Kwon

For information on supporting programs at the McGovern Medical School and the IMM, contact Lise Cameron, 713.500.3602, Allison.H.Cameron@uth.tmc.edu

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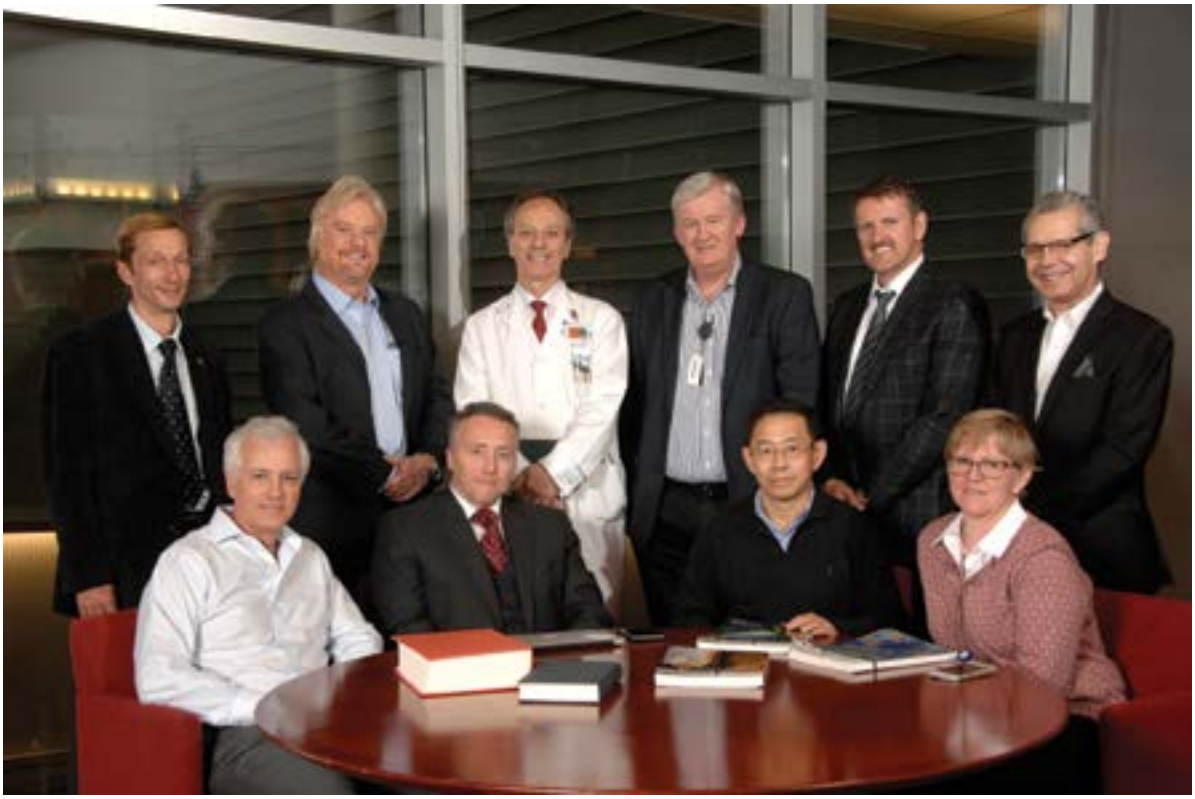

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Director's Message

The IMM has two major objectives:

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

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

I'm pleased to introduce the latest annual IMMpact report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). The IMM is a stand-alone research institute that is embedded within McGovern Medical School. The IMM mission is to deliver translational outcomes from research in molecular medicine that benefits patients. 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.

This year we have recruited additional outstanding new faculty, who bring with them exciting research ideas and innovative technologies. Two of our new recruits, whose stories are featured, also secured prestigious STARs awards from The University of Texas System, which are reserved for the recruitment of highly sought-after scientists. 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 some 30 percent over the preceding year, which in turn had seen a considerable increase over the prior year. 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 foundations, industry collaborations, and, most importantly, the continuing generosity of our friends and donors.

In addition to advancing science and medicine, we 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 delighted to talk with you personally. Alternatively, I would be delighted to see you at our annual IMMpact symposium. Last year 165 guests listened to three 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 on April 18, 2018, and will feature talks on how to use new gene editing technologies to correct inherited diseases. The format will be slightly different from previous years in that we will have an extended question time with an expert panel comprising the speakers plus UTHealth physicians. 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, M.A., M.B., B.Chir., Ph.D., Sc.D.
Executive Director, Institute of Molecular Medicine
John S. Dunn Distinguished University Chair in Physiology and Medicine

Mission

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

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

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

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

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

Our Locations

Fayez S. Sarofim Research Building

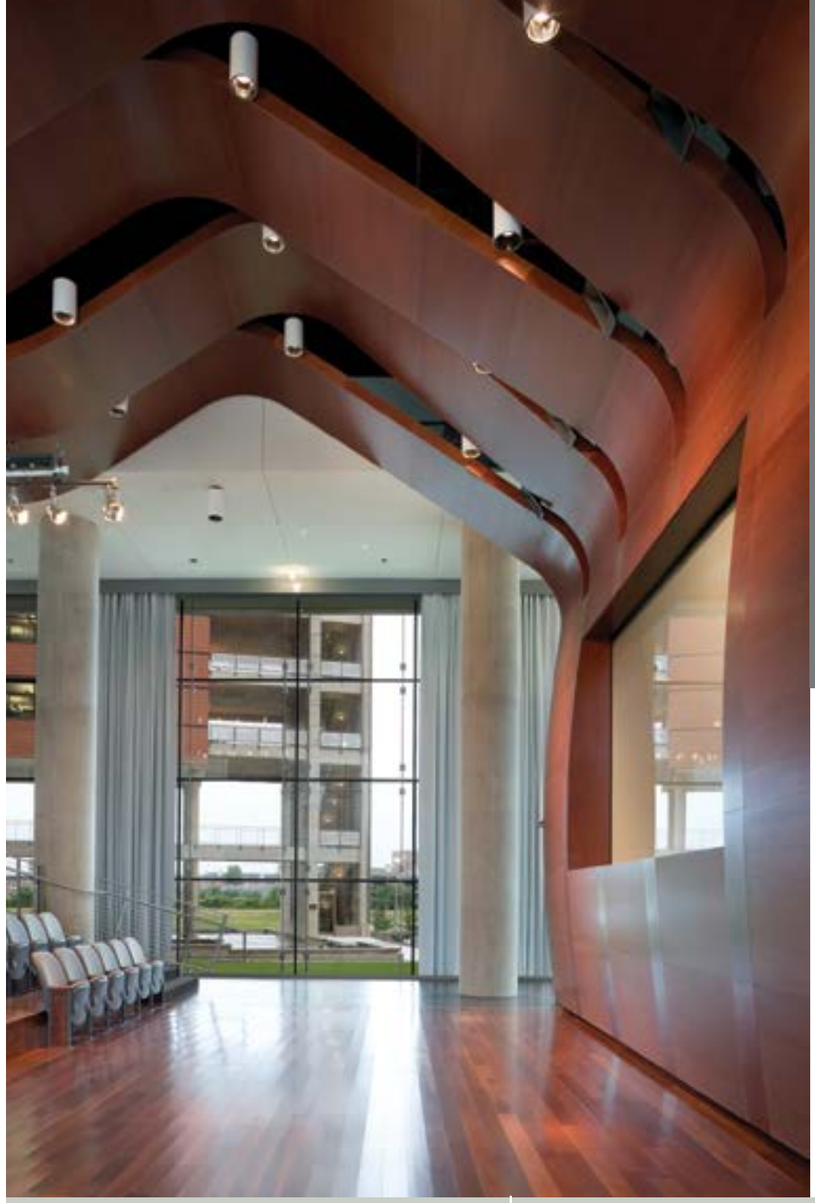
- Primary home of the IMM's faculty, administration, and support staff.
- Located adjacent to the The University of Texas Health Science Center at Houston (UTHealth) University Center Tower within the Texas Medical Center.
- Opened in 2006, the building encompasses 255,748 gross square feet.

South Campus Research Building – 3 (SCRB3)

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

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

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



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

IMMpact Symposium

Wednesday
April 18, 2018
4:00-6:30 p.m.

Fayez S. Sarofim Research Building
1825 Pressler Street

Editing the human genome: the future is now

Cut, Copy, Paste: Gene correction in Cystic Fibrosis

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

Muscular Dystrophies: new hope from gene editing

Radbod Darabi, MD, PhD
Assistant Professor, Center for Stem Cell and Regenerative Medicine

Save the Date

Gene editing – it starts in the lab

All green thumbs know that pruning is key to a healthy garden. Just as all good writers know that editing keeps copy sharp.

Brian Davis, Ph.D., director of the IMM's Center for Stem Cell & Regenerative Medicine, uses these concepts of pruning and editing in the laboratory to modify genes – weeding out the errors in hopes of making patients healthy again.

In simplified terms it goes something like this: Remove cells carrying a genetic disease from a patient. Precisely correct the identified, inherited mutation. Return the corrected cells to the same patient.

Dr. Davis' goal is to be able to complete this process successfully in laboratory animal models so that it can safely move to patients. A patient infused with healthy gene-corrected cells could potentially be cured of the genetic disease.

Dozens of diseases are linked to DNA mutations that are passed down from parent to child, such as sickle cell anemia or early onset Alzheimer's disease. New technology, such as CRISPR-Cas, meganucleases, zinc finger nucleases, and transcription activator-like effector-based nucleases, are the four types of technology known as "molecular scissors," allowing scientists to precisely trim the offending genetic mutation and replacing it with a healthy gene.

Dr. Davis' lab is working to correct damaged lung and blood stem cells. The lung cells are from patients with cystic fibrosis, a genetic lung disease that affects more than 30,000 people in the United States. The blood cells are from immune deficiency patients who suffer from recurrent infections.

"Our goal for the lung is to isolate, expand, and edit the stem cells as they exist in the airway and deliver them back healthy to the patient," Dr. Davis says. "The lung might not be the easiest place to reintroduce a stem cell, so we are also considering targeting gene editing *in vivo*. We don't know which approach will be the best bet."

There are already approved therapies using stem cells in patients. For example, blood stem cell therapy is being used in some cancer patients who receive high doses of chemotherapy or radiation. The renewal of stem cells allows patients to replenish their blood, which can be damaged by cancer treatment. Induced pluripotent stem cells are in clinical trial to treat retinal degenerative diseases, such as macular degeneration, with the goal of saving patients' eyesight. And the nation's first live gene editing took place in California in November in a patient with the rare disease Hunter syndrome.

Dr. Davis says the rapid progress in the field of stem cells has made these advances possible.

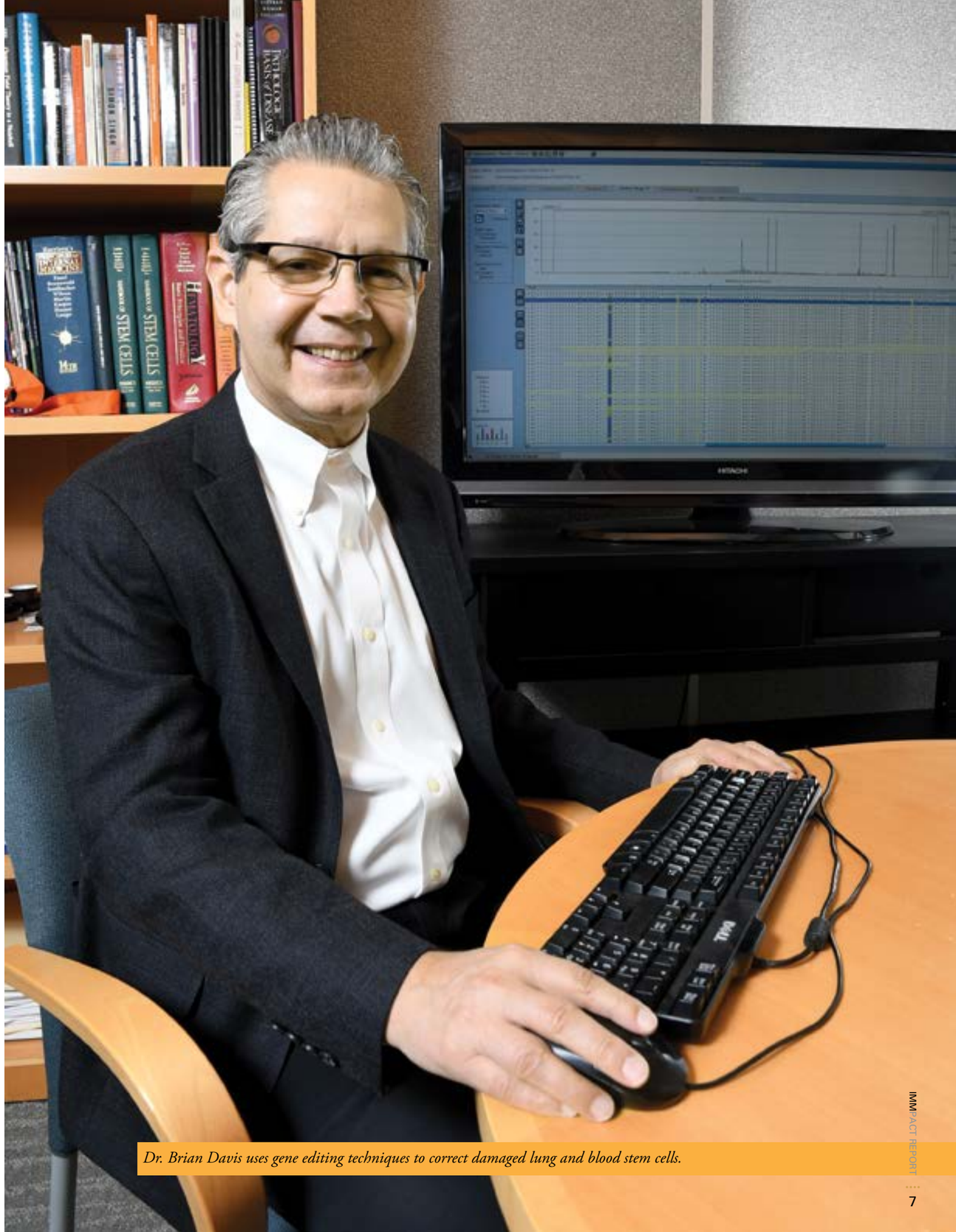
"Ten years ago we did not imagine such a robust method to create stem cells through induced pluripotent stems cells – whereby we can take a cell from an adult hair or blood cell and create the type of stem cell we need and returning organ-specific cells (e.g. lung cells) to that patient," he explains. "This eliminates the need to use donor stem cells from another individual, minimizing the body rejecting the cell."

In addition to creating stem cells, the tools used to specifically correct gene defects are becoming more useful and precise. "We can highlight an area to cut and paste to make a specific genetic correction," Dr. Davis says.

Dr. Davis says he hopes to see gene editing move to clinical applications for patients with genetic lung diseases in 8 to 10 years and even sooner for blood diseases. "It's moving very rapidly," he says. "It's a market of ideas, and there are many teams of scientists around the world working on this. While the technology is moving fast, science and medicine have learned to be as precise as possible not to do something that would adversely affect the chromosomal DNA."

Before the research moves to the patients, funding is required to support the laboratory work.

"Cutting-edge research takes the support of forward-thinking philanthropic donors who see it as a wise development investment, something that will pay off in 5 to 10 years. The National Institutes of Health before providing their grant funding, typically likes to see 'proof of principle' demonstrations – and this is where philanthropic 'seed funding' is most valuable," Dr. Davis explains.



Dr. Brian Davis uses gene editing techniques to correct damaged lung and blood stem cells.

Moving promising therapeutics into practice

Nicknamed the "valley of death" by insiders, the gap between academic discovery and drug development is where most good ideas for new drugs fall to the wayside.

A commercial drug developer turned academic researcher, Zhiqiang An, Ph.D., has worked on both sides.

After 15 years in the pharmaceutical and biotechnology industry, mostly at Merck Research Laboratories, Dr. An was recruited in 2009 to lead the Texas Therapeutics Institute (TTI), an academic drug discovery center at the Institute for Molecular Medicine.

"We're bridging the valley of death at TTI by providing critical drug development technology and expertise to the academic research community," says Dr. An, the institute director. "The TTI has been successful in attracting more than $30 million in funding from federal, state, and industry sources in support of drug discovery."

The institute has established two biotech spinoff companies and so far has licensed out five drug candidates targeting cancer and spinal cord injury to the biotech industry for further development.

The institute's areas of expertise include antibody drug discovery.

"Antibodies are a part of our natural immune system, and they fight infectious diseases and cancer. The rapid rise of antibody-based therapies is largely due to their desirable safety profile, target specificity and efficacy," says Dr. An, the Robert A. Welch Distinguished University Chair in Chemistry.

Therapeutic antibodies represent one of the significant advances in the history of medicine, according to Dr. An, who is recognized worldwide for his research in this area.

In fact, he edited a scientific resource on the topic — "Therapeutic Monoclonal Antibodies From Bench to Clinic."

"We help scientists translate their basic discoveries into new treatments," Dr. An says. "Most of the therapeutic antibodies generated in academic laboratories do not advance beyond the basic discovery stage."

One reason for this limitation, according to Dr. An, is that many researchers do not have access to the highly specialized protein engineering technologies.

The TTI is making these antibody platform technologies available to Texas-based researchers and beyond.

So how do they work?

Antibodies can be engineered to bind to disease targets including receptors on cell surfaces and circulating proteins. In addition, they can be designed to carry toxins and radioisotopes to kill cancer cells.

Picture a miniaturized heat-seeking missile with a drug payload.

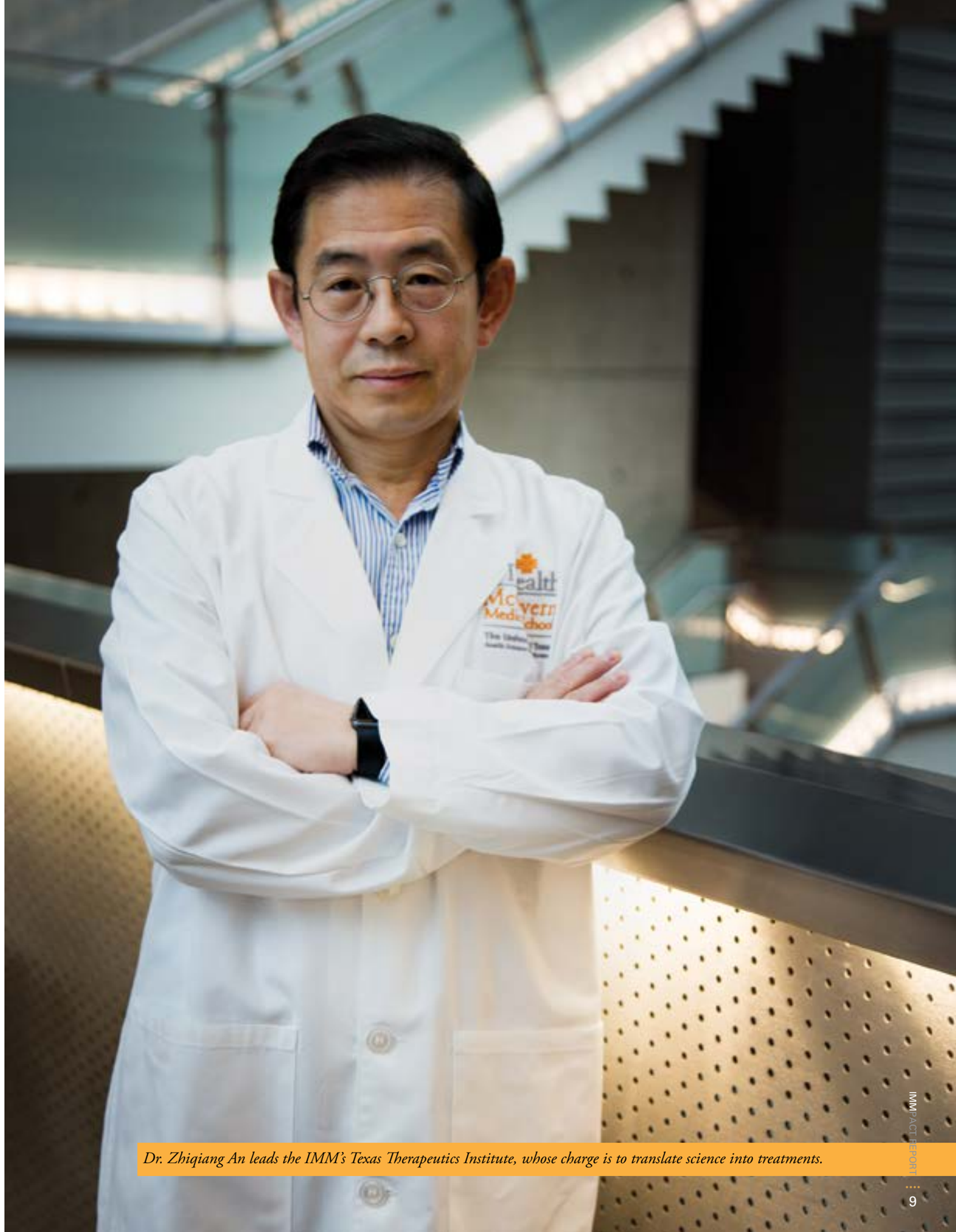
"Not limited to the treatment of disease, antibodies are also used to diagnose medical conditions by detecting disease biomarkers in cells, tissues, and in the human body," Dr. An says. "Antibodies are extremely sensitive and specific to the disease biomarkers."

When it comes to advancing promising treatments, Dr. An sees academia excelling in discovery and innovation.

Pharma and biotech companies on the other hand have the development and commercialization expertise.

"We are confident that TTI will continue to grow and play a critical role in bridging the 'valley of death' in drug discovery and in advancing the biotech industry in Houston and the State of Texas," Dr. An says.

> "The TTI has been successful in attracting more than $30 million in funding from federal, state, and industry sources in support of drug discovery." — Dr. Zhiqiang An



Dr. Zhiqiang An leads the IMM's Texas Therapeutics Institute, whose charge is to translate science into treatments.

Breathing new life into lung disease research

In the battle against lung disease and cancer, scientists are always looking for new platforms to use as a springboard for further research. Now, researchers at the IMM are turning their attention to stem cells as an emerging platform in the fight against diseases.

Sarah Huang, M.B.B.S., Ph.D., assistant professor in the Center for Stem Cell & Regenerative Medicine, understands the difficulties in developing models for lung diseases and stem cell therapy. The challenge for lungs specifically is that the organ has the most complex architecture and involves a considerable number of cell types.

Dr. Huang's lab is researching ways to develop *in vitro* lung tissue models for studies of lung disease and cell therapy and generate clinically applicable cell types for stem cell therapy. The first step in developing this platform was the creation of a step-wise differentiation strategy directing human pluripotent stem cells (hPSCs) to become different types of epithelial cells. Generating cells with the right maturity to be used in a clinical environment and act as a platform for studying diseases was the crux of the research.

"For stem cell therapy, we have to think about the integration of the cells," Dr. Huang says. "How do you put the cells in the body and make sure they can be integrated into the organ? The other challenge people always think about is what is the maturity of the cells that is most suitable for cell therapy? This is up to us to figure out."

Research currently shows that doctors may not need cells that are completely mature, such as post-natal cells, for therapy. Adult progenitor cells have been shown in many cases to be inadequate for cell therapy applications as they don't have high survival rates.

With cells generated from sources like embryonic stem cells, researchers could model whether or not a cell type is the origin for specific types of cancer. Dr. Huang's research during her time at Dr. Hans Snoeck's Laboratory at Columbia University showed the generation of a number of lung and airway epithelial cells from hPSCs, and her work has been successfully adopted by other research groups.

"In combination with CRISPR technology, we could do genome editing, and we could see the source of the mutation of the genes and the origin of the cancer cell," Dr. Huang says.

Dr. Huang received her M.B.B.S. from Xi'an Jiaotong University College of Medicine in China in 2000 and started focusing on lung diseases after joining the master of science program at Peking University Health Science Center in Beijing. Initially, she had been studying cancer, but later found herself drawn to stem cell therapy.

"The link between cancer and stem cells was intriguing to me," she says. "A lot of development pathways that were turned off after birth were turned on after getting cancer."

Dr. Huang has been taking her position at McGovern Medical School in stride and, after spending about a year in Houston, has established a variety of collaborative efforts. They include working with a bio-engineering group from Rice University, MD Anderson Cancer Center's Pulmonary Medicine, and various other collaborations inside the IMM.

"I cannot stop making collaborations," Dr. Huang says.

In the long-term, Dr. Huang has interest in developing a complex, three-dimensional culture of the lung and airway epithelial cells as part of a platform to help characterize how different types of cells could have different susceptibility to the human influenza virus. This also involves comparing different viral strains and following cells in the *in vitro* cultures, labeling them and observing how it proliferates.

"This has been done in mice, but it would be interesting to do it in a human culture," Dr. Huang says.

Dr. Sarah Huang's expertise in human pluripotent stem cells could be key in the fight against lung disease.

Entering uncharted genome territory in the fight against disease

Diving into the world of gene regulation is a demanding task. Our current understanding is far from complete, with much of the post-transcriptional regulation left clouded in relative mystery. Understanding regulation in gene expression can be a way to fundamentally grasp how common diseases arise from genetic variation.

Sidney H. Wang, Ph.D., assistant professor at IMM, aims to change that by exploring the development of new tools to examine RNA binding proteins and by exploring potential new coding genes and how they might function.

Dr. Wang says the field has become more complicated over the last decade with the advent of genome sequencing and the next generation of sequencing technology. Although we have the human genome sequenced, there are still unanswered questions lurking beneath what has already been discovered.

"I'm sure this is cliché to a lot of people, but we still don't know how to read most of the genome sequence," he says.

Researchers are adept at reading the coding regions with the help of the codon table - once you know the frame of the coding gene, researchers can reference the table and look up what amino acid is being encoded and you get the protein sequence. Many think only a small fraction of RNA transcribed by the genome is made into protein, but Dr. Wang's research has led him to have a different belief.

"Most people think that's only 2 percent of the genome, but one thing I found over my postdoc career is that it might be a bit bigger than 2 percent," Dr. Wang says.

By examining patterns of coding genes, Dr. Wang can re-evaluate how many RNAs are actually coding, or by looking closely at so-called non-coding RNA's. Some may still actually be making proteins but may have been overlooked due to strange, "non-typical processes." Part of the reason they have been overlooked is due to how researchers have examined transcripts, and, due to the sheer number of potential proteins, some of these fall outside of the cutoff point, which determines how small is too small to be a protein.

"A lot of these are getting filtered out because of this computation issue," Dr. Wang explains. "If it's smaller than 200 base pair, the open reading frame is not considered real. But what I found is that we can actually find a lot of these tiny regions with signatures of translations, and I was able to verify some portion being made into peptides."

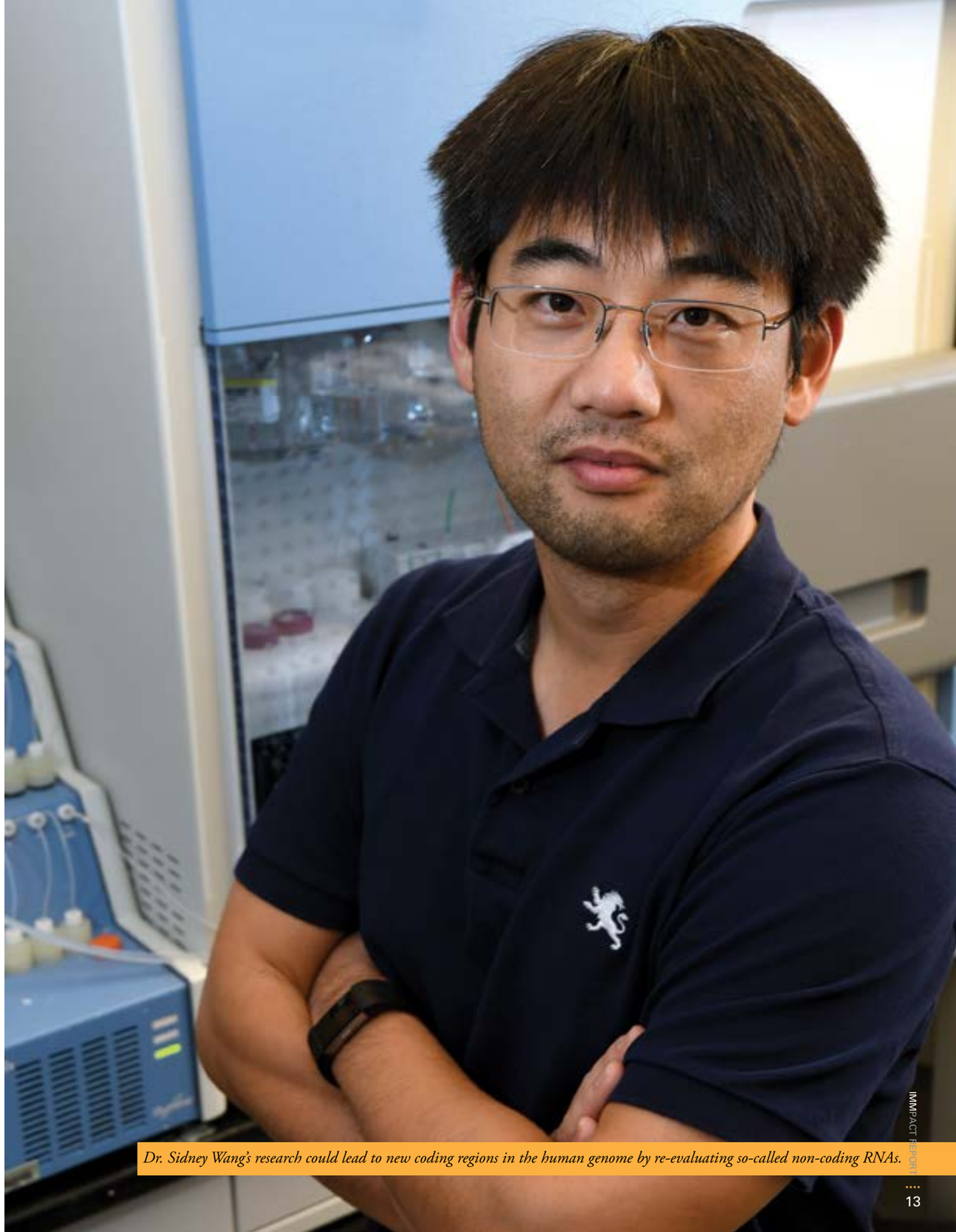
Just from the transcripts already assembled, Dr. Wang and researchers have found almost 5,000 new coding genes. Current estimates show about 25 percent of those coding genes are made into peptides, and verifying that these coding genes are, in fact, functional and what they are doing is one of the main areas of Dr. Wang's research lab at the IMM.

"If you look at the numbers, it's pretty striking because currently there are only 20,000 protein coding genes accepted by most people," he says.

The discovery of new potential coding regions and singling out those which participate in potentially important processes could have implications for human health. However, in focusing on translation rather than transcription, Dr. Wang will be developing new tools that will be effective for his research in studying direct regulation of protein translation.

Dr. Wang graduated from National Dong Hwa University in 2003 with a bachelor's of science in life science and double majored in physics. He obtained a Ph.D in 2012 from Washington University in St. Louis in the laboratory of the distinguished Dr. Sarah Elgin. Dr. Wang says his position with the IMM has been bolstered by a diverse environment, filled with researchers and professors eager to help one another.

"It's just a very collaborative environment in general," he says. "People are always looking for collaborators."



Dr. Sidney Wang's research could lead to new coding regions in the human genome by re-evaluating so-called non-coding RNAs.

Cheering on the IMM

Two former college friends have vaulted their passion for science and technology into a philanthropic mission to support the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM).

Dudley Oldham and John McDonald have known each other since their student days at The University of Texas – where Dudley recalls he took 66 hours of science courses. In more recent years, they have focused their scientific sights on the IMM.

"What I have always admired about the IMM is that it is a place where the best scientists are recruited to work on their driving passion, without bureaucratic oversight, with the goal to make a breakthrough discovery," Oldham says.

Oldham has been involved with the IMM for about a decade – building the institute's first formal advisory council and chairing it for seven years. He then recruited McDonald as a council member a few years ago, realizing he would be the ideal person to succeed him as chair – which he did in September of 2016.

"I knew that his enthusiasm and support for the IMM would be infectious," says Oldham, a retired senior partner at Fulbright & Jaworski.

McDonald, retired managing partner of the Houston office and member of the executive committee of Weaver, LLP, a large Texas accounting firm, says that as chair he wants to help increase the IMM's exposure in the community.

"I view the Advisory Council as the support group for the IMM," McDonald says, "It encourages financial support throughout the community for the innovative research being done and acts as a cheerleader for IMM.

"Awareness is huge for any medical research institute, so we need to 'get the word out' by introducing the IMM and its mission to as many people as we can. We are enlarging the Advisory Council with additional energetic supporters. We have a close working relationship with Dr. John Hancock, and our goal is to support his vision in growing the IMM and attracting the resources necessary to ensure that growth."

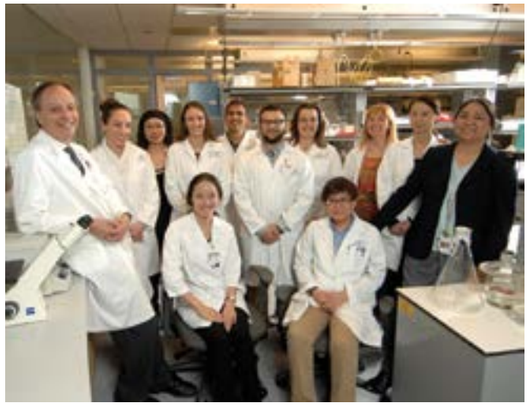
Although Oldham has stepped down as chair, he is still an active member of the IMM Advisory Council. He and his wife, Judy, have established funds to support research, faculty, and students: D. Dudley and Judy White Oldham Research Fund (IMM), D. Dudley and Judy White Oldham Faculty Award (MD Anderson UTHealth Graduate School), and the D. Dudley and Judy White Oldham Scholarship (McGovern Medical School). Oldham also was instrumental as a member of the Anne and Don Fizer Foundation Board in establishing the McGovern Medical School's Fizer Foundation Endowment for Depression Research.

"These gifts are a reflection of our esteem for the outstanding leadership of UTHealth President Dr. Giuseppe Colasurdo, the encouragement and support of the scientists and health care professionals at UTHealth, and our desire to further the advancement of medical research and care," Oldham explains.

"The IMM depends upon its community supporters, and we are grateful to have such leaders as Dudley and John who support our mission and tirelessly advocate on our behalf," says Dr. John Hancock, executive director, IMM.

> "What I have always admired about the IMM is that it is a place where the best scientists are recruited to work on their driving passion, without bureaucratic oversight, with the goal to make a breakthrough discovery."
>
> — Dudley Oldham, Advisory Council member

John McDonald, left, and Dudley Oldham support the IMM through Advisory Council leadership and sharing the IMM vision with the broader Houston community.



