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

IMMpact Report

FISCAL YEAR 2015

About the cover

McGovern Medical School's Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases is led by Dr. John Hancock, center.

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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
John Evans
IMM Faculty

Design:
Roy Prichard

Photography:
Dwight Andrews
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Richard Payne, FAIA, ©2006

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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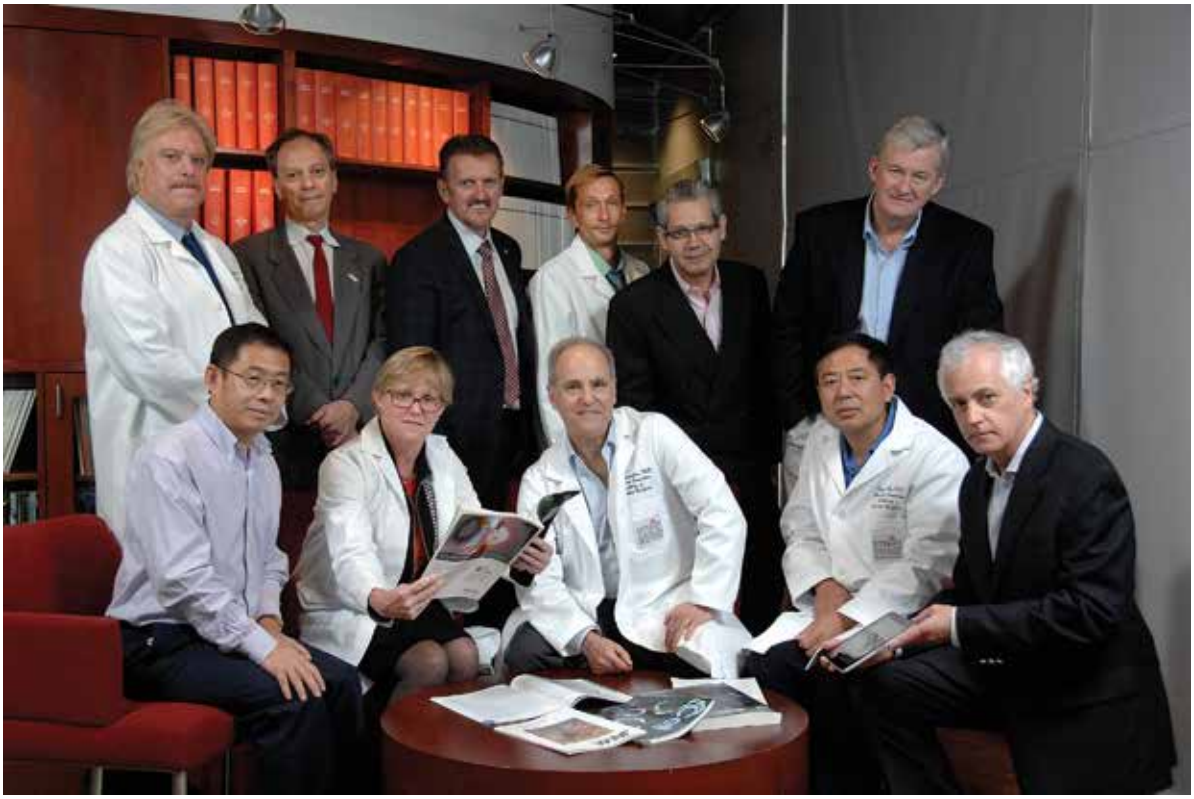

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Director's Message

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 am pleased to introduce our annual IMMpact report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). Inside you will find in-depth feature articles on recent developments and an account from every IMM faculty member describing the innovative research in which they are engaged. I trust that you will find the report interesting and informative.

The IMM is a stand-alone research institute that is embedded within McGovern Medical School. Our 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. The centers for metabolic and degenerative diseases, molecular imaging, and two of our flagship programs in regenerative medicine and drug development, provide excellent examples of these collaborative teams.

This year has seen the successful recruitment of new faculty to IMM, who bring with them with exciting and innovative research programs. This includes the recruitment of Dr. Johnny Huard and his near complete research team from the University of Pittsburg. Dr. Huard will be leading the newly created IMM Center for Tissue Engineering and Aging Research, details of which are described in the report.

I am also pleased to note that despite a persistent challenging environment for scientific research funding, especially from the NIH, IMM faculty continue to be extremely successful. Over the financial year just ended, our new grants and contracts were substantially up again over the preceding year. It is a remarkable testament to the quality and creativity of our scientists that the IMM faculty remains so successful in attracting research funds from what is an ever-diminishing pool. That said, full implementation of our mission remains heavily dependent on attracting support from alternative sources, including research charities, industry collaborations, and, most importantly, the continuing generosity of our friends and donors.

In addition to advancing science and medicine, we therefore wish to further 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, under the leadership of Mr. Dudley Oldham, which plays a key role in the continued growth and development of the IMM.

If you would like to investigate how you can be involved, we would be delighted to talk with you personally, so please feel free to contact us here at the IMM. Also we would be delighted to see you at the annual IMM symposium, which will be held on April 20, 2016. Please mark this date in your calendar because you will hear exciting research stories directly from our faculty and have the opportunity to meet with them and discuss their science and its implications for the future of medicine and health care.

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 Institute of
Molecular Medicine for the
Prevention of Human Diseases

IMMpact Symposium

Wednesday
April 20, 2016
4-6 p.m.

Fayez S. Sarofim Research Building
1825 Pressler Street

Stress and Alzheimer's

Nicholas Justice, Ph.D.
Assistant Professor, Center for Metabolic and Degenerative Diseases

Treating Macular Degeneration

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

Reversing Aging

Johnny Huard, Ph.D.
Distinguished Wallace Professor & Vice Chair for Research
Director, Center for Tissue Engineering and Aging Research

Save the Date

The basic science behind aging

Anti-aging is big business. Just walk into your favorite department store to see the various promises of youth packaged into jars and vials, displayed under glass.

While these products may make claims to turn back the clock, IMM scientists are seeking answers about how we age not to stop time but to help us to maintain our health and strength as we grow older.

Johnny Huard, Ph.D., joined the IMM May 1, 2015, from the University of Pittsburgh, as the director of a new IMM center dedicated to this area of research.

An internationally renowned scientist, Dr. Huard held professor appointments in orthopaedic surgery, microbiology and molecular genetics, bioengineering, pathology, pediatrics, and physical medicine and rehabilitation. Additionally, Dr. Huard served as the vice chair for Musculoskeletal Cellular Therapeutics, Department of Orthopaedics, at Pittsburgh, and was deputy director for Cellular Therapeutic Research at the McGowan Institute for Regenerative Medicine. He is co-founder of Cook MyoSite, Inc., a biotechnology company, and is the chief scientific officer of the Steadman Philippon Research Institute.

At McGovern Medical School, the most immediate goal of the Center for Tissue Engineering and Aging Research, Dr. Huard says, is to establish collaborations with those already working in the field at the university.

"It would be great to combine all of our aging resources together," Dr. Huard says. "I've talked to Dr. Carmel Dyer (director of the Division of Geriatric and Palliative Medicine) who tells me they do not have a lot of basic science work, which is something this center could provide."

The center's scientists seek to answer basic questions, such as: How do we age? What can we do to delay aging?

"As we age, our stem cells become tired and defective, and not as good," explains Dr. Huard, who also is a professor and the vice chair for research in the Department of Orthopaedic Surgery. "We also want to understand the impact of our internal clocks. When we are born, is our clock set at 12 a.m.? When we are 50, is the clock at noon?"

To understand why our stem cells change during the aging process, Dr. Huard and his team use mice who age prematurely. These genetically modified mice have progeria, which causes them to die at 21 days instead of in 2 to 3 years. The progeria mice's stem cells do not proliferate and are limited in number and differentiation compared to a young mouse.

"By injecting young stem cells into these prematurely aging mice, we can increase their lifespan by three times," Dr. Huard says.

The goal for humans, Dr. Huard says, is to collect stem cells at birth that can be used later in life, when we are injured or when we age.

"Think of this as the opposite of kidney dialysis," he explains. "When you have kidney problems and are on dialysis, you are hooked up to a machine that cleans your blood, then replaces it. We see a future where to improve aging you would be hooked up to a machine with your young stem cells, putting the youthful stuff back in."

Beyond collaborators at UTHealth, Dr. Huard and his team work with colleagues at Baylor College of Medicine, Rice University, and A&M University to uncover the basic science behind aging, which could result in retained muscle mass, stronger bones, and sharper mental capabilities.

"Science is a team effort, he says. "Cell, molecular, tissue, gene therapy – this is where we are heading."

> "We see a future where to improve aging you would be hooked up to a machine with your young stem cells, putting the youthful stuff back in."
>
> — Dr. Johnny Huard

Dr. Johnny Huard is working to help us maintain health and strength as we age.

CPRIT funds "rising star" stem cell biologist

With the aid of a program to advance cancer research in the Lone Star State, the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases recently recruited a new researcher who is tackling cancer at the molecular level.

McGovern Medical School was awarded $3.7 million from the Cancer Prevention & Research Institute of Texas (CPRIT) to recruit stem cell investigator Wa Xian, Ph.D. Dr. Xian has developed transformative technologies for the cloning of stem cells from normal, regenerative tissues such as those of the gastrointestinal tract, and has now adapted these technologies for cloning stem cells from the most lethal human cancers.

Dr. Xian comes to McGovern Medical School from the University of Connecticut School of Medicine, where she was a member of its Stem Cell Institute. She is also the co-founder of MultiClonal Therapeutics Inc., an integrated platform of patient-derived stem cells for drug discovery for chronic diseases.

"The CPRIT program has been tremendously important to help us recruit some of the most outstanding young scientists in cancer research who will help us to continue to build excellence in this and related research areas in the future," says George Stancel, Ph.D., executive vice president for academic and research affairs and holder of the Roger J. Bulger, M.D., Distinguished Professorship.

"These are the type of young investigators who will develop into our future institutional leaders in this area and the CPRIT support is critically important to help us recruit them to UTHealth," he adds.

"Dr. Xian brings cutting-edge approaches to tissue-specific stem cells that can be used to develop cell therapies and further the understanding of disease," says Brian Davis, Ph.D., director of the IMM's Center for Stem Cell & Regenerative Medicine.

Dr. Xian received her Ph.D. in molecular genetics from The University of Texas MD Anderson Cancer Center. She completed postdoctoral training as a research associate at Harvard Medical School and as a postdoctoral fellow at Baylor College of Medicine.

This calendar year, the Texas Cancer Registry estimates that 109,053 people in Texas will be newly diagnosed with cancer and that 42,255 will succumb to the disease.

While oncologists can kill the vast majority of ovarian cancer cells with chemotherapy, a tiny number may be resistant to treatment and could cause a recurrence, Dr. Xian says.

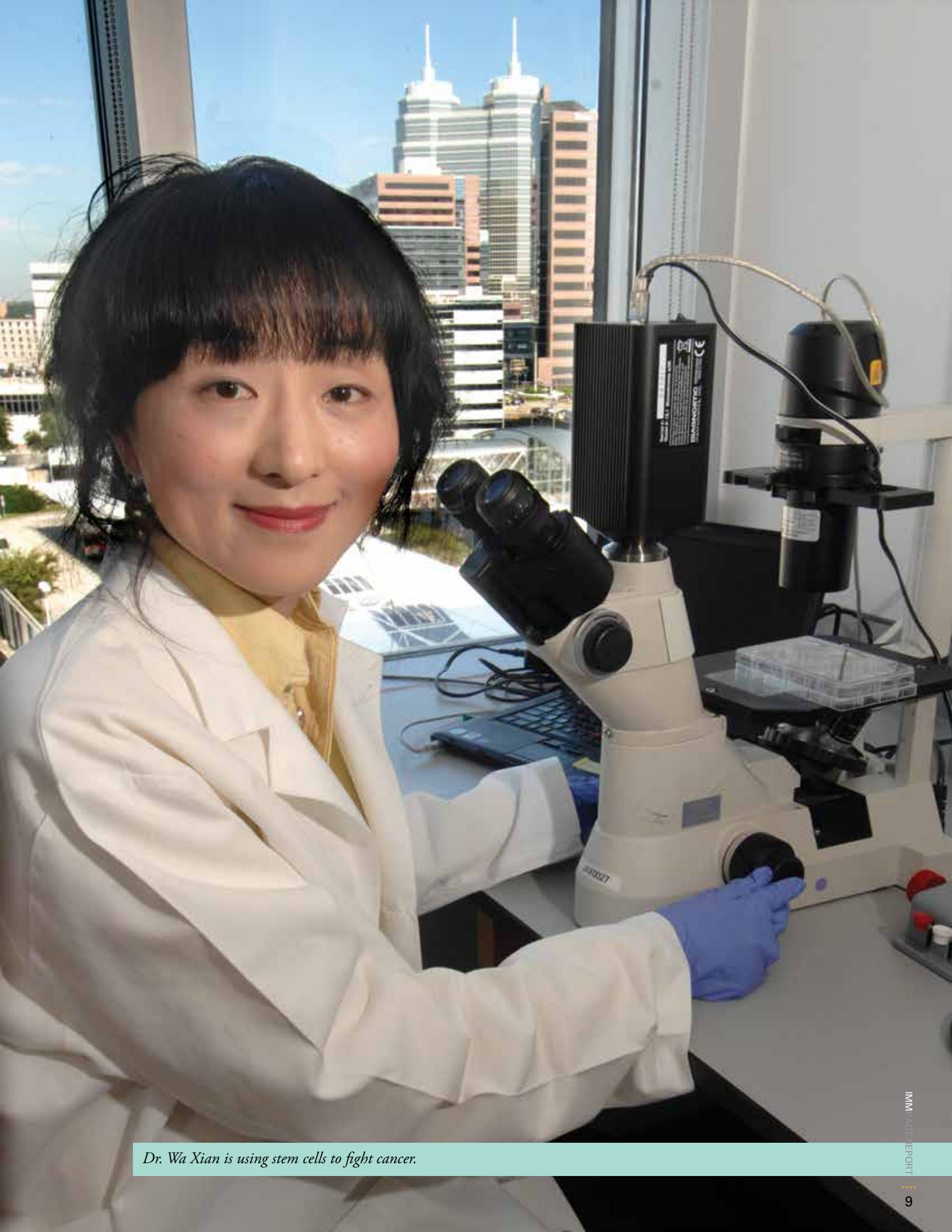
"In my lab, we are generating patient-specific libraries of cancer stem cells to identify and target a particularly nasty subset that survives chemotherapy and comes back as a repeat disease," says Dr. Xian, assistant professor at McGovern Medical School.