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: Raffaella Lombardi, M.D., Ph.D., assistant professor; Priyatansh Gurha, Ph.D., assistant professor; AJ Marian, M.D., professor

General theme of the research programs: The research programs at the Center starts 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:

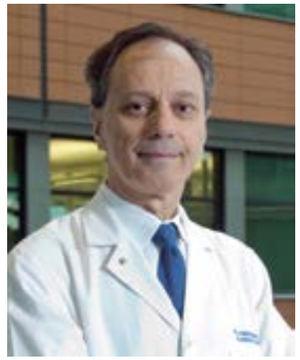
I. Human molecular genetic studies of cardiomyopathies: We have a repository of several hundred cases and their family members with cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy (ACM). Pathogenic and causal variants are 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 included 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, M.D.
Center Director & Professor
James T. Willerson Distinguished
Chair in Cardiovascular Research



AJ Marian, M.D.
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 (ClinicalTrial.Org NCT01537926)
- EXPLORER study: An industry –sponsored clinical trial to test efficacy of ATPase modulator on improve symptoms and exercise tolerance in patients with obstructive hypertrophic cardiomyopathy

KEY PUBLICATIONS

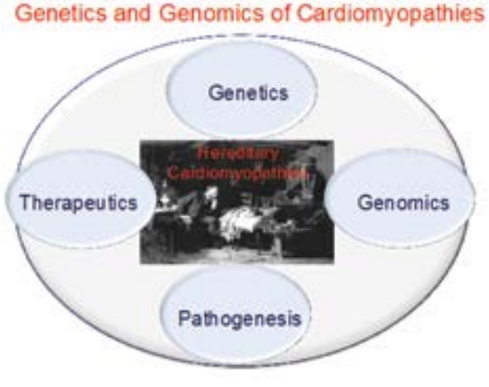
Suppression of Activated FOXO Transcription Factors in the Heart Prolongs Survival in a Mouse Model of Laminopathies. Auguste G, Gurha P, Lombardi R, Coarfa C, Willerson JT, Marian AJ. Circ Res. 2018 Jan 9. doi: 10.1161/*CIRCRESAHA.* 117.312052. PMID: 29317431

A Distinct Cellular Basis for Early Cardiac Arrhythmias, The Cardinal Manifestation of Arrhythmogenic Cardiomyopathy, and the Skin Phenotype of Cardiocutaneous Syndromes. Karmouch J, Zhou QQ, Miyake CY, Lombardi R, Kretzschmar K, Bannier-Hélaouët M, Clevers H, Wehrens XH, Willerson JT, Marian AJ. *Circ Res.* 2017 Oct 10. PMID: 29018034

A Potential Oligogenic Etiology of Hypertrophic Cardiomyopathy, A Classic Single Gene Disorder. Li L, Bainbridge MN, Tan Y, Willerson JT, Marian AJ. *Circ Res.* 2017 Mar 31;120(7): 1084-1090. PMID: 28223422

LAB MEMBERS

Post-doctoral Fellows: Gaelle Auguste, Ph.D.; Jennifer Karmouch, Ph.D.; Sirisha C Marreddy
Research Associate: Grace Czernuszewicz, M.S.
Research and Clinical Nurse: Yanli Tan, RN



Approach to Research Programs on Cardiomyopathies

Priyatansh Gurha, Ph.D.
Assistant Professor

Molecular mechanisms and functions of non-coding RNAs in heart failure

The broad goal of my research is to better understand the role of gene regulatory mechanisms involved in the pathogenesis of cardiomyopathies and heart failure. It is now clear that non-coding RNAs not only play a role in proper heart function but are also involved in pathogenesis of cardiomyopathies and heart failure. Previously, we identified a pathological role of miR-22, one of the most abundant miRNA in the heart. We demonstrated that miR-22 is a key regulator of cardiac hypertrophy and fibrosis. Mechanistically, we identified several miR-22 regulated candidate genes like Purine Rich Binding Protein B (PURB) that play a role in stress-induced cardiac hypertrophy and fibrosis. Furthermore, our research identified several miRNAs that are differentially expressed in Arrhythmogenic Cardiomyopathy (ACM), a primary disease of the myocardium that clinically manifests with cardiac arrhythmias, heart failure, and sudden death. One of the key pathogenic features of ACM is gradual replacement of myocytes by fibro-adipocytes. By integrating RNA-seq data with miRNA expression data we identified miR-184 as the most down-regulated miRNA (~10-fold) in ACM. We show that miR-184 is developmentally down regulated in mice heart and was predominantly expressed in cardiac mesenchymal progenitor cells. We also showed that in ACM an epigenetic network encompassing E2F1 pathway and CpG DNA methylation transcriptionally suppress miR-184 expression in heart. We showed that suppression of miR-184 leads to enhanced adipogenesis and overexpression of this miRNA partially rescue the adipogenic phenotype.

Recently, we have begun to investigate the regulatory role of Lamin (LMNA) in reprogramming the epigenetic code that governs gene transcription ensuing cardiac phenotype in Laminopathies. Objective of this study is to identify and characterize molecular component and mechanistic details that leads to tissue specific disease phenotypes in laminopathies. By studying the human heart with an LMNA mutation, an LMNA-deficient (*Lmna*$^{-/-}$) mouse model, and isolated cardiac myocyte transcriptomic analysis, we identified a diverse set of differentially expressed gene in laminopathies and specifically identified KDM5A and B (or KDM5) as most induced upstream regulator of gene dysregulation. KDM5 is a histone demethylase that removes tri- and di-methylations of lysine 4 of histone H3 (H3K4me3), often leading to suppression of gene expression. The role of KDM5 in heart and in laminopathies has not been documented so far. To determine the causal relation of KDM5 in laminopathies we re-expressed *Lmna*^{WT} in *Lmna*$^{-/-}$ mouse (by AAV9) and found that this was associated with rescue of the KDM5 network and decreased apoptosis and increased overall survival. Currently using wide array of genomic approaches, we are investigating the tissue and cell type specific contribution of KDM5 in laminopathies to ascertain the elusive role of KDM5 in heart and determine if induction of KDM5 is pathogenic in heart failure.

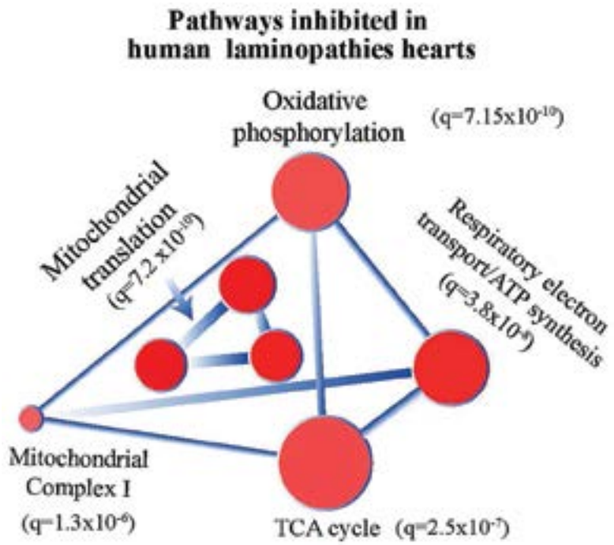
RESEARCH PROJECTS

- Role of lncRNAs in the pathogenesis of AC
- Identification and characterization of molecular mechanisms and functions of long non-coding RNAs and epigenetic regulatory pathways in cardiomyopathies and heart failure.

KEY PUBLICATIONS

Gurha P. MicroRNAs in cardiovascular disease. *Current Opinion in Cardiology*. 2016 May; 31(3): 249-54

Gurha P*[1], Chen X*, Lombardi R, Willerson JT and Marian AJ[1]. Knockdown of Plakophilin 2 Downregulates miR-184 Through CpG Hypermethylation and Suppression of the E2F1 Pathway and Leads to Enhanced Adipogenesis *In Vitro*. *Circulation Research* 119 (6), 731-750. (*Authors contributed equally, 1Corresponding authors).



Pathway analysis of gene suppressed in human DCM that are targets of KDM5 showing enrichment of gene involved in energy metabolism/Mitochondrial function.

Raffaella Lombardi, M.D., Ph.D.
Assistant Professor

Molecular genetics and pathogenesis of hereditary cardiomyopathies

My research focus is the delineation of the pathogenesis of hereditary cardiomyopathies, which are important causes of sudden cardiac death in the young.

In my earlier work, I studied the cardiac physiopathology of patients with Hypertrophic Cardiomyopathy (HCM) and identified novel causal mutations in families with HCM.

Lately I am studying the molecular pathogenesis of Arrhythmogenic Cardiomyopathy (AC), a familiar cardiomyopathy caused by mutation in desmosomal genes. AC is characterized clinically by cardiac arrhythmias, heart failure and sudden cardiac death, and pathologically by the progressive fibrofatty replacement of the myocardium.

My research program is based on the identification of the cellular origin of fibro-adipocytes in the heart of patients with AC. I have generated and characterized a number of lineage tracer mice and successfully shown that a subset of cardiac progenitor cells in the presence of mutant desmosome proteins, differentiate to adipocytes in AC. Because of these discoveries, I was nominated for and won the Louis N and Arnold M. Katz Basic Science Research Award Prize for Young Investigators from the American Heart Association and the William Harvey Award from the Italian Society of Cardiology.

Recently I have shown that cardiac fibroadipocyte progenitors (FAPs) express desmosome proteins and are a major source of adipocytes in AC. The findings expand the cellular spectrum of AC, commonly recognized as a disease of cardiac myocytes, to include non-myocyte cells in the heart. For this project I have received a Beginning Grant in Aid from the American Heart Association.

Recently, I am investigating the role of epicardial progenitor cells [EPCs, identified by the expression of Wt1 (Wilms Tumor 1) transcription factor] in the pathogenesis of AC. The impetus for this study has derived from the observation that fibroadiposis in the patients typically originates from the epicardium.

I have generated a mouse model of AC in which Desmoplakin (Dsp), a known AC causal gene, has been deleted in the EPCs. The transgenic mouse shows epicardial fibrosis, increased adipogenesis, cardiac dysfunction and premature death, resembling the human disease. However, only a small fraction of cardiac fibroblasts and adipocytes in the Wt1Cre: Dsp$^{w/f}$:Eyfp$^{w/f}$ mice directly originate from the mutant EPCs, suggesting that paracrine factors secreted by EPCs may mediate the differentiation of cardiac resident cell types to adipocytes. Hence, I am currently focusing on the identification of the paracrine factors secreted by EPCs which mediate fibrosis and adipogenesis in AC. This study has a high translational potential, as it may lead to the discovery of novel biomarkers and possibly new therapeutic targets.

RESEARCH PROJECTS

- Delineation of the signaling pathways involved in the pathogenesis of hereditary cardiomyopathies
- Identification and molecular characterization of cellular sources of fibro-adipocytes in Arrhythmogenic Cardiomyopathy
- Paracrine role of the epicardium in the context of Arrhythmogenic Cardiomyopathy

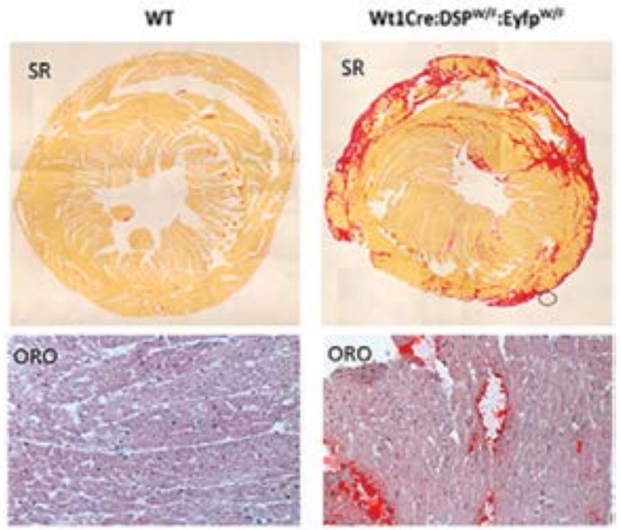
KEY PUBLICATIONS

Lombardi R, Chen SN, Ruggiero A, Gurha P, Czernuszewicz GZ, Willerson JT, Marian AJ. Cardiac fibro-Adipocyte progenitors express desmosome proteins and preferentially differentiate to adipocytes upon deletion of the desmoplakin gene. *Circulation Research*. 2016; 119(1): 41-54.

Chen SN*, Gurha P*, Lombardi R*, Alessandra Ruggiero, Willerson J T, Marian AJ. The Hippo Pathway Is Activated And Is a Causal Mechanism For Adipogenesis in Arrhythmogenic Cardiomyopathy. *Circ Res*. 2014; 114:454-68. (*Authors contributed equally to this work)

LAB MEMBERS

Research Assistant: Grazyna Z Czernuszewicz, MS



Phenotype of the *Wt1-CreETR2: Dsp*$^{w/f}$*: Eyfp*$^{w/f}$ mice. Picrosirius Red (SR) staining in the upper panels shows severe epicardial fibrosis (in red). Oil Red O (ORO) staining in the lower panels shows increased adipogenesis.

The Center for Human Genetics is focused squarely on generating new understanding about the genetic risk for common cardiovascular diseases and using that information to identify effective therapies for these diseases. High blood pressure is a unifying element that amplifies cardiovascular disease risk from stroke, heart and kidney disease. These diseases emerge in middle and later life and so are intricately linked to the normal processes of aging. The genetic variation that makes us unique 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 common in middle and later life and the mechanisms by which variation in these genes re-shape the biological pathways through 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 in our immune responses. In order to be able to adapt to the continuous and rapid mutation of pathogens, 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. We have shown that kidney injury associated with increased blood pressure results from the emergence of auto-antibodies that damage tissues. We have developed understanding of susceptibility to atherosclerosis and the interplay between new drug targets, such as PSCK9, and lipoprotein uptake by cells. Our population geneticists are global leaders in their field, making especially 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. This year we have recruited a new faculty member who is an emerging leader in this field. We are proceeding with a major new initiative to identify additional genetic variation contributing to Alzheimer's disease and age-related neuro-degeneration, extending our studies of the interactions between cardiovascular function and brain disease in this new and critical direction.

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 and from which future medicine will be advanced.

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

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

Genetics of cardiovascular end organ injury

Elevated blood pressure (BP) is the main driver of death from heart and kidney disease and stroke. But BP alone is not enough to create these diseases. Susceptibility also requires genetic factors that permit injury of the organs to advance to disease. We are uncovering which biological processes contain this genetic variation and how this leads to illness. We use rat models of hypertension that differ in susceptibility to BP-induced injury. We have learned that genetic susceptibility to organ injury lies within immune genes. Immune genes provide a defensive arsenal against attacks from pathogens. These pathogens evolve rapidly to overcome our defenses. Our defenses have to have enough pre-wired variety to quickly recognize pathogens and adapt immune responses specifically to them. Imperfect adaptation can allow our tissues to become damage targets. Increased BP triggers this damage by initiating inflammation, but immune genetic variation determines whether the result is an attack on our own organs. Evolution has created a dangerous equilibrium, an arms race, in which our disease resistance to pathogens requires some risk of harming ourselves.

RESEARCH PROJECTS

Mapping of Hypertensive End Organ Disease Loci

One small region of chromosome 17 is responsible for elevation of BP in the injury prone spontaneously hypertensive rat, SHR-A3, compared to the injury resistant SHR-B2. Genome sequencing shows which genes may be involved, and several interesting genes have emerged. One is involved in salt balance. Another is part of the signal mechanism that permits B-lymphocytes to steer antibodies to specific targets. We are subdividing this small region in our animals in order to determine which part of this region of chr 17 contains the actual disease-causing genetic variation.

Testing of Genetic Variants Identified in Disease Pathways

We have discovered a genetic variation that damages a protein vital for immune cell responses. It plays a key role in helping immune cells respond to novel antigens. We believe that this genetic defect also contributes to end organ disease. In particular, this protein seems to help T and B-lymphocytes communicate to prevent development of antibodies of low specificity. The protein alters T and B cell interactions, allowing antibodies that can become reactive to normal tissues and injure them. The genetic variant has several other effects on lymphocyte function.

Structurally Complex Genomic Regions

Our genome contains templates on which effective antibodies can be developed to permit recognition of an infinite variety of antigens. This arsenal must undergo adaptation in B cells. However, the individual arsenals that each of us possess are highly divergent. Similar divergence in the rat models we study alters disease susceptibility. Tools to define these highly complex template regions of the genome are now emerging. We have begun to apply them to describe how these genes vary and how they become adapted in inflammation. We hope to determine which specific template variations create the predisposition toward end organ injury.

KEY PUBLICATIONS

Dhande, I, Y. Zhu, M.C Braun, M.J. Hicks, S.E. Wenderfer and P.A. Doris. Mycophenolate mofetil prevents cerebrovascular injury in stroke-prone spontaneously hypertensive rats. *Physiological Genomics* 49:132-140, 2017.

Dmitrieva, R.I. S. M. Cranford, and P.A. Doris. Genetic control of serum marinobufagenin levels in the spontaneously hypertensive rat and the relationship to blood pressure. *J. Amer. Heart Association.* 6(10): In Press, Aug 2017

Doris, P.A. The genetics of hypertension: an assessment of progress in the spontaneously hypertensive rat. *Physiological Genomics*, 49(11):601-617, 2017.

LAB MEMBERS

Post-doctoral Fellows: Isha S Dhande, Ph.D.
Research Assistants: Yaming Zhu, Sterling Kneedler

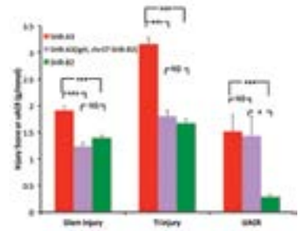


Figure 1: When we replace just 0.7% of the genome of injury susceptible SHR-A3 (red) with equivalent sequences from SHR-B2 (green) two important measures of renal injury (Glom and TI Injury) are reduced to the same level as in resistant SHR-B2 (mauve). The parts of the genome replaced affect a) blood pressure and b) the formation of antibodies.

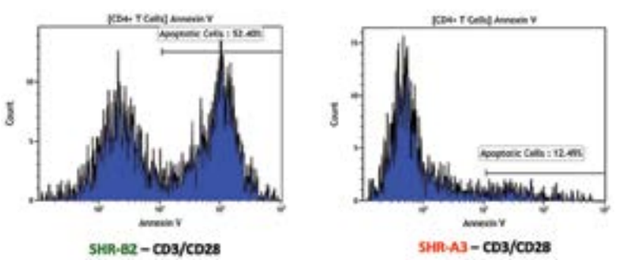


Figure 2: When strongly stimulated, T lymphocytes limit inflammation by spontaneously dying (SHR-B2, left blue peak is live cells, right peak is dying cells). In SHR-A3, loss of gene function causes loss of T lymphocyte death responses, sustaining inflammation(only right blue peak).

Myriam Fornage, Ph.D.
Professor
The Laurence and Johanna Favrot Distinguished Professorship in Cardiology

Molecular epidemiology of brain vascular disease and aging

Diseases of the aging brain, such as stroke and dementia, are among the most significant public health problems of our time. Stroke is the fifth-leading cause of death in the United States and is a major cause of serious long-term disability for adults. Alzheimer's disease is now the sixth-leading cause of death and more than 5 million Americans are living with the disease. By 2050, this number is projected to reach 16 million. There is growing evidence that these disorders begin years, if not over decades before manifestation of symptoms or clinical diagnosis. Indeed, neuroimaging techniques such as magnetic resonance imaging (MRI) have consistently detected brain abnormalities beginning in middle age, which are associated with an increased risk of stroke, cognitive and functional impairment, dementia, and death.

My research program investigates the genetics and genomics of vascular and neurodegenerative disease of the brain both in its clinical and pre-clinical forms in large population samples from young adults to elderly subjects. We use powerful genome technologies to discover novel genes influencing the risk for stroke, Alzheimer's disease, and brain MRI abnormalities. In collaboration with researchers in the United States and Europe, we apply genome sequencing technologies to identify DNA variants, either common or rare, which influence risk for these disorders. Our recent genetic studies have identified new genes influencing white matter lesions on MRI and uncovered a role of glial proliferative pathways and neuroinflammation.

We also study the links between DNA methylation and these diseases. DNA methylation is an epigenetic mechanism used by cells to control gene expression. Unlike DNA sequence variants, DNA methylation marks are not fixed at birth. Some of them can change throughout the lifetime, and in response to environmental influences and aging. Recently, we have used DNA methylation as a marker of a person's biological aging and compared it to his/her chronological age. We have shown that individuals whose biological age estimated from their DNA methylation profile is significantly older than their chronological age are at increased risk of developing brain abnormalities and cognitive function impairment.

These discoveries may yield new insights into disease mechanisms and lead to the development of new therapeutics to prevent or slow disease progression.

RESEARCH PROJECTS

- Discovering common and rare genetic variants influencing MRI-defined brain abnormalities using large-scale genotyping and whole genome sequencing
- Discovering novel epigenetic (DNA methylation) variants that influence risk for brain small vessel disease and its related neurocognitive outcomes
- Discovering novel genetic loci for cardiovascular traits using gene-lifestyle interactions and pathway analysis. In particular, discovering how depression and anxiety interact with genetic variation to influence blood pressure.
- Discovering novel genetic variants influencing cognitive function and Alzheimer's disease in adults of European, African, and Hispanic ancestry

KEY PUBLICATIONS

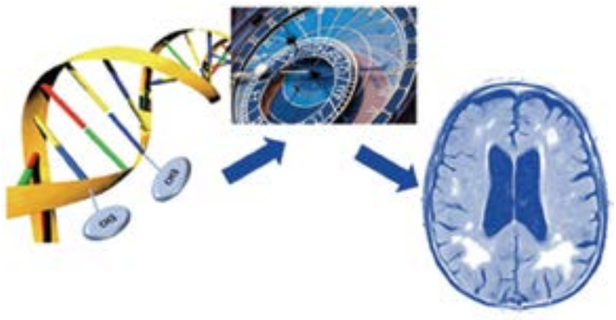
Raina, A., Zhao, X, Grove, M.L., Bressler, J., Gottesman, R.F., Guan, W., Pankow, J.S., Boerwinkle, E., Mosley, T.H., & Fornage, M. Cerebral White Matter Hyperintensities on MRI and Acceleration of Epigenetic Aging: The Atherosclerosis Risk in Communities Study. *Clin. Epigenet.*, 9: 21-29, 2017

Traylor, M., Malik, R., et al. on behalf of META-STROKE, UK Young Lacunar DNA Study, NINDS Stroke Genetics Network, Neurology Working Group of the CHARGE Consortium, & International Stroke Genetics, Consortium. Genetic Variation at 16q24.2 is associated with small vessel stroke. *Ann Neurol* 81: 383-394, 2017

Smith, E.E., Saposnik, G., Biessels, G.J., Doubal, F.N., Fornage, M., Gorelick, P.B., Greenberg, S.M., Higashida, R.T., Kasner, S.E., Seshadri, S.; on behalf of the American Heart Association Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Functional Genomics and Translational Biology; and Council on Hypertension. Prevention of stroke in patients with silent cerebrovascular disease. *Stroke* 48, e44-e71, 2017

LAB MEMBERS

Post-doctoral Fellows: Xueqiu Jian, Ph.D.; Melissa Richard, Ph.D.
Graduate Students: Daokun Sun, B.S.
Research Assistants: Rui Xia, Ph.D., Biostatistician; Ping Wang, Ph.D., Research Associate



DNA methylation profiling can be used to estimate a person's biological age. Individuals whose biological age is significantly older than their chronological age are at increased risk of brain abnormalities on MRI.tion and these brain abnormalities.

Ba-Bie Teng, Ph.D., 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 its 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 made a mouse model that mimics humans with hyperlipidemia by deleting both genes of LDL receptor and an RNA editing enzyme (*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 sever 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 disease. We deleted PCSK9 gene from LDb mice to generate a triple knockout mouse model (*LTp=Ldlr-/-Apobec1-/-Pcsk9-/-*). Using these two mouse models, we showed that PCSK9 modulates atherosclerosis development, apolipoprotein B (apoB) secretion, and endothelial cell function (Cover Figure). We discovered a novel intrahepatic role for PCSK9 - i.e. PCSK9 interacts directly with cytoplasmic apoB in a way that prevents its degradation via the autophagosome/lysosome pathway. This resulted in increased hepatic VLDL (very low density lipoprotein) secretion that is lipolytic processed to increased plasma LDL cholesterol concentrations (Figure 1). PCSK9 regulated autophagy signaling pathway including activation of p-AKT and induction of both AMPK and ULK1 kinase complexes (Figure 2). PCSK9 modulated SREBP-1c lipogenesis, which produced more atherogenic LDL containing elevated levels of cholesteryl ester and phospholipids, resulting in increased atherosclerosis and down regulation of TGF-β in endothelial cells. Thus, PCSK9 contributes to atherosclerosis through its effect on hepatic lipid metabolism and cellular immune function.

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 basis to develop efficient therapeutic approaches to combat progression of diseases

RESEARCH PROJECTS

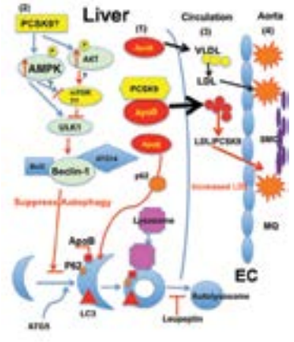
- The role of PCSK9 in autophagy, inflammation, and atherosclerosis.
- Using CRISPR/Cas9 technique to generate IL-17 RC triple knockout mice to study its effect on atherosclerosis.
- Using genetic tools and proteomics to identify genes associated with atherogenesis and to develop genetic therapy for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

KEY PUBLICATIONS

PCSK9 Deficiency Reduces Atherosclerosis, Apolipoprotein B Secretion And Endothelia Dysfunction: Hua Sun, Ph.D.; Ronald M. Krauss, M.D.; Jeffrey T. Chang, Ph.D. and Ba-Bie Teng, Ph.D., 2017, *J. Lipid Research*, in press. (This article was selected as the Cover for J. Lipid Research as shown below). This article was viewed 648 time on the JLR website in Nov and Dec, 2017).

LAB MEMBERS

Research Scientist: Hua Sun, Ph.D.
Visiting Scientist: Yunlong Wang, Ph.D.
Research Assistant: Zhengming Xu
Summer Internship Students: Saxon Hancock; Christine Huang; Nithya Narayana



PCSK9 interacts with ApoB, modulates autophagy, alters LDL and affects atherogenesis. (1). PCSK9 interacts with ApoB, modulates the autophagy degradation pathway by increasing the production of VLDL and altering the LDL compositions (3). The consequence of differences in LDL induces different reaction on endothelial cells, affecting atherogenesis (4). (2). PCSK9 regulates autophagy signaling pathway; it increases p-AKT and p-AMPK and suppresses the autophagy.

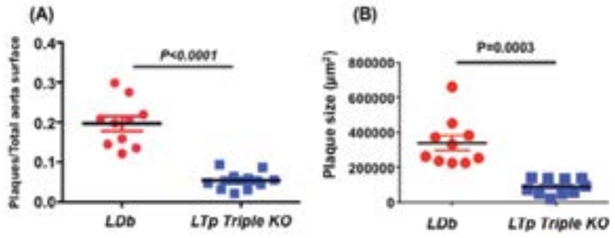


Figure 2. PCSK9 modulates atherosclerosis. LTp mice develop significantly less atherosclerotic lesions compared with LDb mice as determined by (A) en face quantification and (B) aortic sinus quantification.

Sidney Wang, Ph.D.
Assistant Professor

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

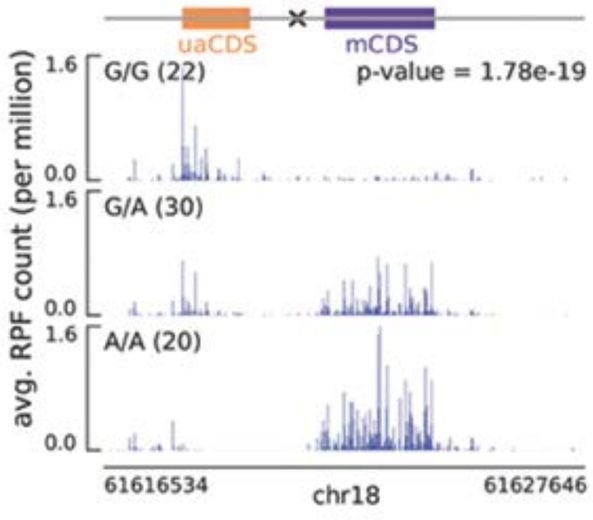
Regulation of gene expression is fundamental to a wide range of biological processes. From cell fate determination during development to malignant transformation during tumorigenesis, precise control of gene expression forms the basis of these processes. Our current understanding of gene regulation is,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

RESEARCH PROJECTS

- Regulation of protein translation by uORF in stress response
- Using RNA binding protein footprint sequencing to investigate translational regulation of protein synthesis
- Identification of functional smORFs across multiple tissues using ribosome profiling and CRISPR-CAS9 knockout screens.

KEY PUBLICATIONS

Raj A, Wang SH, Shim H, Harpak A, Li YI, Engelmann B, Stephens M, Gilad Y, Pritchard JK. 2016. Thousands of novel translated open reading frames in humans inferred by ribosome footprint profiling. *eLife* 5: e13328.



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

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

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

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

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

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

Research interests include:

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

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

Rick Wetsel, Ph.D.
Professor and Director of the Center for Immunology and Autoimmune Diseases
Hans J. Muller-Eberhard, M.D., Ph.D. and Irma Gigli, M.D. 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

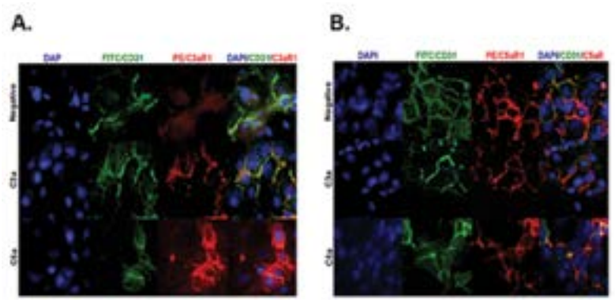
Mueller-Ortiz SL, Calame DG, Shenoi N, Li Y-D, Wetsel RA. The complement anaphylatoxins, C5a and C3a, suppress IFN-β production in response to *Listeria monocytogenes* by inhibition of the cyclic dinucleotide-activated cytosolic surveillance pathway. *J Immunol.* 2017: 198: 3237-3244 (PMID: 28275134).

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

Calame DG, Mueller-Ortiz SL, Wetsel RA. Innate and adaptive immunologic functions of complement in the host response to *Listeria monocytogenes infection*. *Immunobiology* 2016: 221: 1407-1417 (PMID: 27476791).

LAB MEMBERS

Senior Research Scientist: Stacey Mueller-Ortiz, Ph.D.
Research Scientist: Ken Simmons, Ph.D.
Post-doctoral Fellow: John L. Mazzilli, M.D.
Research Instructor: Pooja Shivshankar, Ph.D.
Senior Research Assistant: Yi-Dong Li



Expression of the complement anaphylatoxin receptors (A. C3aR and B. C5aR1) by human vascular endothelial cells following stimulation with either C3a or C5a. Blue-cell nucleus; Green-Endothelial Cells; Red-C3aR or C5aR1.

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

Adenosine signaling and the regulation of chronic lung disease

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

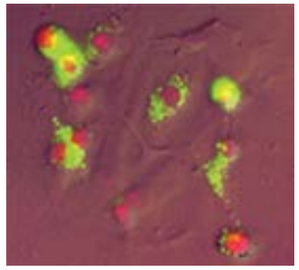
Philip, K., Mills, T. W., Davies, J., Chen, N. Y., Karmouty-Quintana, H., Luo, F., Molina, J. G., Amione-Guerra, J., Sinha, N., Guha, A., Eltzschig, H. K. and Blackburn, M. R. (2017) HIF1A up-regulates the ADORA2B receptor on alternatively activated macrophages and contributes to pulmonary fibrosis.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

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

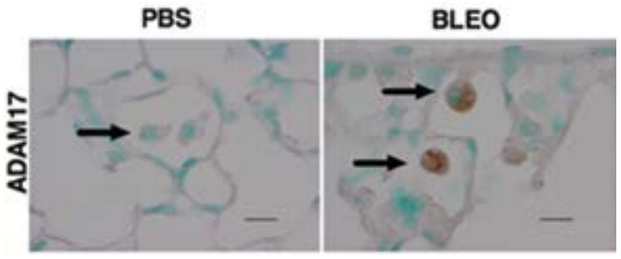
Karmouty-Quintana, H., Philip, K., Chen, N. Y., Weng, T., Molina, J. G., Luo, F., Davies, J., Acero, L., Le, Bao, Bunge, I., Volcik, K., Le, T., Johnston, R. A., Xia, Y., Eltzschig, H. K. and Blackburn, M. R. (2015) Deletion of ADORA2B from myeloid cells dampens lung fibrosis and pulmonary hypertension.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

LAB MEMBERS

Assistant Professor: Tingting Weng, Ph.D.
Senior Research Scientist: Kelly Volcik, Ph.D.
Research Associate: Ning-Yuan Chen
Research Scientist: Jose Molina, Sr.
Graduate Student: Josh Ko, Ph.D.



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, M.D., Ph.D.
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 two 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. This has led us to our recent interest in CRS representing a disease at the crossroads of coagulation and inflammation. Studies are focusing on the elucidating the specifics of the pathways that intersect these two entities as it relates to CRS.

RESEARCH PROJECTS

- Immunologic characterization of important cell types involved in the Th2 immune response as a means of endotyping CRS
- 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

Shaw JL, Ashoori F, Fakhri S, Citardi MJ, and Luong AL. Increased Percentage of Mast Cells within Sinonasal Mucosa of Chronic Rhinosinusitis with Nasal Polyp Patients Independent of Atopy. *International Forum of Allergy Rhinology,* 2012 May; 2(3):233-40. PMID:22344928

Millien VO, Lu W, Shaw J, Yuan X, Mak G, Roberts L, Song LZ, Knight JM, Creighton CJ, Luong A, Kheradmand F, Corry DB. Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science*. 2013 Aug 16;341(6147):792-6.

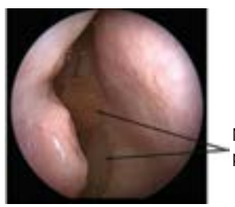
Shaw JL, Fakhri S, Citardi MJ, Porter PC, Corry DB, Kheradmand F, Liu YJ, Luong A. IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med.* 2013 Aug 15;188(4):432-9.

Porter P, Lim DJ, Maskatia ZK, Mak G, Tsai CL, Citardi MJ, Fakhri S, Shaw JL, Fothergil A, Kheradmand F, Corry DB, and Luong A. Airway Surface Mycosis in Chronic Th2-Associated 1 Airway Disease. *J Allergy Clin Immunol.* 2014 Aug 134(2):325-331.

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

LAB MEMBERS

Caroline J. Padro Dietz, Ph.D.
Samantha McMichael, B.A.



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

Dachun Wang, M.D.
Assistant Professor

Lung stem/progenitor cells and diseases

Lung epithelial stem/progenitor cells are critical for the maintenance of homeostasis of airway and alveolar epithelial cell populations that are constantly exposed to injurious stimuli from the environment. There are at least three stem/progenitor cell types responsible for maintaining distal lung epithelial cell populations: 1) alveolar epithelial type II cells; 2) the transient amplifying bronchiolar Clara cells; and 3) a subset of variant Clara cells located at the bronchioalveolar duct junction and the branch point-associated neuroepithelial bodies. Loss of normal functions of any of these stem/progenitor cell types due to injuries or genetic deficiencies is thought to play an important role in the development of chronic or severe pulmonary diseases, including pulmonary fibrosis, asthma, CODP, cystic fibrosis, neonatal respiratory distress syndrome (RDS) and cancer. However little is known regarding the pathogenesis of these pulmonary diseases as well as the corresponding repair mechanisms, since there is no reliable biomedical research model available for studying the biological and disease processes both *in vivo* and *in vitro*. In addition, currently available treatments for those pulmonary diseases at best release symptoms and improve life quality within a limited time range, and the long-term outcome is unfortunately not positive. There is an imperative need for developing of novel therapies to facilitate the treatment of lung diseases. Without doubt, the distal lung stem/progenitor cells represent the key targets for exploring the mechanisms underlying pathogenesis of lung diseases. During the past few years, considerable interest has developed in the therapeutic use of stem cells in the treatment of pulmonary diseases. The embryonic stem (ES) cell/lung disease-specific induced pluripotent stem (iPS) cell derived distal lung stem/progenitor cells are not only a promising source of cells that can be therapeutically used to treat distal lung injuries and genetic disorders, but also provide a good model to study lung disease processes. My research efforts are focused on 1) to isolate and characterize human and mouse ES cell derived distal lung stem/progenitor cell types both *in vitro* and *in vivo*; 2) to generate "clinical grade" lung disease-specific iPS cells for studying pulmonary disease processes and for developing cell-based gene therapeutic strategy for lung tissue regeneration; 3) to identify and characterize factors or regulatory pathways that control distal lung stem/progenitor cell fate during the disease processes for developing a novel strategy for targeted activation of endogenous stem/progenitor cells for lung tissue repair; and 4) to explore lung cancer stem cell-derived exosome miRNA pathways.

RESEARCH PROJECTS

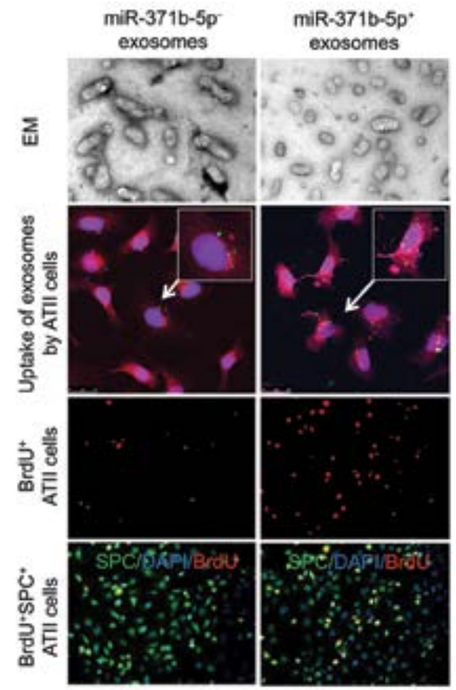
- Isolation and characterization of ES/iPS cell derived distal lung stem/progenitor cells
- Therapeutic potential of ES/lung disease-specific iPS-derived distal lung stem/progenitor cells for the treatment of lung diseases
- Characterization of lung cancer stem cell-derived exosome miRNA pathways controlling cancer cell growth and metastasis

KEY PUBLICATIONS

Quan Y., Wang Z., Gong L., Peng X., Richard M., Zhang J., Fornage M., Alcorn JL., and Wang D. Exosome miR-371b-5p promotes proliferation of lung alveolar progenitor type II cells by using PTEN to orchestrate the PI3K/Akt signaling. *Stem Cell Res Ther.* 2017 Jun 8;8 (1):138-51

LAB MEMBERS

Research Associate: Dr. Yuan Quan



Quan Y., Wang Z., Gong L., Peng X., Richard M., Zhang J., Fornage M., Alcorn JL., and Wang D. Exosome miR-371b-5p promotes proliferation of lung alveolar progenitor type II cells by using PTEN to orchestrate the PI3K/Akt signaling. Stem Cell Res Ther. 2017 Jun 8;8 (1):138-51

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

Transgenic and stem cells core facility

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 800 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.

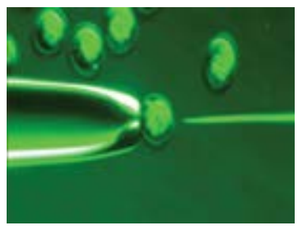
We have derived functional human RPE cells in our laboratory and are currently testing the efficacy and safety of these cells in animal models. In preparation of clinical trials, we will examine the long-term viability of the transplanted cells in murine animal models of ARMD and we will generate transplantable human RPE cells in a GMP-certified facility.

RESEARCH SERVICES

- 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
- CRISPR/Cas9-mediated genome editing
- 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



Pronuclear microinjection of mouse zygotes for the production of genetically-engineered animal models.

RESEARCH PROJECTS

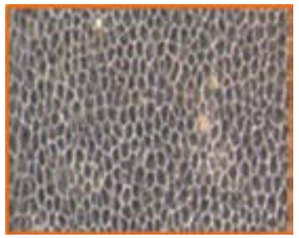
- Stem cell therapy for age-related macular degeneration
- Patient-derived tumor xenograft implantation models

KEY PUBLICATIONS

Mazzilli, J.L., Domozhirov, A.Y., Mueller-Ortiz, S.L., Garcia, C.A., Wetsel, R.A. and Zsigmond, E. M.: Derivation and characterization of the human embryonic stem cell line CR-4: Differentiation to human retinal pigment epithelial cells. *Stem Cell Research*. 18:37-40, 2017.

LAB MEMBERS

Senior Research Associate: Aleksey Domozhirov
Post-doctoral Fellow: John Mazzilli, M.D.



Retinal pigment epithelial cells were derived from the NIH-approved CR-4 human ES cell line. The cells were characterized for molecular markers, tested for correct function and safety in an animal model of age-related macular degeneration.

The Center for Metabolic and Degenerative Diseases integrates eight laboratories investigating aging-associated diseases, including muscle wasting, neurodegeneration, cancer, and type-2 diabetes. Obesity, the underlying medical condition, is a research focus of several groups. Aging and obesity-associated changes in energy metabolism, 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 do progenitor cells in adipose tissue commit to white and brown adipogenesis?
- How does dysfunction of adipose cells promote progression of diseases and aging?
- How does fibrosis and inflammation in adipose tissue affect insulin sensitivity?
- How is angiogenesis implicated in adipose and muscle tissue remodeling?
- Can cells in adipose tissue be targeted for therapeutic purposes?
- What transcriptional pathways can be targeted to treat muscle diseases?
- How does the brain and circadian clock control the body's energy balance?
- How does altered circadian metabolism lead to cancer?
- How does the brain control glucose homeostasis in type 1 and type 2 diabetes?
- What are the functions of the genes mutated in neurodegenerative diseases?
- How does abnormal processing of proteins cause neuronal degeneration?
- How does stress impact Alzheimer's disease pathogenesis?

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, Ph.D.
Center Director & Associate Professor
Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research

Mikhail Kolonin, Ph.D.
Associate 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

Health relies on fat tissue that coordinates the function of other organs. Lipids, the molecules serving the body as an energy source, can cause metabolic disease if fat tissue stops acting like a sponge by absorbing lipids. There are two types of fat cells (adipocytes) that use lipids differently. White adipocytes store lipids and release them in times of energy scarcity, while 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. 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. 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. The future focus of our work is on investigating the role of ASCs in 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. We hypothesize that adipocytes run out because ASCs become 'exhausted' with age and lose replicative potential and that obesity leads to premature exhaustion of ASCs because it involves excessive fat cell number expansion. This hypothesis is now being tested in animal models. To ensure safety of D-WAT, we are performing studies in mouse models to establish the consequences of D-WAT treatment in aging.

RESEARCH PROJECTS

- Adipocyte progenitors: lineages, and function in health, and targeting and 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

Kolonin M.G., et al., Pasqualini R, Arap W. Interaction between tumor cell surface receptor and proteinase 3 mediates prostate cancer metastasis to bone. *Cancer Research*. Priority report, 15 (12), 3144-3150, 2017.

Daquinag A.C. et al., Kolonin M.G. Non-glycanated Decorin is a Drug Target on Human Adipose Stromal Cells, *Molecular Therapy Oncolytics*. 6:1-9, 2017.

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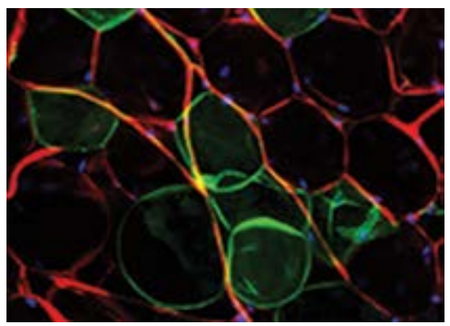
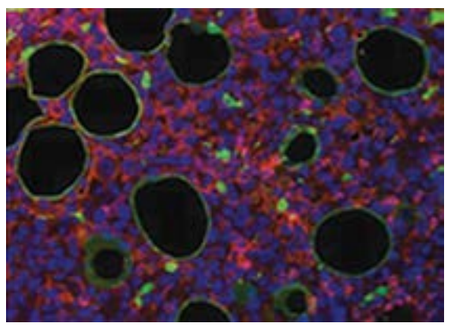
LAB MEMBERS

Senior Research Scientist: Alexis Daquinag
Research Scientist: Zhanguo Gao, Fei Su
Graduate Student: Shraddha Subramanian
Research Assistant: Wanqiao Cao



Distinct lineages of adipocytes identified in mouse fat tissue through lineage tracing of progenitor cells by using green and red reporter genes. Blue: nuclei.



Immunofluorescence of mouse breast adenocarcinoma. Red: malignant cells. Green: intratumoral adipocytes and adipose stromal cells. Blue: nuclei.

Rebecca Berdeaux, Ph.D.
Associate Professor

Regulation of muscle metabolism by cellular signaling pathways

When people chronically overeat and become obese, many changes in hormonal pathways contribute to development of insulin resistance, pre-diabetes and type 2 diabetes. One problem in these individuals is that tissues like muscle and liver become resistant to insulin, which normally reduces blood glucose. On the other hand, in starvation, opposing hormonal pathways regulated by cAMP signaling provide more glucose when needed during starvation or the fight or flight response. Insulin and cAMP-regulated hormonal pathways are normally in a balanced yin-yang relationship. In people with type 2 diabetes, the insulin pathway is reduced and the cAMP pathway is increased. We are studying the molecular intersections of these pathways in major tissues (liver and muscle) that both regulate to blood glucose levels.

One enzyme activated by cAMP signaling that links cAMP with insulin signaling within cells is the enzyme salt inducible kinase 1 (SIK1). After creating mice lacking SIK1, we found that this enzyme acts in skeletal muscle to regulate blood glucose concentration. We discovered that SIK1 does this not through the usual muscle insulin targets. Instead, SIK1 regulates blood glucose levels by a parallel pathway. This is exciting because it provides pre-clinical evidence that SIK1 inhibitors could reduce blood glucose levels even in people with insulin resistance. Our ongoing work addresses how the SIK1 enzyme exerts its effects in skeletal muscle and what influence the cAMP pathway has on SIK1 function in starvation and obesity conditions. In conjunction with this work, we have recently developed new mouse tools to study and visualize cAMP pathway function in different tissues in living mice without use of hormones or drugs that regulate all tissues. Instead, we can control cAMP signaling using an otherwise inert drug. These tools are allowing us to study the intricacies of cAMP signaling in individual tissues and the impact on whole animal metabolism.

RESEARCH PROJECTS

- Identification of the roles of SIK1 in muscle and liver metabolism in obesity
- Development of genetically modified mice to regulate and monitor cAMP pathways in living animals
- Study of how cAMP signaling regulates skeletal muscle regeneration in aging

KEY PUBLICATIONS

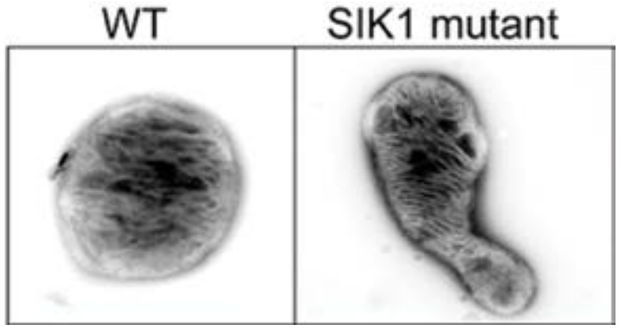
Akhmedov D, Rajendran K, Mendoza-Rodriguez MG, and Berdeaux R. Knock-in luciferase reporter mice for *in vivo* monitoring of CREB activity. *PloS ONE.* 2016 11(6): e0158274.

Akhmedov D, Mendoza-Rodriguez MG, Rajendran K, Rossi M, Wess J, Berdeaux R. Gs-DREADD knock-in mice for tissue-specific, temporal stimulation of cyclic AMP signaling. *Mol Cell Biol*. 2017 37(9): e00584-16.

Kim ER, Fan S, Akhmedov D, Syn K, Lim H, O'Brien W, Xu Y, Mangieri, LR, Zhu Y, Lee CC, Chung Y, Xia Y, Xu Y, Li F, Sun K, Berdeaux R, Tong Q. Red blood cell b-adrenergic receptors contribute to diet-induced energy expenditure by increasing O2 supply. *JCI Insight* 2017 2(14): 93367.

LAB MEMBERS

Research Scientist: Dmitry Akhmedov
Graduate Student: Randi Fitzgibbon
Research Assistants: Maria Mendoza-Rodriguez



Mitochondria use glucose and fat to generate ATP. In diabetes, mitochondria become damaged and their structure changes. Images show muscle mitochondria from wild-type (WT) and SIK1 mutant mice; we propose that shape changes in SIK1 mutants contribute to increased glucose use by muscles.

Kristin Eckel-Mahan, Ph.D.
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. Circadian rhythms, which are endogenous, self-perpetuating oscillations of 24-hr periodicity, are present in all cells of the body. While our sleep/wake cycle, food intake, internal body temperature, hormone section, etc. adapt to and are aligned with the 24-hr. rotation of the earth on its axis, modern technology has provided ample ways to disrupt this alignment. Examples include travel across time zones (jet-lag), working a night shift or rotating shifts, 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 imbalance. We are trying to understand why circadian disruption produces these effects.

While the central pacemaker of the brain is predominantly entrained by light, circadian oscillations in peripheral organs are heavily influenced by other *zeitgebers* ("time-givers") such as food. When circadian clocks across the body are misaligned, risk for metabolic disease increases. Our current experiments include those designed to reveal which *zeitgebers* are most important for tissue-specific clock function and the mechanisms underlying their *zeitgeber* properties. We are also interested in how disrupted peripheral clocks communicate back to the brain and affect the function of the central pacemaker, the suprachiasmatic nucleus.

To date, we have discovered that nutrient quality is a strong determinant of circadian rhythmicity in the liver, adipose, and muscle tissues. For example, a high fat diet can disrupt these clocks, putting them on a different time zone, *per se*, than the brain. Studies to date suggest that this circadian misalignment in humans contributes to weight gain and metabolic diseases such as type II diabetes. High fat diet-induced circadian changes in these tissues can be reversed by restoring whole body insulin sensitivity, indicating that a key link between diet and the circadian clock is insulin signaling in these tissues.

In addition to metabolic disease, our data reveal new links between circadian disruption and liver cancer. Specifically, we have discovered that a particular nuclear receptor isoform, which is expressed only in liver cancer cells but not normal tissue, has unique circadian activity and downregulates one of the key circadian drivers, BMAL1, in the cell. Using human and rodent liver cancer cells, we find that activity of this cancer-specific isoform of hepatocyte nuclear factor 4 (HNF4α) disrupts the cellular homeostasis of cancer cells and promotes the growth of liver tumors. Experimentally restoring expression of the BMAL1 protein results in cell death and impaired tumor growth in a rodent model. Thus, we aim to determine whether therapies could be developed to treat tumors which express this circadian protein based on its unique expression in cancer cells. We are studying whether agents which improve endogenous circadian rhythms in cancer cells, or alternatively, timed application of ligands for this cancer-specific HNF4α isoform might be used to delay or prevent tumor growth altogether.

RESEARCH PROJECTS

- Mechanisms linking nutrients and the circadian clock in metabolic tissues
- Coordination of light- vs. food- driven circadian oscillators and its importance for energy balance
- Mechanisms linking circadian disruption to cancer formation
- Links between weight loss and chronotype in humans

KEY PUBLICATIONS

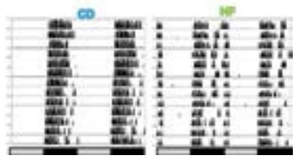
"Circadian Metabolomics in Time and Space" Dyar, KA and Eckel-Mahan KL, *Frontiers in Neuroscience,* Volume 11, Pages 369-378. 2017

"Clocks and Cholesterol: Co-Agonists in Cardiovascular Disease?" Fekry, B and Eckel-Mahan KL, *EBioMedicine*, Volume 20, Pages 5-6. 2017

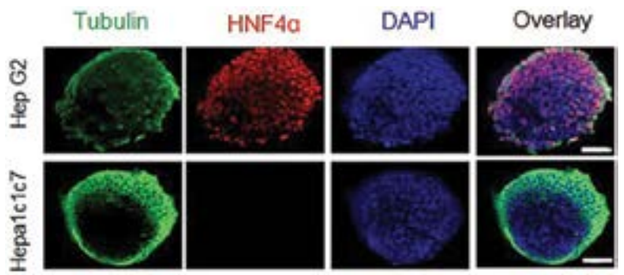
"Interdependence of Nutrient Metabolism and the Circadian Clock System: Importance for Metabolic Health" Ribas-Latre, A and Eckel-Mahan KL, *Molecular Metabolism,* Volume 5, Pages 133-152. 2016

LAB MEMBERS

Post-doctoral Fellows: Baharan Fekry and Aleix Ribas-Latre
Research Assistant: Corrine Baumgartner



Circadian wheel running of mice on a low fat (CD) vs. high fat diet (HF) in "free running" (i.e. constant dark conditions). Gray bars represent the animals' rest period. HF differentially affects the circadian clock of peripheral tissues vs. those of the central nervous system, which control the sleep/wake cycle.



3D organoids grown from human and mouse liver cancer cells reveal heterogeneity in expression of the HNF4α protein (red), which disrupts the circadian clock in those cells. (Green staining=tubulin, Blue staining=DAPI nuclear stain.)

Nicholas Justice, Ph.D.
Assistant Professor

Stress signaling in psychiatric and degenerative disease

My lab studies the neural and endocrine systems that are activated by stress and mediate the bodies' response to stress. These pathways center around the action of the stress neuropeptide Corticotropin Releasing Factor (CRF), and the Hypothalamic-Pituitary-Adrenal (HPA) axis that controls release of the stress hormone, Cortisol. Using mouse genetics, we manipulate select circuits in these stress responsive pathways to understand how the brain produces emotions and memories related to stress. Furthermore, we are attempting to understand how these emotions and memories return as chronic states of anxiety and depression. Determining how neural circuits mediate anxiety states, and the specific molecules and pathways that are activated during chronic anxiety-related diseases will allow the targeting of these pathways to modulate symptoms in human patients.

Chronic stress, anxiety, and depression can also negatively impact other ongoing diseases, including Alzheimer's disease. We and others have shown that stress and excess Cortisol causes Alzheimer's disease to progress faster. However, in parallel experiments we found that early stage Alzheimer's disease perturbs stress pathways causing anxiety and depression before overt cognitive loss. These interacting sources and impacts of stress create a vicious cycle that drives disease progression. We are continuing our work on Alzheimer's disease to determine how late-life neuropsychiatric symptoms might indicate progressing neurodegenerative disease, in the hope that addressing these symptoms might slow progression of the disease. Recently, we have initiated a collaboration with clinical neurologists at UT to investigate the interesting observation that chronic PTSD increases the risk of developing dementia with age. Our experimental and clinical results identify the stress response as a critical influence on neurodegenerative disease progression, and suggest that pharmacological manipulation of stress pathways might be an effective means of slowing down these devastating diseases.

RESEARCH PROJECTS

- Investigations of a new population of hypothalamic neurons that mediate local CRF signaling to impact endocrine, autonomic, and behavioral stress responses
- Neural circuits that transit the basal ganglia to impact movement in response to stress
- The role of stress in the progression of Alzheimer's disease

KEY PUBLICATIONS

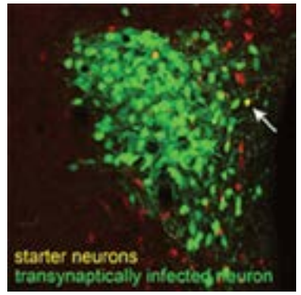
Ramot A, Jiang Z, Tian JB, Nahum T, Kuperman Y, Justice N*, Chen A*. Hypothalamic CRFR1 is essential for HPA axis regulation following chronic stress. *Nat Neurosci*. 2017 Mar;20(3):385-388. doi: 10.1038/nn.4491. Epub 2017 Jan 30. (* - co-corresponding)

Glajch KE, Kelver DA, Hegeman DJ, Cui Q, Xenias HS, Augustine EC, Hernández VM, Verma N, Huang TY, Luo M, Justice NJ, Chan CS. Npas1+ Pallidal Neurons Target Striatal Projection Neurons. *J Neurosci.* 2016 May 18;36(20):5472-88. doi: 10.1523/JNEUROSCI.1720-15.2016.

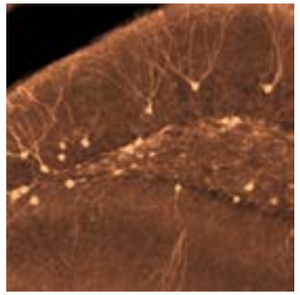
Chen M, Wang J, Jiang J, Zheng X, Justice NJ, Wang K, Ran X, Li Y, Huo Q, Zhang J, Li H, Lu N, Wang Y, Zheng H, Long C, Yang L. APP modulates KCC2 expression and function in hippocampal GABAergic inhibition. *Elife.* 2017 Jan 5;6. pii: e20142. doi: 10.7554/eLife.20142.

LAB MEMBERS

Post-doctoral Fellows: Shivakumar Rajamanickam, Ph.D.; Zhiying Jiang, Ph.D.



Rabies viral tracing of neural circuits. Generation of starter neurons (yellow, arrow), allows infection of all of the neurons that synapse with that neuron (green). In the hypothalamus, CRF neurons are highly interconnected with surrounding CRFR1 neurons (red).



CRF neurons in the hippocampus. The hippocampus, a brain region important for memory formation, responds to stress by activating CRF release. Chronic stress has been linked to age-related memory loss and Alzheimer's disease.

Vihang Narkar, Ph.D.
Associate Professor

Nuclear receptor & co-activator signaling in skeletal muscle

We are investigating the role of nuclear receptors and their co-regulator proteins in skeletal muscle function and diseases. Specifically, we are interested in how these factors control skeletal muscle endurance, size, and regenerative capacity through regulation of gene expression. Our work has therapeutic implications in sports medicine, diabetes, and orphan diseases such as muscular dystrophies, where muscle function and structure is commonly compromised.

RESEARCH PROJECTS

- Regulation of muscle metabolism, vascularization and endurance by ERR-alpha/gamma
- Regulation of apoptosis, autophagy and muscle mass by PGC1-beta
- Therapeutic role of ERR's in ischemic muscle and Duchenne Muscular Dystrophy
- Activation of muscle stem cells by nuclear receptors

KEY PUBLICATIONS

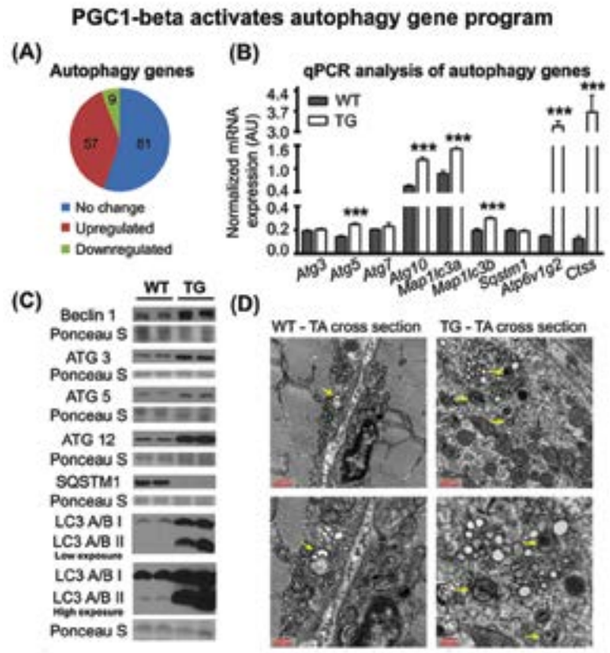
Narkar VA. PGC1α Promoter Methylation and Nucleosome Repositioning: Insights Into Exercise and Metabolic Regulation in Skeletal Muscle. *Endocrinology.* 2017; 158 (7): 2084-2085.

Sopariwala DH, Yadav V, Badin PM, Likhite N, Sheth M, Lorca S, Vila IK, Kim ER, Tong Q, Song MS, Rodney GG, Narkar VA. Long-term PGC1β overexpression leads to apoptosis, autophagy and muscle wasting. *Sci Rep*. 7(1): 10237, 2017

Gallardo-Montejano VI, Saxena G, Kusminski CM, Yang C, McAfee JL, Hahner L, Hoch K, Dubinsky W, Narkar VA, Bickel PE. Nuclear Perilipin 5 integrates lipid droplet lipolysis with PGC-1α/SIRT1-dependent transcriptional regulation of mitochondrial function. *Nat Commun.* 7: 12723, 2016

LAB MEMBERS

Post-doctoral Fellows: Danesh Sopariwala, Neah Likhite
Research Assistant: Megha Sheth



PGC1-beta induces muscle wasting by activating a process called autophagy. Panels (A-D) represent activation of autophagy transcriptional program (A), autophagy biomarker genes (B) and proteins (C), and electron microscopy images of PGC1-beta mediated autophagosome formation (arrows) (D) in muscle.

Kai Sun, M.D., Ph.D.
Assistant Professor

Targeting adipose tissue 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 have published many paradigm-shifting findings about the tight connections between adipose tissue remodeling and obesity development. Specifically, we discovered that obese fat pads are frequently hypoxic and HIF1α 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. Our findings suggest that targeting HIF1α and VEGF-A in adipose tissue may offer the great opportunity for a novel therapeutic approach to prevent and treat the progression of obesity-related metabolic disorders.

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 6α3 and produces endotrophin, which accelerates fibrosis and inflammation, ultimately leading a highly unfavorable microenvironment to sustain metabolic flexibility.

More recently, we use molecular tools and mouse models to study endotrophin. By using a doxycycline-inducible endotrophin overexpression model we demonstrate that endotrophin serves as a powerful co-stimulator of pathologically relevant pathways within the unhealthy adipose tissue milieu, triggering fibrosis and inflammation and ultimately leading to enhanced insulin resistance. We further demonstrate that blocking endotrophin with a neutralizing antibody ameliorates the adverse effects in adipose tissue and effectively reverses metabolic dysfunction induced by high-fat diet. All these exciting observations in our lab highlight endotrophin as an attractive target for obesity and type 2 diabetes.

RESEARCH PROJECTS

- Hypoxia induced pathological changes in adipose tissue
- VEGF-A stimulated metabolic benefits during adipose tissue healthy expansion
- Reversibility of adipose tissue fibrosis by novel anti-fibrotic therapies

KEY PUBLICATIONS

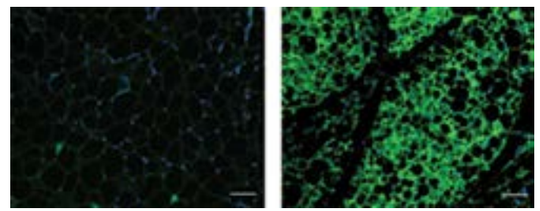
Zhao YS, Gu X, Zhang NY, Kolonin MK, An ZQ, Sun K. Divergent Functions of Endotrophin on Different Cell Populations in Adipose Tissue. *AJP-Endo & Metab.* 2016 Dec 1;311(6):E952-E963.

Park J*, Kim M*, Sun K*, An YA, Gu X, Scherer PE. VEGF-A-Expressing Adipose Tissue Shows Rapid Beiging and Enhanced Survival After Transplantation and Confers IL-4-Independent Metabolic Improvements (*contributed equally). *Diabetes.* 2017 Jun; 66(6):1479-1490.

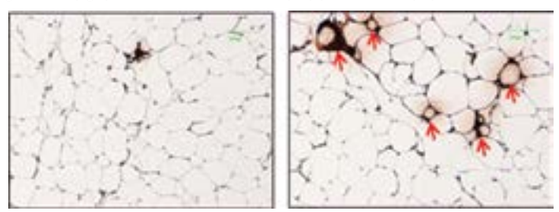
Kim ER, Fan S, Akhmedov D, Sun K, Lim H, O'Brien W, Xu Y, Mangieri LR, Zhu Y, Lee CC, Chung Y, Xia Y, Xu Y, Li F, Sun K, Berdeaux R, Tong Q. Red blood cell β-adrenergic receptors contribute to diet-induced energy expenditure by increasing O2 supply. *JCI Insight.* 2017 Jul 20;2(14).

LAB MEMBERS

Post-doctoral Fellows: Yueshui Zhao, Xin Li
Sr. Research Assistant: Xue Gu
Visiting Scholar: Shuhong Peng



Adipose Tissue-derived VEGF-A Induces Browning of White Adipocytes: Immunofluorescent (IF) staining by anti-UCP-$_1$ antibody (the marker of brown adipocytes) in subcutaneous adipose tissue of VEGF-A transgenic mice (right) and their littermate controls (left) (Scale bars, 50 μm).



Adipose Tissue-derived Endotrophin Stimulates Local Macrophage Accumulation: Immunohistochemical (IHC) staining by anti-Mac2 antibody (the marker of macrophages) in subcutaneous adipose tissue of endotrophin transgenic mice (right) and their littermate controls (left) (Scale bars, 100 μm). The arrows indicate "crown-like" structures formed by macrophage accumulation.

Qingchun Tong, Ph.D.
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 obesity and its associated diabetes. Various animal models in combination with the state of the art techniques including optogenetics, chemogenetics and *in vivo* live imaging are used in the lab.

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
- Erythrocytes as a novel therapeutic target for metabolic disorders

KEY PUBLICATIONS

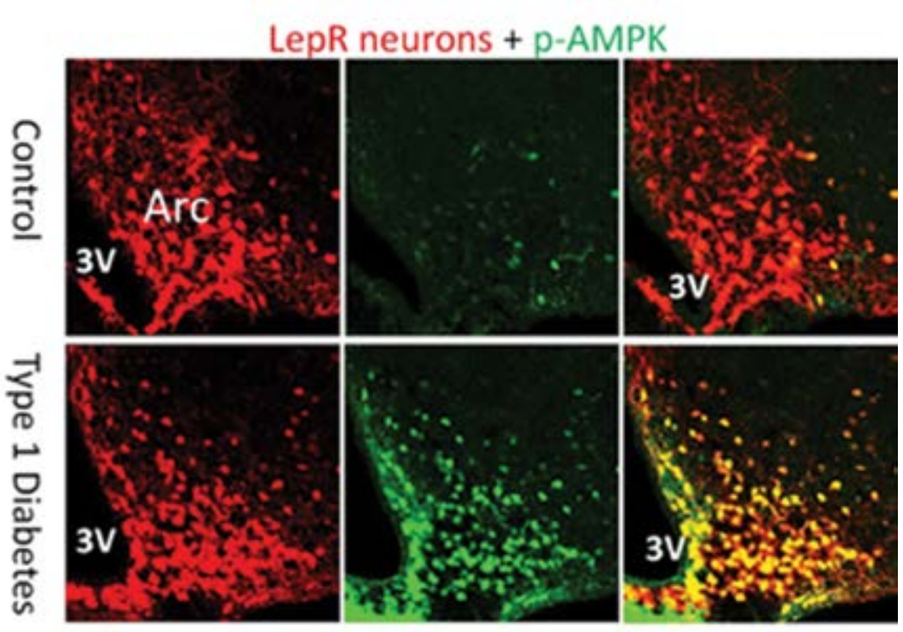
Xu Y, Lu Y, Xu P, Mangieri LR, Isingrini E, Xu Y, Giros B and Tong Q. Dopamine Release from Midbrain Leptin Receptor Neurons in High-fat Diet Feeding Regulation. *eNeuro*, 2017 May 26;4(3). pii: ENEURO.0083-17.2017.

Kim ER, Fan S, Akhmedov D, Sun K, Lim H, O'Brien W, Xu Y,. Mangieri LR, Zhu Y, Lee CC, Chung Y, Xia Y, Xu Y, Li F, Sun K, Berdeaux R and Tong Q. Red Blood Cell β-adrenergic Receptors Contribute to Diet-induced Energy Expenditure by Increasing O2 Supply. *JCI Insight*, 2017 Jul 20;2(14). pii: 93367. doi: 10.1172/jci.insight.93367

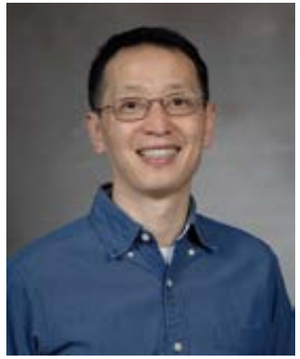
Mangieri LR, Lu Y, Xu Y, Cassidy RM, Xu Y, Arenkiel BR and Tong Q. Antagonistic Control of Feeding and Self-grooming Behaviors by GABAergic and Glutamatergic LH→PVH Projections. *Nature Communications*, 2017, accepted.

LAB MEMBERS

Post-doctoral Fellows: Yungang Lu, Ph.D.; Xugen Huang, Ph.D. (visiting)
Graduate Students: Leandra Mangieri, Ryan Cassidy, Canjun Zhu (visiting)
Instructor: Yuanzhong Xu, M.D., Ph.D.



Dramatic increase of p-AMP kinase signal (green) in the hypothalamic neurons of the brain that express leptin receptor (red) in mice with type 1 diabetes (bottom panels) compared to controls (top panels). Arc: arcuate region of the hypothalamus. 3V: the third ventricle. LepR: leptin receptor.



Sheng Zhang, Ph.D.
Assistant Professor
Becker Family Foundation Professor in Diabetes Research

Molecular mechanisms of neurodegenerative diseases

As our society is enjoying an unprecedented longer life expectancy, more people are being affected by aging-related brain degenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease. Currently, there is no effective cure or preventions against any of these debilitating maladies, which inflict unbearably high emotional and financial toll to patients and their families and pose a pressing threat to the well-being of our society. To help discover effective treatment strategies, our lab is studying the molecular machineries that neurons normally utilize to stay healthy but are disrupted in these brain diseases.

Neurodegenerative disorders are due to significant loss of neurons and their functional connections that underlie our senses, reasoning, and responses. Unlike most other cells in the body, such as skin, that are constantly dividing and being replenished each day, neurons face one particular challenge: once they are born and mature into interconnected functional units, they lose their ability to regenerate and cannot be replaced for the rest of their life. Accordingly, these long-lived neurons rely on robust self-clearance machineries inside the cell to stay health and ward off internal crisis and external insults for decades to come.

Indeed, one common pathological hallmark of almost all the neurodegenerative diseases is the presence of abnormal protein deposits, often known as tangles and plaques, in the affected brains. Cells normally operate several robust self-maintenance machines, including *chaperones* that facilitate proteins to stay in shape, and *autophagy* (meaning "self-eating" in Greek) that cleans up and recycles worn-out or toxic cellular materials. Both neurodegenerative diseases and formation of pathogenic deposits have been linked to these self-protective mechanisms, which often become inefficient or nonfunctional in aging neurons.

Using biochemical, cell biology, and genetic tools from model organism *Drosophila* to mammalian systems, we study how *chaperones* and *autophagy* operate in the cell to recognize and efficiently clear toxic materials, while sparing and protecting normal constituents. Our goal is to develop a strategy to employ these internal protection machineries to fight against aging-related diseases.

Protein folding, aggregation and clearance on neuronal survival

Chaperone Hsp110 is one of the most abundant proteins in the brain and is also a major component of the potent molecular machine called *disaggregase* that is capable of dismantling large and tightly packed protein aggregates. We are studying how this chaperone machinery works inside neurons to preserve cellular healthy and longevity.

Huntington's disease is a devastating fatal brain disorder, caused by a unique type of mutations (polyglutamine expansion) in Huntingtin protein. Recently, we found that Huntingtin itself plays an important role in a subtype of autophagy called *selective autophagy*. This raises an intriguing possibility that the disease-causing mutations can interfere with this protective mechanism, and correction of such abnormality might offer an effective therapeutic approach.

Biogenesis of specialized cellular organelles and their dysfunction in brain diseases

In neurons, specialized cellular organelles, such as synaptic vesicles (SVs) and lysosome-related organelles (LROs), control diverse aspects of cellular functions, and their disruption leads to a spectrum of disorders including AD, PD, and schizophrenia. Signal carriers, such as dopamine that is produced in neurons affected in PD, can be highly unstable chemicals normally packaged inside the membrane-enclosed SVs for their proper storage and function. We are studying the formation and regulation of these specialized cellular organelles and their links to brain diseases.

RESEARCH PROJECTS

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

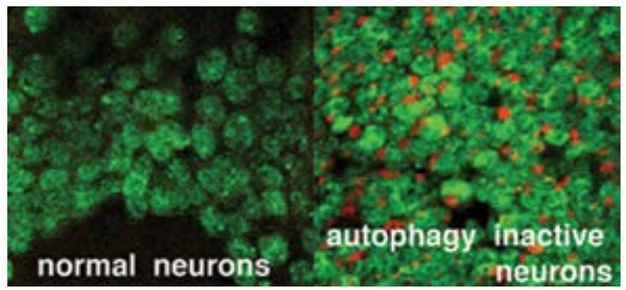
KEY PUBLICATIONS

Gabriela David-Morrison, Zhen Xu, Yan-Ning Rui, Wu-Lin Charng, Manish Jaiswa, Shinya Yamamoto, Bo Xiong, Ke Zhang, Hector Sandoval, Lita Duraine, Sheng Zhang*, Hugo J. Bellen*. "WAC Regulates mTOR Activity by Acting as an Adaptor for the TTT and Pontin/Reptin Complexes." (2016); *Dev Cell.* 36(2):139-51.

Antonio J Tito, Shebna Cheema, Mian Jiang, Sheng Zhang*."A simple one-step dissection protocol for whole-mount preparation of adult Drosophila brains". (2016) *The Journal of Visualized Experiments* (118). doi: 10.3791/55128.

LAB MEMBERS

Instructor: Shiyu Xu, Ph.D.
Post-doctoral Fellows: Boli Hu, Ph.D., Gang Li, Ph.D., Antonio Tito, Ph.D.
Research Assistants: Juan Chen, Ph.D., Mrs. Lili Ye



In neurons (green) of *Drosophila* brains, abnormal aggregates (red dots) similar as that found in human disease brains are prominent in neurons with disrupted autophagy but are completely absent in neurons with normal autophagy.

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 to medicine.

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 in 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. Translational activities further explore visualization of brain function in neonates,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 different 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, Ph.D.
Center Director & Professor
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research
Director, Center in the NCI Network for Translational Research

CENTER FOR **MOLECULAR IMAGING**

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

Diagnostic Imaging and Delivery of Therapeutics directed by NIRF imaging

The Center for Molecular Imaging (CMI) consists of an interdisciplinary team of scientists and engineers who focus upon multi-modality molecular imaging including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and our specialty, near-infrared fluorescence (NIRF) to enable new understandings in several disease states. Between 2005-2007, my team was the first to dual label imaging agents with nuclear and NIR fluorescence agents to demonstrate the potential for pre-surgical PET imaging and intraoperative NIRF imaging for reduced tumor burden in surgical oncology. In addition, my team adapted NIRF tomography within the CT gantry of a PET/CT device to demonstrate hybrid small animal PET/CT/NIRF tomography. Finally, we translated into the clinical setting unique NIRF instrumentation and trace dose of NIRF Imaging agent to demonstrate for the first time to visualize the active "pumping" of the lymphatic vasculature, which mediates immune response and maintains fluid homeostasis (Figures 1). We actively collaborate with clinical scientists in Pediatrics, Interventional Radiology, the UTHealth Vascular Anomalies Clinic, Pathology, and Otorhinolaryngology, as well as engineers and scientists at Rice, Baylor College of Medicine, and the Methodist Hospital. Our team effectively translates new NIR imaging technologies literally from "bench-to-bedside" and back again in order to make discoveries in translational NIR. Fluorescence lymphatic imaging studies conducted by the CMI team include identifying key signaling pathways and regulators associated with aberrant processes of lymphangiogenesis and lymphatic stasis in human diseases and in animal models of human disease.