Dr. Xian anticipates a time in which patient-specific cancer stem cell libraries will be standard-of-care to assist oncologists to optimize personalized treatments for cancer patients.

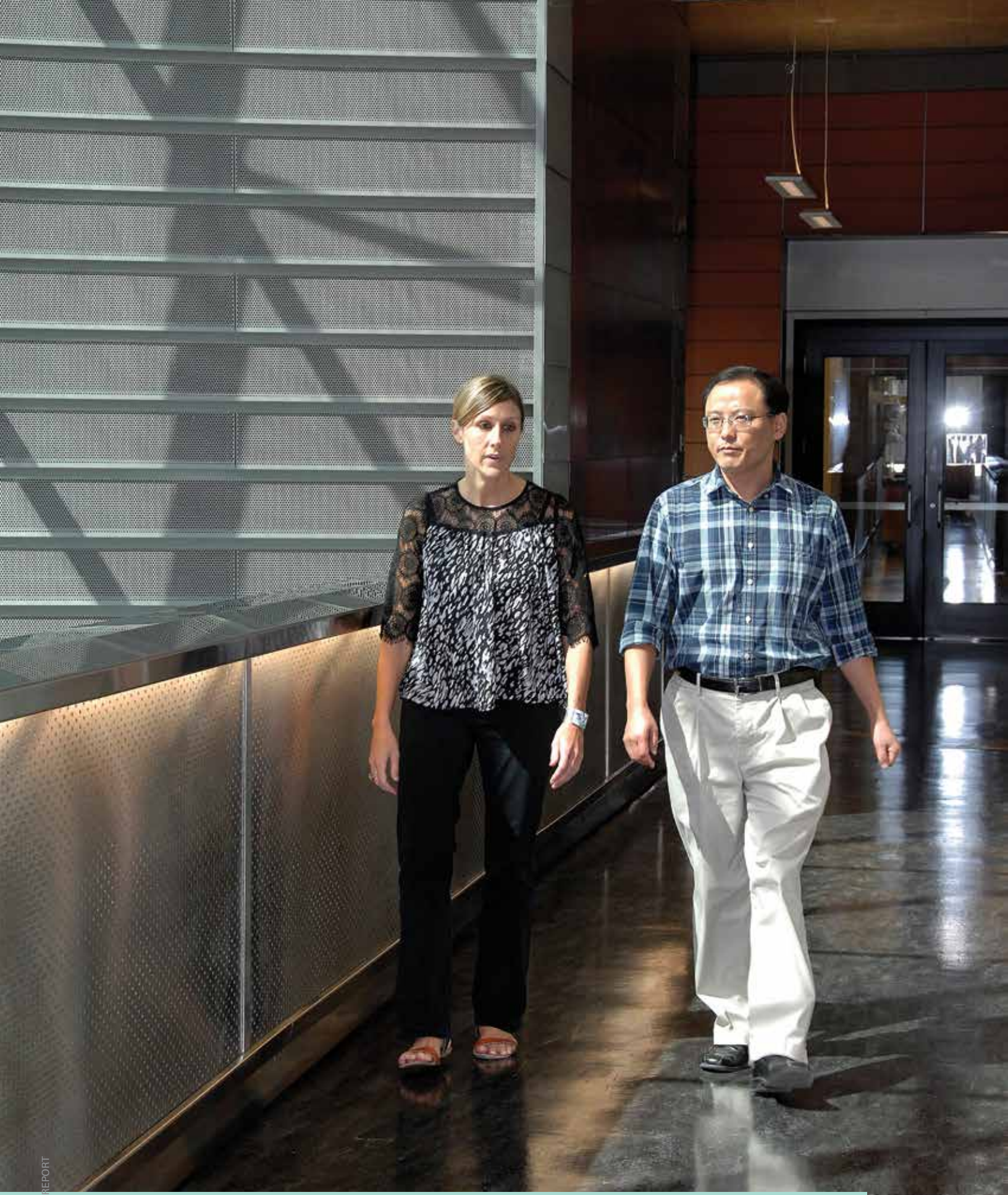
CPRIT was established by Texas voters in 2007, authorizing the state to issue $3 billion in bonds to fund groundbreaking cancer research and prevention programs and services in Texas. Beginning operations in 2009, CPRIT has to date awarded $1.35 billion in grants to Texas researchers, institutions and organizations.

CPRIT provides funding through its academic research, prevention and product development research programs.

> "Dr. Xian brings cutting-edge approaches to tissue-specific stem cells that can be used to develop cell therapies and further the understanding of disease."
>
> — Dr. Brian Davis



Dr. Wa Xian is using stem cells to fight cancer.



Dr. Kristin Eckel-Mahan and Dr. Kai Sun are the newest members of the Center for Metabolic and Degenerative Diseases.

Two investigators join Center for Metabolic and Degenerative Diseases

Charged with understanding metabolic health and disease, the IMM's Center for Metabolic and Degenerative Diseases welcomes two new faculty members. These investigators stood out as uniquely qualified from nearly 100 candidates.

"It was an interesting challenge identifying those applicants whose portfolio would resonate with every principal investigator," says Center for Metabolic and Degenerative Diseases Director Mikhail Kolonin, Ph.D.

The new talented faculty are Kai Sun, M.D., Ph.D., whose research interests are in obesity and diabetes, and Kristin Eckel-Mahan, Ph.D., who studies the role of the body's circadian clock on health.

"Not only have they been trained by top laboratories and published in first-tier journals, they also engaged every current faculty member as potential collaborators that could bridge the distinct scientific interests of individual groups composing the center," explains Dr. Kolonin, the Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research.

Dr. Sun, who joined the IMM in March 2015 from the University of Texas Southwestern in Dallas as an assistant professor, says the collaborative atmosphere has been key.

"I have received critical input and support for hiring and lab organization," he says, adding that the available research equipment helps him work efficiently – without delay of sending samples to another source and waiting on results.

Dr. Sun's research focuses on the relationship between fat tissue pathological remodeling and type 2 diabetes. He tries to identify critical factors with clinical significance in the pathophysiology of human obesity and insulin resistance.

"Inflammation and fibrosis are hallmarks of unhealthy fat," he explains, adding that white fat is his research focus.

A graduate of University of California, Riverside, Dr. Sun has discovered a molecule called endotrophin that accelerates the fibrosis and inflammation in obese fat tissue.

"In my lab we want to discover when and how this molecule is created – could it be a biomarker for obesity? Could it be targeted for treatment of obesity and type 2 diabetes?" Dr. Sun asks.

Joining the IMM in May 2015, Dr. Eckel-Mahan received her Ph.D. from the University of Washington and continued her training at the University of California, Irvine. Her research focus is the role of the 24-hour circadian clock in health and disease.

We have clocks everywhere, Dr. Eckel-Mahan explains. The "master" brain clock and the peripheral tissue clocks are located throughout the body.

The brain clock responds primarily to the 24-hour light and dark cycle induced by the earth's rotation on its axis. Other cues, such as food, strongly influence the body's peripheral clocks.

"I study the clock from the perspective that a stricter adherence to our internal 24-hour biological clock is important for metabolic health," she explains.

Many scientific studies point to health issues resulting from disruption of the circadian clock – insulin resistance, obesity, diabetes, and even cancer.

"One goal of an organism, metabolically speaking, is for its clocks to be in synchrony," she says.

Dr. Eckel-Mahan started her focus on circadian clocks in graduate school.

"I saw it mattered when we were testing animals – what time of day it was. We also discovered that feeding an animal a high fat diet dramatically reprograms the liver clock," she adds.

One area of her studies is night eating syndrome, which is when someone eats 25 percent or more of their calories during a typical rest period, after their evening meal.

Dr. Kolonin says the addition of these new recruits will help bridge the center's research efforts.

"Both the established and the new investigators are excited about the clear cohesion of our efforts that will synergize in the individual strides of every group toward important discoveries," he says.

Brandishing math, physics in the war against cancer

What do cancer tumors have to do with physics?

That's what one outraged audience member once asked Vittorio Cristini, Ph.D., after he delivered a lecture on the topic at a prestigious cancer institute.

"I was so thrilled to be there. It was very early in my career, and this guy stood up and said I was crazy. That physics has nothing to do with cancer," he recalls with a smile.

"So I ask him – when he is removing a cancerous tumor from a patient during an operation, what happens if he takes that tumor in his hand and then releases it. He said, 'It falls to the ground, of course.'

"Well, then, it seems that cancer indeed obeys the laws of physics, I suggested."

Dr. Cristini, professor in the Center for Proteomics and Systems Biology, is one of the pioneers in this innovative field of research, which applies the laws of physics with mathematical models to cancerous tissues.

Named as one of the World's Most Influential Scientific Minds in 2014 by Thomson Reuters, Dr. Cristini is a Yale-trained chemical engineer, who started his career studying complex materials and fluids.

"I was doing research in my engineering field at the University of Minnesota when by chance I became friends with a pharmacologist," Dr. Cristini recalls. "He showed me the morphology of cancerous tumors in mice, and I noticed similarities with what I was studying. The physical laws governing how tumors infiltrate were similar to the physical properties of the complex materials and fluids, such as governing the growth of crystals."

That was the lightbulb moment that took Dr. Cristini down a new research path – applying physics to cancer.

"I am interested in the physics of cancer and the physics of tissue in general," he says. "Cancer is as much of a physics problem as a biological problem."

Dr. Cristini and his colleagues study how cancerous tumors are transported through tissue and how they respond to pharmaceuticals.

"We are looking at the transport properties and finding how they correlate with clinical outcomes by developing mathematical models in cancer patients – both prospective and retrospective trials," he says. "What we are finding are novel physics-derived cancer biomarkers: usually, when the transport properties are poor, the outcomes are poor."

Vice chair of the Department of Nanomedicine and Biomedical Engineering, a professor of the Department of Imaging Physics at The University of Texas MD Anderson Cancer Center, and on the faculty of the Graduate School of Biomedical Sciences, Dr. Cristini came to McGovern Medical School from the University of New Mexico, where he held an endowed professorship and was the director of computational biology.

"We have an extensive network of collaboration in this field, and many of the researchers are right here in Houston," he adds.

The investigators are focused now on confirming their theory on how individual patients respond to cancer drugs, based on tissue-specific mathematical formulas.

"The math models are based on the unique tissue properties of that patient, and blood perfusion plays an important role," Dr. Cristini explains.

The goal is to create a cancer treatment that is optimized for a specific patient.

"We have identified different regions in the same tumor that respond differently to the same medication. These differences correspond to transport properties," Dr. Cristini explains. "Biology is the underlying cause of different physical properties. But if we can directly manage the physics, we can change the outcome and the biology. The laws of physics are universal."

Dr. Vittorio Cristini applies the laws of physics to cancerous tissues.

Dr. Irma Gigli, second from left, helps cuts the ribbon during the IMM's dedication ceremony in 2006.

IMM pioneer creates a legacy of support

One of the founding leaders of the Institute for Molecular Medicine is also one of its strongest supporters, who has overcome barriers to establish herself as a pioneer in the field of translational medicine.

The innovative spirit of Professor Emerita Irma Gigli, M.D., led to the creation of the first program in immunodermatology sponsored by the National Institutes of Health – a historic feat in a male-dominated environment.

"There were institutions where at the time you could count the number of women on one hand," she says.

Growing up in Argentina during the 1940s, Dr. Gigli was determined to become a physician. After graduating from medical school, she traveled to the United States to pursue her joint research interests in dermatology and immunology. Her career took off, rising from her first medical residency at Cook County Hospital in Chicago to the Howard Hughes Medical Institute in Miami, and then on the faculty of Harvard Medical School.

Dr. Gigli went on to become the director of her own laboratory, chair of the Department of Dermatology at the University of California at San Diego, and elected to the prestigious Institute of Medicine of the National Academy of Sciences and the American Academy of Arts and Sciences.