RESEARCH PROJECTS

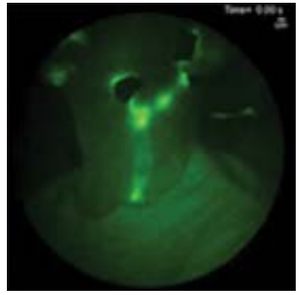
- Imaging chylo-and lymphothorax in children with congenital heart defects
- Molecular Imaging of MMP-targeted viral gene delivery vectors for treatment of heart disease
- Lymphatic delivery of therapeutics targeting the immune system
- Imaging cancer-positive lymph nodes with a cancer-specific near-infrared fluorescent molecular imaging agent to guide intraoperative lymph node dissection
- Imaging lymphatic responses to progressive rheumatoid arthritis and its treatment with biologics
- Imaging lymphatic responses to radiation and surgery in head and neck cancer
- Assessing CSF outflow into the lymphatics under microgravity conditions
- Small animal imaging and tomography

KEY PUBLICATIONS

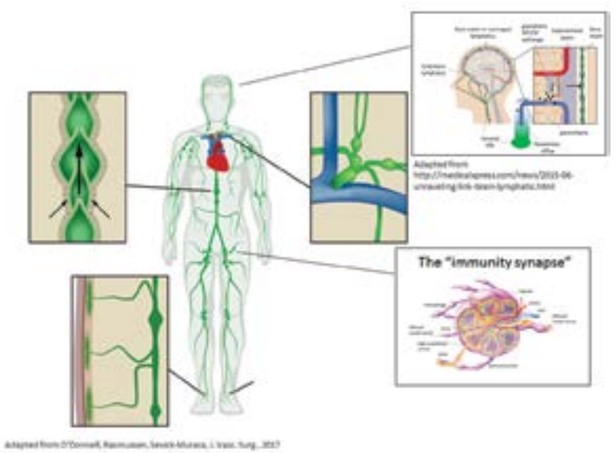
O'Donnell, T.F., Rasmussen, J.C., and E.M. Sevick-Muraca, "New diagnostic modalities in the evaluation of lymphedema," *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, Mar; 5(2): 261-273, 2017 PMID: 28214496.

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.

Sevick-Muraca, E.M., Kwon, S.K., and J.C., Rasmussen, "Emerging lymphatic imaging Technologies for mouse and man," *Journal of Clinical Investigation*, 124(3): 905-14, 2014. PMID: 24590275



Lymphatic outflow imaged in head and neck cancer. Reproduced from Rasmussen, J.C., Tan, I.C., Naqvi, S., Aldrich, M.B., Maus, E.A., Blanco, A.I., Karni, R.J., and E.M. Sevick-Muraca, "Longitudinal monitoring of head and neck lymphatics in response to surgery and radiation," Head and Neck, 39(6): 1177-1188, 2017 PMID: 28263428.



The lymphatics in health and disease. Reproduced in part from O'Donnell, T.F., Rasmussen, J.C., and E.M. Sevick-Muraca, "New diagnostic modalities in the evaluation of lymphedema," *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, Mar; 5(2): 261-273, 2017 PMID: 28214496.

Melissa B. Aldrich, M.B.A., Ph.D.
Assistant Professor

Lymphatic functional and immune aberrations in lymphedema

The lymphatic system is the "other" circulatory system and works with the blood circulatory system to ensure fluid homeostasis, dietary lipid absorption, immune surveillance, and immune cell transit. After the heart pumps blood to tissues, 90% of the resulting fluid in interstitial spaces returns to the blood vascular system via venous capillary uptake. Lymph forms when the other 10% of interstitial fluid, containing cellular waste, molecules that are too large to enter venous capillaries, and immune cells, enters the unidirectional, blind-ended lymphatic capillaries. Lymph then proceeds to larger collector lymph vessels, and passes through numerous lymph nodes before reuniting with the blood circulatory system through the subclavian veins and thoracic duct.

An average-sized human adult body contains 12 liters of interstitial fluid, and 4 liters of lymph pass through the thoracic duct daily. When lymphatic transit is disrupted, as happens after cancer treatment, for example, swelling, pain, infection, and other morbidities arise, and a permanent disease called lymphedema (LE) results. Over 15 million people in the U.S. currently are burdened with LE, and there is only palliative treatment (daily, time-consuming compression bandaging and massage)—no cure is available.

My colleagues recently developed near-infrared fluorescence lymphatic imaging (NIRFLI), the only imaging system in the world that can provide real-time movies of lymphatic pumping. This imaging modality has been translated (FDA approval for research) to the bedside, and I am now surveying changes in lymphatic function and blood immune cells in breast cancer patients at the Nellie B. Connally Breast Cancer Center at MD Anderson. 40% of these patients are a high risk for developing LE, and I hope to identify imaging and serum biomarkers of early LE development. Several studies by others have shown that early treatment of LE can often deter and even reverse LE development, and identification of serum biomarkers could suggest appropriate oral therapeutics for LE.

RESEARCH PROJECTS

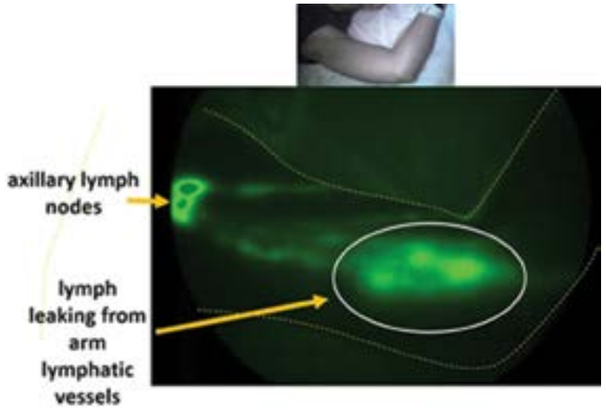
- 5-year longitudinal, prospective study of breast cancer patients at high risk for LE (NIH funded)
- Phase 1 clinical study of rheumatoid arthritis patients receiving an anti-inflammatory medication via lymphatic delivery
- Clinical study of lymphatic dysfunction in peripheral venous disease
- Clinical study of pneumatic compression treatment efficacy in LE in head and neck cancer survivors, primary/genetic LE patients, and cancer-associated LE patients

KEY PUBLICATIONS

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

Greives M.R., Aldrich M.B., Sevick-Muraca E.M., and Rasmussen J.C. Near-infrared fluorescence lymphatic imaging of a toddler with congenital lymphedema. *Pediatrics* 139(4):e20154456 (PMID: 28356336), 2017.



Near-infrared fluorescence lymphatic image (NIRFLI) of axillary lymph node basin/upper arm in a breast cancer patient. Leaky lymphatics indicate early lymphedema development.

Ali Azhdarinia, Ph.D.
Assistant 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

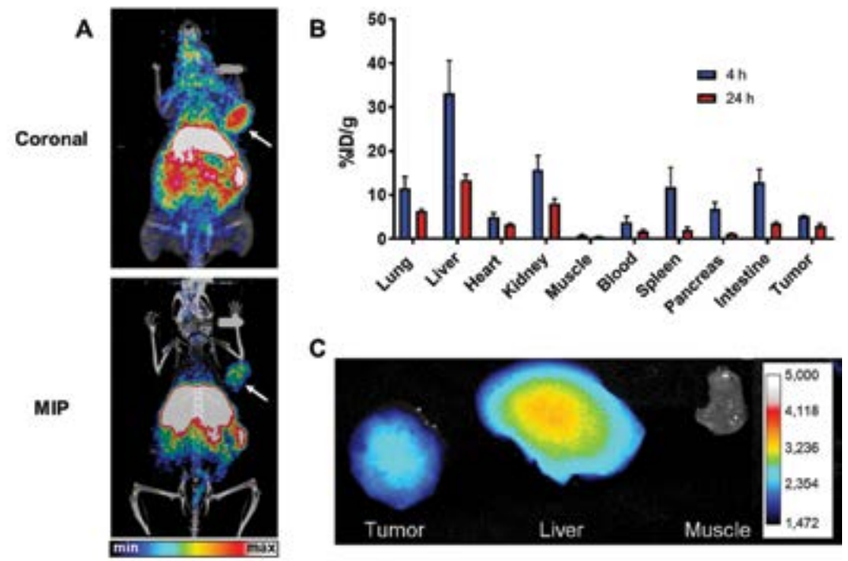
Lim, H.J., Perera, H., Wilems, T.S., Ghosh, S., Zheng, Y., Azhdarinia, A., Caoab, Q., Smith Callahan, L.A. Response to di-functionalized hyaluronic acid with orthogonal chemistry grafting at independent modification sites in rodent models of neural differentiation and spinal cord injury. *J. Mater. Chem. B,* 4, 6865, 2016.

Ghosh, S.C., Rodriguez, 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.* 2017 [Epub ahead of print]. PMID:28572490.

Ghosh, S.C., Hernandez Vargas, S., Rodriguez, M., Kossatz, S., Voss, J., Carmon, K.S., Reiner, T., Schonbrunn, A., Azhdarinia, A.* Synthesis of a Fluorescently Labeled 68Ga-DOTA-TOC Analog for Somatostatin Receptor Targeting. *ACS Med Chem Lett.* 6;8(7):720-725, 2017. PMID:28740605

LAB MEMBERS

Graduate Students: Servando Hernandez Vargas
Research Assistant: Julie Voss
Research Scientist: Sukhen Ghosh



Nuclear imaging of a tumor-bearing mouse injected with a multimodality, tumor-seeking contrast agent (A), corresponding uptake in key tissues (B) and near-infrared fluorescence imaging of resected tissues (C).

Barrett Rowland Harvey, Ph.D.
Assistant Professor

Development of diagnostic imaging agents for cancer and infectious disease

Engineered to recognize and specifically target disease, antibodies are powerful tools for both basic and translational research. 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 infection. Taking advantage of antibody specificity and technological achievements in antibody engineering, our lab also develops antibody-based imaging 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 surgeons in the removal of disease.

RESEARCH PROJECTS

- Antibody agent development for molecular imaging of cancer and infectious disease
- Virulence factor regulation governing bacterial infection

KEY PUBLICATIONS

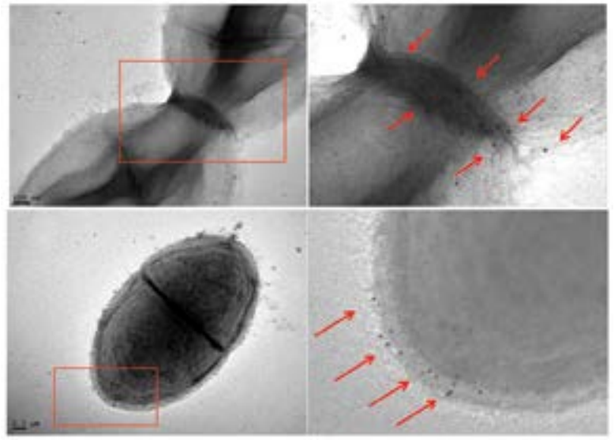
Processing of the major autolysin of *E. faecalis*, AtlA, by the zinc-metalloprotease, GelE, impacts AtlA septal localization and cell separation. Stinemetz EK, Gao P, Pinkston KL, Montealegre MC, Murray BE, Harvey BR. PLoS One. 2017 Oct 19;12(10):e0186706. doi: 10.1371/journal.pone. 0186706. *eCollection* 2017. PMID: 29049345

Antibody Guided Molecular Imaging of Infective Endocarditis. Pinkston KL, Gao P, Singh KV, Azhdarinia A, Murray BE, Sevick-Muraca EM, Harvey BR. *Methods Mol Biol.* 2017;1535:229-241. PMID: 27914083

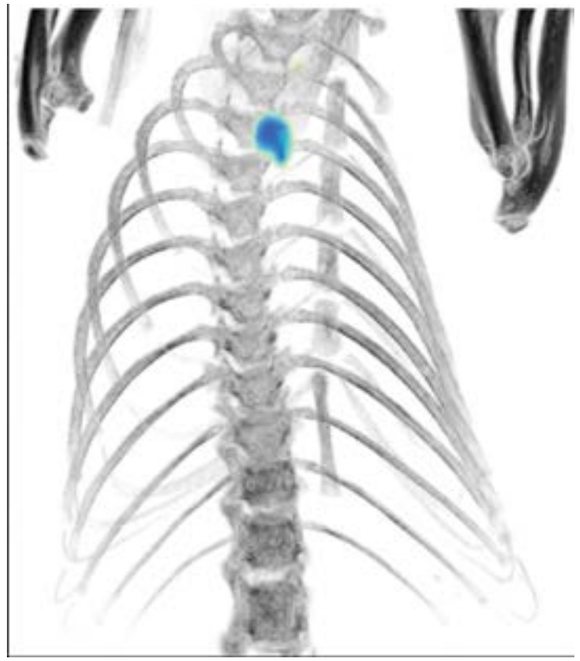
Functional studies of *E. faecalis* RNase J2 and its role in virulence and fitness. Gao P, Pinkston KL, Bourgogne A, Murray BE, van Hoof A, Harvey BR. PLoS One. 2017 Apr 6;12(4):e0175212. doi: 10.1371/journal.pone.0175212. *eCollection* 2017. PMID: 28384222

LAB MEMBERS

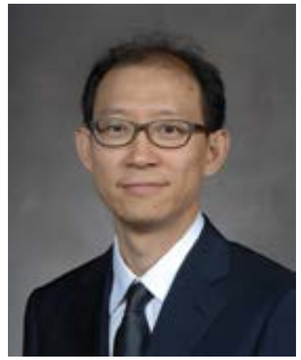
Research Instructor: Peng Gao, Ph.D.



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



Live animal imaging to diagnose enterococcal endocarditis infection within the heart valve of a rat.



Sun Kuk Kwon, Ph.D.
Assistant Professor

Functional lymphatic imaging in animal models of lymphovascular disorders

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, I've 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.

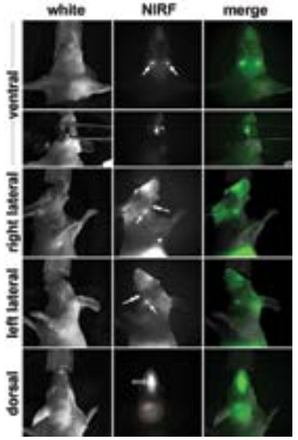
Recently, CMI demonstrated that direct infusion of an immune-attenuating biologic into the lymphatics can result in improved local and systemic responses when compared to conventional routes of administration in an arthritis rat model. Recently, we used the novel lymphatic infusion device for immunotherapy to maximize drug exposure to tumor-draining LNs and reduce toxicity by localizing immune stimulation to the regional lymphatics for systemic anti-tumor immunity. We 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. We 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 functional lymphatic imaging to show the efficacy of drug delivery into the lymph nodes of animal models of lymph node metastasis and autoimmune disorders
- Assess brain lymphatics and cerebrospinal fluid (CSF) outflow into the peripheral lymphatics in Alzheimer disease (AD) transgenic mice
- Develop new methods for assessing CSF drainage in mice and swine for translation with intrathecal delivery of drugs as a viable delivery route
- Develop PET/CT methodology for quantifying infarct size in gene therapy trials of myocardial infarct in mice



White light, NIR fluorescent, and merge images in the ventral, right and left lateral, and dorsal view of a mouse showing CSF drainage to the cisterna magna and supracerebellar cistern (red circle), endoturbinate (open arrow), and finally to the MLNs (arrow) after intrathecal injection. Double arrows, lymphatic vessels. Arrowhead, liver. Asterisk, upper hard palate in the mouth.

KEY PUBLICATIONS

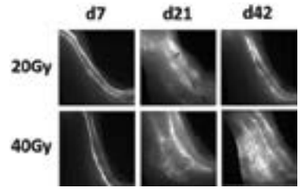
Sunkuk Kwon and Price, R.E. "Characterization of internodal collecting lymphatic vessel function after surgical removal of an axillary lymph node in mice," *Biomedical Optics Express*, 7; 1100-1115, 2016.

Sunkuk Kwon and Eva M. Sevick-Muraca. "Effect of lidocaine with and without epinephrine on lymphatic contractile activity in mice *in vivo*," *Journal of Anesthesia*, 30; 1091-1094, 2016.

Sunkuk Kwon and Eva M. Sevick-Muraca, "Effects of Depilation-Induced Skin Pigmentation and Diet-Induced Fluorescence on *In Vivo* Fluorescence Imaging," *Contrast Media & Molecular Imaging*, vol. 2017, Article ID 7659242, 7 pages, 2017. doi:10.1155/2017/7659242

Melissa B. Aldrich, Fred C. Velasquez, Sunkuk Kwon, Ali Azhdarinia, Kenneth Pinkston, Barrett R. Harvey, Wenyaw Chan, John C. Rasmussen, Russell F. Ross, Caroline E. Fife, and E. M. Sevick-Muraca. "Lymphatic delivery of etanercept via nanotopography improves response to collagen-induced arthritis," *Arthritis Research & Therapy*, 19; 116, 2017.

Sunkuk Kwon, Christopher F. Janssen, Fred Christian Velasquez, and Eva M. Sevick-Muraca, "Fluorescence imaging of lymphatic outflow of cerebrospinal fluid in mice," In press, *Journal of Immunological Methods*, 2017.



Magnified near-infrared fluorescent images in the foot of mice 7, 21, and 42 days after radiation. Our preliminary data showed significant changes of lymphatic vasculature in mice 21 days after radiation with 20 and 40Gy; however, only mice treated with 40Gy showed persistent lymphatic abnormality as compared to mice with 20Gy.

John Rasmussen, Ph.D.
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. 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. We are currently imaging additional subjects with early venous or arterial disease to determine at what stage of disease the abnormal lymphatic anatomy and function appear, and whether these lymphatics changes are a result of or contribute to the development of the vascular disease. A better understanding of the role of the lymphatics in early vascular disease may enable the development of more efficacious therapeutic approaches. To date, we have observed abnormal lymphatic anatomy and/or function in all subjects with early disease. Additional studies focus on the relationship between the lymphatics and vascular malformations.

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 are currently using NIRF imaging to assess the lymphatic response to a new advanced pneumatic compression device developed specifically for subjects with head and neck lymphedema and have observed a reduction in the extent of abnormal lymphatics in as little as two weeks.

We continue the development of this technology, including 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

Rasmussen, J.C.,* Tan, I.,* Naqvi, S., Aldrich, M.B., Maus, E.A., Blanco, A.I., Karni, R.J., and Sevick-Muraca, E.M., "Longitudinal monitoring of the head and neck lymphatics in response to surgery and radiation," *Head and Neck,* 39(6):1177-1188, 2017 (PMID: 28263428).

Rasmussen, J.C.,* Zvavanjanja, R.C.,* Aldrich, M.B., Greives, M.R., and Sevick-Muraca, E.M., "Near-infrared fluorescence lymphatic imaging of Klippel-Trénaunay syndrome," Journal of vascular surgery: *Venous and lymphatic disorders*, 5(4):533-537, 2017 (PMID: 28623992).

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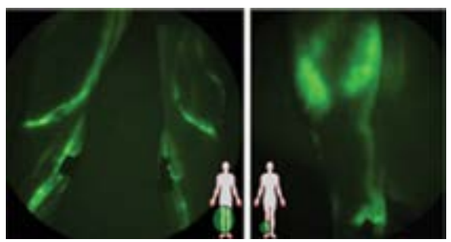
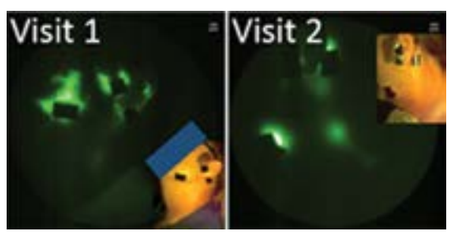


Normal lymphatic vessels in a healthy volunteer (left) and abnormal lymphatics in a patient with stage C4 venous disease (right)



Reduction of abnormal lymphatics after two weeks of advance pneumatic compression therapy in a patient with head and neck lymphedema (Reproduced from Rasmussen, et al., *Head and Neck*, 2017).

Banghe Zhu, Ph.D.
Assistant Professor

High-resolution diffuse optical tomography for brain functional imaging in children with brain tumor; and assessment of delivery and infection of protease activated virus in animal model of myocardial infarction

High-resolution diffuse optical tomography for brain functional mapping in children with brain tumor: Brain functions, including cognitive functions, are frequently disturbed in brain tumor patients. These disturbances may result from the tumor itself and also from the treatment directed against the tumor. Surgery, radiotherapy, and chemotherapy all may affect cerebral functioning, both in a positive as well as in a negative way. Apart from the anti-tumor treatment, glioma patients often receive glucocorticoids and anti-epileptic drugs, which both also have influence on brain functioning. Childhood cancers, such as leukemia, also have evidence of cognitive decline after treatment. Childhood brain tumors represent an anatomically and biologically diverse group of neoplasms that can present with both common and unusual symptoms; chemotherapy and radiation treatment are made on basis of optimizing tumor treatment and minimizing brain network dysfunction side effects. Currently, we are developing a high resolution, multi-pixel technique based Functional Near Infrared Spectroscopy - Diffuse Optical Tomography (fNIRS-DOT) imaging system with a computationally-efficient tomographic algorithm to assess brain network dysfunction in children with brain tumors.

Assessment of delivery and infection of protease activated virus in animal model of myocardial infarction (MI): Heart disease is the leading cause of death in the U.S. and close to 5 million Americans have heart failure. Gene therapy has the potential to treat a number of cardiac diseases. The Suh lab at the Department of Biomedical Engineering at Rice University was the first to create adeno-associated virus (AAV) vectors that could be activated by extracellular proteases that are biomarkers of tissue remodeling. Validation of *in vivo* AAV targeting and infection strategy depends upon quantitatively associating MMP activity with vector delivery and transgene expression. Use of far-red fluorescent protein (iRFP) represents a method to assess transgene expression in preclinical studies. While molecular imaging of MMP could provide an important companion diagnostic in clinical gene therapy trials that employ MMP targeted therapies, fluorescence-based techniques are not suited for the deep location of the heart in humans. Previously, we developed a dual-labeled imaging agent targeting active gelatinases (MMP -2/-9) for non-invasive, *in vivo* microPET/CT and NIRF imaging. We use *in vivo* microPET/CT imaging of mice with induced myocardial infarct to detect MMP activity and *ex vivo* iRFP and NIRF fluorescence measurements calibrated in Standard International (SI) units for assessing accuracy of protease-activatable virus (PAV) targeting.

RESEARCH PROJECTS

- Develop high-resolution diffuse optical imaging system
- Develop 3-D tomography algorithm for brain imaging
- Assess the gene therapy using iRFP gene reporter
- Participate in peripheral vascular disease clinical studies
- Participate in head and neck surgery clinical studies

KEY PUBLICATIONS

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.

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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., and Sevick-Muraca, E.M., "Update on clinical near-infrared fluorescence imaging: A review of devices, performance, and applications," *British Journal of Radiology,* 88:20140547(2015).

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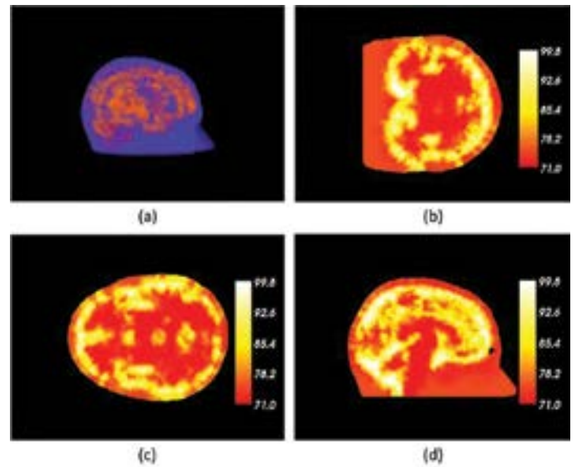
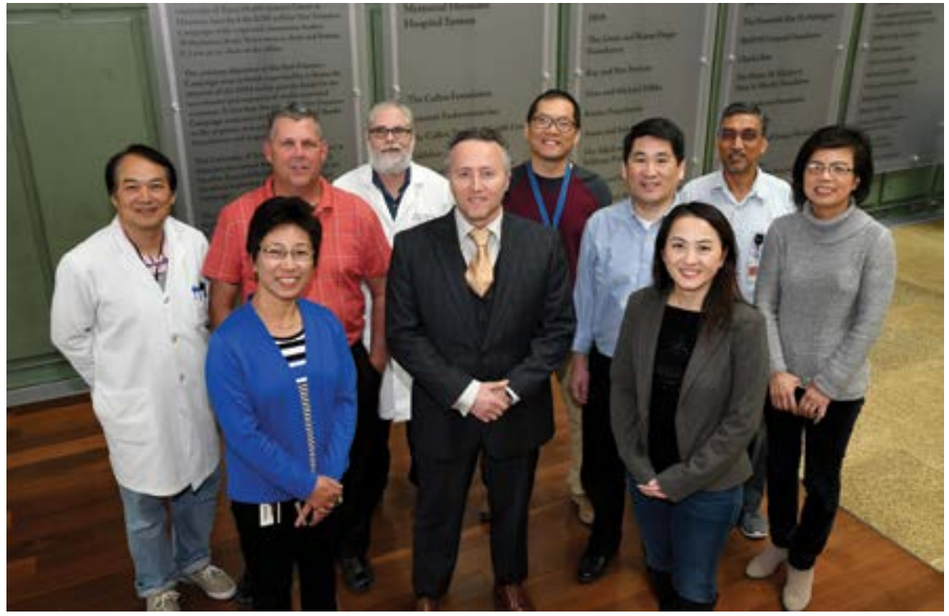


3-D (a), Coronal (b), Transverse (c), and Sagittal (d) cross-sections of the reconstructed oxygen saturation of the children's brain.



The Center for Precision Biomedicine focuses on developing the mathematical, experimental, and analytical technologies that will deliver precise medication to the correct tissues. This is accomplished by understanding the underlying physiological problems, the proteomic and genomic biomarkers indicative of disease, selective targeting of tissues or toxins, the use of targeted nanoparticles, and mechanistic mathematical models of tissue and vasculature to predict and overcome biological barriers to tissue penetration.

These efforts connect us with collaborators across UTHealth; institutions within the Texas Medical Center, such as Baylor, Methodist and MD Anderson; and across Texas through interactions with the centers/programs for Clinical and Translational Science, nanomedicine researchers, and faculties studying disease mechanisms using proteomics, genomics, and bioinformatics. At the IMM, we have state-of-the-art mass spectrometers, providing in-depth proteomic analysis of cells, tissues, or biological fluids, leading to the discovery of novel targets for drug development and biomarkers for early detection and personalized precision medicine. We also develop new aptamers and multi-functional nanoparticle therapeutics for targeting pathological tissues, such as cancer. Using custom-built NIRS/Ramen spectrometers, we provide novel ways to investigate the structures of diseased bones and ulcerative colitis. 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. We provide multiscale mathematical and computational modeling to aid current prospective clinical trials focusing on understanding drug penetration barriers in tumors and improving tumor response and patient outcomes.

Hubs of Research Collaboration with the Center include:

- Clinical and Translational Proteomics Core Laboratory
- Nanochemistry/3D-printing Service Center
- NCI Programs in Computational Cancer Biology and Nanomedicine
- UT System-wide Proteomics Core Facility Network
- UTHealth/MDACC Clinical and Translational Center for Translational Technologies

Vittorio Cristini, Ph.D.
Professor and Director, Center for Precision Biomedicine, Institute for Molecular Medicine
Rochelle and Max Levit Chair in the Neurosciences
University of Texas System STAR Fellow

Vittorio Cristini, Ph.D.
Professor and Director
Rochelle and Max Levit Chair in the Neurosciences

Translational biomedicine

Our research program focuses on developing mechanistic biophysical models for predicting tumor response to various treatment methods (e.g., chemotherapy, targeted therapy, nanomedicine, and immunotherapy) in individual patients using standard clinical diagnostic measurements, such as histopathology, CT, and MRI. We have taken a multidisciplinary approach in our projects, which has resulted in novel mathematical modeling algorithms and insights into how and why cancer behaves the way it does in each patient. Our ultimate goal is to bring our models to the clinic so that patient outcomes can be improved. Currently, we have three major research areas.

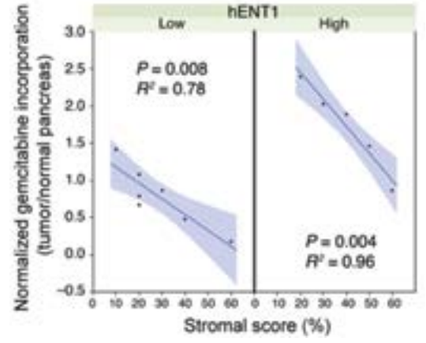
Translational physical oncology. Physical processes such as transport mechanisms for drug molecules within tissue and the forces exchanged by cancer cells with the surrounding tissue determine cancer growth and treatment outcome. We apply engineering and physical sciences approaches to the modeling of complex normal and pathologic biological tissue. Toward clinical translation of the mathematical models, we have been investigating the effects of diffusion, perfusion, and other transport mechanisms on the rate at which tumors grow and spread and on resistance to drug and other systemic therapies, based on input from experimental and patient data. We have produced a series of pioneering modeling work on describing and quantifying physical mechanisms that play fundamental roles in the growth of cancer and in response to therapies. Through our joint work with pathologists and oncologists, we have made important discoveries on the role of physical transport in patient drug resistance. We just published a book (monograph) in this area: *An Introduction to Physical Oncology,* CRC Press.

Multiscale modeling. Biological processes can occur across physical time and space scales, forming a complex system with multiple feedback and feed-forward loops. Advanced multiscale methods are therefore needed to simulate and predict the behavior of complex biological systems. We are developing methods to address a significant challenge in multiscale modeling, i.e., bridging the gaps between different modeling methods and between models at different scales, from the molecular, to the cellular and tissue scales, based on "dynamic density functional theory," a technique implemented in the physical sciences.

Systems pharmacology and drug pharmacokinetic-pharmacodynamic (PKPD) modeling. Many PKPD models based on ordinary differential equations (ODEs) have been developed to describe the temporal response of tumor and normal cells to chemotherapy or other therapeutics. However, drug resistance sometimes occurs due to limited penetration of drugs deep into the tumor, implying that not only "time" but also "space" factors have an impact on drug efficacy in both normal and tumor tissue. We are investigating a combined PKPD and spatiotemporal tumor modeling approach to study tumor response to chemotherapy.

RESEARCH PROJECTS

- Biophysical theories to predict the growth and invasion and drug response in local and metastatic cancers
- Mechanistic modeling of tumor response to immunotherapy
- Upscaling and downscaling framework (i.e., functionally linking biological behaviors at different scales)



A mass transport model of pancreatic ductal adenocarcinoma (PDAC) reveals that gemcitabine incorporation in the tumors is highly variable and correlates inversely with the amount of stroma, after accounting for hENT1 levels.

KEY PUBLICATIONS

Hosoya H, Dobroff AS, Driessen WH, Cristini V, Brinker LM, Staquicini FI, Cardo-Vila M, D'Angelo S, Ferrara F, Proneth B, Lin YS, Dunphy DR, Dogra P, Melancon MP, Stafford RJ, Miyazono K, Gelovani JG, Kataoka K, Brinker CJ, Sidman RL, Arap W, Pasqualini R. Integrated nanotechnology platform for tumor-targeted multimodal imaging and therapeutic cargo release. *Proc Natl Acad Sci U S A* 113:1877-82, 2016

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LAB MEMBERS

Post-doctoral Fellows: Joseph Butner, Ph.D.
Graduate Students: Prashant Dogra, Terisse Brocato, Naomi Hasegawa, Jorge Tito, Oluwadara Coker
Research Scientists: Satyanarayan Nandi, Ph.D.

Jeffrey Chang, Ph.D.
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. We wish to understand the events that lead tumor cells to acquire a metastatic state, whether through acquired mutations or transdifferentiation processes. 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. We have identified a selection of natural compounds and FDA-approved drugs targeting novel pathways that have shown the ability to inhibit metastasis in preclinical models.

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 backwards-chaining expert system that leverages a knowledge base containing descriptions of common bioinformatics algorithms.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

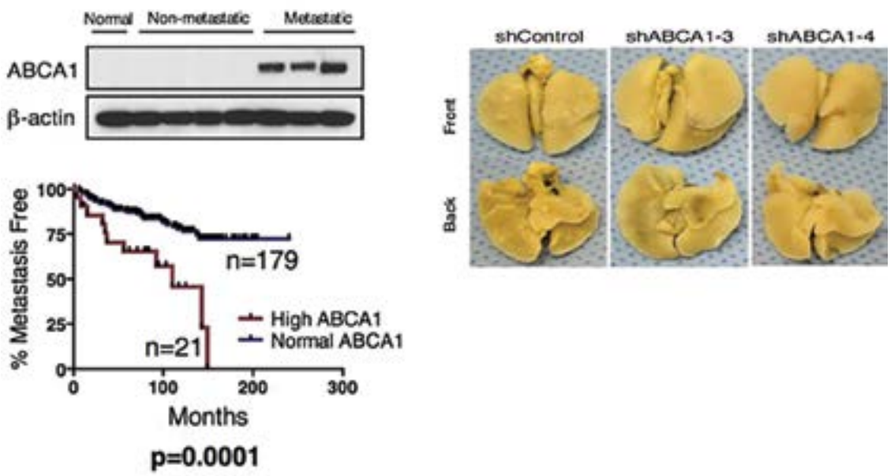
Zhao W., Prijic S., Urban B., Tisza M.J., Li L., Tan Z., Chen X., Mani S.A., and Chang J.T.: Candidate anti-metastasis drugs suppress the metastatic capacity of breast cancer cells by reducing membrane fluidity. *Cancer Research* 76(7):2037-49, 2016.

Tisza M.J., Sjol J.S., Chen X., Levental I.*, and Chang J.T.*: Motility and stem cell properties induced by the Epithelial-Mesenchymal Transition require destabilization of lipid rafts. *Oncotarget*, 2016.
* Co-Corresponding Authors

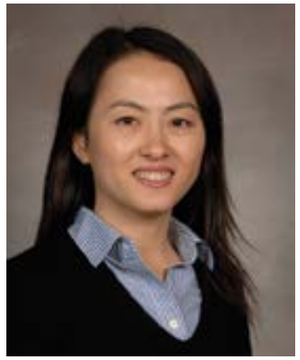
Chen X and Chang J.T.: Planning bioinformatics workflows using an expert system. *Bioinformatics* 33(8), 2017.

LAB MEMBERS

Post-doctoral Fellows: Weina Zhao, Ph.D., Sarah Prjic, Ph.D.. Huan Qiu, Ph.D.
Graduate Student: Kevin Zhu
Research Assistant: Aurnab Baidya
Visiting Scientists: Ying Luo, Juhua Dan



We have identified a cholesterol efflux channel that is expressed at high levels in metastatic breast cancer cell lines (top left panel). In human breast cancer tumors, high expression of ABCA1 is associated with 100% chance of metastasis, while only about a quarter of the patients with normal ABCA1 metastasize (bottom left panel). In a mouse model, knockout of ABCA1 leads to a significant reduction of lung metastases in a breast cancer model (right panel).



Xiaohong Bi, Ph.D.
Assistant Professor

Optical spectroscopy and imaging for medicine

Optical spectroscopy and imaging techniques have demonstrated great potential in providing noninvasive *in situ* diagnosis. Our research focuses on developing optical tools, especially Raman spectroscopy (RS), for clinical problems, such as early disease diagnosis, therapy response evaluation, and guidance of surgery.

RS exploits subtle changes in the molecular composition of tissue and is sensitive to disease- and aging- associated biochemical changes in tissue environment. We are currently using an RS fiber optic system to test patients with inflammatory bowel disease (IBD) in clinics. *In vitro* RS studies on colon biopsies have shown over 99.7% accuracy in differentiating the two distinct yet often indeterminate forms of IBD: ulcerative colitis and Crohn's colitis. The incorporation of RS to colonoscopy is expected to improve diagnosis accuracy *in situ*. Further application of RS in cancer diagnosis and surgical margin assessment is also being explored in our laboratory.

We have extensive experience in quantifying bone quality, which are important determinants of fracture resistance. The effect of genetic variations and disease on bone compositional properties and mechanical function is constantly studied in the lab. In addition, we have developed RS spectral markers that are related to breast and prostate cancers induced bone alterations. These markers can be used to assess bone quality and to evaluate the response of metastatic bone to treatment. A noninvasive *in vivo* Raman system has been developed to transcutaneously evaluate bone quality *in vivo*. Currently this system is being applied for translational studies in clinics, investigating changes in bone quality with diabetes and aging.

Another area of research involves developing targeted imaging and biosensing methods using surface enhanced Raman spectroscopy (SERS). By combining RS and nanotechnology, such SERS methods can detect biomarkers in body fluid in up to fm scale.

RESEARCH PROJECTS

- Develop and apply noninvasive endoscopic Raman spectroscopy to provide diagnostic information for inflammatory bowel disease, colorectal cancer, Barrett's esophagus, and other diseases in gastrointestinal tract
- Evaluate changes in bone quality and mechanical functions with aging, genetic defect, diabetes, and other bone metabolic disorders in preclinical models and patients
- Develop and apply *in vivo* Raman techniques to evaluate bone quality in patients and to predict the fracture risk
- Cancer-targeted imaging using ultrasensitive SERS imaging technique and targeted nanoparticles

KEY PUBLICATIONS

H. Ding, A. W. Dupont, P. Wang, S. Singhal, L. D. Scott, S. Guha, M. Younes, X. Bi, *In vivo* analysis of mucosal lipids reveals histological disease activity in ulcerative colitis using endoscopic Raman spectroscopy, 2017, *Biomedical Optics Express,* 2017, 8(7): 3426-3439.

H. Ding, A. W. Dupont, S. Singhal, L. D. Scott, S. Guha, M. Younes, Y. Ye, X. Bi, Effect of physiological factors on the biochemical properties of colon tissue – An *in vivo* Raman spectroscopy study, *Journal of Raman spectroscopy,* 2017, 48(7): 902-909

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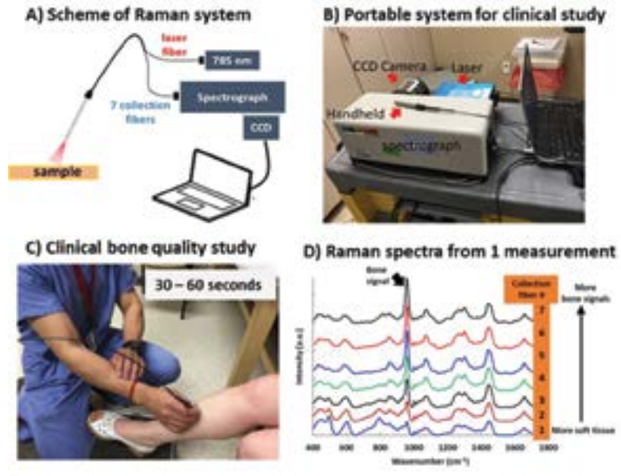
Q. Zhang, X. Sun, J. Yang, H. Ding, D. Lebrun, K. Ding, C. Houchen, RG Postier, CG Ambrose, Z. Li, X. Bi, M. Li, ZIP4 silencing improves bone loss in pancreatic cancer, 2015, *Oncotarget,* 2015, 6(28):26041-51 (2015)

H. Ding, J.S. Nyman, J.A. Sterling, D.S. Perrien, A. Mahadevan-Jasen, and X. Bi, Development of Raman Spectral Markers to Assess Metastatic Bone in Breast Cancer, *Journal of Biomedical Optics,* 19(11): 111606 (2014)

LAB MEMBERS

Research Assistant: Guijin Lu



The scheme (A) and the picture of the portable Raman system for transcutaneous *in vivo* detection of bone composition, which is indicative of bone quality. C) Ongoing clinical application for fracture risk assessment. D) Representative spectra collected concurrently from one measurement, showing increasing bone signals from depths.

Sheng Pan, Ph.D.
Associate Professor

Proteomics in deciphering proteome alterations associated with diseases

Proteins are essential functional biomolecules that are involved in all sorts of cellular physiologic activities and have been important targets for drug development and early detection of diseases. Proteomics, especially quantitative proteomics, has been emerging as a powerful tool in 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, post-translational modifications (PTMs), and protein-protein interactions, which may all lead to malfunction of cellular processes. In our lab, mass spectrometry based proteomics technologies are applied to study pancreatic cancer and other diseases. These studies are carried out with various goals, aiming to better understand the molecular mechanisms underlying tumorigenesis, to investigate changes in PTM status associated with diseases, and to identify cancer associated protein biomarkers to improve diagnosis or therapeutic treatment. The samples analyzed include a variety of disease and control specimens, including tumor tissues, blood and other bodily fluids, as well as isolated cells from various clinical specimens. Our current research interests include: 1) discovery of protein biomarkers and drug targets in pancreatic ductal adenocarcinoma (PDAC), especially proteome changes associated with early malignant signals in PDAC precursors; 2) Investigation of protein glycosylation alterations in malignances, chronic inflammation and neurodegenerative diseases; 3) mass spectrometric characterization of protein glycation adducts in association with cancer and other diseases; 4) innovation of proteomics technologies for clinical applications. Currently, our disease focuses are pancreatic cancer and other GI-tract diseases. In addition, through collaborative efforts, our lab also supports proteomics investigation of chronic inflammation, neurodegenerative diseases, GI-tract microbiome, HIV and other diseases. Systems biology and bioinformatics are important components in our study for analysis of protein interaction networks and regulatory pathways associated with cancer and other disease mechanisms to facilitate biomarker discovery for early detection, treatment selection and therapeutic targets.

RESEARCH PROJECTS

- Development of proteomic signatures for early detection of pancreatic cancer in diabetics
- Characterization of protein glycation end-products (AGEs) linked to pancreatic cancer in diabetics
- Investigation of the macroheterogeneity of protein N-glycosylation to elucidate malignancy associated alterations in N-glycosylation pathways
- Characterization of proteome alterations in plasma/serum associated with drug treatment response in pancreatic cancer
- Quantitative proteomics to support biomarker development for ulcerative colitis-associated colorectal cancer
- Development of quantitative proteomics and bioinformatics for digital biobanking

KEY PUBLICATIONS

Chen R, Lai LA, Sullivan Y, Wong M, Wang L, Riddell J, Jung L, Pillarisetty VG, Brentnall1 TA, Pan S, "Disrupting glutamine metabolic pathways to sensitize gemcitabine-resistant pancreatic cancer", *Scientific Reports,* 2017, ;7(1):7950.

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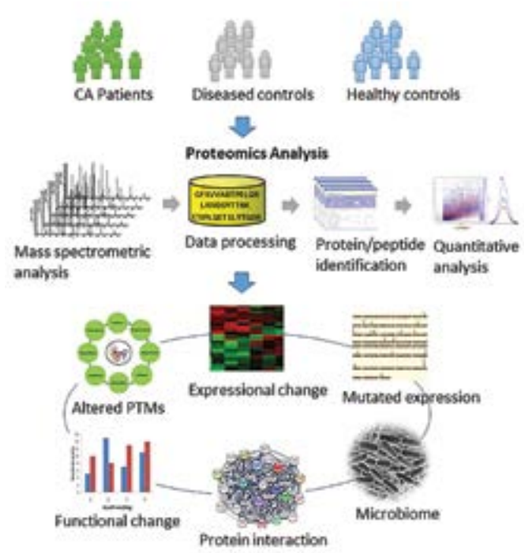
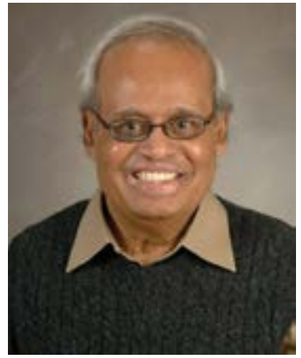
LAB MEMBERS

Post-doctoral Fellow: Hong Peng, Ph.D.
Research Scientist: Yumi Sullivan
Visiting Scientist: Lei Wang, M.D./Ph.D.
Undergraduate Research Internship: Kristina Stevanovic, Azhar Khandekar



Proteomics in clinical and translational studies



Satyanarayan Nandi, Ph.D.
Adjunct Professor

Understanding universal drug resistance for targeted therapy and immunotherapy for cancers using the laws of physics

The targeted therapy in the past 12 years, and more recently the immunotherapy in the past 5 years, have been quite successful in treating several cancers and certainly extended the life of many patients. However, for both of these treatments, only about a third of the patients responds, and those responding develop drug resistance in about a year on the average. Two major questions are: 1) Can we determine a way to separate the responders from the non-responders before the treatment begins? In the traditional biological approach, intense research /studies are going on to out such biomarkers: genetic mutation pattern and pathways of escape. The plan of my research to try to find out such biological mechanism using the principles of physics. 2) Can we figure out the mechanism for the universal drug resistance that develops for the patients who do respond? What are the intrinsic resistance, and the acquired and adaptive resistance the cancer cells develop? So far, together with Dr. V. Cristini, I have developed a mathematical model which describe both the response and universal drug resistance using physics ideas. The ordinary differential equation describing the model can be written as dy/dt = ay – b y^2 + ct. Here, y is the normalized tumor volume at time t = 0.

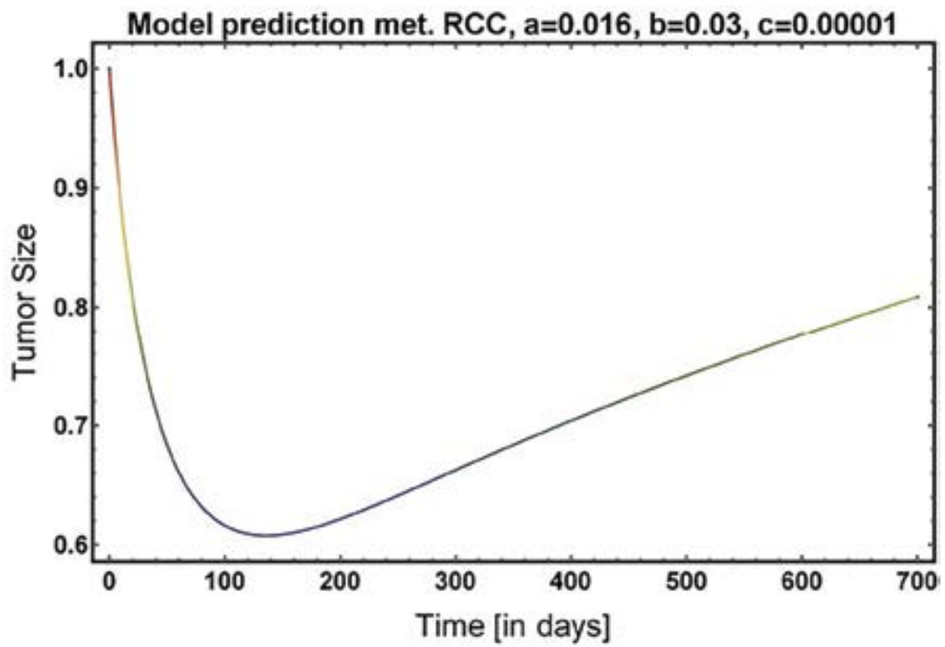
The first term represents the growth of the tumor in the absence of any therapy, the 2nd term represent the effect of the therapy, and 3rd term represent intrinsic resistance of the tumor to the therapy. We are now trying to collaborate with the oncologist to get redacted patient data to validate the model, correlate the parameters a, b and c to set of biological variables, as well as make predictions. The model does give the progression free survival, duration of survival, and the universal drug resistance. The deficiency of the model is it is two components: assumes the dominant component of the mutations response to the drug, but there is a much smaller component, which is intrinsically resistance to the therapy. Once validated, we plan to extend the model to include more complex situations. If this approach is successful, this will greatly benefit patients. It will give some guidance to the oncologist which therapy has better probability to get a response. Also, it will let us know how long the therapy should be continued and associated risk factors. In another project, I am also trying to understand the cancer metastasis, and build quantitative models.

RESEARCH PROJECTS

- Understanding universal drug resistance to targeted therapy and immunotherapy in cancer treatments
- Understanding cancer metastasis using physical and biological laws: physical biology

LAB MEMBERS

Post-doctoral Fellow: Joseph Butner, Ph.D.



David Volk, Ph.D.
Assistant Professor

Targeting cancer with X-aptamers and nanoparticles

The major focus of my lab is to develop targeting agents and smart particles, which attack specific diseased tissues, mainly cancers, or infectious organisms, such as tuberculosis. Current treatments are often ineffective or create harsh side effects, which are deleterious for patients. We use modified DNA hybrids, which are coupled with drug-like or protein-like attachments (X-aptamers). X-aptamers can be used alone or can be coupled to particles containing anti-cancer agents to act as a targeting agent for the particles. Such smart particles can be used alone or can be loaded into large silicon particles to create a slow sustained release of the smart nanoparticles.

Aptamer Development - In recent years our translational team of chemists, biologists, and oncologists has developed a number of aptamers targeting breast and ovarian cancer cells, including those targeting the proteins E-selectin, CD44, Annexin A2, and AXL. Such aptamers can greatly reduce the burden of cancer in a dose-dependent manner, as we recently showed in two breast cancer cell lines using the E-selectin aptamer called ESTA1. However, aptamers are even more effective when used in combination therapy together chemotherapeutics 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 shown our method is safe and effective, with desirable pharmacokinetic and pharmacodynamic properties. Our recent cancer-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 (Liu et al. 2018) targeting the endothelium of lymphoma in bone marrow.

Previously, we developed an aptamer targeting the dengue 2 virus, and current work on infectious diseases includes aptamers and smart particles targeting *Clostridium difficile* (C. Diff), influenza and *Mycobacterium 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. Other ongoing work focuses on the targeting of Bruton's Tyrosine Kinase, C. diff toxins and cancer checkpoint proteins.

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. NGS data files often contain tens of millions of DNA sequences, and the analysis of them is not trivial. We therefore developed Aptaligner (Lu et al. 2014), a completely automated program with easy-to-use graphical user interfaces, noise-reduction filters, DNA length error filters, and statistical analysis packages for the analysis of many X-aptamer projects contained in a single NGS data file.

A recent project in the lab is the creation of specialty software for the analysis of the significant DNA sequence changes (recombination) in the *vslE* surface protein as a function of time during B. burgdorfori infections, the cause of Lyme disease. A poorly understood process called antigenic variation leads to large-scale changes in the bacteria's *vlsE* locus, and thus the bacteria's protein surface, which leads to escape from the host's immune system. Antigenic variation is thought to be the cause of long-term Lyme disease infection and post-infection deficits.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

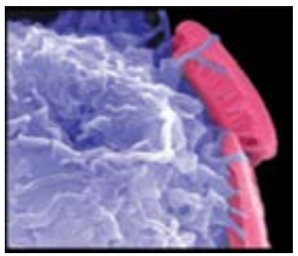
Therapeutic Targeting of AXL Receptor Tyrosine Kinase Inhibits Tumor Growth and Intraperitoneal Metastasis in Ovarian Cancer Models. Kanlikilicer, P., et al. Volk, D.E., et al. *Molecular Therapy Nucleic Acids* (2017) 9:251-262.

Thioaptamer Targeted Discoidal Microparticles Increase Self Immunity and Reduce Mycobacterium Tuberculosis Burden in Mice. Leonard, F., et al., Volk, D.E., et al, *Molecules* (2017) 22:1355.

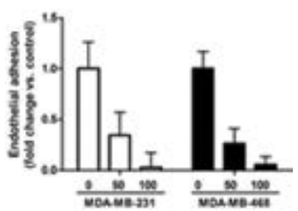
A Novel DNA Aptamer for Dual Targeting of Polymorphonuclear Myeloid-derived Suppressor Cells and Tumor Cells. Liu, H., et al., Volk, D.E. et al. *Theranostics* (2018) 8:31-44.

LAB MEMBERS

Research Scientists: Lokesh Rao, Ph.D., Ana Maria Zaske, Ph.D.
Research Associates: Xin Li, M.S.
Medical Students: Andrea Costello (MSIII), Brenda Saucedo (MSIII)
Visiting Students: Sindhu Gangula, Sommer Luu



Smart slow release particles attacking cancer.



ESTA1 aptamer provides dose-dependent cancer reduction.

Hongyu Wang, M.D., Ph.D.
Assistant Professor

Cancer biomarker discovery and targeted therapy

Biomarker discovery and targeted therapy are impotent parts of precision medicine. Validated biomarkers are essential for clinical diagnosis, prognosis, and prediction of response to therapy. Aptamer mediated biomarker discovery and targeted therapy are attractive approaches for precision cancer treatment. Aptamers are oligonucleic acid or peptide molecules that can bind to a specific target with high affinity and specificity. DNA aptamers are attractive alternatives to antibodies on targeted therapy, and offer many significant advantages over monoclonal antibodies in terms of feasibility, low cost, non-immunogenicity, and facile modification for various applications.

We created a combinatorial DNA aptamer library that has been modified with thiophosphate substitutions of the phosphate ester backbone (TA) for enhanced nuclease resistance and binding. We further developed bead-based X-aptamer (XA) libraries containing chemically-modified DNA bases and backbones enabling rapid selection of XA affinity reagents to a target biomolecule in solution.

Immune checkpoint antibodies are emerging as highly promising therapeutic agents to restore latent anti-tumor immunity and control cancer growth. However, the major adverse events with immunomodulatory antibodies are inflammatory pathologies. Modified aptamers are attractive alternate for immunomodulatory antibodies with no immunogenicity. Using the bead-based X-aptamer library, we have successfully identified immune checkpoint protein programmed cell death protein 1 (PD-1) / programmed death-ligand 1 (PD-L1) specific X-aptamers. Compared to PD-1 and PD-L1 antibodies, XA-PD1 and XA-PDL1 demonstrated similar cell binding intensity. XA-PDL1 has shown the same capacity as PD-L1 antibody binding to PD-L1 expression on pancreatic tumor tissue. These X-aptamers provide a synthetic alternative to antibodies for use in research, diagnostic assays, and potentially as a therapeutic.

Beyond selection for single target protein, we used a cell-based SELEX and tissue morphology based (Morph-X-Select) aptamer selection processes to identify a panel of thioaptamers (TAs) against patient-derived endothelial cells or tumor cells and successfully identified TAs that bind specifically to those target cells. Sequence Endo28 was characterized as an Annexin A2-specific aptamer that allows for selective delivery of therapeutic agents to the tumor vasculature. Targeted delivery of Annexin A2 siRNA resulted in profound anti-angiogenesis and anti-tumor effects. These methods of aptamer selection and nanoparticle assembly offer a rapid and highly specific development of new targeted treatment for cancer.

The identified thioaptamer/X-aptamer can specifically bind biomarkers on cancer cells and applied in clinical diagnosis and prognosis. The technologies developed in those projects will build up a new platform for biomarker discovery, imaging and targeted therapy, and can be extended to many other cancers or diseases.

RESEARCH PROJECTS

- Targeted cancer therapy with aptamer mediated nanoparticle–drug delivery
- Proteomics biomarker discovery
- Develop immune-checkpoint blockade X-aptamers for cancer immunotherapy

KEY PUBLICATIONS

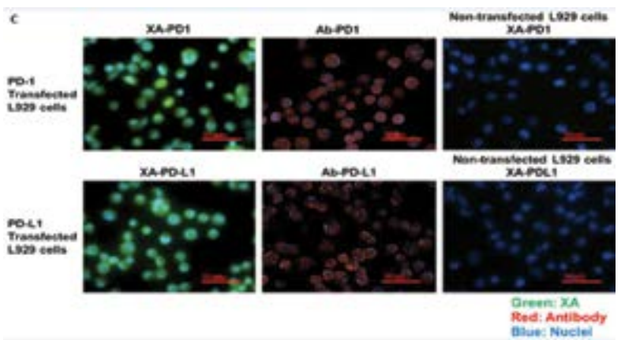
*Mangala LS, *Wang H, *Jiang D, Wu SY, Somasunderam A, Volk DE, Lokesh GL, Li X, Pradeep S, Yang X, Haemmerle M, Rodriguez-Aguayo C, Nagaraja AS, Rupaimoole R, Bayraktar E, Bayraktar R, Li L, Tanaka T, Hu W, Ivan C, Gharpure KM, McGuire MH, Thiviyanathan V, Zhang X, Maiti SN, Bulayeva N, Choi HJ, Dorniak PL, Cooper LJ, Rosenblatt KP, Lopez-Berestein G, Gorenstein DG, Sood AK. Improving vascular maturation using noncoding RNAs increases antitumor effect of chemotherapy. *JCI Insight*. 2016;1(17):e87754. doi: 10.1172/jci.insight.87754. PubMed PMID: 27777972; PMCID: PMC5070952. (*equal contributions)

Wang H, Lam CH, Li X, West DL, Yang X. Selection of PD1/PD-L1 X-Aptamers. *Biochimie*. 2017 Sep 11. pii: S0300-9084(17)30230-4. doi: 10.1016/j.biochi.2017.09.006. [Epub ahead of print]; PMID: 28912094

Lokesh GL, Wang H, Lam CH, Thiviyanathan V, Ward N, Gorenstein DG, Volk DE. X-Aptamer Selection and Validation. *Methods Mol Biol*. 2017;1632:151-174. doi: 10.1007/978-1-4939-7138-1_10. PubMed PMID: 28730438.

LAB MEMBERS

Research Associate: Xin Li
Research Associate: Li Li, M.S.



Binding affinity of selected XAs. Selected XA-PD1-78 and XA-PDL1-82 were incubated with PD-1 or PD-L1 expression cells and their binding affinity were analyzed by flow cytometry (A), dissociation constant (Kd) (B) and in comparison to appropriate antibodies (C).

Zhihui (Bill) Wang, Ph.D.
Associate Professor

Quantitative biology and medicine

Our research program focuses on integrating mathematical, physical, and statistical methods with experimental investigations and patient data analysis to quantitatively study tumor progression and invasion. We are working to use our models to help biologists and medical scientists to simulate experimental procedures, optimize and predict clinical therapies and outcomes, and test and refine their biological/medical hypotheses. We have three specific research areas.

Multiscale cancer modeling. Cancer growth is an emergent, integrated phenomenon that spans multiple spatial and temporal biological scales resulting from dynamic interactions between individual cells, and between cells and their constantly changing environment. Our research projects address a challenging part of current systems modeling of cancer: bridging the gaps between, and linking, the molecular, cellular, multicellular, and tissue scales. We also have successfully integrated a combination of *in vitro* and *in vivo* experiments paired with patient data analysis with the mathematical models. These models examine how changes occurring at the molecular level percolate across and affect tumor growth behaviors at the tissue and tumor scales.

Cross-scale drug target discovery. Most mathematical models used in identifying cancer drug targets to date focus on the molecular level (i.e., on genes, proteins, and large-scale signaling networks). However, selection and identification of drug targets that account for molecular-, multicellular-, and tumor-scale behaviors is potentially more realistic and hence more powerful than focusing only on cell signaling. We are developing cross-scale drug target evaluation methods for identifying potential drug targets and multi-target therapeutics (high in both efficacy and safety while minimizing unintended adverse effects), based on single- and multiple-parameter perturbation algorithms.

Translational cancer modeling. We are developing practical (relatively simple yet powerful) mathematical tools based on biophysical theories to correlate physical properties of drug transport with tumor progression and treatment outcome. Together with experimental/clinical investigators, we use ODE- and PDE-based models to predict treatment outcome for each individual patient prior to actual treatment. Since these tools are derived based on fundamental principles of mass transport, they are broadly applicable to the clinical sciences. The concept of this approach is also likely to be useful beyond the context of cancer in any case where drug delivery relies on local diffusion properties, demonstrating the general applicability and broader impact of his modeling method.

RESEARCH PROJECTS

- A hybrid multiscale modeling approach to study normal mammary gland development and breast cancer initiation
- Development of dynamic molecular target selection, identification, and validation methods with multiscale modeling
- Predictive modeling of cancer treatment

KEY PUBLICATIONS

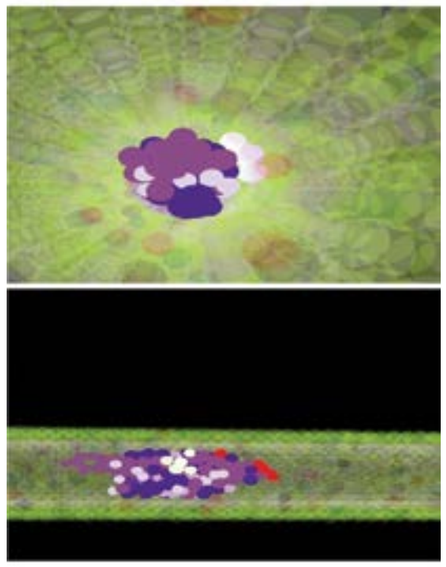
Vittorio Cristini, Eugene J. Koay, Zhihui Wang. An Introduction to Physical Oncology: How Mechanistic Mathematical Modeling Can Improve Cancer Therapy Outcomes. *CRC Press, Taylor & Francis Group.* July 2017

Wang Z, Kerketta R, Chuang YL, Dogra P, Butner JD, Brocato TA, Day A, Xu R, Shen H, Simbawa E, Al-Fhaid AS, Mahmoud SR, Curley SA, Ferrari M, Koay EJ, Cristini V. Theory and Experimental Validation of a Spatio-temporal Model of Chemotherapy Transport to Enhance Tumor Cell Kill. *PLoS Comput Biol* 12:e1004969, 2016

Butner JD, Chuang YL, Simbawa E, Al-Fhaid AS, Mahmoud SR, Cristini V, Wang Z. A hybrid agent-based model of the developing mammary terminal end bud. *J Theor Biol* 407:259-270, 2016

LAB MEMBERS

Post-doctoral Fellows: Joseph Butner, Ph.D.
Graduate Students: Prashant Dogra, Terisse Brocato, Naomi Hasegawa, Jorge Tito



Simulation examples of a multiscale hybrid model for simulating ductal carcinoma *in situ* (DCIS) growth.

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 (or normal) stem cells is motivated by their essential role in both normal development – from the fertilized egg to fully developed organism – 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. This therapeutic potential of stem cells has already been demonstrated for several decades in which blood stem cells (obtained from either bone marrow, mobilized peripheral blood, or umbilical cord blood) have been utilized to regenerate the complete blood system in cancer patients undergoing chemotherapy for cancer. There is accumulating evidence that certain stem cells, such as mesenchymal stem cells, may facilitate repair of tissue damage by reducing inflammation. 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.

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 are lung involved in active regeneration of cells and tissues lost due to normal cell turn-over, 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. Presently, this approach is being investigated within the center for muscle (muscular dystrophies), lung (Cystic Fibrosis), and blood (immune deficiencies).

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 ovarian cancer and lymphoma.

Brian R. Davis, Ph.D.
Associate Professor and Center Director
The C. Harold and Lorine G. Wallace Distinguished University Chair

Brian Davis, Ph.D.
Associate Professor
Director of the Center for Stem Cell and Regenerative Medicine
C. Harold and Lorine G. Wallace Distinguished University Chair

Genetically corrected stem cells for treatment of inherited blood and lung diseases

My 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/progenitor 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 recently reported introduction of lung-specific fluorescent reporters into iPS cells and utilized to specifically isolate early lung progenitors 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 – or iPS cells with subsequent differentiation to blood stem cells for transplantation. 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. The third laboratory project focuses on "natural gene correction," that is when spontaneous mutations arising in blood cells bearing inherited genetic mutations result in functional restoration of the defective gene, followed by *in vivo* selection for the revertant corrected cells. This gives rise to the phenomenon of revertant somatic mosaicism. We are presently examining this natural gene correction particularly as it occurs *in vivo* in patients with the Wiskott-Aldrich Syndrome.

RESEARCH PROJECTS

- Correction of iPS and airway stem cells from Cystic Fibrosis patients
- Correction of iPS and blood stem cells from Wiskott-Aldrich Syndrome patients
- Characterization of spontaneous gene mutations resulting in correction of inherited Wiskott-Aldrich Syndrome defects

KEY PUBLICATIONS

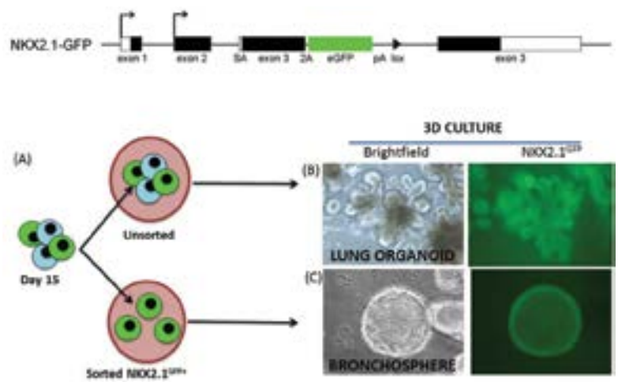
T.J. Laskowski, Y.V. Caeneghem, R. Pourebrahim, C. Ma, Z. Ni, Z. Garate, A.M. Crane, X.S. Li, W. Liao, M. Gonzalez-Garay, J.C. Segovia, D.E. Paschon, E.J. Rebar, M.C. Holmes, D. Kaufman, B. Vandekerckhove, B.R. Davis: Genetic correction of induced pluripotent stem cells from a Wiskott-Aldrich Syndrome patient normalizes the lymphoid developmental and functional defects. *Stem Cell Reports*. 2016, 7:139-148.

F. Hawkins, P. Kramer, A. Jacob, I. Driver, D. Thomas, K.A. Benson, N. Skvir, A.M. Crane, A. A. Kurmann, A. N. Hollenberg, S. Nguyen, B.G. Wong, A.S. Khalil, S.X.L. Huang, S. Guttentag, J.R. Rock, J.M. Shannon, B.R. Davis, D.N. Kotton. Prospective Isolation of NKX2.1-expressing Human Lung Progenitors Derived from Pluripotent Stem Cells. *Journal of Clinical Investigation* 2017, 127:2277-2294.

A. Jacob, M. Morley, F. Hawkins, K.B. McCauley, J.C. Jean, H. Heins, C-L Na, T.E. Weaver, M. Vedaie, K. Hurley, A. Hinds, S.J. Russo, S. Kook, W. Zacharias, M. Ochs, K. Traber, L.J. Quinton, A. Crane, B.R. Davis, F.V. White, J. Wambach, J.A. Whitsett, F.S. Cole, E.E. Morrisey, S.H. Guttentag, M.F. Beers, D.N. Kotton. Differentiation of human pluripotent stem cells into functional lung alveolar epithelial cells. *Cell Stem Cell* 2017, 21:1-17.

LAB MEMBERS

Post-doctoral Fellows: Dr. Leila Rouhigharabaei
Graduate Students: Varada Anirudhan
Medical Students: Nicholas King
Research Staff: Dr. Ana M. Crane, Dr. Nadine Matthias



NKX2.1 GFP Reporter Line. We are utilizing an NKX2.1 GFP reporter to specifically identify and isolate lung cells derived from Cystic Fibrosis and corrected iPS cells. As shown in the right hand panels, we can use such GFP-expressing cells to generate lung-specific organoids and bronchospheres in the laboratory (in collaboration with D. Kotton and F. Hawkins).

Laura A. Smith Callahan, Ph.D.
Assistant Professor

Tissue engineering approaches for the treatment of CNS injuries

The research in my laboratory focuses on developing tissue engineering approaches toward 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 and the expansion of clinically relevant cell sources for use in stem cell therapy.

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 will be integrated into advanced hybrid matrices. These matrices maximize the advantages of both synthetic (consistency in fabrication and cellular response) and natural (native bioactive signaling) polymers, while mitigating their disadvantages, namely lack of bioactive signaling and batch to batch inconsistency in scaffold properties and cellular response, respectively. When combined with additional bioactive signaling and controlled architecture, these hybrid matrices can begin to emulate the native tissue microenvironment and support tissue development far better than traditional matrices. Preliminary studies have focused on formulating matrices to facilitate the extension of axons from the host across spinal cord lesion cavities in subacute rat models so spinal cord injury.

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

RESEARCH PROJECTS

- Optimization of culture surfaces for the differentiation of human induced pluripotent stem cells to neural stem cells and oligodendrocyte progenitor cells
- Identification of optimal artificial matrix properties such as bioactive signaling moiety concentration or mechanical properties using combinatorial approaches

KEY PUBLICATIONS

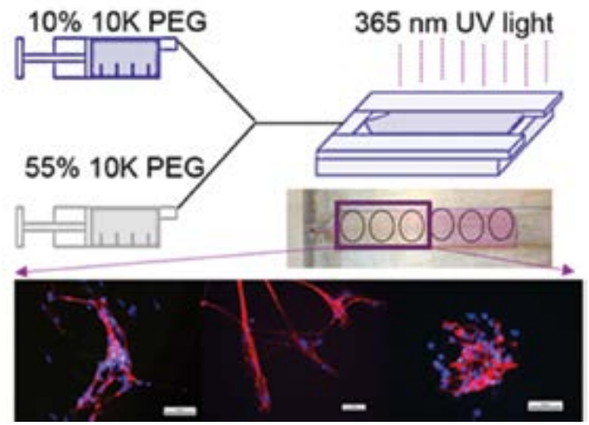
Mosley MC, Lim HJ, Chen J, Yang Y-H, Li S, Liu Y, Smith Callahan LA. Neurite extension and neuronal differentiation of human induced pluripotent stem cell derived neural stem cells on a polyethylene glycol hydrogels containing a continuous Young's Modulus gradient. *Journal of Biomedical Materials Research: Part A.* 105 (3): 824-833, 2017.

Lim HJ, Mosley MC, Kurosu Y, Smith Callahan LA. Concentration dependent survival and neural differentiation of murine embryonic stem cells cultured on polyethylene glycol dimethacrylate hydrogels possessing a continuous concentration gradient of n-cadherin derived peptide His-Ala-Val-Asp-Lle. *Acta Biomaterialia.* 56: 153-160, 2017.

Lim HJ, Khan Z, Wilems TS, Li X, Perera TH, Kurosu YE, Ravivarapu K, Mosley MC, Smith Callahan LA. Human induced pluripotent stem cell derived neural stem cell survival and neural differentiation on polyethylene glycol dimethacrylate hydrogels containing a continuous concentration gradient of n-cadherin derived peptide His-Ala-Val-Asp-Ile. *ACS Biomaterials Science & Engineering*. 3 (5): 776-781, 2017.

LAB MEMBERS

Post-doctoral Fellows: T. Hiran Perera; Xi Lu



Schematic of hydrogel with a continuous gradient in mechanical properties and response of human induced pluripotent stem cell derived neural stem cell cytoskeletal organization (red) and neural network organization in response to the change in stiffness of the hydrogel culture substrate. Nuclear staining is blue. Scale bars= 50µm.

Qi Lin Cao, M.D.
Associate 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 cell 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 glias 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
- Human iPSC-derived neural stem or precursor cells for spinal cord injury and stroke

KEY PUBLICATIONS

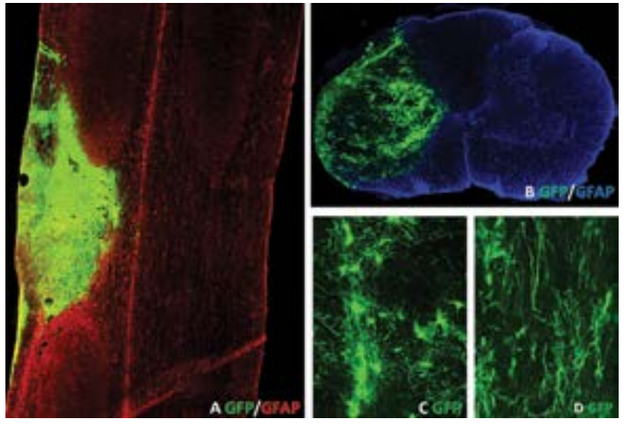
Liu Y, Zheng YY, Li SL, Xue HP, Schmitt K, Hergenroeder GW, Wu JQ, Zhang YY, Kim DH, and Cao QL (2017). Human neural progenitors derived from integration-free iPSCs for SCI therapy. *Stem Cell Res.* 9:55-64. doi: 10.1016. PMID: 28073086

Cuevas-Diaz Duran R, Yan H, Zheng YY, Huang XF, Grill R, Kim DH, Cao QL* and Wu JQ* (2017). The systematic analysis of coding and long non-coding RNAs in the sub-chronic and chronic stages of spinal cord injury. *Sci Rep.* 7:41008. doi: 10.1038. PMID: 28106101 (* co-corresponding authors).

Apolipoprotein E as a novel therapeutic neuroprotection target after traumatic spinal cord injury, Cheng X, Zheng Y, Bu P, Qi X, Fan C, Li F, Kim DH, Cao Q., *Experimental neurology*. 2018; 299(Pt A):97-108.

LAB MEMBERS

Post-doctoral Fellows: Yiyan Zheng; Xiuquan He
Research Assistants: Jun Li; Chrystine Gallegos



Survival of grafted hiPSC-derived neural stem cells. Human iPSC-derived NSCs were transplanted at 2 week after moderate C5 unilateral contusion SCI (IH 180 kdyne) in rude rats. Robust survival of grafted NSCs was observed in all animals received hiPSC-NSC graft at 2 months after transplantation (A, B). The grafted NSCs filled the cavity and mainly remained in areas around the injury epicenter at the injury side (A, B). Grafted hiPSC-NSCs differentiated into cells with morphology of neurons (C) with long processes extending to the spared host spinal cord (D).