One of the early physician-scientists to bridge basic immunology with clinical dermatology, she has published over 160 original manuscripts and book chapters, many of which identify basic mechanisms involved in host defense and in the development of skin diseases.

It was in 1995, when she and her late husband Professor Hans J. Muller-Eberhard were recruited by Dr. James T. Willerson to start the IMM.

Dr. Gigli says the IMM's direction has always been clear. "We were trying to put together areas that had connections in medical biology to understand the basics of disease," she says. "In many ways, I am romantic about science – you do science because you love science."

Dr. Gigli's dedication to science and the founding principles of the IMM have been unwavering.

When her husband passed away in 1998, Dr. Gigli, director emerita of the IMM Center for Immunology and Autoimmune Diseases, created The Hans J. Muller-Eberhard Chair in Immunology at the IMM in his memory to advance the research to which he dedicated his life.

She recently infused the Muller-Eberhard Memorial Lecture Series with an additional $50,000, which was matched by the UTHealth Game Changers Initiative.

Dr. Gigli says her late husband would be happy with the progress the IMM has achieved over these decades.

Dr. Gigli also recently made the extraordinary decision to leave an estate commitment of $2 million to establish The Luis Gigli and Irma Gigli, MD Endowment in Immunology at the IMM.

The endowment is funded by money Dr. Gigli and her brother inherited from their parents.

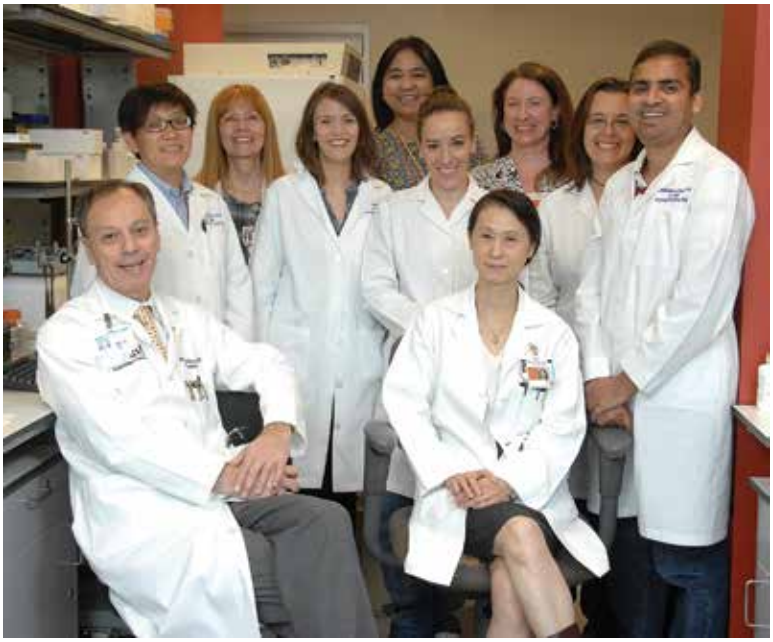
"My father was a firm believer in philanthropy and in education because he was an orphan at age 7 and did not have the opportunity of higher education," she explains, adding that although her father, who died at age 59, provided well for the family, he did not provide them with luxury. Dr. Gigli's brother, Luis, died suddenly from a stroke in 2014.

"I wanted to do something specific for students that has a relationship to my family," she says. "Supporting young scientists is extremely important, and immunology is what both my husband and I had worked on our whole scientific life."

Her generosity will make a difference to the IMM.

"It is so meaningful to have one of the IMM's founding leaders give back in such a way," says Dr. John F. Hancock, executive director of the IMM. "These generous gifts help put us on the level of much larger institutions in terms of resources for faculty and student recruitment."

CENTER FOR **CARDIOVASCULAR GENETICS**



The IMM Center for Cardiovascular Genetics focuses on elucidation of molecular genetics and pathogenesis of cardiovascular diseases in humans. Located on the ninth floor of the Denton A. Cooley Building at the Texas Heart Institute at St. Luke's Health, the center provides specialized clinical services to patients with genetic cardiovascular disorders through the Cardiovascular Genetic Clinic at the Texas Heart Institute Outpatient Clinic. The Center also has a Research Clinic, which is utilized for clinical research activities.

Our mission is to prevent cardiovascular diseases in humans through elucidating their molecular genetic causes. Genetic studies afford the opportunity to prevent the disease prior to the development of clinical manifestations of the disease. Delineation of the molecular pathways that link the mutations to the phenotype enables interventions to reverse or attenuate the evolving phenotype in those who already have developed the disease.

The research programs at the Center entail human molecular genetic studies through recruitment of the probands and family members, phenotypic characterization and molecular genetic studies. The main objective of these activities is to identify the causal genes for hereditary cardiovascular diseases, primarily cardiomyopathies. Genetic discoveries are complemented with genomic analysis of cardiac tissue to determine changes in the regulation of gene expression. The findings are then applied in cell culture systems and animal models in order to delineate the molecular links between the causal variants and the phenotype. Upon elucidation of the molecular links between the genetic mutations and the clinical phenotypes, genetic and pharmacological interventions are pursued to block the linking pathways in order to prevent and reverse the phenotype. The initial intervention studies are pursued in cell and animal models and then extended to humans through pilot randomized placebo-control trials.

Specific themes of research include:

I. Human molecular genetics/genomics Studies: These studies are designed to delineate the molecular genetic and genomic basis of cardiovascular diseases in humans with a specific focus on three most common forms of hereditary cardiomyopathies; namely Hypertrophic Cardiomyopathy, Dilated Cardiomyopathy, and Arrhythmogenic Cardiomyopathy.

II. Functional characterization of the genetic variants identified in humans with cardiovascular diseases: These studies are conducted in cardiac myocytes through gene transfer studies and in genetically engineered animal models

III. Experimental Therapies: Upon delineation of the molecular mechanisms specific pathways that are responsible for the induction of the phenotype are pharmacologically and genetically targeted in myocytes through in vitro and in vivo studies. Likewise, gene therapy approaches are used to correct the deficiency of the proteins in the heart.

IV. Clinical Studies: The discoveries at the bench are extended to human patients to test the beneficial effects of experimental therapies in human patients with hereditary cardiovascular diseases. Genetic information garnered through the above studies are applied to the practice of cardiovascular medicine to guide appropriate medical interventions.

AJ Marian, M.D.
Center Director & Professor
George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research

AJ Marian, M.D.
Professor and Director of the Center for Cardiovascular Genetics
George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research

Molecular genetics and pathogenesis of hereditary cardiomyopathies

Our long-standing research objectives have been to delineate the molecular genetics 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 (AC): AC 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 AC.

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 genomic DNA sequencing to identify the causal genes and mutations, genomic characterization to define epigenetic regulation of gene expression, and transcriptomic analysis to link the epigenetic changes to function. Genetic and genomic discoveries are then pursued through molecular mechanistic studies, including in genetically modified animal models and cultured cells to identify the mechanisms that link the causal mutations to the disease phenotype. The mechanistic discoveries are complemented with preventive and therapeutic approach, utilizing genetic and pharmacological approaches that target the pathogenic pathways. These studies are initially pursued in the animal models and subsequently, in humans. The latter is tested through randomized placebo-controlled pilot clinical trials to set the stage for 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)
- LIBERTY-HCM: An industry –sponsored clinical trial to improve symptoms and exercise tolerance in patients with hypertrophic cardiomyopathy

KEY PUBLICATIONS

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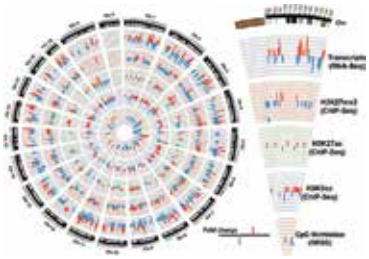
LAB MEMBERS

Post-doctoral Fellows: Gaelle Auguste, Ph.D., Gaelle Auguste, Ph.D., Jennifer Karmouch, Ph.D.
Research Associate: Grace Czernuszewicz, M.S.
Faculty – Assistant Professors: Priyatansh Gurha, Ph.D., Raffaella Lombardi, M.D., Ph.D.



Integrated genomic analysis in a mouse model of dilated cardiomyopathy: Genomic data includes genome-wide CpG methylation, H3K9ac, H3K27ac, and H3K27me3 histone modifications, along with transcriptomic data assigned to the mouse chromosomes.

Priyatansh Gurha, Ph.D.
Assistant Professor

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

The main focus of my research is to understand the molecular basis of heart failure. The objective of this research is to identify and modulate gene regulatory networks that lead to heart failure and to target these to alleviate disease symptoms and progression of heart failure. Current studies have highlighted the role of non-coding RNAs in regulation of gene expression and heart failure. Recently, I identified miR-22 (one of the most abundant miRNA in the heart) as a key regulator of cardiac hypertrophy and fibrosis. Furthermore using global gene expression analysis and biochemical approaches we identified novel targets of miR-22 with potential pathogenic role in stress induced cardiac hypertrophy and fibrosis.

Working on similar lines we also characterized the role of differentially expressed microRNAs (miRNAs) in Arrhythmogenic Cardiomyopathy (AC). AC is a primary disease of the myocardium that clinically manifests with cardiac arrhythmias, heart failure, sudden death and pathologically by a gradual replacement of myocytes by fibro-adipocytes. Using whole transcriptome RNA-seq we initially identified the pathogenic role of gene networks/ pathways namely canonical Wnt and Hippo in AC. Next by integrating RNA-seq data with miRNA expression data we identified miR-184 as the most down-regulated miRNA (~10-fold) in AC. Given that AC represents a unique phenotype of excess myocardial adipocytes, we characterized the role of miR-184 in adipogenesis in AC. We demonstrate that reduced levels of miR-184 induces adipogenesis while enhanced levels of miR-184 partially mitigates adipogenesis. Based on these and other findings we establish the role of miRNA-184 in the pathogenesis of AC.

Using wide array of genomic approach we are now investigating the role of other miRNAs and long non-coding RNAs in AC and other forms of heart failure.

RESEARCH PROJECTS

- Role of miRNAs in the pathogenesis of AC
- Identification and characterization of long non-coding RNAs and molecular mechanisms/ function in cardiomyopathies and heart failure.

KEY PUBLICATIONS

Gurha P, Abreu-Goodger C, Wang T, Ramirez MO, Drumond AL, van Dongen S, Chen Y, Bartonicek N, Enright AJ, Lee B, Kelm RJ Jr, Reddy AK, Taffet GE, Bradley A, Wehrens XH, Entman ML, Rodriguez A. Targeted deletion of microRNA-22 promotes stress-induced cardiac dilation and contractile dysfunction. *Circulation*, 2012 Jun 5; 125(22): 2751-61, PMID: 22570371.

Suet Nee Chen*, Priyatansh Gurha*, Raffaella Lombardi*, Alessandra Ruggiero, James T. Willerson, A.J. Marian. The Hippo Pathway Is Activated And Is a Causal Mechanism For Adipogenesis in Arrhythmogenic Cardiomyopathy. *Authors contributed equally. *Circulation Research*, 2014 Jan 31; 114(3): 454-68. PMID: 24276085.

Gurha P, Wang T, Larimore AH, Sassi Y, Abreu-Goodger C, Ramirez MO, Reddy AK, Engelhardt S, Taffet GE, Wehrens XH, Entman ML, Rodriguez A. microRNA-22 promotes heart failure through coordinate suppression of PPAR/ERR-nuclear hormone receptor transcription. *PLoS One,* 2013 Sep 27; 8(9): e75882, PMID: 24086656.

Kalsotra A, Singh RK, Gurha P, Ward AJ, Creighton CJ, Cooper TA .The Mef2 transcription network is disrupted in myotonic dystrophy heart tissue, dramatically altering miRNA and mRNA expression. *Cell Reports*, 2014 Jan 30; 6(2): 336-45. PMID: 24412363.

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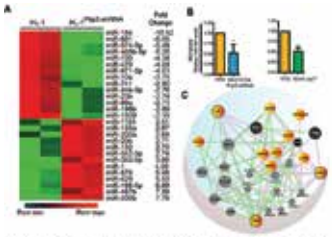
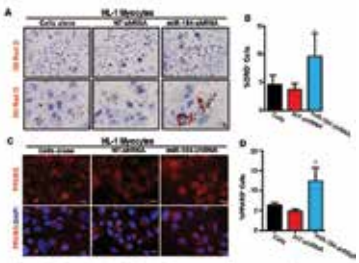


Differentially expressed miRNAs and miR-184 target gene network involved in adipogenesis.



Knock down of miR-184 enhances adipogenesis.

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

Molecular genetics and pathogenesis of hereditary cardiomyopathies

The focus of my research is the delineation of the molecular pathogenesis of genetic cardiomyopathies, which are important causes of sudden cardiac death in the young, with an emphasis on Arrhythmogenic Cardiomyopathy (AC), which is caused by mutations in desmosomal genes.

Although significant progress has been accomplished over the past two decades in revealing the causal genes, at the present time there is no effective pharmacological or non-pharmacological therapy for this disorder.