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

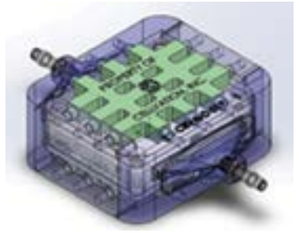
Jackson ML, Srivastava A, Cox CS. Pre-clinical progenitor cell therapy in traumatic brain injury: a Meta-Analysis. *J Surg Res* 214:38-48, 2017. PMID: 28624058

Liao GP, Aertker BA, Kota DJ, Prabhakara KS, Smith PA, Hetz RA, Xue H, Bedi S, Olson SD, Cox CS. Assessing blood brain barrier permeability in traumatic brain injury research. *ADMET & DMPK*. 3(3):182-189, 2015.

Cox CS, Hetz RA, Liao GP, Aertker BM, Ewing-Cobbs L, Juranek J, Savitz SI, Jackson ML, Romanowska-Pawliczek A, Triolo F, Dash PK, Pedroza C, Lee DA, Worth L, Aisiku I, Choi HA, Holcomb JB, Kitagawa R. Treatment of severe adult traumatic brain injury using bone marrow mononuclear cells. *Stem Cells* 35:1065-1079, 2016. PMID: 27800660, PMCID: PMC5367945

LAB MEMBERS

Steven Kosmach, MSN, RN, CCRC-TBI Clinical
Joiya Arrington, MSN, RN-TBI Clinical
Yidao Cai-Programmer Analyst
Akshita 'Jade' Kumar, M.D.-TBI-Clinical and Cell Therapy
Louis Carrillo, M.D.-TBI-Clinical and Cell Therapy
Mitchell George, M.D.-TBI-Clinical and Cell Therapy
Scott Olson, Ph.D. - Assistant Professor
Katherine Ruppert, Ph.D.-Sr Research Associate
Karthik Prabhakara-Sr Research Assistant
Cecilia Martin, Ph.D.-Research Associate
Supinder Bedi, Ph.D.-Assistant Professor
Amit Srivastava, Ph.D.-Assistant Professor
Naama Toledo-Furman, Ph.D.-Flow Cytometry/innate immunity
Hasen Xue, M.D.-Research Associate
Fabio Triolo, Ph.D.-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 bio-reactor for stem cell production.

Radbod Darabi M.D., Ph.D.
Assistant Professor

Skeletal muscle regeneration using human stem cells

My lab's main interest is using pluripotent stem cells for skeletal muscle regeneration. During the last few years, we have developed novel methods for using human embryonic stem cells (ES cells) as well as induced pluripotent stem cells (iPS cells) for cell therapy in mice models for different types of muscular dystrophies.

Here at IMM, by using cutting-edge gene editing technologies (such as CRISPR/Cas9 system) our lab has successfully generated knock-in human ES/iPS reporter cell lines for early myogenic genes such as PAX7 and MYFF5. This will allow studying the emergence of early myogenic progenitors from human ES/iPS cells; a crucial step to identify and isolate myogenic progenitors for future cell based therapies. Other major goals of the lab include using high throughput screening (HTS) to identify important inducers of myogenesis in human stem cells and evaluation of *in vivo* regeneration potential of these cells in mice models of muscular dystrophies and muscle mass injuries.

Our research team also works on derivation of iPS cells from muscular dystrophy patients; *in vitro* gene correction of iPS cells; optimizing cell delivery and engraftment; study mechanisms involved in cell homing into the muscle after systemic/arterial cell delivery; as well as exploring the effect of local tissue perfusion in cell survival and engraftment.

Our lab is currently funded by a NIH (RO-1) grant award from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) to study myogenic differentiation of human ES/iPS cells using knock-in reporters for myogenic genes.

RESEARCH PROJECTS

- Generation of knock-in human ES/iPS reporter cell lines for early myogenic genes (PAX3, PAX7, MYF5)
- Gene correction of muscular dystrophy iPS cells using CRISPR/Cas9 system
- High throughput screening (HTS) for myogenic induction of human iPS reporter cells
- Systemic/arterial cell delivery approaches for cell therapy in muscular dystrophies
- Using bio-scaffolds for cell delivery in mice models for muscle mass loss injuries

KEY PUBLICATIONS

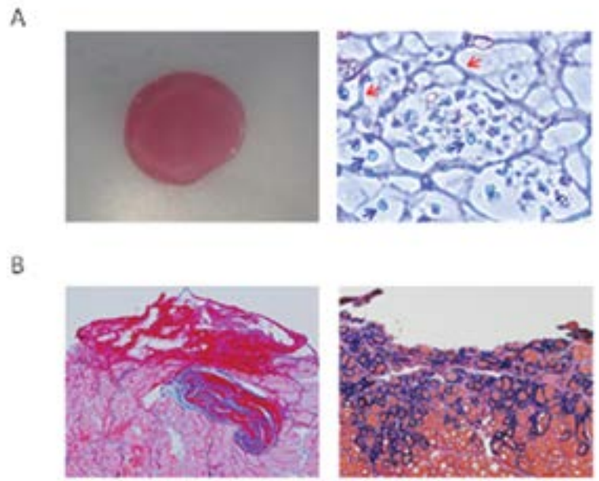
Skoglund G, Lainé J, Darabi R, Fournier E, Perlingeiro R, Tabti N. Physiological and ultrastructural features of human induced pluripotent and embryonic stem cell-derived skeletal myocytes *in vitro*. *Proceedings of the National Academy of Sciences of the USA (PNAS).* 2014 Jun 3; 111(22):8275-80.

Wu J, Hunt SD, Xue H, Liu Y, Darabi R. Generation and Characterization of a MYF5 Reporter Human iPS Cell Line Using CRISPR/Cas9 Mediated Homologous Recombination. *Scientific Reports.* 2016 Jan 5; 6:18759. doi: 10.1038/srep18759.

Wu J, Hunt SD, Xue H, Liu Y, Darabi R. Generation and Validation of PAX7 Reporter Lines from Human iPS Cells Using CRISPR/Cas9 Technology. *Stem Cell Research.* 2016 Mar; 16(2):220-8.

LAB MEMBERS

Post-doctoral Fellow: Jianbo Wu
Research Associate: Nadine Matthias
Master Student (GSBS): Jose L. Ortiz-Vitali
Undergraduate Student (Rice University): Jonathan Lo



Muscle injury repair using bio-scaffold seeded with stem cells to repair muscle mass loss injury
A. Hydrogel seeded with LacZ labeled stem cells demonstrates good cell survival within the scaffold.
B. Severe muscle mass loss injury with massive fibrosis (left) is repaired using bio-scaffold/stem cell transplantation. LacZ+ donor-derived fibers (blue) replace the fibrosis one month after transplantation.

Pramod Dash, Ph.D.
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 for persons of all ages and sexes. According to the Centers for Disease Control, approximately 2.6 million Americans sustain a documented brain injury each year (i.e., hospitalization, emergency room or other physician contacts), 87% of which can be classified as concussion. Recent studies have challenged the perception that the consequences of concussion are restricted to the traumatic incident. Mounting evidence indicates that a concussion is a progressive disease with both short-term and long-lasting consequences.

One of the most common long-term psychological consequences of brain injury is anxiety and other stress-related disorders. An inability to extinguish the memory of a traumatic event lies at the core of many stress-related disorders. When a person experiences a traumatic event, the subject can form an association between an otherwise innocuous stimuli (referred as the conditioned stimulus) such as a sound, a smell and/or the context in which the event occurred, and the harmful event (referred to as the unconditioned stimulus). This association is learned rapidly and the memory for the traumatic event is robust and can be long-lasting. When the person is subsequently exposed to the conditioned stimulus alone (referred to as the trigger), it causes a fear response referred to as the conditioned response. Repeated exposure to the conditioned stimulus in the absence of the unconditioned stimulus results in a gradual reduction of the fear response through extinction. Although epidemiological studies have reported comorbidity between TBI and stress-related disorders, especially post-traumatic stress disorder (PTSD), the cellular and neurochemical mechanism(s) that underlie this association are largely unknown.

The amygdala is a key structure that is essential for the formation of fear memories and for mediating fear responses. The neurons of the central nucleus of amygdala (CeA) project to structures including the periaqueductal grey (controls the freezing behavior), the lateral hypothalamus (increases blood pressure) and the paraventricular nucleus of the hypothalamus (mediates release of stress hormones). The output from the central nucleus, in turn, is controlled by the projections from pyramidal neurons within the infralimbic (IL) cortex of the medial prefrontal cortex (Figure 1). When the activity of IL neurons is blocked, animals can learn the association between the conditioned and unconditional stimuli, however, the memory of extinction is impaired. In contrast, stimulation of IL neurons facilitates extinction learning and the formation of extinction memory.

Using a rodent model, we have found that a single mTBI can cause prolonged impairments in the ability of injured animals to extinguish a fearful memory in spite of repeated exposure to the conditioned stimulus. Our cellular analysis revealed that this dysfunction is not caused by a loss of neurons in the IL. Interestingly, quantification of dendritic spines showed a significant reduction in the spine density of layer II/III pyramidal neurons. As spines are potential sites for neuronal communication, these results suggest that the ability of IL neurons to inhibit the activity of the central nucleus of the amygdala is reduced. Further, these results suggest that molecular strategies to block the loss of spines may prevent the development of stress-related disorders after concussion. Alternatively, strategies to increase spine density in the chronic stage of injury may enhance the ability of the IL to reduce amygdala output and stress-related disorders.

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

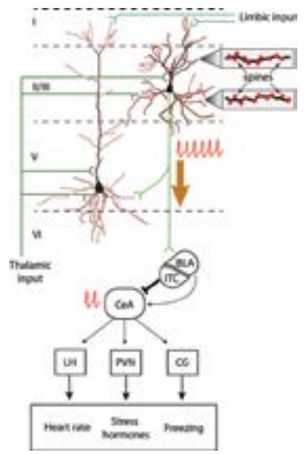
Zhao J, Huynh J, Hylin MJ, O'Malley JJ, Perez A, Moore AN, Dash PK. Mild Traumatic Brain Injury Reduces Spine Density of Projection Neurons in the Medial Prefrontal Cortex and Impairs Extinction of Contextual Fear Memory. *J Neurotrauma.* 2017 [Epub ahead of print]

Dash PK, Zhao J, Kobori N, Redell JB, Hylin MJ, Hood KN, Moore AN. Activation of Alpha 7 Cholinergic Nicotinic Receptors Reduce Blood-Brain Barrier Permeability following Experimental Traumatic Brain Injury. *J Neurosci.* 36(9):2809-18, 2016.

Fischer TD, Hylin MJ, Zhao J, Moore AN, Waxham MN, Dash PK. Altered Mitochondrial Dynamics and TBI Pathophysiology. *Front Syst Neurosci.* 10:29, 2016.

COLLABORATORS

Dr. James McCarthy: chair 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



Schematic drawing showing how neurons in the infralimbic cortex inhibit amygdala output and block stress-related disorders.

Sarah Xuelian Huang, M.B.B.S., Ph.D.
Assistant Professor

Human pluripotent stem cells for lung regeneration and disease modeling

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

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 (including the additional difficulty of obtaining airway and alveolar cells from the same donor).

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

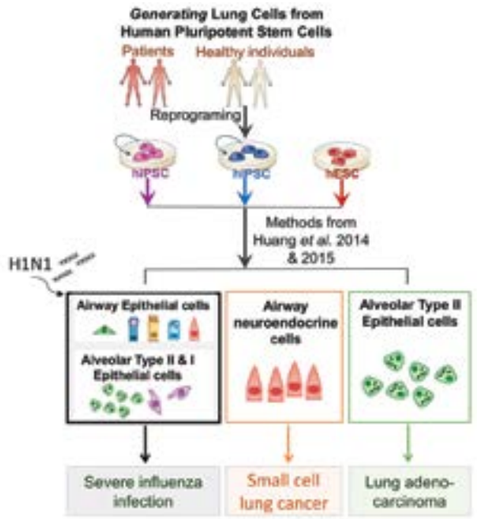
RESEARCH PROJECTS

- Use patient hiPSC differentiated lung epithelial cells to examine TLR3 mediated intrinsic immunity and test rare mutations in TLR3 as causative for severe influenza infection induced by H1N1 virus
- Mapping the pathogen recognition patterns and the cellular and molecular responses of the lung epithelial cells to pathogen infection
- Understanding the basic mechanisms of lung lineage specification from $NKX2.1^+SOX2^+SOX9^+$ $NKX2.1^+SOX2^+P63^+$ human lung and airway progenitors using molecular, genetic and epigenetic approaches

KEY PUBLICATIONS

N. Valerio Dorrello, Brandon A. Guenthart, John D. O'Neill, Jinho Kim, Katherine Cunningham, Ya-Wen Chen, Mauer Biscotti, Theresa Swayne, Holly M. Wobma, Sarah X.L. Huang, Hans-Willem Snoeck, Matthew Bacchetta, and Gordana Vunjak-Novakovic. Functional vascularized lung grafts for lung bioengineering. *Sci Adv.* 2017 Aug 30;3(8):e1700521

Finn Hawkins, Philipp Kramer, Anjali Jacob, Ian Driver, Dylan C. Thomas, Katherine B. McCauley, Nicholas Skvir, Ana M. Crane, Anita A. Kurmann, Anthony N. Hollenberg, Sinead Nguyen, Brandon G. Wong, Ahmad S. Khalil, Sarah X.L. Huang, Susan Guttentag, Jason R. Rock, John M. Shannon, Brian R. Davis, and Darrell N. Kotton. Prospective isolation and single cell profiling of NKX2-1+/CD47+ human lung progenitors derived from pluripotent stem cells. *J Clin Invest.* 2017 Jun 1;127(6):2277-2294. doi: 10.1172/JCI89950. Epub 2017 May 2.



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

Dong Kim, M.D.
Professor and Chair
Vivian L. Smith Department of Neurosurgery
Director, Mischer Neuroscience Institute
Memorial Hermann–TMC

Advancing the field of neuroscience

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

Our 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 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.

I specialize in the following diseases:

- Intracranial aneurysms
- Brain tumors, benign and malignant
- Skull base tumors and meningiomas
- Trigeminal neuralgia
- Chiari malformations

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Santiago-Sim T, Fang X, Hennessy M, Nalbach 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.

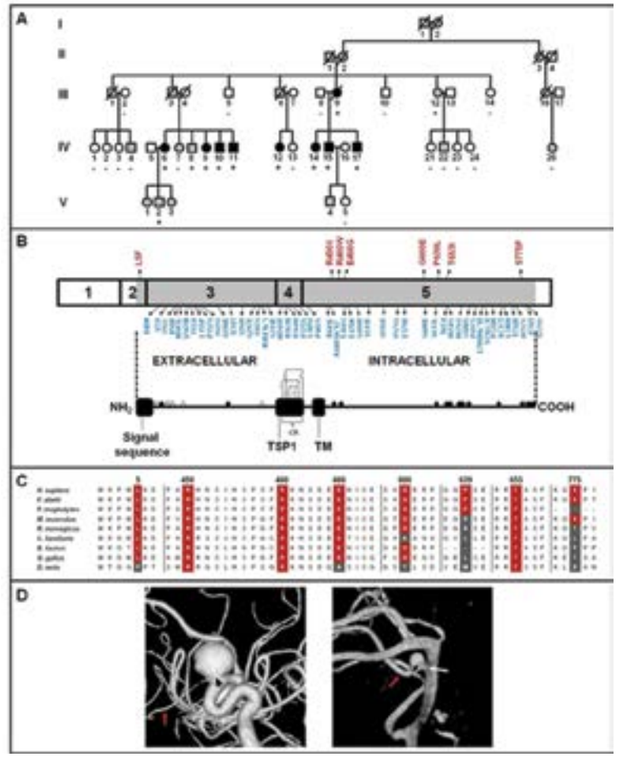
Santiago-Sim T, Fang X, Hennessy M, Nalbach S, DePalma S, et al. THSD1 (Thrombospondin Type 1 Domain Containing Protein 1) Mutation in the Pathogenesis of Intracranial Aneurysm and Subarachnoid Hemorrhage. *Stroke.* 2016 Dec;47(12):3005-3013. Epub 2016 Nov 15.

Ying Liu, Yiyan Zheng, Shenglan Li, Haipeng Xue, Georgene W. Hergenroeder, Jiaqian Wu, Yuanyuan Zhang, Dong H. Kim, Qilin Cao: Human neural progenitors derived from integration-free iPSCs for SCI therapy. 2016; *Stem Cell Res.* 2017 Jan 5;19:55-64.

Duran RC, Yan H, Zheng Y, Huang X, Grill R, Kim DH, Cao Q, Wu JQ. The systematic analysis of coding and long non-coding RNAs in the subchronic and chronic stages of spinal cord injury. *Scientific Reports.* 2017 Jan 20;7:41008.

Levi AD, Okonkwo D, Park P, Jenkins A, Kurpad S, Parr A, Ganju A, Aarabi B, Kim D, Casha S, Fehlings M, Anderson KD, Gage A, Hsieh J, Huhn S, Curt A, Guzman R. Emerging safety of intramedullary transplantation of human neural stem cells in chronic cervical and thoracic spinal cord injury. *Neurosurgery.* 24 May 2017.

Figure 1



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

Momoko Yoshimoto Kobayashi, M.D., Ph.D.
Associate Professor

Elucidating the mechanisms of multiple waves of hematopoiesis in the mouse embryo

My lab is trying to understand how multiple waves of various hematopoietic cells are produced from "hemogenic endothelial cells" before the first hematopoietic stem cells (HSCs) are produced in the mouse embryo. Specifically, we are asking questions 1) innate B-1 cell progenitors are produced independent from HSCs and persist into post-natal life, and 2) how pre-HSCs 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. We have recently reported the presence of HSC-independent B-1 progenitors in HSC-deficient embryos. Our data and others' publication showed lack of B-1 cell potential in highly purified HSCs in adult bone marrow and fetal liver, suggesting that HSC-independent B-1 progenitors are produced somewhere in the mouse embryo and contribute to producing B-1 cell pool that persists to postnatal life. 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.

At the same time when B-1 progenitors are produced in the embryo, pre-HSC and adult repopulating HSCs are produced from the para-aortic region of the embryo. Pre-HSCs acquire adult-repopulating ability within one day and its mechanism has yet to be elucidated. We are trying to clarify the molecular mechanisms through which pre-HSCs gain efficient repopulating ability by single-cell RNA sequencing.

Knowledge obtained from above projects will help us to understand the mechanism of HSC and B-1 cell production *in vivo*, and to produce those human counterparts from human iPS cells *in vitro*, which might open a path of cell therapy for hematological disorders and immune deficient patients.

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 multiple waves of hematopoiesis and HSC production in the mouse embryo
- Identify important molecules for HSC maturation in the mouse embryo utilizing single-cell RNA-sequencing

KEY PUBLICATIONS

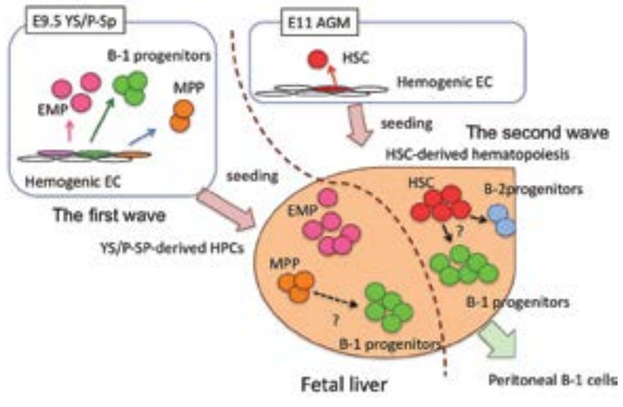
Yoon J, Wang H, Kim YC, Yoshimoto M, Abbasi S, Morse Iii HC. Plasma cell alloantigen ENPP1 is expressed by a subset of human B cells with potential regulatory functions. *Immunol Cell Biol.* 94(8):719-728, 2016.

Kobayashi M, Nabinger SC, Bai Y, Yoshimoto M, Gao R, Chen S, Yao C, Dong Y, Zhang L, Rodriguez S, Yashiro-Ohtani Y, Pear WS, Carlesso N, Yoder MC, Kapur R, Kaplan MH, Daniel Lacorazza H, Zhang ZY, Liu Y. Protein Tyrosine Phosphatase PRL2 Mediates Notch and Kit Signals in Early T Cell Progenitors. *Stem Cells*.35:1053-1064, 2017.

Hadland BK, Varnum-Finney B, Mandal PK, Rossi DJ, Poulos MG, Butler JM, Rafii S, Yoder MC, Yoshimoto M, Bernstein ID. A Common Origin for B-1a and B-2 Lymphocytes in Clonal Pre-Hematopoietic Stem Cells. *Stem Cell Reports.* 8:1563-1572, 2017.

LAB MEMBERS

Assistant Professor: Michihiro Kobayashi M.D., Ph.D.



Hypothesis: The first wave of B-1 cell develops from yolk sac hemogenic endothelial cells in the mouse embryo and contributes to B-1 progenitors in the fetal liver and postnatal B-1a cell pool in the adult peritoneal cavity. Hemogenic endothelial cells (ECs) in the yolk sac (YS) and para-aortic splanchnopleura (P-Sp) produce B-1 progenitor cells prior to HSC emergence. These progenitor cells seed fetal liver. Around at E11, the first HSCs emerge from hemogenic ECs at AGM region and also seed fetal liver and expand. Fetal liver B-1 progenitor cells derived from YS hemogenic EC and HSC mature into the peritoneal B-1 cells after birth. (Ann NY Acad Sci 2015)

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

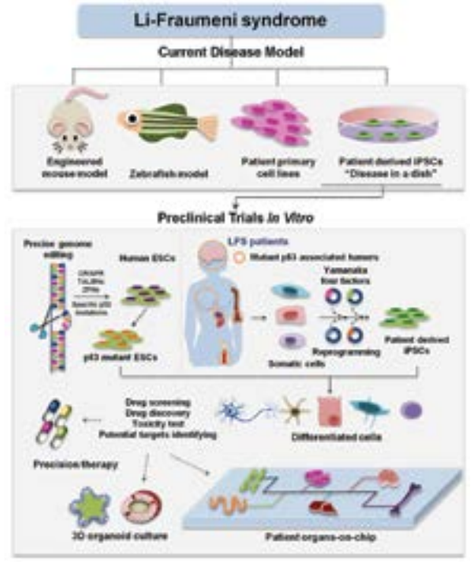
Jian Tu, Zijun Huo, Julian Gingold, Ruing Zhao, Jianan Shen, Dung-Fang Lee. The histogenesis of Ewing sarcoma. *Cancer Rep Rev.* 1(2): 1, 2017.

Yu-Hsuan Lin, Brittany E Jewell, Julian Gingold, Linchao Lu, Ruiying Zhao, Lisa L Wang, Dung-Fang Lee. Osteosarcoma: Molecular pathogenesis and iPSC modeling. *Trends Mol Med.* 23(8):737-755, 2017.

Ruoji Zhou, An Xu A, Julian Gingold, Louise C Strong, Ruiying Zhao, Dung-Fang Lee. Li-Fraumeni syndrome disease model: A platform to develop precision cancer therapy targeting oncogenic p53. *Trends Pharmacol Sci.* 38(10):908-927, 2017.

LAB MEMBERS

Post-doctoral Fellows: An Xu, Mo Liu, Dandan Zhu
Students: Ruoji Zhou, Brittany E Jewell
Technicians: Ying Liu
Visiting Scholars: Jian Tu, Donghui Wang



The application of LFS iPSC model to drug development for LFS and *p53* mutation-associated tumors. LFS iPSC model overcomes the limitations of current LFS disease models like mouse model, zebrafish model and primary cell lines, and holds potentials in modeling LFS associated cancers and facilitating clinical trials in a dish. Precise genome editing techniques make it possible to expand the bank of PSCs with different *p53* mutations, which provides valuable resource for precision cancer medicine. Integration of 3D organoid and organs-on-chip with LFS iPSC disease model offers exciting opportunities for testing existing both WT and mutant *p53*-associated pathway related drugs and discovering new therapeutic compounds.

Ying Liu, Ph.D.
Assistant Professor

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

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. Our research entails the use of combined genetic and molecular and cellular biological approaches applied to *in vitro* and *in vivo* models. We focus on dissecting the neural developmental pathways and the corresponding pathogenesis in spinal cord injury and stroke. Our long-term goal is to identify therapeutic targets for the treatment of CNS diseases.

By transient overexpression of four transcription factors, OCT4, SOX2, KLF4 and C-MYC, somatic cells such as dermal fibroblasts, keratinocytes, and blood cells, can be reprogrammed to human induced pluripotent stem cells (iPSCs). Most critically, 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. Recently we have adapted the highly efficient genome editing tool CRISPR/Cas9 system in creation of neural lineage reporters and gene corrections of patient iPSCs. These neural lineage specific cells are applied to in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

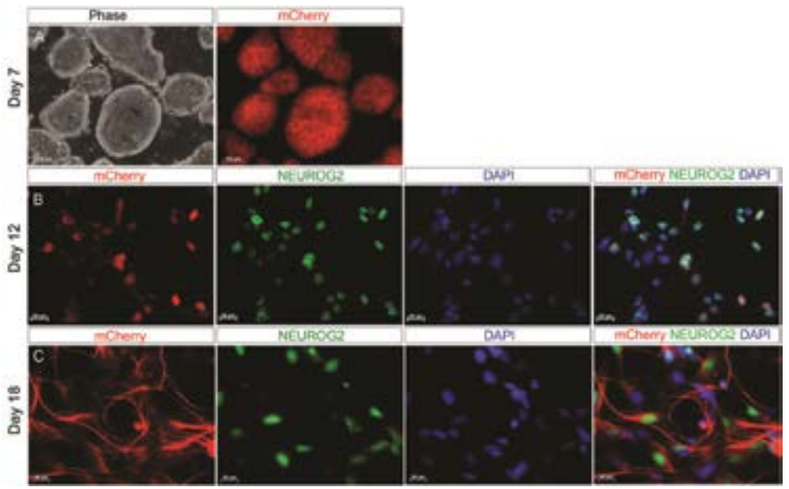
Liu, Y.*, Zheng, Y., Li, S., Xue, H., Schmitt, K., Hergenroeder, G.W., Wu, J., Zhang, Y., Kim, D.H., Cao, Q*. (2017) Human neural progenitors derived from integration-free iPSCs for SCI therapy. *Stem Cell Res.* 2017 Jan 5;19:55-64. doi: 10.1016/j.scr.2017.01.004. [Epub ahead of print] (*corresponding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

Li, S., Zhang, A., Xue, H., Li, D., Liu, Y. (2017) One-step piggyBac transposon-based CRISPR/Cas9 activation of multiple genes. *Mol Ther Nucleic Acids.* 15 September 2017. 8:64–76, doi. http://dx.doi.org/10.1016/j.omtn.2017.06.007. PMID: 28918057 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 8, 2017. Article ID 9182748, https://doi.org/10.1155/2017/9182748 [Epub ahead of print]

LAB MEMBERS

Post-doctoral Fellow: Shenglan Li
Visiting Graduate Student: Di Jia
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).

Nami McCarty, Ph.D.
Associate Professor
Jerold B. Katz Distinguished Professorship in Stem Cell Research

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 3991 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 a research delineating roles of the quiescent multiple myeloma 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 led 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.
- Delineating transcription factor networks on drug resistant lymphomas: We will continue to address roles for PAX5-BACH2 signaling networks in mantle cell lymphomas. We will also closely work with collaborators at clinicians to determine whether BACH2 sub-cellular localization in the cell determine drug resistance outcome and patient survival.

KEY PUBLICATIONS

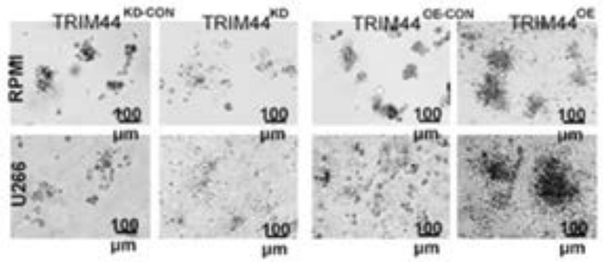
Zhang, H., and McCarty, N. Tampering with cancer chemoresistance by targeting the TGM2-IL6-autophagy regulatory network. *Autophagy* 13:627-628, 2017.

Zhang, H., Chen, Z., Miranda, R.N., Medeiros, L.J., and McCarty, N. Bifurcated BACH2 control coordinates mantle cell lymphoma survival and dispersal during hypoxia. *Blood* 130:763-776. 2017. This article was featured in "this week in Blood" as an Editor's pick.

Chen, Z., Lin, T-C., Bi, X., Lu, G., Dawson, B.C., McNiece, I and McCarty, N. TRIM44 promotes quiescent multiple myeloma cell 2 occupancy and survival in the osteoblastic niche via HIF-1α stabilization. *Leukemia,* 2017, Under revision.

LAB MEMBERS

Post-doctoral Fellows: Tsung Chin Lin, Ph.D., Lyn Liu, Ph.D., Quach Tu Hue, Ph.D.
Graduate Students: Farah Ladha
Research Assistants: Zheng Chen, M.S.



TRIM44 over-expressed cells (TRIM44^{OE}) increased colony formation in methylcellulose medium. Cells were seeded and after 21 days, cell groupings of >40 cells were counted as a colony. The pictures were taken by an inverted microscope (x10).

Naoki Nakayama, Ph.D.
Associate Professor
Annie & Bob Graham Distinguished Chair in Stem Cell Biology

Pluripotent stem cell differentiation and lineage specification

The cartilage of joints is not spontaneously repaired after injury in humans. There has been considerable interest in the clinical application of stem cells to the repair of damaged cartilage; however, current cell therapies using chondrocytes and adult stem cells such as mesenchymal stromal cells face the problems of low yield of cells and their tendency to yield unsuitable and/or unstable cartilage after expansion. Joint cartilage is a permanent cartilage. In contrast, growth-plate cartilage in the limb and vertebra, is a transient cartilage, destined to form bone. The joint and growth-plate cartilage are developed from distinct populations of precursor cells during embryogenesis. Therefore, we hypothesize that the embryonic cell-types responsible for joint formation: i.e. joint progenitor would be the best for the regeneration of adult joint cartilage. Pluripotent stem cells (PSCs), whether derived from an embryo, or induced from adult cells, are expected to differentiate into any cell-types of human body in culture through processes that mimic embryogenesis, making human (h) PSCs a promising source of embryonic cells for regenerative medicine.

What is the molecular basis of 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-like cartilage in culture. However, most of them are mineralized and turned into bone when transplanted into immunocompromised mice. In order to establish methods to generate cartilage that stays as cartilage permanently after transplantation (i.e., joint cartilage-like permanent cartilage), 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 determine that controlling cAMP signaling leads to cartilage made from the standard chondroprogenitors displaying very limited bone forming capacity after transplantation (i.e. pseudo-permanent cartilage). 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 additional signaling mechanisms for the formation of permanent cartilage.

Large quantity of articular cartilage-forming cells; long-term expansion of PSC-derived human chondroprogenitors: 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 future.

RESEARCH PROJECTS

- Further defining the process of chondrogenesis from the hPSC-derived chondroprogenitors and joint progenitors
- 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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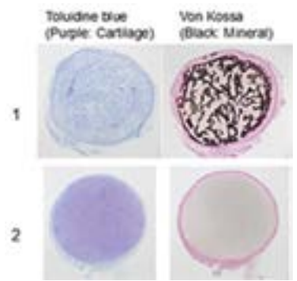
Li, H., Lu, A., Tang, Y., Beckman, S., Nakayama, N., Poddar, M., Hogan, M., and Huard, J. (2016) "The Superior Regenerative Potential of Muscle Derived Stem Cells for Articular Cartilage Repair is Attributed to High Cell Survival and Chondrogenic potential" *Mol. Ther. Methods Clin. Dev.*, 3:16065.

Lee, J.Y., Matthias, N., Pothiawala, A., Ang, B.K., Yan, Q., Pigeot, S., Martin, I., Huard, J. and Nakayama, N. (2017) "Pre-transplantational control of the post-transplantational fate of human pluripotent stem cell-derived cartilage" *Stem Cell Reports*, in revision.

LAB MEMBERS

Research Assistants: Azim Pothiawala
Senior Research Associate and Animal Specialist: Nadine Matthias



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

Pamela Wenzel, Ph.D.
Assistant Professor

Biomechanical force regulates cell potential and cancer metastasis

Our lab studies how biomechanical force generated by blood flow in the vasculature and lymph flow in the lymphatics impacts cell potential and behavior.

One arm of our research is designed to address how frictional force promotes stem cell function during embryogenesis, in the adult, and in the context of disease and injury. We are especially interested in how we might use this information in the laboratory to expand improved sources of hematopoietic stem cells and other cellular therapies for treatment of hematologic disorders and neurological trauma. A number of genetic and biochemical pathways are currently under investigation as key players mediating the signaling cascade downstream of fluid force that potentiate stem cell potential and immunomodulatory function of stem cells. We employ various tools and approaches to evaluate stem cell function, including microfluidics, pharmacology, mouse genetics, and transplantation assays.

Fluid flow and hydrostatic pressure also have been implicated in tumor biology, but it remains unclear what role lymphatic or vascular shear stresses may play in regulating metastatic potential of cancer cells. Using biomimetic microchips designed to model the lymphatic vasculature, we modulate the shear stress 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

- Mechanobiology of hematopoietic and mesenchymal stem cells
- Fluid flow in initiation of metastasis

KEY PUBLICATIONS

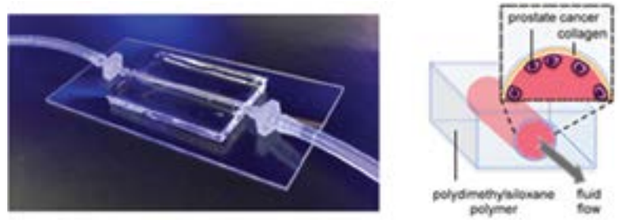
Diaz, M.F., Vaidya, A.B., Evans, S.M., Lee, H.J., Aertker, B.M., Alexander, A.J., Price, K.M., Ozuna, J.A., Liao, G.P., Aroom, K.R., Xue, H., Gu, L., Omichi, R., Bedi, S., Olson, S.D., Cox, C.S., & Wenzel, P.L. Biomechanical forces promote immune regulatory function of bone marrow mesenchymal stromal cells. *Stem Cells*. 35:1259-1272, 2017.

Lee, H.J., Diaz, M.F., Price, K.M., Ozuna, J.A., Zhang, S., Sevick-Muraca, E.M., Hagan, J.P., & Wenzel, P.L. Fluid shear stress activates YAP1 to promote cancer cell motility. *Nature Communications*. 8:14122, 2017. This work was featured in a news story at UTHealth: https://www.uth.edu/media/story.htm?id=e2ea8aec-975e-4ee1-af8e-8d975d8fe8e6. Recommended in F1000Prime as being of special significance in its field.

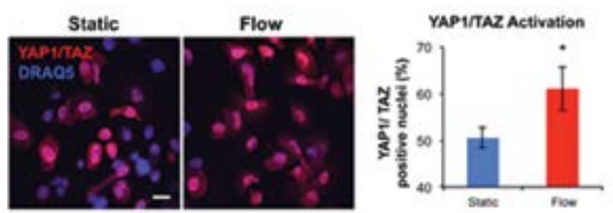
Lee, H.J., Diaz, M.F., Ewere, A., Olson, S.D., Cox, C.S., & Wenzel, P.L. Focal adhesion kinase signaling regulates anti-inflammatory function of bone marrow mesenchymal stromal cells induced by biomechanical force. *Cellular Signalling*. 38:1-9. doi: 10.1016/j.cellsig.2017.06.012, 2017.

LAB MEMBERS

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.



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 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, Ph.D.
Associate Professor

Gene transcription and regulation of stem cell differentiation and neural injuries

My laboratory combines stem cell biology and systems-based approaches involving genomics, bioinformatics and functional assays to unravel gene transcription and regulatory mechanisms governing stem cell differentiation. One major focus of the group is investigating stem cell neural differentiation and developing effective and safe treatment for spinal cord injury and neurological diseases. We are studying gene expression and the regulation of transcription factors and regulatory RNAs using next-generation sequencing technologies. These studies are crucial in understanding the molecular mechanism of stem cell neural differentiation and its clinical implications. The goal is to identify and modulate key regulators as therapeutic targets to direct the differentiation of stem cells into desired neural cell types more efficiently, and to increase transplantation safety. The other area of her research interest lies in the studies of the regulatory networks of hematopoietic precursor cell self-renewal and differentiation using multipotent EML (erythroid, myeloid, and lymphocytic) cell as a model system. We are using integrated genomic and proteomic approaches to identify key components that control the switch. We have identified that TCF7, together with RUNX1, are important regulators in this process. Currently we are generating a global interaction network and a novel and comprehensive view of the regulation of early stages of hematopoietic precursor self-renewal and differentiation. This study can serve as a model for the analysis of cell self-renewal and differentiation in general and provide insight for efficient expanding and manipulating hematopoietic precursor and stem cells.

RESEARCH PROJECTS

- Investigate gene expression and regulatory mechanisms during stem cell differentiation
- Characterize molecular signatures and identify therapeutic targets for spinal cord injury and neurological diseases
- Pinpoint key transcription factors and regulatory RNAs, and modulate key regulators to steer the direction of stem cell differentiation and improve efficiency

KEY PUBLICATIONS

Wu, J. Q. (ed.) (2016). Transcriptomics and Gene Regulation. *Springer Publisher Dordrecht* 2016.

The Systematic analysis of coding and long non-coding RNAs in the sub-chronic and chronic stages of spinal cord injury. Cuevas-Diaz Duran, R., Yan, H., Zheng, Y., Huang, X., Grill, R., Kim, D.H., Cao, Q., and Wu, JQ. *Sci Rep.* Jan 20;7:41008. doi: 10.1038/srep41008. 2017

Single-cell RNA-sequencing in the brain. Cuevas-Diaz Duran, R., Wei, H.C., and Wu, J.Q. *Clin Trans Med* (2017) 6:20. Dec;6(1):20. doi: 10.1186/s40169-017-0150-9. Epub 2017 Jun 8.

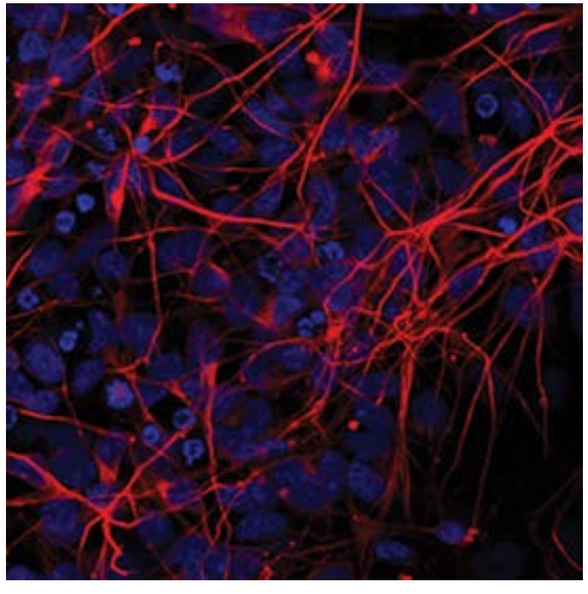
Human Neural Progenitors Derived from Integration-Free iPSCs for SCI therapy. Liu, Y., Zheng, Y., Li, S., Xue, H., Schmitt, K., Hergenroeder, G.W., Wu, JQ., Zhang, Y., Kim, D.H., and Cao, Q. *Stem Cell Research.* doi: 10.1016/j.scr.2017.01.004. 2017

Single-cell RNA-sequencing in the brain. Cuevas-Diaz Duran, R., Wei, H.C., and Wu, J.Q. *Clin Trans Med* (2017) 6:20. Dec;6(1):20. doi: 10.1186/s40169-017-0150-9. Epub 2017 Jun 8.

Exosomes Mediate Epithelium–Mesenchyme Crosstalk in Organ Development. Jiang, N., Xiang, L., He, L., Yang, G., Zheng, J., Wang, C., Zhang, Y., Wang, S., Zhou, Y., Sheu, T., Wu, J., Chen, K., Coelho, P.G., Tovar, N.M., Kim, S.H., Chen, M., Zhou, Y-H., and Mao, *J. ACS Nano.* 2017 Jul 27. doi: 10.1021/acsnano.7b01087. PMID: 28727410

LAB MEMBERS

Post-doctoral Fellows: Yanan You, Raquel Cuevas Diaz Duran, Haichao Wei, Xiaomin Dong
Undergraduate Student: Vy Hong



Immunofluorescence labeling of neurons derived from H1 human embryonic stem cells (hESCs). beta-tubulin (TujIII red) labels both immature and mature neurons. Nuclei (blue) are stained by DAPI.

Wa Xian, Ph.D.
Assistant Professor
CPRIT Scholar

Stem cells of regenerative and malignant epithelia

My laboratory develops new technology for capturing the stem cells of highly regenerative tissues such as the gastrointestinal tract, the lung, and organs such as liver, pancreas, and kidney. These stem cells are quite remarkable as we can grow them for years in a test tube as immature cells and yet on command trigger them to form the organ from which they were derived. Importantly, we are finding that many of the major chronic inflammatory diseases of these organs, such as inflammatory bowel disease, lung diseases, such as asthma and COPD, are driven by permanent changes in the stem cell population in these patients. As such, patient-derived stem cells will drive future development of relevant disease models and drug discovery to mitigate these conditions.

A second but related direction of the laboratory is to use our stem cell cloning technology to identify and capture stem cells of highly lethal cancers such as those of the ovaries, pancreas, and esophagus. Remarkably, about 1:2000 tumor cells of a given cancer is a true "cancer stem cell," whereas the rest cannot determining the future of the tumor. Our goal is to understand this minor population of cells in the tumor and how we can target them with drugs that exploit their unique properties.

RESEARCH PROJECTS

- **Intestinal stem cell variation underlying inflammatory bowel disease**
  Inflammatory bowel disease, including Crohn's and ulcerative colitis, remain serious and difficult to manage conditions affecting 1.6 million Americans. The etiology has a genetic component but seems dominated by environmental factors consistent with an interplay between the immune system, the microbiome of the gut, and the intervening intestinal epithelial barrier. We have applied the stem cell technology we developed to identify unusual stem cells in the colons of these patients, which may key for understanding the pathology and chronic features of IBD and identifying new therapeutic targets for resolving this disease.
- **Cloning and targeting stem cells of precancerous lesions: Barrett's esophagus**
  Barrett's esophagus is a precancerous lesion that increases the risk for the development of esophageal adenocarcinoma by 30-100-fold. Given the poor 5-year survival rates for this cancer, we are focused on identifying drugs that eliminate the stem cells of Barrett's esophagus before they have a chance to evolve into dysplasia and malignant cancer.
- **Intratumor heterogeneity among stem cells of high-grade ovarian cancer**
  High-grade ovarian cancer (HGOC) is remarkably sensitive to chemotherapy with many patients achieving a complete response only to suffer relapses 6-24 months later typically with resistant properties. To understand the cellular and molecular basis of this resistance phenomenon, we are generating large libraries of cancer stem cells from each patient to examine intratumor heterogeneity and how these differences relate to resistant clones.

KEY PUBLICATIONS

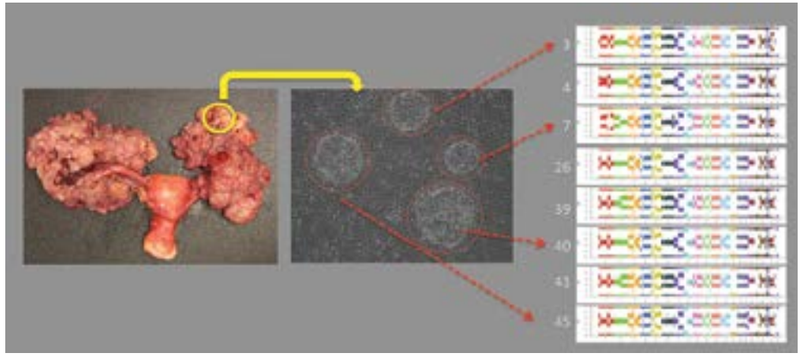
Wang X, Yamamoto Y, Wilson LH, Zhang T, Howitt B, Farrow MA, Kern F, Ning G, Hong, Y, Khor CC, Chevalier B, Bertrand D, Nagarajan, N., Sylvester,,FA, Hyams, JS, Devers, T, Bronson, R, Lacy, DB, Ho, KY, Crum, CP, McKeon, F, and Xian, W. (2015). Cloning and variation of ground state intestinal stem cells. *Nature* 522, 173-178.

Yamamoto Y, Wang X, Bertrand D, Kern F, Zhang T, Hu YY, Deluba M, Srivastava S, Ming T, Khor CC, Wilson L, Blaszyk H, Rolshud D, Liu JJ, Howitt B, Crum CP, Nagarajan N, Ho KY, McKeon F, and Xian W. Mutational spectrum of Barrett's stem sells suggests paths to initiation and progression of a precancerous lesion. *Nature Comm.* 2016 Jan 19;7:10380

Xian W, McKeon F. (2017). Barrett's Stem Cells as a Unique and Targetable Entity. *Cell Mol Gastroenterol Hepatol.* 4, 161-164.

LAB MEMBERS

Post-doctoral Fellow: Wei Rao, Ph.D.
Senior Research Scientist: Brian Wang, M.D.



Derivation of patient-specific cancer stem cell (CSC) library of clones from high-grade ovarian cancer case (left). Four CSC clones sampled for expansion and CNV analysis (right).

The demographics of the American population are shifting toward an increasing elderly population, placing extraordinary demands on our health care system. Aging results in the progressive attrition of homeostasis and functional reserve of all organ systems. As a consequence, the incidence of numerous debilitating diseases, including neurodegeneration, osteoporosis, sarcopenia, sensorineural defects, cardiovascular disease, diabetes, and cancer increases with age. The major focus of the center is to understand the molecular basis of aging and developing strategies for preventing or delaying age-associated diseases, which represent fundamental and pressing challenges that the medical research community faces today. The precise nature of the damage that is responsible for aging-related degenerative changes remains ill-defined, but may include mitochondrial damage, telomere attrition, nuclear dysmorphology, accumulation of genetic mutations, and cumulative DNA, protein or membrane damage. A universal characteristic of aging is the loss of tissue regenerative potential during the progressive depletion of stem cells, which leads to an impaired ability to respond to stress, and as a consequence, dramatically increases the risk of morbidity and mortality. This and the exponentially increased incidence of numerous degenerative diseases in the elderly has led to the hypothesis that aging is caused, in part, by the loss of functional stem cells necessary for tissue rejuvenation.

Our research program is currently focusing on determining the pathway(s) through which stem cells become dysfunctional with age. We are currently examining the intrinsic, cell autonomous mechanisms, as well as the effects of the microenvironment (muscle, vascularity, blood vessel) or systemic factors in driving stem cells dysfunction through non cell autonomous mechanisms. In addition, we are trying to determine the mechanism(s) underlying the dramatic therapeutic effects observed following systemic injection of functional, young, but not aged, stem cells have on healthspan and lifespan in mouse models of accelerated aging. In addition, we are performing proteomics to identify the therapeutic factors secreted by young, functional stem cells. The successful completion of this research will result in the development of novel approaches for the use of stem cells or rejuvenating factors, derived from functional stem cells, to extend human health and lifespan. We are also establishing numerous investigators in the area of aging research so we can synergize our efforts on tissue engineering and aging research.

Johnny Huard, Ph.D.
Professor & Vice Chair for Orthopedic Research
Department of Orthopedic Surgery
Director, IMM Center for Tissue Engineering and Aging Research

Muscle-derived stem/progenitor cell applications

The focus of my research is in the areas of gene therapy, tissue engineering, and regenerative medicine applications based on the use of muscle-derived stem/progenitor cells (MDSPCs). My research team's primary areas of interest include basic stem cell biology and molecular techniques for gene editing and tissue engineering, and application of these techniques for translation to the clinic to aid in repair and regeneration of a variety of tissues. Our research efforts involve investigating applications of MDSPCs for treatment of a variety of diseases, conditions, and injuries that affect the musculoskeletal system, including those resulting from natural and accelerated aging processes. My team has received national and international recognition, and technologies that we have developed have been licensed to industry. Muscle-derived cells that have been isolated by my team are currently being utilized in clinical trials for the treatment of stress urinary incontinence and myocardial infarction. More than 400 women suffering with SUI in Canada and the U.S. have volunteered for this stem cell therapy (Phase III Clinical Trial).

RESEARCH PROJECTS

- Muscle stem cells reprogrammed through genome engineering for autonomously regulated anti-fibrotic therapy, and cell autonomous and non-autonomous mechanisms of stem defects with aging
- Development of biological approaches to improve functional recovery after compartment syndrome injury
- Bone abnormalities and healing defects in muscular dystrophy, and biomimetic coacervate delivery of muscle stem cells to improve cardiac repair

KEY PUBLICATIONS

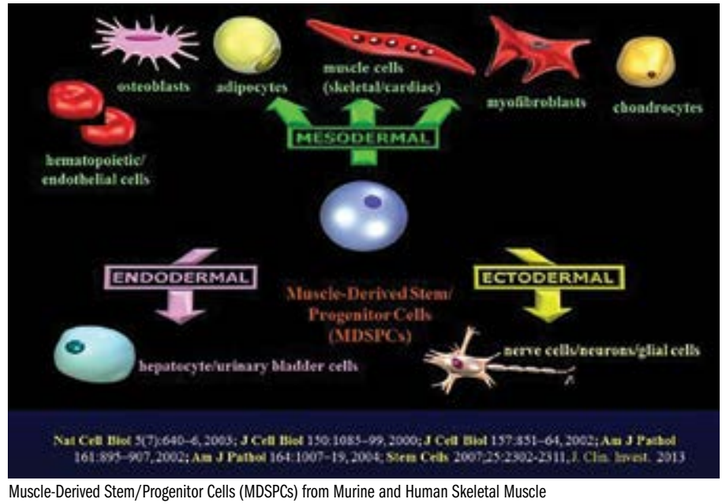
Murray IR, Gonzalez González Galofre ZN, Baily J, Dobie R, Wallace RJ, Mackinnon AC, Smith JR, Greenhalgh SN, Thompson AI, Conroy KP, Griggs DW, Ruminski PG, Gray GA, Singh M, Campbell MA, Kendall TJ, Dai XJ, Li Y, Iredale JP, Simpson H, Huard J, Peault B, Henderson NC. αv integrins on perivascular mesenchymal 1 cells critically regulate skeletal and cardiac muscle fibrosis. Nature Communications. *Nat Commun.* 2017 Oct 24;8(1):1118. PMID: 29061963.

Mu X, Tang Y, Takayama K, Chen W, Lu A, Wang B, Weiss K, Huard J. RhoA/ROCK inhibition improves the beneficial effects of glucocorticoid treatment in dystrophic muscle: implications for stem cell depletion. *Hum Mol Genet.* 2017 Aug 1;26(15):2813-2824. PMID: 28549178.

Takayama K, Kawakami Y, Lavasani M, Mu X, Cummins JH, Yurube T, Kuroda R, Kurosaka M, Fu FH, Robbins PD, Niedernhofer LJ, Huard J. mTOR signaling plays a critical role in the defects observed in muscle-derived stem/progenitor cells isolated from a murine model of accelerated aging. *J Orthop Res.* 2017 Jul;35(7):1375-1382. Epub 2016 Sep 22. PMID: 27572850.

LAB MEMBERS

Assistant Professors: Ping Guo, Ph.D., Aiping Lu, M.D., Xiaodong Mu, Ph.D., Xueqin Gao, M.D., Ph.D., Krishna Sinha, Ph.D.
Post-doctoral Research Fellows; Shanshan (Ellen) Gao, Ph.D., Chieh (Judy) Tseng, Ph.D., Xuying Sun, Ph.D., William Sealy Hambright, Ph.D.
Senior Research Scientists: Polina Matre, M.B.A., Ph.D., Yan Cui, M.D.
Visiting Scientist: Fan Yang, Ph.D.
Visiting Students: Zhenhan (Stephen) Deng, Chih-Yi (Amy) Lin
Research Team: Haiying Pan, Haizi Cheng, M.D., Ling (Jeannie) Zhong, Martha Pena, Sarah Amra, Michelle Ramirez, Mary Hall, M.B.A., Ph.D., Barbara Lipari



Muscle-Derived Stem/Progenitor Cells (MDSPCs) from Murine and Human Skeletal Muscle

Xueqin Gao, M.D., Ph.D.
Assistant Professor

Muscle-derived stem cells for bone and cartilage regeneration and repair

I am a member of Dr. Johnny Huard's research team. My research focuses on using muscle-derived stem cells (MDSCs) and gene therapy for bone and cartilage repair. I conduct translational studies to use MDSCs for the treatment of bone defects, non-union fractures, and age-related bone and cartilage conditions, such as osteoporosis and osteoarthritis. I am also investigating bone biology in a disease model of muscular dystrophy.

Human muscle-derived stem cells for bone regeneration

Large segmental bone defects and non-union fractures caused by traumatic injury or cancer resection represent major issues in clinical orthopaedics. We are investigating new vectors and growth factors to mediate *ex vivo* gene therapy and using biomaterials to deliver growth factors to enhance human muscle-derived stem cell (hMDSC)-mediated bone regeneration. We are also investigating the effects of the age of both donor hMDSCs and hosts on hMDSC-mediated bone repair.

Human muscle-derived stem cells for age-related cartilage injury or osteoarthritis

The application of stem cells, including murine muscle-derived stem cells (mMDSCs) in murine models, for treating osteochondral defects or for osteoarthritis repair are clinically translational. In this project, we are using viral vectors and biomaterials to deliver growth factors for hMDSC-mediated cartilage repair, particularly for the treatment of monoiodoacetate (MIA)-induced osteoarthritis in a murine model.

Investigation of interactions between muscle and bone in a muscular dystrophy model

Duchenne muscular dystrophy (DMD) is a deadly muscle disease that inflicts about 1 in 3,000 boys. Patients often become wheelchair-bound in their second decade of life. We have found bone abnormalities in a dystrophin/utrophin double knock out (dko) model that closely mimics the clinical manifestations of human DMD. For this project, we are investigating how muscular dystrophy affects the bone quality and defect healing, and how muscle and bone interact in this mouse model in order to unveil mechanisms which may be translated into new strategies to improve the life quality of DMD patients.

RESEARCH PROJECTS

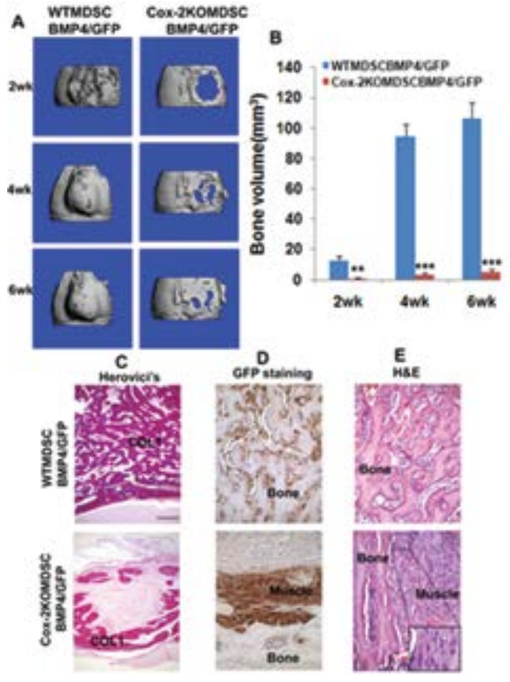
- Utilizing human muscle-derived stem cells and gene therapy for bone tissue repair
- Using human muscle-derived stem cells for cartilage and osteoarthritis repair using *ex vivo* gene therapy and biomaterial scaffolding.
- Bone abnormalities in muscular dystrophy (NIH RO1 awarded to Dr. Huard)

KEY PUBLICATIONS

Gao X, Usas A, Lu A, Kozemchak A, Tang Y, Poddar M, Sun X, Cummins JH1, Huard J. Cyclooxygenase-2 deficiency impairs muscle-derived stem cell-mediated bone regeneration via cellular autonomous and non-autonomous mechanisms. *Hum Mol Genet.* 2016 Jun 27. pii: ddw172. [Epub ahead of print], PMID:27354351

Gao X, Usas A, Tang Y, Lu A, Tan J, Schneppendahl J, Kozemchak AM, Wang B, Cummins JH, Tuan RS, Huard J. A comparison of bone regeneration with human mesenchymal stem cells and muscle-derived stem cells and the critical role of BMP. *Biomaterials.* 2014 Aug;35(25):6859-70. PMID:24856105

Gao X, Usas A, Proto JD, Lu A, Cummins JH, Proctor A, Chen CW, Huard J. Role of donor and host cells in muscle-derived stem cell-mediated bone repair: differentiation vs. paracrine effects. *FASEB J.* 2014 Aug;28(8):3792-809. PMID:24843069



Cox-2 -deficient MDSCBMP4/GFP-mediated bone regeneration is impaired *in vivo* using a critical-size bone defect model.

Ping Guo, Ph.D.
Assistant Professor

Stem cell/Gene therapy and tissue engineering for treating sports related diseases

Our lab is focusing on the discovery and development of gene modification and stem cell therapy for treating human sport related diseases. Currently, we have three major areas of research.

RESEARCH PROJECTS

Biomimetic coacervate delivery of muscle stem cells for cardiac repair and regeneration.

Cellular cardiomyoplasty (CCM), which involves the transplantation of exogenous cells into the heart, is a promising approach to repair injured myocardium and improve cardiac function. We have successfully expanded human muscle derived stem cells (MDSCs), to clinically relevant numbers in culture. More importantly, human MDSCs have already entered the clinical arena for the treatment of bladder dysfuntion & myocardial infarction, confirming that MDSCs represent a viable therapeutic cell source for CCM. However, several limitations, such as a poor approaches for delivering the cells (direct intramyocardial injection in PBS) which leads to limited cell retention and survival. Moreover, MDSCs low cardiomyogenic potential limits the cardiac regenerative potential of the MDSCs. The use of cytokines, or growth factor-coacervate, as a novel delivery vehicle for the MDSCs, represents a new area of research that could not only promote cell retention, survival, and the cardiac regenerative potential of the MDSCs, but also synergistically enhance angiogenesis through the release of cytokines or growth factors.

The use of biomimetic microspheres to improve the beneficial effect of platelet rich plasma for musculoskeletal tissue repair.

Platelet-rich plasma (PRP) has significant advantages over other potential biologic treatment modalities since it contains an abundance of autologous growth factors and is both easy to obtain and manipulate. PRP contains many important growth factors that may accelerate tissue healing (including PDGF, VEGF, IGF, and TGF-β1, among many others). However, PRP also contains large quantities of substances that are known to promote further inflammation and subsequent tissue damage. Further complicating this issue, certain growth factors may promote tissue healing and regeneration in one type of tissue but produce detrimental effects in other types of tissues. For example, angiogenic growth factors are beneficial for skeletal muscle healing but have been shown to stimulate blood vessel formation and cartilage degeneration. We hypothesize that inhibition of VEGF will enhance the beneficial effects of PRP on chondral injuries via the suppression of angiogenesis and subsequent fibrocartilage formation.

Stem cells and regeneration in digestive tract organs.

The pancreas is a vital part of the digestive system and a critical controller of blood sugar levels. Diabetes develops when the pancreas beta-cells fail to produce sufficient quantities of insulin. Stem cells hold tremendous potential because they have the potential to become virtually any kind of cell and hence could be a source of insulin-producing cells that could be placed in a Biohub, which is a bioengineered "mini organ" that mimics the native pancreas.

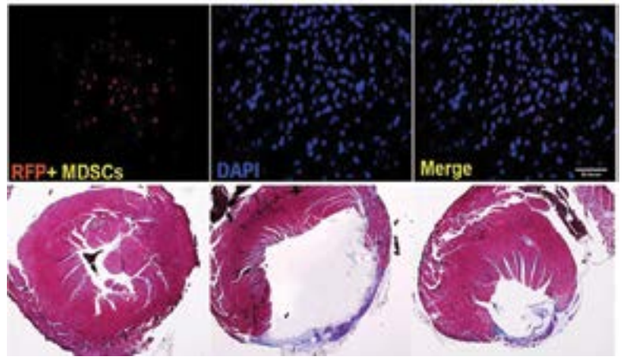
KEY PUBLICATIONS

Chiyo Shiota, Krishna Prasadan, Ping Guo, Joseph Fusco, Xiangwei Xiao, George Gittes. GcgCreERT2 Knockin Mice as A Toll for Genetic Manipulation in Pancreatic Alpha Cells. *Diabetologia*, 2017, Sept. 7, published online.

Johnny Huard, Aiping Lu, Xiaodong Mu, Ping Guo, Yong Li, Muscle Injuries and Repair: What's New on the Horizon! *Cells Tissues Organs,* 2016, 202:227-236.

LAB MEMBERS

Post-doctoral Fellows: Shanshan Gao
Graduate Students: Isaac Castillo
Research Assistants: Elizabeth Morris, Sabrina Gonzalez.



Transplantation of muscle derived stem cells into the myocardial infarcted heart. Engraftment of MDSCs (with RFP tag) was detected by anti-RFP antibody (top panel). We also have tested co-injection of MDSCs with TGFβ1 siRNA. Four weeks after injection, MDSCs reduced the infarcted area and partially preserved the normal tissue structure in the infarcted zone as we reported previously and reduced scar formation (blue in Trichrome staining) when co-injected with TGFβ1 siRNA (bottom panel).

Aiping Lu, M.D.
Assistant Professor

Identification of therapeutic muscle derived stem cells for treatment of muscular disease and accelerate aging

Our group focuses on identification of therapeutic muscle derived stem cells for treatment of muscle injury, muscular disease, and accelerate aging.

Pregnancy Improves Muscle Healing and Myogenic Differentiation of Myogenic Progenitor Cells (MPCs). Pregnancy represents a unique biological model of a shared circulatory system between the mother and fetus. We have demonstrated that pregnant mice exhibit accelerated muscle healing following cardiotoxin injury when compared to non-pregnant control mice. We also found that myogenic differentiation capacity of MPCs isolated from pregnant mice was significantly improved when compared to that of non-pregnant mice. In addition, MPCs from the non-pregnant mice displayed enhanced myogenic capacity when cultured in the presence of serum obtained from pregnant mice. Our results suggest that circulating factors present during pregnancy may stimulate MPCs and improve their myogenic potential.

Enhance stem cell therapeutic outcome by improving microenvironment for treatment of muscular dystrophy. Duchenne muscular dystrophy (DMD) is lethal genetic disease and there is still no effective cure for DMD. Transplantation of muscle progenitor cells from healthy donors for treatment in DMD has been widely investigated but with unsatisfied outcome due to the harmful macroenvironment in the muscle of DMD patients. We hypothesized blood exchange between young/or pregnant WT and dystrophic mice will improve this environment, this will not only enhance the outcome of stem cell transplantation from donor, but also have a beneficial effect for the function of muscle stem cells in the host. The results obtained from this study will develop novel and clinical relevant therapies to alleviate stem cell dysfunction and improve the histopathologies in muscle dystrophy.

RESEARCH PROJECTS

- To analyze the cell non-autonomous effects induced by aging and stress on MDSPCs using heterochronic parabiosis and tissue-specific inactivation of ERCC1 (NIH, PO1)
- To investigate the cell- cell interaction between myogenic cells and non-myogenic cells
- The role of pregnancy in muscle injury and wound healing
- Parabotic pairing between dystrophic mice and WT mice for improving muscle and heart histopathology

KEY PUBLICATIONS

Proto JD, Lu A, Dorronsoro A, Scibetta A, Robbins PD, Niedernhofer LJ, Huard *J. PLoS One.* 2017 Jun 22;12(6):e0179270.

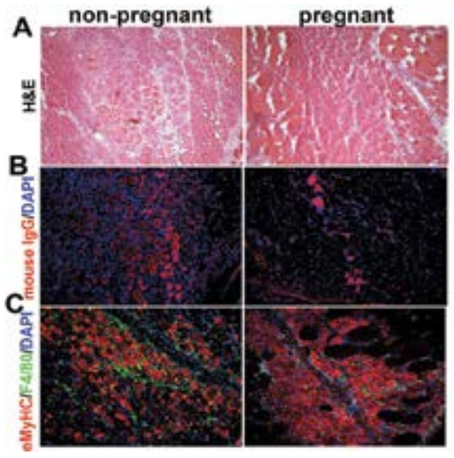
Mu X, Tang Y, Takayama K, Chen W, Lu A, Wang B, Weiss K, Huard *J. Hum Mol Genet.* 2017 Aug 1;26 (15):2813-2824.

Li H, Lu A, Tang Y, Beckman S, Nakayama N, Poddar M, Hogan MV, Huard *J. Mol Ther Methods Clin Dev.* 2016 Nov 30;3:16065. eCollection 2016.

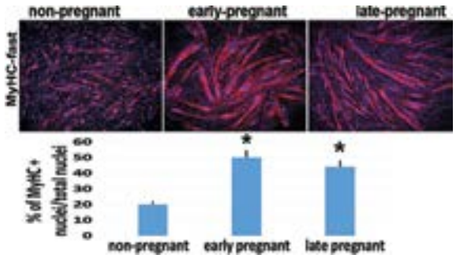
Huard J, Lu A, Mu X, Guo P, Li Y. *Cells Tissues Organs.* 2016;202(3-4):227-236.

Gao X, Usas A, Lu A, Kozemchak A, Tang Y, Poddar M, Sun X, Cummins JH, Huard *J. Hum Mol Genet.* 2016 Aug 1;25(15):3216-3231.

Huard J, Mu X, Lu A. *Clin Pharmacol Ther.* 2016 Aug; 100(2):142-6.



Improvement of muscle healing after injury in pregnant mice.



Muscle progenitor cells (MPCs) isolated from non-pregnant mice exhibit improved myogenic differentiation capacity after stimulation with serum from pregnant mice.

Xiaodong Mu, Ph.D.
Assistant Professor

Inhibition of NF-κB signaling with novel small molecule inhibitors delays muscle stem cell senescence and rescues defective muscle phenotypes in progeroid mice

Our "Stem Cells in Aging and Cancer" research team is part of Dr. Johnny Huard's research group in the Center for Tissue Engineering and Aging Research and the Department of Orthopaedic Surgery, and our studies involve stem cell biology, wound regeneration, fibrosis prevention, muscular dystrophy, premature aging, and cancer biology (osteosarcoma). Currently, we are especially interested in studying the mechanisms of stem cell senescence and premature aging, and the mechanisms of various musculoskeletal disorders and conditions (i.e., Duchenne Muscular Dystrophy, cancer cachexia-related muscle atrophy, heterotopic ossification, and osteosarcoma).

Currently, I am focusing my research in the following areas:

1) Understanding the cellular and molecular regulatory mechanisms of muscle stem cell defects in diseased and aged muscles in an effort to reduce fibrosis and improve function of regenerating muscles.

Accelerated exhaustion, senescence, and loss of regenerative potential of stem cells have been observed in diseased skeletal muscle, such as that resulting from progeria and muscular dystrophic disease. Some key stem cell regulators: Notch, Wnt, RhoA, and mTOR signaling pathways have been shown to be important regulators of tissue aging and cell senescence.

2) Investigating the mechanisms of muscle atrophy in osteosarcoma-induced cancer cachexia, and potential effects of muscle stem cells on reducing muscle atrophy.

Skeletal muscle atrophy is frequently associated with cancer cachexia and results in reduced endurance of patients to clinical treatments. We are studying the role of muscle stem cells in mediating muscle atrophy in an osteosarcoma mouse model, and expect to find ways to improve the function of muscle stem cells and reduce muscle atrophy. Potential methods include the inhibition of Wnt, ALDH, or Notch signaling pathways, and muscle stem cell transplantation.

3) Improvement of wound healing by reducing fibrosis or application of stem cells.

We previously reported the effects of the hormone relaxin and MMPs in the prevention of fibrosis during the healing process of injured skeletal muscle or amputated digits. Since healing of diseased skeletal muscle (i.e., dystrophic muscle) is usually accompanied by excessive fibrosis, we are investigating how to enhance regeneration of diseased soft tissues with the application of relaxin, MMPs, and multipotent stem cells.

RESEARCH PROJECTS

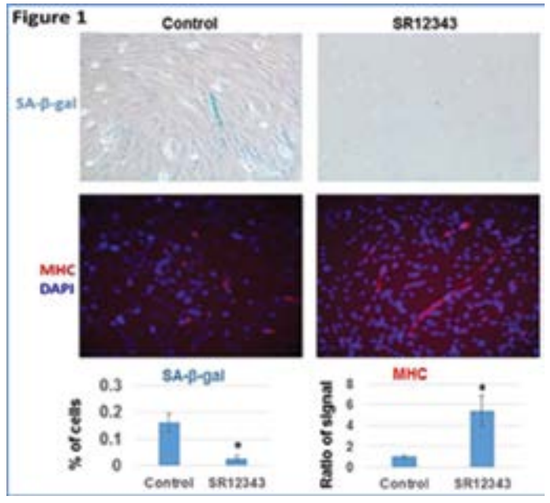
- Understanding of the cellular and molecular regulatory mechanisms of muscle stem cell defects in diseased and aged muscles, including the roles of NF-κB, Notch, and Wnt signaling in regulating stem cell senescence of accelerated aging in contrast to normal aging, as well as roles of cancer stem cells
- Investigating the mechanisms of muscle atrophy in osteosarcoma-induced cancer cachexia and potential effects of muscle stem cells on reducing muscle atrophy
- Improvement of soft tissue wound healing by reducing fibrosis or application of stem cells

KEY PUBLICATIONS

Mu X, Tang Y, Takayama K, Chen W, Lu A, Wang B, Weiss K, Huard J. RhoA/ROCK inhibition improves the beneficial effects of glucocorticoid treatment in dystrophic muscle: implications for stem cell depletion. *Hum Mol Genet.* 2017 Aug 1;26(15):2813-2824. PMID: 28549178.

Mu X, Agarwal R, March D, Rothenberg A, Voigt C, Tebbets J, Huard J, Weiss K. Notch Signaling Mediates Skeletal Muscle Atrophy in Cancer Cachexia Caused by Osteosarcoma. *Sarcoma.* 2016.

Takayama K, Kawakami Y, Lavasani M, Mu X, Cummins JH, Yurube T, Kuroda R, Kurosaka M, Fu FH, Robbins PD, Niedernhofer LJ, Huard J. mTOR signaling plays a critical role in the defects observed in muscle-derived stem/progenitor cells isolated from a murine model of accelerated aging. *J Orthop Res.* 2016 Aug 30



Treatment of Z24$^{-/-}$ MPCs with NF-κB inhibitor decreased the ratio of senescent cells and increased the myogenesis potential. Z24$^{-/-}$ MPCs were treated with novel NF-κB inhibitor (50 μM) for 4 days, and the cell senescence assay revealed a decrease in SA-β-gal+ cells; the myogenesis assay also revealed increased myotube formation (Myosin Heavy Chain/MHC+) after NF-κB inhibition when Z24$^{-/-}$ MPCs were maintained in myogenic differentiation medium.

Krishna Sinha, Ph.D.
Assistant Professor

Musculoskeletal tissue regeneration using adult stem cell therapy in malignant and non-malignant skeletal disorders

My research interests are focused on epigenetic control of cell fate determination, specifically on critical factors responsible for bone formation and bone homeostasis as well as prostate cancer-induced skeletal metastases. Our long-term research plan is to identify and study gene function in age-related bone disorders, in order to provide pivotal information that will inform the development of preventive measures and treatments for bone disorders. Since joining Dr. Huard's research group, I have broadened my research interests to include gene therapy approaches that use multipotent skeletal muscle-derived stem cells in the regeneration of musculoskeletal tissues during aging and disease.

Epigenetic control in multipotent potentials of skeletal muscle-derived stem cells.

Skeletal muscle derived progenitor/stem cells (MPCs) are highly regenerative and multipotent that have ability to differentiate into various cell types including skeletal muscle, bone and cartilage. Epigenetic mechanisms play important role in self-renewal capacity of adult stem cells through transcription control of genes that are involved in stemness of stem cells. My current research focuses on the epigenetic regulation by histone modification in control of those genes which are involved in the multipotent abilities of MPCs and their regenerative capacity. We are using chemical compound inhibitors and CRISPR/Cas9 gene editing tools to ablate the function of epigenetic regulators. MPCs are modified for expression of osteoblast specific transcription factor, Osterix (Osx) to improve their bone regenerative capacity. Osx plays a critical role in turning on expression of osteoblast genes during differentiation (Figure).

The histone demethylase NO66 has an oncogenic role in PCa and bone metastasis.

Bone is the most susceptible organ for metastases by nearly all types of cancer, particularly prostate and breast cancers, and skeletal metastases lead to severe defects in bone architectures during aging. NO66 is upregulated in lung cancer. We have found that NO66 levels are elevated in prostate cancer patient samples and xenografted samples. Our data indicate that NO66 overexpression promotes the proliferation and invasion of prostate cancer cells. In xenograft studies, femurs of male SCID mice implanted with NO66-overexpressing PC3 cells have significant bone loss compared with mice with control PC3 cells, suggesting that NO66 plays an oncogenic role in PCa progression and bone metastasis. In addition, we are also exploring research avenues to test the potential of muscle stem cell therapy in treating PCa and skeletal lesions.

RESEARCH PROJECTS

- To study epigenetic mechanisms of self-renewal and depletion of skeletal muscle-derived stem cells, and in the repair of musculoskeletal tissues using next-gen sequencing approaches
- To study post-translational modification of Osterix by lysine methylation in differentiation of skeletal muscle stem cells into osteoblasts and bone formation
- To investigate the oncogenic function of NO66 in PCa progression and bone metastasis, and develop stem cell therapy to inhibit the development of PCa in bone
- Proteomic approach to identify factors responsible for high healing capacity of *MRL/MpJ* (super-healer) mouse strain to understand the mechanisms of tissue regeneration

The long-term goal of our research program is to understand the role of epigenetic regulators and develop therapies for better bone health in age-related osteoporosis and other bone disorders.