I have developed cell culture models as well as genetically modified animal models of AC and have utilized these models to understand the molecular links between the mutant desmosome proteins and cardiac fibro-adipogenesis, which is a unique and enigmatic phenotype of AC. The mechanistic discoveries in these models have been complemented with genetic and pharmacological intervention targeting the implicated pathways in order to prevent and reverse the phenotype. I have specifically focused on developing an independent research career on elucidating the molecular pathogenesis of the unique phenotype of fibro-fatty replacement of cardiac myocytes in AC and the cellular origin of excess adipocytes. I have implicated suppression of the canonical Wnt signaling, in part by nuclear relocalization of the desmosome junction protein plakoglobin (PG or JUP), in the pathogenesis of AC. Together with my colleagues at the Center for Cardiovascular Genetics, I have shown that mutations in the desmosome proteins affect the structure as well as the signaling functions of the intercalated disks (IDs), which result in activation of the Hippo cascade.

Activation of the Hippo kinase cascade suppresses the canonical Wnt signaling pathway and contributes to fibro-adipogenesis in AC. More recently, the focus of my studies has been the identification of the cellular origin of adipocytes in the heart of patients with AC. Through genetic fate-mapping experiments I have identified a subset of cardiac progenitor cells (CPC) from the second heart field as a cell source of adipocytes in AC. Furthermore I have identified molecular key pathways implicated in the differentiation of cardiac progenitor cells to adipocytes. Recently I have shown that a subset of cardiac cells expressing the platelet-derived growth factor receptor-alpha protein (PDGFRA+) express desmosome proteins and are able to differentiate to adipocytes; hence they represent an attractive novel candidate cell type that differentiate to adipocytes in AC (unpublished data).

These findings could lead to the development of new therapies aimed at preventing cardiac precursor cells from switching from a muscle cell fate to a fat cell fate.

For my studies on cardiomyopathies I was awarded with the "The 2008 Louis N. and Arnold M. Katz award" from the American Heart Association, which is the most prestigious award given to young investigators in the cardiovascular field.

RESEARCH PROJECTS

- Delineation of the signaling pathways involved in the pathogenesis of primary cardiomyopathies
- Identification and molecular characterization of cellular sources of fibro-adiposis in Arrhythmogenic Cardiomyopathy
- Molecular pathogenesis of cardiac involvement in laminopathies

KEY PUBLICATIONS

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)

Lombardi R. Genetics and Sudden Death. *Curr Opin Cardiol* 2013; 28 (3): 272-81.

Lombardi R, Marian AJ. Molecular genetics and pathogenesis of arrhythmogenic right ventricular cardiomyopathy: a disease of cardiac stem cells. *Pediatr Cardiol.* 2011 Mar; 32(3):360-5.

Lombardi R, da Graca Cabreira-Hansen M, Bell A, Fromm RR, Willerson JT, Marian AJ. Nuclear plakoglobin is essential for differentiation of cardiac progenitor cells to adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res.* 2011 Dec 9; 109(12):1342-53.

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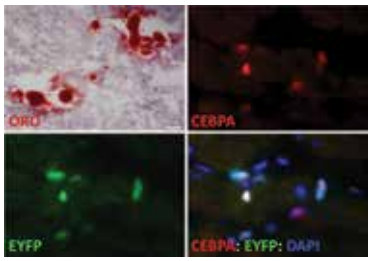
Lombardi R, Bell A, Senthil V, Sidhu J, Noseda M, Roberts R and Marian AJ. Differential interactions of thin filament proteins in two cardiac troponin T mouse models of hypertrophic and dilated cardiomyopathies. *Cardiovasc Res.* 2008; 79(1):109-17.

Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ. Resolution of Established Cardiac Hypertrophy and Fibrosis and Prevention of Heart Failure in the β-Myosin Heavy Chain-Q403 Transgenic Rabbits with N-Acetylcysteine. *Circulation* 2009 Mar 17; 119(10):1398-407.

LAB MEMBERS

Assistant Professor: Priyatansh Gurha, Ph.D.
Post-doctoral Fellows: Suet Nee Chen, Ph.D., Karmouch Jennifer, Ph.D., Auguste Gaelle, Ph.D.
Research Associate: Grazyna Z. Czernuszewicz, M.S.



Infiltration of fat tissue (ORO staining) in the heart of a mouse model of Arrhythmogenic Cardiomyopathy. In vivo labeling of adipocytes (identified by CEBPA, in red) with the fluorescent protein EYFP (in green) in the heart of the transgenic mouse.

Heart and kidney disease, high blood pressure and stroke are linked together and together have a larger impact on the health of our population than any other disease process. These are diseases that emerge in middle and later life and so are intricately linked to the normal processes of aging. Heredity impacts our risk of these diseases as well as shaping how we age. The work of our center targets the identification of genes that contribute to cardiovascular disease and how these interact with aging and how genetic influences arising through our life history can modulate disease risk.

An important emerging concept that is developing in our laboratories is that an important element of chronic disease of the cardiovascular system is that these diseases produce a chronic istate of inflammation. For example, the blood vessel wall in atherosclerosis is invaded by cells of our immune system and as injury to blood vessels progresses, so the genetic factors shaping our immune responses may modulate disease outcome. Understanding these processes of "sterile inflammation" to uncover what genes are involved and how genetic variation in them is a new challenge. This challenge is made more complex by the fact that the extent of genetic variation in our immune responses is very high. Our immune systems have been evolving in large part through competition with the pathogens in the world around us. As simpler organisms, these can evolve rapidly and one counter strategy embedded in our immune response is the preservation of genetic variation that enhances the adaptability of our immune response.

Progress in the laboratories of our investigators has provided important new understanding of susceptibility to atherosclerosis, coronary artery disease, progressive kidney disease, stroke and high blood pressure. We have a major new initiative to identify 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.

The advances we pursue will allow doctors to target treatments to the underlying cause of disease in each affected individual, not just the symptoms. It will allow us to see how different elements of lifestyle and environment shape the processing and expression of information contained within our genomes. Our work is focused on understanding how genes and disease shape our "healthspan" and how we can use such understanding to prevent disease and extend the healthspan.

Eric Boerwinkle, Ph.D.
Center Director & Professor
Kozmetsky Family Chair in Human Genetics

Eric Boerwinkle, Ph.D.
Professor and Director, IMM Center for Human Genetics
Kozmetsky Family Chair in Human Genetics
Dean, Professor and Chair, Department of Epidemiology, School of Public Health

I received my B.S. in Biology from the University of Cincinnati in 1980, an M.A. in Statistics (1984), and M.S. and Ph.D. in Human Genetics (1985) from the University of Michigan, Ann Arbor, where I served as senior research associate in the Department of Human Genetics from 1985-1986. I joined the University of Texas-Houston Center for Demographic/ Population Genetics in 1986 as a research assistant and became assistant professor in the same year. In 1991, I worked to move the renamed Human Genetics Center to the School of Public Health, University of Texas-Houston Health Science Center as associate professor. In 1996, I was promoted to professor, and in 1997, director of the Human Genetics Center. I became a faculty member of the Institute of Molecular Medicine in 1996 and became professor and director of the Research Center for Human Genetics. In 2011, I became the associate director of the Human Genome Sequencing Center at Baylor College of Medicine.

My research interests encompass the genetic analysis of common chronic diseases in humans, including coronary artery disease, hypertension, and Alzheimer's Disease. This work includes localizing genes, which contribute to disease risk, identification of potentially functional mutations within these genes, testing these candidate functional mutations in experimental systems, defining the impact of gene variation on the epidemiology of disease, and determining the extent to which these genes interact with environmental factors to contribute to disease risk. Activities include both statistical analysis and laboratory work. A large part of my current research effort consists of localizing genes contributing to disease risk using modern exome (the protein encoding part of the genome) and whole genome sequencing methods. Success depends on keeping up with the latest genomic technical advances. The laboratory is set up and operating as a high through-put sequencing and genotyping facility in which speed, accuracy, and efficiency are monitored continuously.

Genetic analysis of the common chronic diseases in humans, including coronary artery disease, hypertension, and Alzheimer's disease

We have completed the most comprehensive genome-wide analyses for a variety of heart disease-related risk factors, including blood pressure and cholesterol levels. These investigations have led to the identification of novel susceptibility genes in both cases. As sequencing the personal genomes of individuals has become more accessible and less expensive, our group has focused on the analysis of the vast information contained in the human genome. We are internationally recognized in the analysis of large-scale genomic data. Such "Big Data" problems are facilitated by growing our research program in a "Cloud."

Developing an internationally recognized team of investigators targeting the genetics of cardiovascular disease and its risk factors ensures a productive future and further discoveries.

RESEARCH PROJECTS

- Obtaining the DNA sequence of 100,000 individuals to study the determinants of health and disease

KEY PUBLICATIONS

Tennessen J.A., Bigham A.W., O'Connor T.D., Fu W., Kenny E.E., Gravel S., McGee S, Do R., Liu X., Jun G., Kang H.M., Jordan D, Leal S.M., Gabriel S., Rieder M.J., Abecasis G, Altshuler D., Nickerson D.A., Boerwinkle E., Sunyaev S., Bustamante C.D., Bamshad M.J., Akey J.M.; Broad G.O.; Seattle G.O.; on behalf of the NHLBI Exome Sequencing Project. (2012) Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science* 337(6090):64-9.

Fu W., O'Connor T.D., Jun G., Kang H.M., Abecasis G., Leal S.M., Gabriel S., Altshuler D., Shendure J., Nickerson D.A., Bamshad M.J.; NHLBI Exome Sequencing Project, Akey J.M. (2013) Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants *Nature* 493(7431):216-20.

Crosby J., Peloso G.M., Auer P.L., Crosslin D.R., Stitziel N.O., Lange L.A., Lu Y., Tang Z.Z., Zhang H., Hindy G., Masca N., Stirrups K., Kanoni S., Do R., Jun G., Hu Y., Kang H.M., Xue C., Goel A., Farrall M., Duga S., Merlini P.A., Asselta R., Girelli D., Olivieri O., Martinelli N., Yin W., Reilly D., Speliotes E., Fox C.S., Hveem K., Holmen O.L., Nikpay M., Farlow D.N., Assimes T.L., Franceschini N., Robinson J., North K.E., Martin L.W., DePristo M., Gupta N., Escher S.A., Jansson J.H., Van Zuydam N., Palmer C.N., Wareham N., Koch W., Meitinger T., Peters A., Lieb W., Erbel R., Konig I.R., Kruppa J., Degenhardt F., Gottesman O., Bottinger E.P., O'Donnell C.J., Psaty B.M., Ballantyne C.M., Abecasis G., Ordovas J.M., Melander O., Watkins H., Orho-Melander M., Ardissino D. Loos R.J., McPherson R., Willer C.J., Erdmann J., Hall A.S., Samani N.J., Deloukas P., Schunkert H., Wilson J.G., Kooperberg C., Rich S.S., Tracy R.P., Lin D.Y., Altshuler D., Gabriel S., Nickerson D.A., Jarvik G.P., Cupples L.A., Reiner A.P., Boerwinkle E., Kathiresan S. (2014) Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med* 371(1):22-31.

Li A.H., Morrison A.C., Kovar C., Cupples L.A., Brody J.A., Polfus L.M., Yu B., Metcalf G., Muzny D., Veeraraghavan N., Liu X., Lumley T., Mosley T.H., Gibbs R.A., Boerwinkle E. (2015) Analysis of loss-of-function variants and 20 risk factor phenotypes in 8,554 individuals identifies loci influencing chronic disease. *Nat Genet* 47(6):640-642.

Polfus L.M., Gibbs R.A., Boerwinkle E. (2015) Coronary heart disease and genetic variants with low phospholipase A2 activity. *N Engl J Med* 372(3):295-296.

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

Out-living our kidneys: Studies to understand the decline of kidney function as we age and its relationship to blood pressure and immune function

Our kidneys occupy a rough neighborhood in our bodies. Although they receive a sizeable fraction of the blood our hearts pump out, they are constantly on the verge of oxygen deficiency. Indeed, this characteristic has been adapted as a "feature" rather than a "bug" by evolution: it is our kidneys that are used as a monitor whether we need to increase the number of red cells in our blood. Our kidneys control the production of red cells by the bone marrow and determine the oxygen-carrying capacity of the blood. Since the kidneys are also our excretory organs, their neighborhood is made tougher by the continuous filtration and concentration of waste products that must be eliminated from our blood. It is not so surprising then, that as we age, our kidney function declines. As medicine advances and we live longer lives, a growing portion of society is outliving their kidneys and face long-term renal dialysis or transplant for survival. We seek to understand what causes progressive loss of renal function and how this process is amplified by high blood pressure.

The best predictor of whether an individual will lose enough kidney function to require dialysis is whether they have a first or second degree relative who has reached this "end stage" of renal disease. Familial aggregation indicates that inherited factors shape risk of renal disease. At present, the mechanism of renal functional decline is not known. Kidneys are difficult to study in humans because they lie deep within the body and their functional units, the glomeruli and nephrons, are microscopic structures. Increased blood pressure is a major risk factor that amplifies the age-related decline in renal function. We study this disease process in a rodent genetic model in which separate genetic factors influence both blood pressure and kidney disease. We are working to identify the genes involved and the pathways that are disturbed by effects arising in these genes.

Our recent work reveals that genetic variation in each of several immune system genes combine to play a key role in susceptibility to injury. Blood pressure elevation exceeds the ability of the kidney to match blood flow to the local needs for oxygen and nutrients in the kidney tissue. This triggers an inflammatory process in which immune responses are provoked by damage to the tissue. Genetic variation appears to determine whether the outcome is injury and repair or injury leading to sustained inflammation and further tissue damage. We can modify the course of disease by modifying the immune response. We now have identified a key set of genes containing variation that regulates the shape of the immune response. Our immediate goal is to understand which immune cells are expressing these genes and which aspects of their function are altered so as to create a self-damaging immune response. With this information in hand we will be able to seek and narrowly target therapeutic approaches that can sustain kidney function while allowing the normal protective functions of the immune system to be preserved.

RESEARCH PROJECTS

- Inherited susceptibility to renal and cardiovascular end organ disease
- Genetic mechanisms of elevated blood pressure
- Immune cell genetics and signaling

KEY PUBLICATIONS

Bell, R., S.M. Herring, N. Gokul, M. Monita, M.L. Grove, E. Boerwinkle, P.A. Doris. High resolution identity by descent mapping uncovers the genetic basis for blood pressure differences between SHR lines. *Circulation* (Cardiovascular Genetics). 4:223-31, 2011

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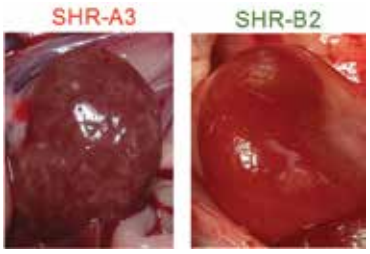
Braun, M.C. S.M. Herring, N. Gokul, M. Monita, R.J. Bell, S.E. Wenderfer and P.A. Doris. Hypertensive Renal Disease: Susceptibility And Resistance In Inbred Hypertensive Rat Lines. *J. Hypertension* 31:2050-2059, 2013

Gonzalez-Garay, M.L., S.M. Cranford, M.C. Braun, and P.A. Doris. Diversity in the pre-immune immunoglobulin repertoire of SHR lines susceptible and resistant to end organ injury, Genes and Immunity, 15(8):528-533, 2014.

Braun, M.C. S.M. Herring, N. Gokul, M. Monita, R.J. Bell, S.E. Wenderfer. M.L. Gonzalez-Garay, Y. Zhu and P.A. Doris. Hypertensive renal injury is associated with gene variation affecting immune signaling. *Circulation* (Cardiovascular Genetics) 7:903-910, 2014

LAB MEMBERS

Collaborating Faculty: Eric Boerwinkle (IMM), Myriam Fornage (IMM), Manuel Gonzalez-Garay (IMM), Oleh Pochynyuk (MMS), Michael Braun (Baylor College of Medicine), Scott Wenderfer (Baylor College of Medicine), M. John Hicks (Baylor College of Medicine), Roland Buelow (Open Monoclonal Technology Inc)
Technician: Yaming Zhu
Post-doctoral Fellow: Isha Dhande, Ph.D.
Clinical Fellow: Xiaoyan Wu, M.D., Ph.D.



Different susceptibility to renal injury is visible at the macroscopic level in the kidneys of 30 week old SHR-A3 (injury-prone) and SHR-B2 (injury-resistant) rats. These inbred rat lines are very close genetic relatives of one another with 87% of their genomes identical (i.e. they are between fraternal and identical twins in their genetic similarity). This closeness has allowed us to pinpoint the genomic regions contributing to differences in disease susceptibility within the remaining 13% of the genomes that are not identical.

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

Genetic basis of brain vascular disease and brain aging

My research interests focus on the genetic basis of common chronic diseases with an emphasis on vascular disease of the brain and brain aging. Patients with acute stroke and dementia represent the easily recognized "tip of the iceberg," but the deleterious effects of aging on the brain begin well before clinical symptoms become apparent. Brain abnormalities, readily detectable by magnetic resonance imaging (MRI), are common in asymptomatic populations beginning in middle age. My research program investigates the genetics and genomics of vascular and neurodegenerative disease of the brain both in its clinical and preclinical forms in well characterized populations from young adulthood to old age. Research strategies combine genetic epidemiology and functional genomic approaches using the latest genome resources and technologies. I use powerful genome-wide association and sequencing studies in collaboration with researchers in the United States and Europe to discover novel genes influencing the risk for stroke, Alzheimer's disease, and brain MRI abnormalities. 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 white matter lesions and other MRI traits related to brain vascular disease and dementia using large-scale genotyping and exome sequencing (AG033193, AG049506, and NS087541)
- Discovering novel epigenetic variants that influence risk for brain small vessel disease and its related neurocognitive outcomes (NS087541)
- Discovering common and rare genetic variants influencing risk for ischemic stroke and its etiologic subtypes in well-characterized clinical samples from the NINDS Stroke Genetics Consortium (NS069208)
- Discovering common and rare genetic loci influencing cardiovascular traits (lipids and blood pressure) in diverse ethnic groups as part of the NHGRI Population Architecture and Genomic Epidemiology (PAGE) consortium (HG007416)
- Discovering novel genetic loci for cardiovascular traits using gene-lifestyle interactions and pathway analysis (HL118305, HL105756, and HL120393)
- Discovering novel genetic variants influencing cognitive function and decline in middle-aged adults of European, African, and Hispanic ancestry (HL122658 and AG048642)

KEY PUBLICATIONS

Verhaaren BF, Debette S, Bis JC, Smith JA, Ikram MK, et al., Multiethnic genome-wide association study of cerebral white matter hyperintensities on MRI. *Circ Cardiovasc Genet.* 2015; 8:398-409.

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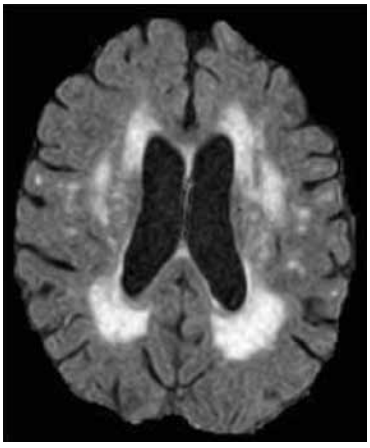
Chauhan G, Adams HH, Bis JC, Weinstein G, Yu L, et al. Association of Alzheimer's disease GWAS loci with MRI markers of brain aging. *Neurobiol Aging*. 2015; 36:1765.e7-16.

Gottesman RF, Fornage M, Knopman DS, Mosley TH. Brain Aging in African-Americans: The Atherosclerosis Risk in Communities (ARIC) Experience. *Curr Alzheimer Res.* 2015; 12:607-613.

LAB MEMBERS

Post-doctoral Fellows: Xueqiu Jian, Ph.D., Melissa Richard, Ph.D.
Ph.D. Student: Li-An Lin, BS
Biostatistician: Xiaopeng Liu
Research Associate: Ping Wang, Ph.D.
Scientific Programmer: Xiaoping Zhao



Brain magnetic resonance image showing subcortical white matter hyperintensity, atrophy of gray matter, and enlarged ventricles.

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

- Identify disease markers by metabolomics and miRNAs profiling
- Development of viral vectors and endothelial cells for therapeutics

Atherosclerosis is an inflammatory disease in the aorta that increases in severity as we age. The disease includes imbalance lipid metabolism that leads to hyperlipidemia and maladaptive immune responses that affect the arterial vasculature. To understand the development of atherosclerosis and to elucidate the cross-regulation between atherosclerosis and immunity, we develop genetic animal models. We have made a mouse model that mimics humans with hyperlipidemia by deleting both the 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 the atherosclerosis development.

Based on LDb mice, we deleted a recent discovered causative gene of hyperlipidemia (PCSK9) from LDb mice to generate a triple knockout mouse model (LTp; *Ldlr-/-Apobec1-/-Pcsk9-/-*). We demonstrated a delay and reversal of atherosclerosis development in LTp mice (see Figure, triple knockout mice had significant less lesions than LDb mice). Importantly, absence of PCSK9 gene resulted to reduced pro-inflammatory response to pathogenic LDL.

Therefore, examination of cellular and molecular mechanisms by which proatherogenic factors modulate disease development will provide insight into the understanding of physiological and pathological development process. It will provide a basis to develop efficient therapeutic approaches to combat progression of disease. Importantly, the understanding of the interaction of genetics and environment on us would allow us to age gracefully.

RESEARCH PROJECTS

- The role of PCSK9 (proprotein convertase subtilisin/kexin type 9) in autophagy, inflammation, and atherosclerosis
- The regulation of PCSK9 miRNAs in the brain
- Investigating the action of novel Ribozyme molecules in regulating the production of apolipoprotine B and lipoprotein-associated phospholipase A2 (Lp-PLA2) mRNAs

KEY PUBLICATIONS

Lu Xu, Xiaoyuan Dai Perrard, Jerry L. Perrad, Donglin Yang, Xinhua Xiao, Ba-Bie teng, Scott I. Simon, Christie M. Ballantyne, and Huaizhu Wu. Foamy monocytes form early and contribute to nascent atherosclerosis in mice with hypercholesterolemia. (2015) *Arterioscler Thromb Vasc Biol*; 35: 1787-1797. PMID: 26112011

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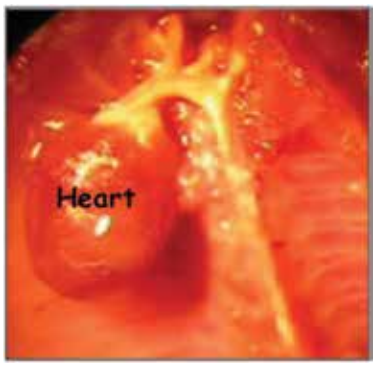
Hersharan Nischal, Hua Sun, Yuchun Wang, David A. Ford, Ying Cao, Peng Wei, and Ba-Bie Teng. Long-term expression of apolipoprotein B mRNA-specific hammerhead ribozyme via scAAV8.2 vector inhibits atherosclerosis in mice. (2013) *Molecular Therapy-Nucleic Acids*; 2: e125. PMID: 24084845

Decha Pinkaew, Rachel J. Le, Yanjie Chen, Mahmoud Eltorky, Ba-Bie Teng, and Ken Fujise. Fortilin reduces apotosis in macrophages and promotes atherosclerosis. (2013) *Am J Physiol Heart Circ Physiol*; 305: H1668-1679. PMID: 24043250

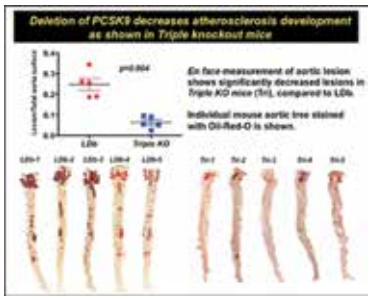
Hua Sun, Amin Samarghandi, Ningyan Zhang, Zemin Yao, Momiao Xiong, and Ba-Bie Teng. Proprotein Convertase Subtilisin/Kexin Type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. (2012) *Arterioscler Thromb Vasc Biol;* 32: 1585-1595. PMID: 22580899

LAB MEMBERS

Mentor: Pei-Ying Chung, Ph.D.
(Assistant professor at School of Nursing)
Research Assistant: Michael Tan
Research Scientist: Hua Sun, Ph.D.
Summer Intern: William Li, High school student



The severe atherosclerotic lesions are shown in the aorta of an *LDb* mouse. *LDb* mice are developed in Dr. Teng's laboratory. They are excellent model for studying the pathogenesis of atherosclerosis.



Deletion of PCSK9 gene from LDb mice decreases the development of atherosclerosis. We used an en-face measurement to determine the formation of atherosclerotic lesions in mouse aorta. As shown, triple knockout mice (Triple KO; Ldlr-/-Apobec1-/-Pcsk9-/-) mice have significantly less atherosclerotic lesions than that of LDb mice (8.8%±3.5 vs. 23.7%±3.3, p=0.004).

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.

As part of its interest in pulmonary immunity, 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 macular degeneration and diabetic retinopathy.

The Center's scientists also are actively pursuing the generation of genetically engineered stem cell lines that will avoid immune mediated graft rejection during transplantation procedures.

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

Rick Wetsel, Ph.D.
Center Director & Professor
Hans J. Muller-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 pulmonary regenerative medicine, and stem cell therapeutics

Intractable respiratory diseases are a leading cause of mortality and morbidity worldwide. There are over 35 million Americans with lung disease, and it is the number three killer (behind heart disease and cancer) in the United States, accounting for approximately 400,000 deaths per year. It is also a major cause of death in babies under 1 year of age, accounting for approximately 20 percent of infant mortality. Current treatments for lung disease at best provide symptomatic relief but offer no prospect of cure or disease reversal. Lung transplantation is the only viable option for patients with severe chronic lung disease. Lung disease is commonly caused by viral and bacterial infections (Pneumonia), environmental toxins (Chronic Obstructive Pulmonary Diseases-emphysema), allergies (Asthma), and genetic mutations (Cystic Fibrosis-Surfactant Deficiencies). Robust and well-regulated immune, inflammatory, and cellular repair responses are critical in controlling the severity of lung disease as well as preventing the development of irreversible chronic lung pathologies. However, the paucity of cellular and molecular knowledge regarding lung immunity and tissue regeneration has slowed the development of novel therapeutics that could be used for the effective treatment of lung disease.