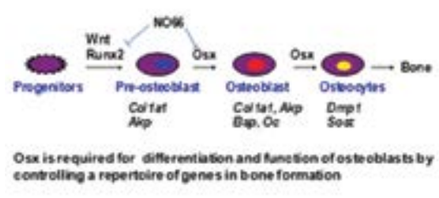
KEY PUBLICATIONS

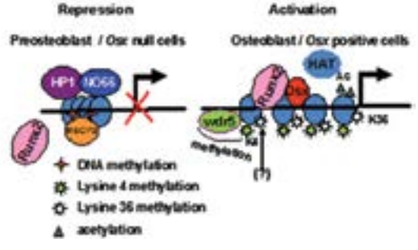
Brien et al. (2012). The Polycomb PHF19 binds H3K36me3 and recruits PRC2 and the NO66 demethylase to hitherto active genes during embryonic stem cell differentiation. *Nature Structure and Molecular Biology,* 2012 Dec;19(12):1273-81.

Yue Tao, Minhao Wu, Xing Zhou, Dandan Zhang, Benoit deCrombrugghe, Krishna M Sinha, Jianye Zang (2013). Structural Insights into the Interactions of Histone Demethylase NO66 with Osterix to Repress Gene Transcription. *Journal of Biological Chemistry* 2013, 288, 16430-16437.

Krishna M Sinha, Hideyo Yasuda, Xin Zhou and Benoit deCrombrugghe (2013). Osterix and NO66 histone demethylase control the chromatin architecture of Osterix target genes during osteoblast differentiation. *J Bone Mineral Research.* Epub 2013 Sep 23.

Q Chen, K Sinha, J Deng, H Yasuda, R Krahe, R Behringer and B de Crombrugghe (2015). Mesenchymal Deletion of Histone Demethylase NO66 in Mice Promotes Bone Formation. *J Bone Mineral Research,* Epub 2015 Feb 26.





Osterix serves as molecular switch for gene activation in osteoblasts.

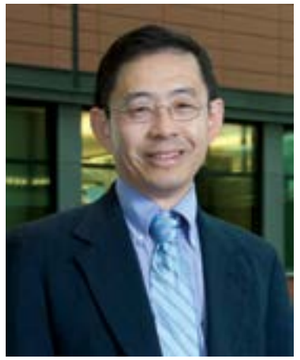
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston for the discovery, development, and commercialization 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 investigators have brought in significant funding from pharmaceutical and biotechnology companies, including Johnson & Johnson, and Merck, and from the National Institutes of Health, the Cancer Prevention and Research Institute of Texas, and the Department of Energy. TTI researchers have made significant scientific discoveries in the areas of cancer biology, fungal natural products, and cancer antibody drug development.

Current research activities at TTI 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. On the translational side, research from TTI has resulted in the establishment of two UT-spin off biotech companies and granted licenses for multiple biologic therapeutics.

In addition to basic and translational research programs, TTI is building two major drug discovery platforms: 1) the Therapeutic Monoclonal Antibody Lead Optimization and Development Platform and 2) the Natural Products and Small Molecular Drug Discovery Platform. The drug discovery platforms not only support TTI internal projects, they also support collaborative projects with other scientists at IMM, Texas based institutions, and other institutions around the country and around the world.

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



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

Discovery and development of therapeutic antibodies and antibiotics

Our group focuses on the discovery and development of therapeutic antibodies and antibiotics against human diseases including cancer and infectious diseases. Currently, we have four 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.
- **HER3 mediated cell signaling and HER3 targeting antibodies for cancer therapy.** Ablated regulation in the HER/ErbB family receptor signaling has been implicated in various cancer types. Agents targeting EGFR and HER2 exhibited clinical benefits for the treatment of some cancer types, but drug resistance is widespread. Current understanding of the drug resistance mechanisms is limited and HER3 has been implicated in the resistance to current EGFR and HER2 therapies. Our group is working on: 1) HER3 mediated cell signaling; 2) the role HER3 plays in resistance to current anti-HER2 and EGFR antibody therapies; and 3) generation of HER3 targeting antibodies and understanding their mode of actions.
- **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 approach to developing effective viral vaccine. We have an ongoing project to aid the design of HCMV and dengue vaccines by profiling antibody response to the experimental vaccines in rhesus monkeys 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

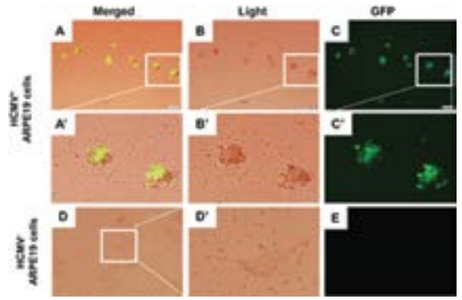
Weixu Meng et al, 2017. Targeting Human Cytomegalovirus-Infected Cells by Redirecting T Cells Using an Anti-CD3/Anti-gB Bispecific Antibody. Antimicrob. *Agents Chemother.* doi:10.1128/AAC.01719-17.

Lin Xia et al. 2017 Active evolution of memory B-cells specific to viral gH/gL/pUL128/130/131 pentameric complex in healthy subjects with silent human cytomegalovirus infection. *Oncotarget.* 8(43): 73654–73669.

Sha Ha et al. 2017. Neutralization of Diverse Human Cytomegalovirus Strains Conferred by Antibodies Targeting Viral gH/gL/pUL128-131 *Pentameric Complex. Journal of Virology* 91(7) e02033-16.

LAB MEMBERS

Post-doctoral Fellows: Xun (Mark) Gui, Zhiqiang Ku, Leike (Simon) Li, Wenxin Luo, Qihui Wang, Yanhong Wang, Yixiang Xu, Xiaohua Ye
Graduate Students: Yuanzhi Chen
Research Coordinator: Georgina T. Salazar
Research Scientist: Ahmad S. Salameh



Light microscopy of T cells incubated with ARPE-19 cells with or without HCMV infection in the presence of bispecific antibody. T cells and bispecific antibody were added to a 6-well plate that was seeded with ARPE-19 cells with or without HCMV infection as indicated by GFP. Images A' and B' shows that T cells migrated towards the HCMV-infected ARPE-19 cells. T-cells are evenly dispersed in the wells with uninfected ARPE-19 cells (image D'). Magnification is 10X for A, B, C, D and E, 40X for A', B', C', and D'. Antimicrob. Agents Chemother. doi:10.1128/AAC.01719-17

EM 3D reconstruction of pentamer bound by Fabs. Structures of the pentamer bound with Fab 2-15, Fab 1-85, or Fab 1-103 and their overlay, which was rotated 180° horizontally with respect to the individual structures. The random conical tilt (RCT) method was used to reconstruct the 3D structures. The surface rendering was generated using the Chimera visualization package. Glycoprotein gH is colored gray, and gL is colored yellow. Journal of Virology 2017,91(7) e02033-16.

Gerald Bills, Ph.D.
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.

Our lab employs genomics to interpret and predict genetically encoded chemical diversity of microorganisms using filamentous fungi as model organisms, especially 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 B_0, the starting molecule for the antifungal drug CANCIDAS. 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*, the causal agent of *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 pathogen. 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

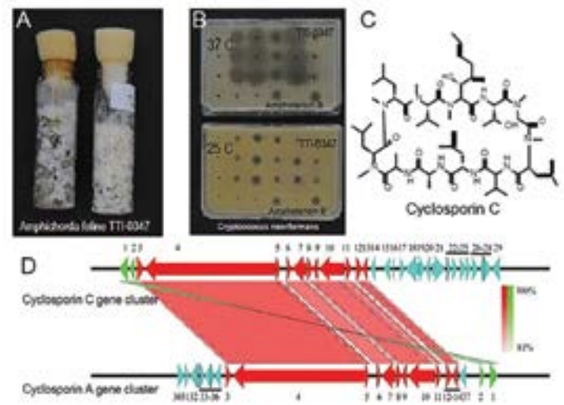
Li, Y., R. Scott, A.R. Hooper, G.A. Bartholomeusz, A.V. Kornienko & G.F. Bills. 2017. *Aspergillus candidus* is a newly recognized source of sphaeropsidin A: Isolation, semi-synthetic derivatization and anticancer evaluation. *Bioorganic & Medicinal Chemistry Letters* https://doi.org/10.1016/j.bmcl.2017.11.001.

Li, Y., Q. Yue, J. Dinith, D. Swenson, G. Bartholomeusz, Z. An, J. Gloer & G. Bills. 2017. Anti-Cryptococcus phenalenones and cyclic tetrapeptides from *Auxarthron pseudauxarthron*. *Journal of Natural Products.* 80:2101–2109.

Bills, G.F. & J.B. Gloer. 2016. Biologically active secondary metabolites from the Fungi. *Microbiology Spectrum* 4: doi:10.1128/microbiolspec.FUNK-0009-2016.

LAB MEMBERS

Post-doctoral Fellow: Dr. Nan Lan.
Visiting Professor: Prof. Meichun Xiang (Chinese Academy of Sciences, Institute of Microbiology).



Cylcosporin C disrupts the growth of the *Cryptococcus* meningitis pathogen at human body temperature. A. Cylcosporin C-producing fungus, *Amphichorda felina*, grown on wheat fermentation medium (12 ml). B. Extracts of *A. felina* containing cyclosporin C disrupt growth of *Cryptoccocus neoformans* at 37°C, but not at ambient temperature (25°C). Antifungal activity of amphotericin B is temperature independent. C. Chemical structure of cyclosporin C. D. Comparison of biosynthetic gene clusters encoding cyclosporine C and A.

Kendra S. Carmon, Ph.D.
Assistant Professor

Therapeutic targeting and mechanisms of LGR5 in colon tumors and cancer stem cells

Emerging evidence has shown that within several different malignant tumor 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 unequivocally established that the adult stem cell marker LGR5 (Leucine-rich repeat-containing, G protein-coupled Receptor 5) is highly expressed in primary colon tumors and that only LGR5-positive colon CSCs 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 carcinomas. However, the function and mechanism of LGR5 in CSCs is still relatively unknown. My previous work in Dr. Qingyun's (Jim) Liu's laboratory led to the discovery that LGR5 functions as a receptor of the secreted growth factors R-spondins to potentiate Wnt signaling, a key regulatory pathway in stem cell survival and tumorigenesis. More recently, we have found that LGR5 interacts with the scaffold protein IQGAP1 (IQ Motif Containing GTPase Activating Protein 1) to recruit the small G protein Rac1 and increase cell-cell adhesion in colon cancer cells. Altogether, these findings suggest that LGR5 plays an important role in cancer and could serve as a promising new target for the development of CSC-based therapies.

My research is focused on the development of innovative antibody-drug conjugates (ADCs) that will 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 tumor cells which express high levels of target antigen. We have successfully generated LGR5-targeted ADCs that incorporate the cytotoxin monomethyl-auristatin E (MMAE) and showed they could destroy colon cancer cells and xenografts. We are currently taking novel approaches to modify and improve the efficacy of our LGR5-targeting ADCs and are also identifying and characterizing new cancer targets for future ADC development. In collaboration with Dr. Ali Azhdarinia's group, we are using PET/CT imaging to evaluate our antibodies as diagnostics and measure tumor uptake and biodistribution in mice prior to antibody-drug conjugation. Furthermore, we are continuing to investigate the signaling mechanism(s) of LGR5 and its role in the control of stemness and drug resistance of CSCs. 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.

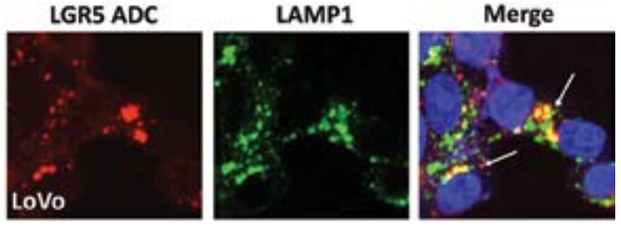
RESEARCH PROJECTS

- Development of antibody-drug conjugates to target colon tumors and cancer stem cells
- Investigation of the LGR5 signaling mechanism and its role in cancer stem cells and drug resistance

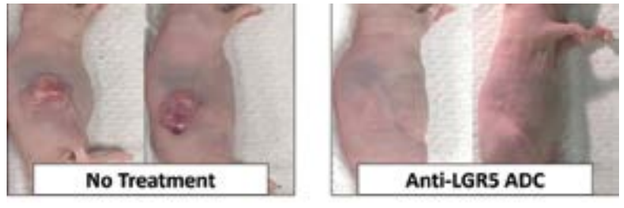
KEY PUBLICATIONS

Carmon K.S., Gong X., Yi J., Wu L., Thomas A., Moore C.M., Masuho I., Timson D.J., Martemyanov K.A., Liu Q.J. LGR5 receptor promotes cell-cell adhesion in stem cells and colon cancer cells via the IQGAP1-Rac1 pathway. *J Biol Chem.* 292(36):14989-15001, 2017.

Gong, X., Azhdarinia, A., Ghosh, S.C., Xiong, W., An, Z., Liu, Q., and Carmon, K.S. LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence, *Mol. Cancer Ther.* 15(7):1580-90, 2016.



Anti-LGR5 ADC (red) binds LoVo colon cancer cells, internalizes, and is transported to the lysosome (LAMP1, green) where drug is released to destroy the cancer cells (ADC/LAMP1 co-localization, yellow).



Treatment with anti-LGR5 ADCs eradicates tumors in a xenograft model of colon cancer.

Xiaodong Cheng, Ph.D.
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 important for the development of human diseases. We apply multidisciplinary approaches, coupling biochemistry, biophysics and cell biology with pharmacology and chemical biology, to understand the structure and function of a family of cAMP sensors: exchange proteins directly activated by cAMP (EPAC). Our goals are to unravel the signaling intricacies of EPAC proteins and to design pathway specific modulators for these important signaling molecules so that their functions can be exploited and controlled pharmaceutically for the treatment of human diseases. We have developed first-in-class EPAC selective inhibitors and EPAC knockout mouse models to study the physiological functions and diseases relevance of this family of important signaling molecules. Recently, we have identified a potential use of EPAC inhibitors in the prevention and treatment of 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 cancer, chronic pain, and heart failure.

RESEARCH PROJECTS

- Structural and functional analyses of the exchange proteins directly activated by cAMP (EPAC), funded by NIH
- Preclinical development of novel drug candidates targeting EPAC for the treatment of microbial infections caused by tick-borne bacteria Rickettsia, funded by NIH
- Examine the roles of EPAC proteins in major human diseases, such as cancer, chronic pain, and heart failure, using EPAC knockout mouse models and pharmacological inhibitors

KEY PUBLICATIONS

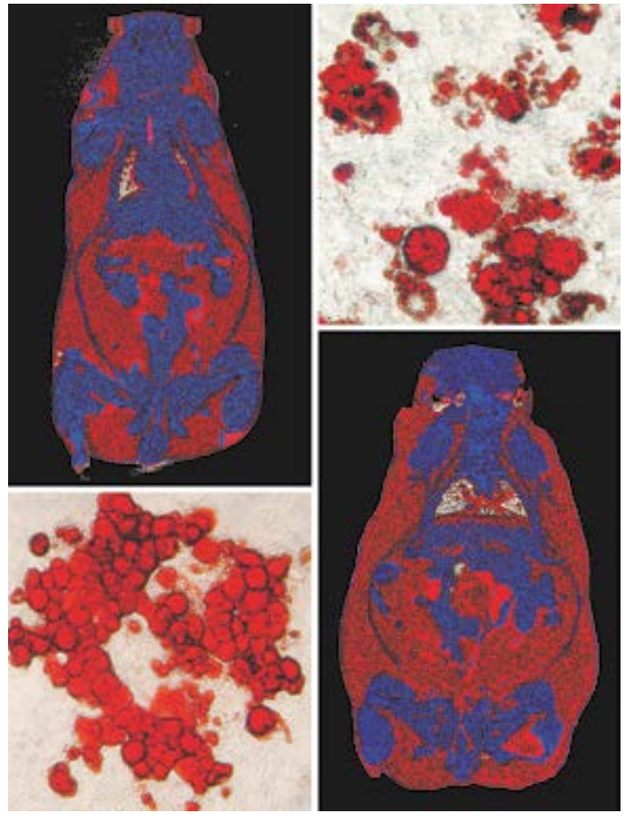
Hu, Y., Robichaux, W. G., Kim, E. R., Mei, F. C., Wang, H., Tong, Q., Xu, M., Chen, J., and Cheng, X. Role of exchange protein directly activated by cAMP isoform 1 in energy homeostasis: regulation of leptin expression and secretion in white adipose tissue. *Molecular Cellular Biology.* 36:2440-1250, 2016. <MCB Spotlight article> http://mcb.asm.org.ezproxyhost.library.tmc.edu/content/36/19/2431.full

Wang, H., Robichaux, W. G., Wang, Z., Mei, F. C., Cai, M., Du, G., Chen, J. and Cheng, X. Inhibition of Epac1 suppresses mitochondrial fission and prevents neointima induced by vascular injury. *Scientific Reports.* 6:36552, 2016.

Zhu, Y., Mei, F. and Cheng, X. A cell-based, quantitative and isoform-specific assay for exchange proteins directly activated by cAMP. *Scientific Reports.* 7:6200, 2017.

LAB MEMBERS

Research Assistant Professor: Fang Mei
Research Scientist: Wenli Yang
Instructor: Yue Li
Post-doctoral Fellows: William Robichaux
Research Assistants: Pei Luo



Loss of EPAC1 increases leptin sensitivity and protects mice from high-fat diet induced obesity

Wenliang Li, Ph.D.
Assistant Professor

Studying and targeting cancer metastasis and drug resistance

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

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

Another research topics in the lab is to investigate the mechanisms of cancer cell plasticity and drug resistance. In particular, we study how prostate cancers become resistance to new generation of androgen receptor pathway inhibitors (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 is no effective treatments to prevent or overcome drug resistance related to NED.

RESEARCH PROJECTS

- Targeting new metastasis kinases that we discovered to inhibit progression of prostate and breast cancers
- Studying and inhibiting key epigenetic regulators to overcome drug resistance in cancer
- Precision oncology, based on molecular profiles and drug sensitivities of patient tumors and patient-derived-xenografts in mice

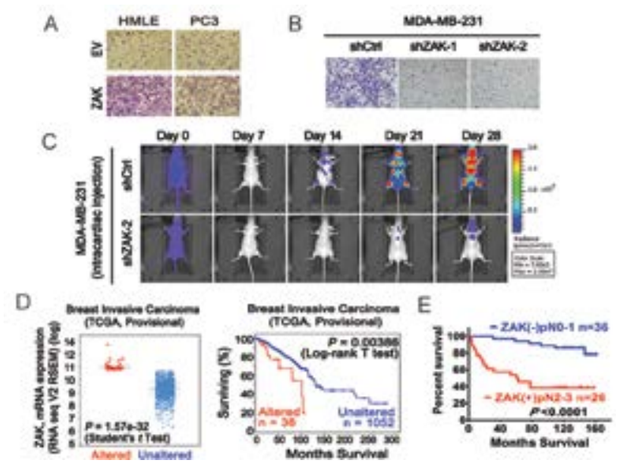
KEY PUBLICATIONS

Hulsurkar, M., Li, Z., Li, X., Zhang, Y., Zheng, D., Li, W*. Beta-adrenergic signaling promotes tumor angiogenesis and prostate cancer progression through HDAC2-mediated suppression of thrombospondin-1. *Oncogene* 36(11):1525-1536, 2017 Mar.

Li, L., Su, N., Zhou, T., Zheng, D., Wang, Z., Yuan, S., Li, W*. Mixed lineage kinase ZAK promotes epithelial-mesenchymal transition in cancer. *Cell Death & Disease* (in press).

LAB MEMBERS

Post-doctoral Fellows: Zheng Wang, Ning Su, Ying Liu
Visiting Scholar: Ting Zhou



We discovered a new critical metastatic-promoting protein call ZAK kinase. ZAK is crucial for cancer cell invasion in culture and bone metastasis in mouse xenografts (A-C). Higher level of ZAK in human breast cancers is significantly associated with worse outcomes (D-TCGA data, and E-breast cancer tissue microarray).

Qingyun (Jim) Liu, Ph.D.
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 elucidated 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 ant-LGR5 antibodies showed excellent anti-tumor efficacy in preclinical models of colon cancer. We also have 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.

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

KEY PUBLICATIONS

Carmon, K.S., Gong, X, Lin, Q., Thomas, A., and Liu, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A,* 108:11452-11457 (2011).

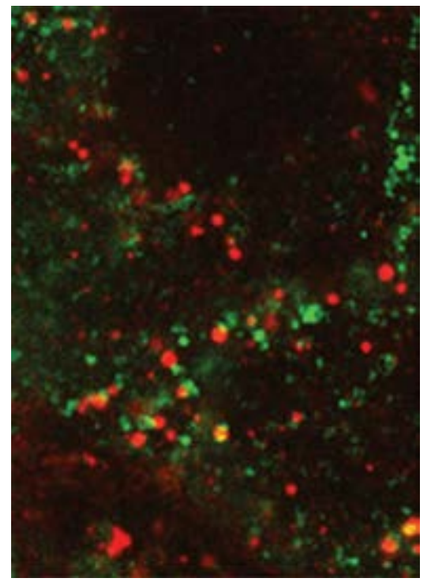
Carmon, K. S., Gong, X., Yi, J., Thomas, A., Liu, Q. RSPO-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proc Natl Acad Sci U S A.* 111: E1221-E1229 (2014).

Gong, X., Azhdarinia, A., Ghosh, S., Xiong, W., An, Z., Liu, Q., Carmon, K.S. (2016). LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol Cancer Ther.* 15:1580-1590 (2016).

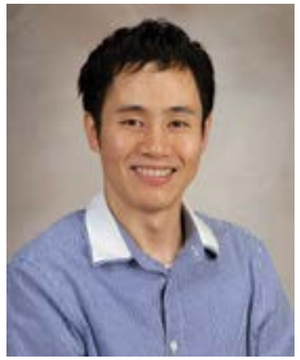
Carmon, K.S., Gong, X., Yi, J., Wu, L., Thomas, A., Moore, C.M., Masuho, I., Timson, D.J., Martemyanov, K.A., Liu, Q. J.: LGR5 receptor promotes cell-cell adhesion in stem cells and colon cancer cells via the IQGAP1 -Rac1 pathway. *J Biol Chem.* 292:14989-15001 (2017).

LAB MEMBERS

Post-doctoral Fellow: Soohyun Park, Jianghua Tu
Research Associates: Wangsheng Alice Yu, Ling Wu



Internalization and localization of anti-LGR4 antibody into ovarian cancer cells. LGR4 antibody (Red) is internalized into the lysosome (Green) in cancer cells.



Kyoji Tsuchikama, Ph.D.
Assistant Professor

Branched linker technology for constructing efficacious antibody-drug conjugates (ADCs) toward innovative cancer therapeutics

Antibody-Drug Conjugates (ADCs) represent a rapidly growing and extensively potent class of anticancer therapeutics. As demonstrated with the 4 FDA-approved ADCs (Adcetris®, Kadcyla®, Besponsa®, and Mylotarg®) and more than 60 promising ADCs in clinical trials (as of 2017), successful treatment outcomes using ADCs have inspired scientists and clinicians to further advance this new molecular platform for developing effective cancer therapeutics. ADCs deliver potent anticancer drugs selectively to tumors while avoiding healthy tissues, enabling the use of highly active drugs that have been too toxic to use for cancer treatment. An appropriate chemical linker between the antibody and the highly active drug provides a specific bridge, enabling selective delivery and precise release of the highly active drug only at the tumor sites. Thus, the use of proper ADC linkers is a key for successful implementation of ADC-based chemotherapy.

Our primary interest is in the development of novel chemical ADC linkers by taking advantage of the power of organic chemistry, medicinal chemistry, and chemical biology. Despite extensive efforts to improve ADC linker technology, most ADC linkers developed to date load only single cytotoxic drugs. The clinical potential of ADC linkers that can load two or more drugs remains unexplored because of the lack of efficient and versatile methods. Recently, we developed an efficient method using dual-loading ADC linkers enabling simple and easy installation of two molecules onto an antibody. We have revealed that our dual-loading ADCs are more effective in cancer cell killing tests than those constructed with traditional single-loading linkers. Based on this success, we are currently advancing the multi-loading strategy to produce next-generation ADCs for combating the cancer drug resistance and heterogeneity issues. The drug resistance and tumor heterogeneity are unsolved issues in cancer chemotherapy leading to discontinuation of medication and recurrence of malignancy. We envisage that our multi-loading ADC technology will provide an innovative approach for overcoming such unsolved issues and establish a novel ADC platform providing a number of valuable additions to the current list of drug candidates for the future clinical studies.

RESEARCH PROJECTS

- Design, synthesis, and evaluation of novel branched chemical linkers for constructing multi-loading antibody-drug conjugates (ADCs)
- Structural optimization of ADC linkers for high plasma stability and rapid drug release
- Quorum sensing-guided drug delivery

KEY PUBLICATIONS

Tsuchikama, K.*, An, Z. (2016) Antibody-drug conjugates: recent advances in conjugation and linker chemistries, *Protein & Cell*, DOI:10.1007/s13238-016-0323-0.

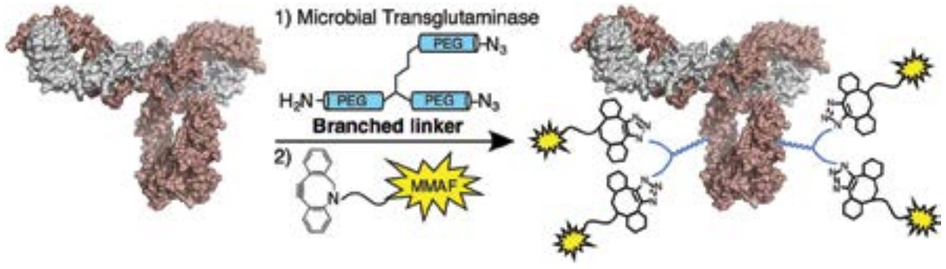
Tsuchikama, K.*, Shimamoto, Y., Anami, Y. (2017) Truncated Autoinducing Peptide Conjugates Selectively Recognize and Kill Staphylococcus aureus. *ACS Infectious Diseases,* 3(6), 406–410.

Anami, Y., Xiong, W., Gui, X., Deng, M., Zhang, C. C., Zhang, N., An, Z., Tsuchikama, K.* (2017) Enzymatic Conjugation Using Branched Linkers for Constructing Homogeneous Antibody–Drug Conjugates with High Potency. *Organic & Biomolecular Chemistry,* 15, 5635–5642.

LAB MEMBERS

Post-doctoral Fellows: Yasuaki Anami, Ph.D., Chisato Tsuchikama, Ph.D.

The Tsuchikama lab has an open position for Postdoctoral Fellow with experience in organic chemistry, medicinal chemistry, and/or pharmacology.



Enzymatic conjugation of a cancer-specific antibody, a branched linker developed in our group, and the highly cytotoxic drug MMAF. The resulting antibody-drug conjugate (ADC) shows enhanced cell killing potency against human cancer cells compared to ADCs constructed using traditional linear linkers.

Ningyan Zhang, Ph.D.
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 development of 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 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 using *in vitro* and *in vivo* models. The long-term goal of our research is to understand the role of antibodies in dynamic interaction between cancer cells and host immunity in the tumor microenvironment and to identify key molecular targets for development of effective anticancer immunotherapies.

RESEARCH PROJECTS

- Determine the role of proteolytic hinge cleavage of antibodies in cancer immune suppression.
- Understand mechanisms of cancer evasion of antibody immunotherapeutics.

KEY PUBLICATIONS

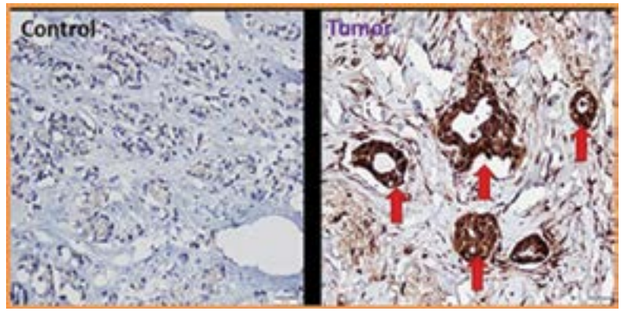
Sha Ha, Fengsheng Li, Matrhew C. Troutman, Daniel C. Freed, Aiming Tang, John W. Loughney, Dai Wang, I-Ming Wang, Josef Vlassak, David C. Nickle, Richard R. Rustaandi, Melissa Hamm, Pete A. Dephillips, Ningyan Zhang, Jason S. McLelian, Hua Zhu, Stuart P. Adler, Michael A. MaVoy, Zhiqiang An, Tong-Ming Fu (2017) Neutralization of diverse human cytomegalovirus strains conferred by antibodies targeting viral gH/gL/pUL128-131 pentameric complex. *Journal of Virolog*. Vol 91, e02033-16.

Anami, Yasuaki, Xiong, Wei, Gui, Xun, Deng, Mi, Zhang, Cheng, Zhang, Ningyan, An, Zhiqiang, Tsuchikama, Kyoji. (2017) Enzymatic Conjugation Using Branched Linkers for Constructing Homogeneous Antibody-Drug Conjugates with High Potency. *Organic & Biomolecular Chemistry.* DOI: 10.1039/c7ob01027c.

Weixu Meng, Aimin Tang, Xiaohua Ye, Xun Gui, Leike Li, Xuejun Fan, Robbie D. Schultz, Daniel Freed, Sha Ha, Dr. Dai Wang, Ningyan Zhang, Tong-Ming Fu, Zhiqiang An. (2017) Targeting Human Cytomegalovirus-Infected Cells by Redirecting T Cells Using an Anti-CD3/Anti-gB Bispecific Antibody. *Antimicrobial Agents and Chemotherapy.* doi:10.1128/AAC.01719-17

LAB MEMBERS

Research Associate/Scientists: Hui Deng, M.S., Xuejun Fan, M.D., Ph.D., Wei Xiong, Ph.D.
Post-doctoral Fellows: Yanhong Wang, Ph.D.



Detection of proteolytic hinge cleavage of antibodies in tumor tissues from breast cancer patients by immunohistochemistry method (IHC), see Clinical Cancer Research, 2015; doi: 10.1158/1078-0432. CCR-15-1057.

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 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 will 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

Current trends in biomedical research are increasingly focused on translational studies not only for the understanding of disease processes and therapies, but also for disease diagnosis and the evaluation of therapeutic efficacy. These studies often require extensive analyses of research and clinical specimens to identify steady or perturbation-induced proteome alterations associated with a disease status or biological state. Such proteome alterations may include changes in protein composition and expression, post-translational modifications (PTMs), protein functions, as well as protein interaction with proteins and other biomolecules. Our service center provides state-of-the-art proteomics services to the entire UT Health Science Center at Houston, Texas Medical Center research community, and other external organizations.

The basic services provided are designed to identify and quantitate proteins and their PTMs in a broad range of research specimens from purified protein samples to complex mixtures such as cell and tissue extracts, plasma/serum, and/or other biofluids. We also provide advanced supports, such as biomarker discovery and verification, development of targeted proteomics assays, and metaproteomics for microbiome profiling. The service center contains the cutting-edge instrumentation and trained personnel to provide an integrated proteomics analysis, including sample preparation, mass spectrometric analysis, and bioinformatics data processing.

Collaboration Imaging Service Center

The IMM Center for Molecular Imaging is a facility that all researchers at UTHealth who are or wish to be involved in small animal/ translational imaging studies should be acquainted with. The center is directed by Dr. Eva Sevick and led by seven engineering and basic science faculty members whose research focuses on different aspects of molecular imaging, including new instrumentation, design, and chemistry of targeted probes, innovative algorithms, and pioneering translation of new imaging technologies into clinical trials.

The newly formed Molecular Imaging "collaboration" center utilizes this existing expertise to interact with clinicians, clinician-scientists, as well as academic and industry researchers across the nation on translational projects in cancer, drug discovery, autoimmune disorders, gastrointestinal disorders, nanotechnology, chronic wound care, peripheral vascular disease, and others. Facilities include a Siemens hybrid PET/CT small animal scanner with custom fluorescence tomography capabilities and an array of custom bioluminescence and fluorescence instrumentation that is paired with unique imaging agents/gene reporter systems. Generalized protocols are available to investigators to maximize benefit from the latest developments in molecular imaging.

Flow Cytometry Service Center

Flow cytometry is a technique used to analyze the characteristics of cells 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 then be measured to determine properties of individual cells such as relative size, complexity, and cell type.

Thousands of cells can be analyzed per second as they pass through liquid in front of the lasers. These instruments allow scientists to evaluate a large number of samples in a short timeframe and gather information on very rare populations of cells and additionally isolate cell populations to be sorted. The service center provides training, instrumentation, and technical expertise for both analysis and cell sorting. These instruments are available on a fee-for-services charge to all research investigators from UTHealth and external organizations.

Transgenic and Stem Cell Service Center

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

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

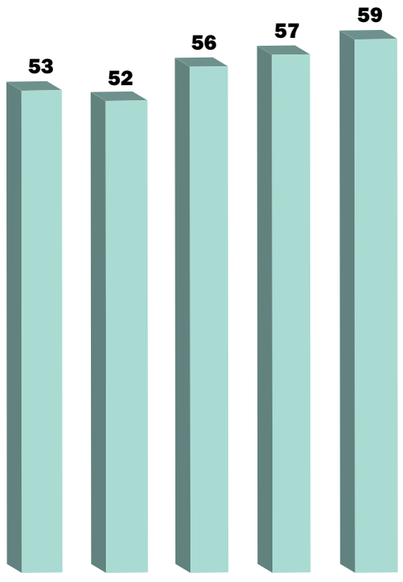
Nanochemistry Service Center

The Nanochemistry Service Center was established in 2012 and is located on the third and fourth floors of the Fayez S. Sarofim Research Building.

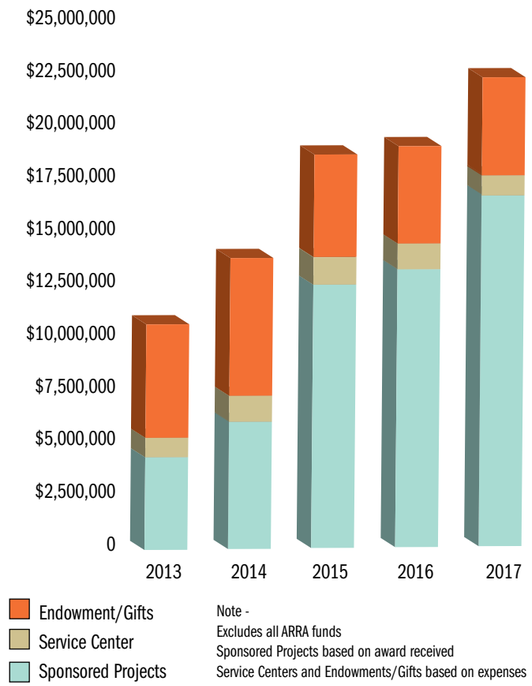
It specializes in the discovery, synthesis, and purification of DNA aptamers, X-Aptamers, and RNA for specific targeting of proteins for the delivery of chemotherapeutic agents or the down-regulation of the protein.

While most of our projects target cancers, such as ovarian, breast, and pancreatic cancers, we also develop aptamers for the modulation of other diseases. The center also offers nanoparticle production, and chemical conjugation services. Recently, we have added two large-scale, high-resolution (14 microns) 3D printers capable of producing both prototype models and final products for both industry and academics. The 3D printers can use standard STL files and medical imaging files such as MRI data.

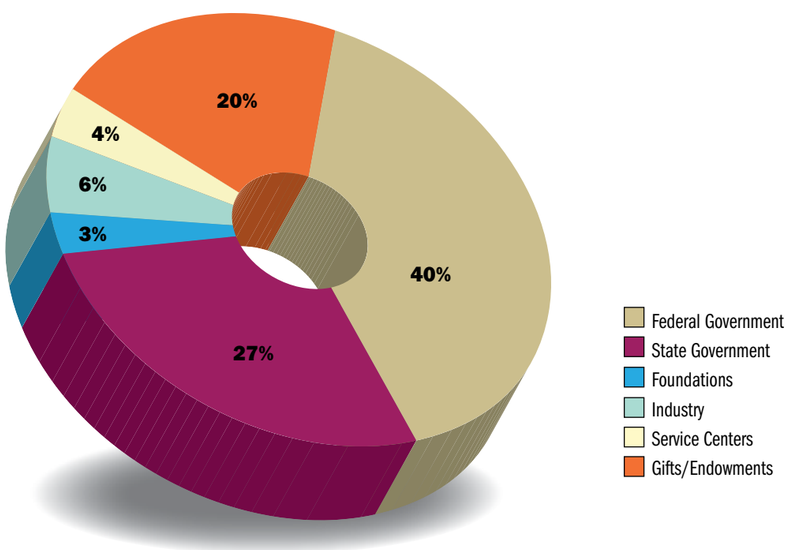
Number of Faculty



Total Funds Supporting Research



Total Expenses Supporting Research



Gift Report

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