Our laboratory for the past several years has focused on delineating key molecules responsible for mediating the inflammatory and immune responses in the lung during both normal and pathological conditions. Much of this research has involved studies of the complement anaphylatoxins (C3a and C5a) and their specific receptors (C3aR and C5aR). These receptors are seven-transmembrane G-protein coupled receptors that mediate numerous biological responses in inflammation and immunity, including smooth muscle contraction, histamine release from mast cells, vasodilation, and directed migration of numerous peripheral blood leukocytes. To examine the requisite role of the anaphylatoxin receptors in lung disease, our laboratory has generated numerous "knock-out" mice in which the genes encoding these receptors, their ligands, and carboxypeptidase regulators have been selectively ablated by gene targeting and homologous recombination methods. The generation of these mice has facilitated the discovery of numerous biological roles of the anayphylatoxins in the pathogenesis of lung disease. For example, studies using mice in which the C3a receptor has been deleted have demonstrated that C3aR is a important mediator of key hallmarks of asthma, including airway hyperresponsiveness, mucus production, lung cellular inflammation, and the CD4+ Th2 cytokine response.

We also are investigating the therapeutic use of embryonic (ES) and induced pluripotent (iPS) stem cell derived progenitor cells. Part of this program has focused on the development of stem cell therapeutics for the regeneration of lung epithelium destroyed by acute lung injury as well as by chronic lung diseases such as COPD. This research has led to the generation of the first pure population of lung alveolar epithelial type II cells from human ES cells. These cells were recently demonstrated to abrogate lung epithelial damage in an acute lung injury model in mice. In addition, we are exploring the therapeutic potential of gene corrected patient specific iPS cells for the treatment of genetic diseases affecting the lung such as surfactant protein B deficiency.

RESEARCH PROJECTS

- Delineate the molecular mechanisms by which complement anaphylatoxins modulate adaptive immunity during allergic and infectious diseases
- Determine the biological role of the complement anaphylatoxins on lung epithelial injury and tissue regeneration
- Evaluate the therapeutic potential of gene corrected iPS cell-derived lung progenitor cells for surfactant deficiencies
- Identify and characterize lung progenitor cells important in tissue regeneration
- Generate embryonic stem cell lines that can be differentiated into transplantable progenitor cells that avoid graft rejection

KEY PUBLICATIONS

Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the β2-microblobulin gene minimizes the immunogenicity of human embryonic stem cells. *Stem Cells Transl. Med.* 2015 Oct; 4(10): 1234-1245..

Calame DG, Mueller-Ortiz SL, Morales JE, Wetsel RA. The C5a anaphylatoxin receptor (C5aR1) protects against Listeria monocytogenes infection by inhibiting type I IFN expression. *J. Immunol.* 2014, Nov 15; 193 (10): 5099-5107.

Mueller-Ortiz SL, Morales JE, Wetsel RA. The receptor for the complement C3a anaphylatoxin (C3aR) provides host protection against Listeria monocytogenes-induced apoptosis. *J. Immunol.* 2014, 193:1278-1289.

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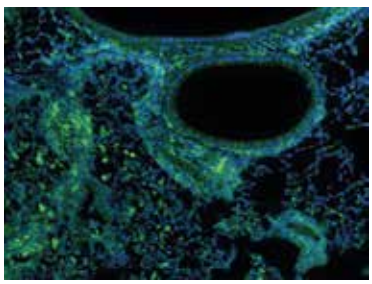
Wang D, Morales JE, Calame DG, Alcorn JL, Wetsel RA. Transplantation of human embryonic stem cell derived alveolar epithelial type II cells abrogates acute lung injury in mice. *Molecular Therapy.* 2010, 18: 625-634.

Wang D, Haviland DL, Burns AR, Zsigmond E, Wetsel RA. A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *Proc Natl Acad Sci.* 2007, 104:4449-4454.

LAB MEMBERS

Senior Research Scientist: Dr. Stacey Mueller-Ortiz
Senior Research Associate: Dr. Pooja Shivshankar
Post-doctoral Fellows: Dr. Young Uk-Kim, Dr. John Mazzilli
Senior Research Assistant: Yi-Dong Li
M.D./Ph.D. Graduate Student: Daniel Calame



Expression of the C3a receptor (green color) on inflammatory cells and lung epithelial cells in a mouse model of asthma.

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

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

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Le, T. T. T., Karmouty-Quintana, H., Melicoff, E., Le, T. T. T., Weng, T., Chen, N. Y., Pedroza, M., George, A. T., Garcia-Morales, L. J., Bunge, R. R., Bruckner, B. A., Loebe, M., Seethamraju, H., Agarwal, S. K. and Blackburn, M. R. (2014) Blockade of interleukin-6 trans signaling attenuates pulmonary fibrosis. *J. Immunol.* 193, 3755-3768. PMID: 25172494

Weng, T., Karmouty-Quintana, H., Garcia-Morales, L. J., Molina, J. G., Pedroza, M., Bunge, R. R., Bruckner, B. A., Loebe, M., Seethamraju, H. and Blackburn, M. R. (2013) Hypoxia-induced deoxycytidine kinase expression contributes to apoptosis in chronic lung disease. *FASEB J.* 27, 2013-2026. PMID: 2339349

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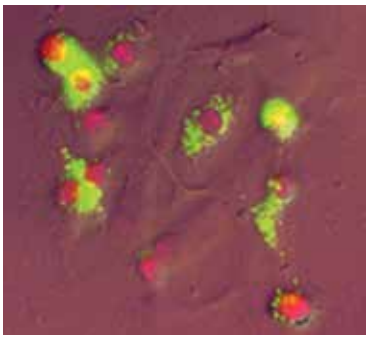
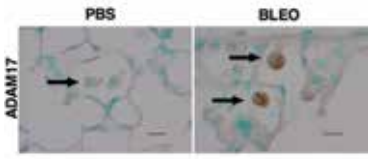
LAB MEMBERS

Assistant Professor: Tingting Weng, Ph.D.
Senior Research Scientist: Kelly Volcik, Ph.D.
Post-doctoral Fellow: Frank Lou, Ph.D.
Ph.D. Student: Kemly Philip, M.D.
Research Associate: Ning-Yuan Chen
Sr. Research Scientist: Jose Molina
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 2 groups defined by the absence or presence of nasal polyps (see image 1). This clinical classification has been supported by immunologic profiles of the inflamed sinus tissue in which CRS without nasal polyps are characterized by predominance of neutrophils and elevated T helper cell type 1 (Th1) cytokines while CRS with nasal polyps (CRSwNP) have high presence of eosinophils, mast cells, and basophils and expression of type 2 cytokines such as IL-4, IL-5, and IL-13.

Allergic fungal rhinosinusitis (AFRS) is a 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.

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

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

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

In addition, my lab is interested in the molecular characterization of fungi-mediated signaling pathway(s) and the fungal component responsible for signaling in the inflammatory response in some CRS subtypes. We have shown that fungal proteases seem to play an important role. Ongoing studies are focusing on the elucidating the specifics of this pathway as it relates to CRS.

RESEARCH PROJECTS

- Immunologic characterization of important cell types involved in the Th2 immune response
- 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

Porter PC, Yan, T, Luong A, Delclos GL, Abramson SL, Kheradmand F, and Corry DB. Proteinases as Molecular Adjuvants in Allergic Airway Disease. *Biochim Biophys Acta.* 2011 Nov;1810(11):1059-65. PMID: 21712069

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

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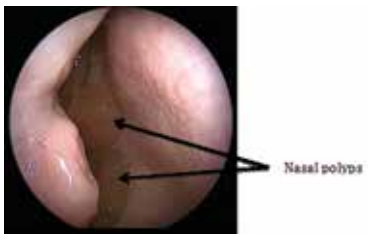

Nasal polyps seen by nasal endoscopy within nasal cavity of CRSwNP patient.

Dat. Q. Tran, M.D.
Associate Professor
Allergy/Immunology & Rheumatology,
Pediatric Research Center, Pulmonary Medicine

Immune modulation for the treatment of allergy, infectious diseases, cancer and autoimmunity

Our group focuses on the novel strategies to harness the immune system for 1) induction of immune tolerance to treat autoimmunity, inflammation and allergy and 2) enhancement of immunity against infections and cancer. Our research primarily focuses on Foxp3+ regulatory T cells (Tregs), a critical subset of CD4+ T cells that play a vital role in orchestrating immune homeostasis. Strategies that optimize the number and function of Tregs would shift the balance toward immune tolerance; abrogating Tregs would boost immunity. On a similar branch of research, we also concentrate our efforts to understand the different pathways of TGFbeta in order to develop therapeutic targets to modulate immune responses.

Our primary research projects funded by an R01 from NHLBI/NIH:

Aim 1: Assess the Treg repertoire, subsets and function in patients post allogeneic stem cell transplant.

Hypothesis: Patients who develop chronic GVHD will have restricted TCR repertoire, decreased Helios+ and LAP+ Tregs and decreased bona fide Tregs based on Treg-specific demethylated region (TSDR).

Impact: This study will provide critical biomarkers for predicting the development of cGVHD and a justification for novel LAP+ Treg immunotherapy to prevent or treat this debilitating condition.

Aim 2: Elucidate the function of IL1 receptor type 1 (CD121a) and type 2 (CD121b) on LAP+ Tregs.

Hypothesis: The unique and temporal expression of CD121a and CD121b enhances Treg function, stability and regulation of Th17 differentiation.

Impact: This study will provide invaluable insights into the critical role of IL1 receptors on LAP+ Tregs and a rationale for therapeutic use of LAP+ Tregs to induce tolerance.

Aim 3: Determine TCR diversity, stability and functionality of expanded LAP+ Tregs.

Hypothesis: LAP+ Tregs represent a highly purified, stable, and potent population, resulting in a more effective and safe product for immunotherapy to treat autoimmune and transplant-related conditions.

Impact: This outcome will provide the necessary preclinical data for transition into a clinical trial with LAP+ Tregs to prevent or treat GVHD and other autoimmune diseases.

RESEARCH PROJECTS

- Optimizing fever and hyperthermia to modulate immunity toward allergy, cancer and vaccine responses
- Investigating Tregs and their role in mucosal immunity, particularly with probiotics (Collaboration: Dr. Marc Rhoads and Dr. Yuying Liu)
- Deciphering the contribution of Tregs in pulmonary inflammation and allergic lung diseases associated with obesity (Collaboration: Dr. Richard Johnston)

KEY PUBLICATIONS

Forkhead box protein 3(+) regulatory T cells and Helios(+) subset in perinatally acquired HIV. Degaffe G, Zakhour R, Zhang W, Contreras GA, Bell CS, Rodriguez G, Del Bianco G, Pérez N, Benjamins LJ, Murphy JR, Heresi GP, Tran DQ. *Clin Exp Immunol.* 2015 Apr;180(1):108-17. doi: 10.1111/cei.12560. PMID: 25425428

Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT, Schickel JN, Tran DQ, Stoddard J, Zhang Y, Frucht DM, Dumitriu B, Scheinberg P, Folio LR, Frein CA, Price S, Koh C, Heller T, Seroogy CM, Huttenlocher A, Rao VK, Su HC, Kleiner D, Notarangelo LD, Rampertaap Y, Olivier KN, McElwee J, Hughes J, Pittaluga S, Oliveira JB, Meffre E, Fleisher TA, Holland SM, Lenardo MJ, Tangye SG, Uzel G. *Science*. 2014 Sep 26;345(6204):1623-7. doi: 10.1126/science.1255904. Epub 2014 Sep 11. PMID: 25213377

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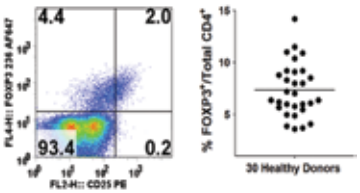
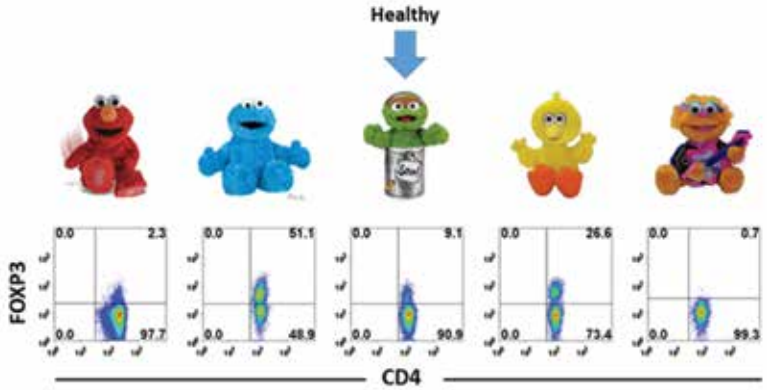
LAB MEMBERS

Research Techician I: Rachel Song and Colby Hofferek.
MDACC Clinical Hem/Onc Fellow: Dr. Ossama M. Maher



Flow cytometric plot demonstrating intracellular expression of Foxp3 transcription factor and its correlation to the cell surface IL2 receptor alpha chain (CD25) within CD4+ T cells (left figure). Right figure shows the percentages of Foxp3+ Tregs within CD4+ T cells from healthy donors.



Varying frequencies of Tregs in health and diseases. The plots show the percentages of Foxp3+ Tregs within CD4+ T cells.

Dachun Wang, M.D.
Assistant Professor

Lung stem/progenitor cells and tissue regeneration

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, and neonatal respiratory distress syndrome (RDS). 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 to develop novel therapies to facilitate the regeneration or repair of injured distal lung epithelia. Without doubt, the distal lung stem/progenitor cells represent the key targets for exploring the pathogenesis of lung diseases and the mechanisms of repair from injury. During the past few years, considerable interest has developed in the potential clinical 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 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 therapy strategy for lung tissue regeneration; and 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.

RESEARCH PROJECTS

- Isolation and characterization of embryonic stem cell derived distal lung stem/progenitor cells
- Pathways to regular distal lung stem/progenitor cell fate
- Therapeutic potential of ES/lung disease-specific iPS-derived distal lung stem/progenitor cells for the treatment of lung diseases
- Generation and characterization of HLA-1 deficient human ES cell line for tissue regeneration

KEY PUBLICATIONS

Wang D#., Quan Y., Yan Q., Morales JE, Wetsel RA., Targeted Disruption of the β2-Microglobulin Gene Minimizes the Immunogenicity of Human Embryonic Stem Cells. *Stem Cell Transl Med.* 2015 Aug. pii: sctm.2015-0049.
Co-corresponding author

Quan Y. and Wang D*. An invited review: Clinical potentials of human pluripotent stem cells in lung diseases. *Clinical and Translational Medicine.* 2014, Jun 17; 3:15 doi:10.1186/2001-1326-3-15. (* corresponding author)

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Yan Q., Quan Y., Sun H., Peng X., Zou Z., Alcorn J.L., Wetsel R.A., and Wang D*. A site-specific genetic modification for induction of pluripotency and subsequent isolation of derived lung alveolar epithelial type II cells. *STEM CELLS.* 2014 Feb;32(2):402-13. (* corresponding author)

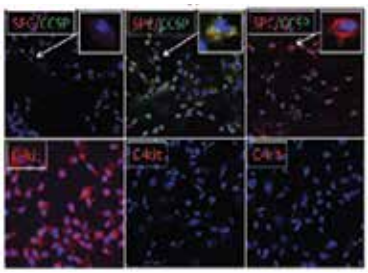
Wang D., Morales J.E., Calame D.G., Alcorn J.L., and Wetsel R.A. Transplantation of Human Embryonic Stem Cell-Derived Alveolar Epithelial Type II Cells Abrogates Acute Lung Injury in Mice. *Molecular Therapy*. 2010; 18: 3,625-634 mar.

Wang D., Haviland D.L., Burns A.R., Zsigmond E., and Wetsel R.A. A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *PNAS*, Mar 13;104(11):4449-54 (2007).

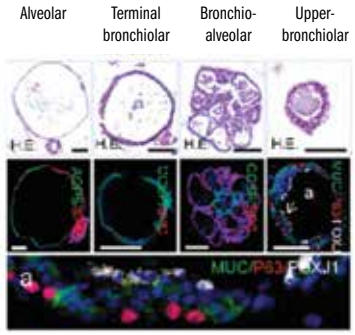
LAB MEMBERS

Senior research assistant: Dr. Yuan Quan

Pluripotent Stem cell-derived lung stem/progenitor cell types.



Lung stem cells | Bronchioalveolar stem cells | Alveolar epithelial progenitor type II cells



Alveolar-, terminal bronchiolar-, bronchioalveolar-, and upper bronchiolar-like structures derived from lung stem cells in 3D selective cultures

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

Transgenic and stem cells core facility

The Transgenic and Stem Cells Core Facility was established in 1998 and since that time, it has generated over seven hundred new transgenic, knock-out and knock-in animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions.

The mouse embryonic stem (ES) cell lines derived in the Core Laboratory are highly effective for the generation of knock-out and knock-in mice and for studies involving cellular differentiation.

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.

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 in mice
- Gene targeting, selection, expansion, cryo-preservation 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

- Generation of more than 750 transgenic, knock-out and knock-in animal models
- 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 more than 20 mouse and human cell lines, including human ES cells approved for NIH-funded research

KEY PUBLICATIONS

Nonaka, N., Zsigmond, E., Kudo, A., Kawakami, H., Yoshida, K., Yoshida, M., Kawano, N., Miyado, K., Nonaka, M. and Wetsel, R. : Epididymal C4b-binding protein is processed and degraded during transit through the duct and is not essential for fertility. *Immunobiology*, 220(4): 467-75, 2015.

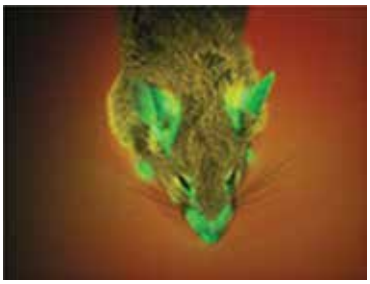
Shegog, R., Lazarus, M. M., Murray, N.G., Diamond, P. M., Sessions, N., and Zsigmond, E. Using a molecular biology simulation to impact student academic achievement and attitudes. *Res. Sci. Educ.* DO 10.11007/s11165, 2011.

Zsigmond, E.: Transfection of mouse and human embryonic stem cells by electroporation. Transfection, *Bio-Rad Labs. Tech Note:* 5904, 2009.

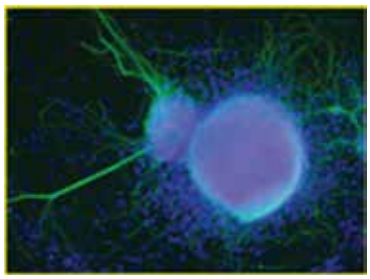
Wang. D., Haviland, D. L., Burns, A.L., Zsigmond, E. and Wetsel, R.A.: A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *PNAS*. 104:4449-4454, 2007.

LAB MEMBERS

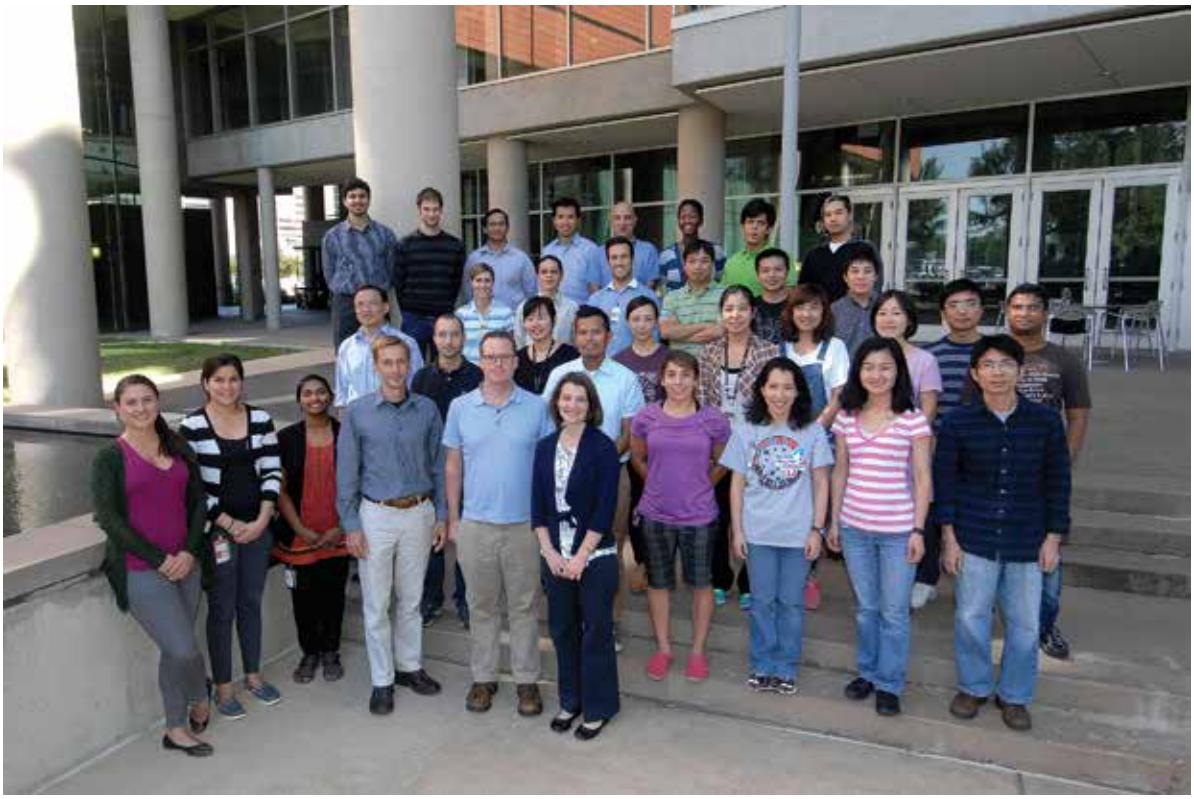
Manager: Aleksey Domozhirov



Genetically-engineered mouse expressing GFP



Neural differentiation of mouse embryonic stem cells



The Center for Metabolic and Degenerative Diseases is a diverse group that takes an integrative approach to tackle some of the most pressing health challenges of our time: obesity and the associated diseases such as diabetes, and cancer, as well as muscle wasting and neurodegenerative diseases. These different health conditions involve defects in multiple related cell signaling pathways and physiological processes. The guiding vision for the Center has been to recruit investigators with focus on complementary aspects of energy metabolism, cell signaling and cell fate determination. Key questions being addressed by the Center's faculty include the following:

- How do white and brown adipocyte progenitors determine adipose tissue function?
- How does adipose tissue promote progression of cancers and other diseases?
- Can pharmacological depletion of cells in adipose tissue be used therapeutically?
- How does fibrosis and inflammation in adipose tissue affect insulin sensitivity?
- How is angiogenesis implicated in adipose and muscle tissue remodeling?
- What transcriptional pathways can be targeted to treat muscle diseases?
- How does the brain and circadian clocks control the body's energy balance?
- 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?

To address these questions, the Center employs state-of-the-art methods in model organisms, including the mouse and the fruit fly. Collaboration among the Center's laboratories promotes research synergy, thereby increasing productivity and innovation. The Center's faculty also collaborate with 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
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

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

Function and targeting of adipocyte progenitor cells in disease

Research in my laboratory is investigating the mechanisms linking adipose tissue expansion and dysfunction in obesity with life-threatening diseases such as type-2 diabetes and cancer. Specifically, we are focused on pathological functions of adipose stromal / progenitor cells and on approaches to suppress them. We have discovered that stromal cells from white fat tissue serve as the mechanistic link between fat tissue overgrowth and obesity pathogenesis. We have shown that white adipocyte progenitors traffic to tumors and stimulate cancer progression. This year we reported that mobilization of these cells from fat tissue is regulated by the SPARC protein and are now elucidated the molecular mechanisms of their trafficking to tumors. We have also developed a compound D-WAT that targets white adipocyte progenitors. This year we reported in a series of publications that depletion of white adipocyte progenitors prevents obesity and cancer progression in mouse models. D-WAT has been licensed for further pre-clinical development. We are also characterizing a distinct population of progenitors giving rise to brown fat, which metabolically counteracts the function of white fat in animal models. These studies are now further carried out based on patient specimens in collaboration with clinicians.

RESEARCH PROJECTS

- Progenitors and lineages of white and brown adipocytes in health and disease
- Experimental therapies targeting white adipocyte progenitors in disease
- Mechanisms of adipose stromal cell trafficking and function in tumor microenvironment
- Adipose tissue cell markers and mechanisms of intercellular communication

KEY PUBLICATIONS

PRUNE2 is a human prostate cancer suppressor regulated by the intronic long noncoding RNA PCA3. Salameh A, et al., Kolonin MG, et al. *Proceedings of the National Academy of Sciences of the USA.* 2015; 112(27):8403-8.

Stem cells from adipose tissue and breast cancer: hype, risks and hope. Bertolini F, Petit JY and Kolonin MG. British Journal of Cancer. 2015; 112(3):419-23.

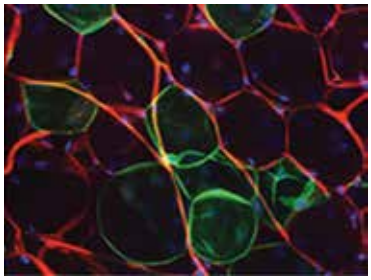
Depletion of white adipocyte progenitors induces beige adipocyte differentiation and suppresses obesity development. Daquinag AC, Tseng C, et al. and Kolonin MG. *Cell Death and Differentiation.* 2015; 22(2):351-63.

Targeted Pro-apoptotic Peptides Depleting Adipose Stromal Cells Inhibit Tumor Growth. Daquinag AC, Tseng C, et al. and Kolonin MG. *Molecular Therapy*. In press.

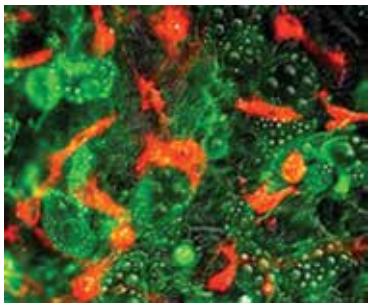
Proteolytic Isoforms of SPARC Induce Adipose Stromal Cell Mobilization in Obesity. Tseng C and Kolonin MG. *Stem Cells.* In press.

LAB MEMBERS

Sr. Research Scientists: Alexis Daquinag, Zhanguo Gao
Post-doctoral Fellow: Zhang Tao
Ph.D. Candidate: Chieh Tseng
Sr. Research Assistant: Ali Dadbin



Distinct adipocyte populations genetically tracked with green and red reporter fluorophores in adipose tissue. Nuclei are blue.



Adipocytes differentiating from progenitor cells (green) and stromal/vascular cells (red) in tissue culture. Lipid droplets are white.

Rebecca Berdeaux, Ph.D.
Associate Professor

Harnessing new pathways to improve muscle metabolism and muscle growth

Our laboratory is dedicated to understanding how cells respond and adapt to stress-induced hormonal changes and how those pathways might become inappropriately activated or inhibited in disease. We focus on hormone-induced changes in gene regulation and the impact of those newly expressed genes on physiology.

How does insulin resistance develop? Humans require a constant glucose supply to maintain heart and brain function even when food is scarce. On the other hand, excess circulating glucose is detrimental and underlies development of type 2 diabetes. In type 2 diabetes, blood glucose becomes too high in part because liver, muscle, and fat tissue become resistant to the hormone insulin. "Insulin resistance" occurs in individuals with clinical pre-diabetes, which affects ~30% of adults in the US, most of whom are undiagnosed. In spite of the prevalence of this disease, few FDA approved drugs attack insulin resistance. Thus, there is an urgent need to identify "drug-able" proteins to increase the therapeutic options for pre-diabetes.

Our laboratory studies an enzyme (salt inducible kinase 1, or SIK1) that is present throughout the body and participates in fine-tuning hormonal responses. In liver, SIK1 was previously thought to inhibit a key pathway that stimulates new glucose synthesis. To visually monitor this pathway in living animals, we engineered mice that glow in the dark when the pathway is turned on. Surprisingly, deletion of the SIK1 gene in mice had no affect on liver glucose synthesis. Instead, SIK1-deficient mice were strongly protected from hyperglycemia when fed a high fat diet, even though the mice became just as obese as control animals. We now know that SIK1 is turned up in skeletal muscle of fat mice and that SIK1 inhibits the actions of insulin. This makes SIK1 a very promising target for therapeutic development. We are currently investigating why SIK1 abundance is increased in obesity and how this enzyme inhibits insulin action.

How do hormones regulate muscle growth and strength? One aspect of aging is loss of skeletal muscle mass and strength, which impacts metabolic health as well maintenance of normal daily activities. Our laboratory is undertaking a multi-faceted approach to identify pathways that could be targeted with drugs to help maintain muscle mass through activation of stem cells or promotion of growth, or hypertrophy, of existing muscle. First, we are analyzing the effects of SIK1 on muscle mass because SIK1 responds to hormones that promote muscle growth. We found that mice lacking SIK1 have much larger muscle cells than control animals. We are now determining how SIK1 puts the brakes on muscle growth and testing the impact of SIK1 inhibition on muscle strength and exercise ability with aging. Second, we have developed tools to characterize how novel factors released by muscle activate muscle stem cells and study the impact of these pathways on muscle stem cell function and ultimately muscle mass during aging. In addition, we created mice in which we can mimic hormonal pathways that stimulate muscle hypertrophy using an otherwise inert chemical compound. Using these mice and isolated muscle stem cells, we are working to establish a signature of genes and proteins associated with muscle stem cell activity and muscle growth. Ultimately we expect to uncover new pathways that could be targeted to promote muscle growth and strength in aging individuals.

RESEARCH PROJECTS

- Role of SIK1 in development and severity of type 2 diabetes
- Regulation of exercise performance by SIK1
- Chemical-genetic methods to stimulate muscle stem cell proliferation and muscle regeneration to uncover new pathways that promote muscle regeneration and hypertrophy

KEY PUBLICATIONS

Berdeaux R, Goebel N, Banaszynski L, Takemori H, Wandless T, Shelton GD, and Montminy M. (2007) SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. *Nature Medicine*. 13(5): 597-603. (Cover illustration)

Stewart R, Flechner L, Montminy M, and Berdeaux R. (2011) CREB is activated by muscle injury and promotes muscle regeneration. *PloS ONE* 6(9): e24714.

Berdeaux R and Stewart R. (2012) cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism and regeneration. *Am J Physiol: Endo and Metab.* 303(1): E1-17. (Cover illustration)

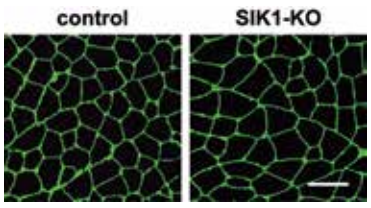
Stewart R*, Akhmedov D*, Robb C, Leiter C and Berdeaux R. (2013) Regulation of SIK1 abundance and stability is critical for myogenesis. *PNAS,* 110(1): 117-22.

Akhmedov D and Berdeaux R. (2013) The effects of obesity on skeletal muscle regeneration. Front. Physiol., 4: 371.

Clark, R.I., Tan, S.W.S., Péan, C., Roostalu, U., Vivancos, V., Bronda, K., Pilátová, M., Fu, J., Walker, D.W., Berdeaux, R., Geissmann, F., and Dionne M.S. (2013) Mef2 is an in vivo immune-metabolic switch. *Cell*, 155(2): 455-447.

LAB MEMBERS

AHA Post-doctoral Fellow: Dmitry Akhmedov, Ph.D.
Doctoral Student: Randi Fitzgibbon, B.S., 2015 Dean's Research Award Recipient
Senior Research Assistants: Maria Mendoza, M.S., Kavitha Rajendran, M.S.



Muscle fiber boundaries outlined with a fluorescent protein to measure the cross sectional area (size) of individual muscle cells in control (left) and SIK1-knockout (right) mice.

Kristin Eckel Mahan, Ph.D.
Assistant Professor

The circadian clock in health and disease

The internal circadian (24-hour) clock is tethered to our environment and functions as an ever-present regulator of our human physiology. Examples of circadian oscillations are the sleep/wake cycle, food seeking and intake, and hormone production and release. There is now substantial evidence that disrupting our endogenous circadian clocks (such as occurs during night shift work, traveling across time zones, or taking certain drugs and medications) has deleterious effects when it comes to metabolism, influencing processes as disparate as body weight regulation and cognitive efficiency. A surprising number of metabolically-related disturbances, including obesity, diabetes, cancer and dementia have all been associated with an impaired circadian clock in either the brain or the peripheral organs. Diseases associated with clock disruption are disparate in their phenotype, as the circadian clock is present in all tissues of the body and is ultimately supported by time keeping in individual cells (such as individual neurons of the hippocampus) that are synchronized and coordinating within and sometimes even across tissues.

Interests of our lab center on how specific cues (or "zeitgebers") control the circadian clock in a tissue-specific way to direct cellular metabolism. In particular, we are interested in what happens to the normal function of tissues, such as the liver and the brain, when the clock breaks down. Understanding the mechanisms underlying the diseases associated with clock disturbance, including cancer, obesity, and cognitive decline will shed light onto potential behavioral or pharmacological steps that might be taken to preserve, restore, or better synchronize our internal circadian clocks.

Recent work has proved that even simple behavioral deviations, such as the consumption of a high fat diet, profoundly affects the circadian clock of the liver, reprogramming it in a manner in which oscillations in gene expression or protein activity are either lost, phase-shifted, or there is a genesis of oscillations where they should not take place. Thus, nutrients themselves appear to function as "zeitgebers" for the circadian clock. This nutrient-sensing capability of cells is mediated in part by critical nuclear receptors that control 24-hour rhythmicity and when defective, appear to be responsible for a fatty liver phenotype among other metabolic problems such as cancer progression.

The clock is as important in what it keeps oscillating throughout the circadian cycle as what it prevents from oscillating during the circadian cycle. Thus, the appropriate levels of gene or protein activity throughout the circadian cycle is essential for optimal metabolic activity and is controlled in a tissue-specific way. Understanding the mechanisms underlying this control is necessary for discovering why perturbation of the clock at the environmental and behavioral levels may lead to specific diseases, including obesity and cancer.

RESEARCH PROJECTS

- Mechanisms underlying nutritional regulation of peripheral vs. central clocks
- Role of the circadian clock in rhythmic energy intake
- Circadian mechanisms underlying Night Eating Syndrome (NES)
- Regulation of hepatic cell division and tumor initiation by CLOCK-associated tumor suppressors

KEY PUBLICATIONS

Eckel-Mahan, KL, Sassone-Corsi P Phenotyping Circadian Rhythms in Mice *Current Protocols in Mouse Biology,* 2015 September

Eckel-Mahan KL, Patel VR, de Mateo S, Ceglia NJ, Sahar SS, Dilag S, Dyar KA, Orozco-Solis R, Baldi P, and Sassone-Corsi P. Reprogramming of the Circadian Clock by Nutritional Challenge *Cell*, 2013 December 19, 155(7), 1464-1478

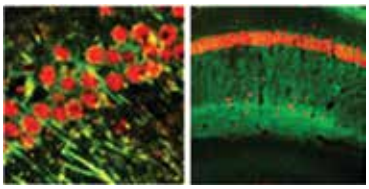
Eckel-Mahan KL and Sassone-Corsi P Metabolism and Circadian Rhythmicity Converge *Physiological Reviews*, 2013 Jan;93(1):107-35

Eckel-Mahan KL, Patel VR, Vignola KS, Mohney RP, Baldi P and Sassone-Corsi P. A Map of the Circadian Metabolome Reveals the Organized Interface between Metabolism and Transcription in the Liver *Proc Natl Acad Sci* U S A. 2012 Apr 3;109(14):5541-6

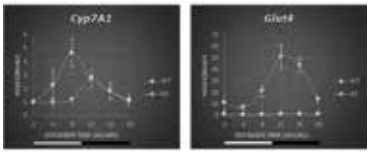
Eckel-Mahan KL, Phan TH, Han S, Wang HB, Chan GC-K, Scheiner ZS, Storm DR. Circadian Oscillation in MAPK Activity and cAMP in the Hippocampus: Implications for Memory Persistence *Nature Neuroscience* 2008 Sept Vol. 11(9): 1074-1082.

LAB MEMBERS

Post-doctoral Fellows: Aleix Ribas Latre, Baharan Fekry
Research Assistant: Christopher Kwok



Robust expression of circadian clock proteins in individual nuclei of CA1 neurons of the hippocampus, a region of the brain essential for long term memory formation.



Knocking out a specific circadian protein-interacting partner ("KO") in the liver dampens oscillation of the expression of *Cyp7A1*, a gene important for cholesterol removal from the body, while inducing rhythmicity of the insulin-sensitive glucose transporter, *Glut4* (a gene not normally expressed at high levels in the liver). These livers become extremely fatty relative to a normal, control liver.

Nicholas Justice, Ph.D.
Assistant Professor

Stress-related disease and the impact of stress on neurodegenerative disease progression

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 cause 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

- How does stress impact the progression of Alzheimer's Disease
- How does Post Traumatic Stress Disorder accelerate the progression of Alzheimer's Disease
- Local neural circuits that regulate Hypothalamic-Pituitary-Adrenal axis output
- Functional characterization of neural circuits that respond to stress
- Modeling Amyotrophic Lateral Sclerosis (ALS) in mouse using a newly discovered expansion in the c9orf72 locus

KEY PUBLICATIONS

Posttraumatic stress disorder-like induction elevates β-amyloid levels, which directly activates corticotropin-releasing factor neurons to exacerbate stress responses. Justice NJ, Huang L, Tian JB, Cole A, Pruski M, Hunt AJ Jr, Flores R, Zhu MX, Arenkiel BR, Zheng H. *J Neurosci*. 2015 Feb 11;35(6):2612-23.

Sex differences in NMDA GluN1 plasticity in rostral ventrolateral medulla neurons containing corticotropin-releasing factor type 1 receptor following slow-pressor angiotensin II hypertension. Van Kempen TA, Dodos M, Woods C, Marques-Lopes J, Justice NJ, Iadecola C, Pickel VM, Glass MJ, Milner TA. *Neuroscience.* 2015 Aug 22. S0306-4522(15)00761-7.

Parvalbumin+ Neurons and Npas1+ Neurons Are Distinct Neuron Classes in the Mouse External Globus Pallidus. Hernández VM, Hegeman DJ, Cui Q, Kelver DA, Fiske MP, Glajch KE, Pitt JE, Huang TY, Justice NJ, Chan CS. *J Neurosci.* 2015 Aug 26;35(34):11830-47.

Local CRH Signaling Promotes Synaptogenesis and Circuit Integration of Adult-Born Neurons. Garcia I, Quast KB, Huang L, Herman AM, Selever J, Deussing JM, Justice NJ, Arenkiel BR. *Dev Cell.* 2014 Sep 29;30(6):645-59. doi: 10.1016/j.devcel.2014.07.001. Epub 2014 Sep 4.

Novel subunit-specific tonic GABA currents and differential effects of ethanol in the central amygdala of CRF receptor-1 reporter mice. Herman MA, Contet C, Justice NJ, Vale W, Roberto M. *J Neurosci.* 2013 Feb 20;33(8):3284-98.

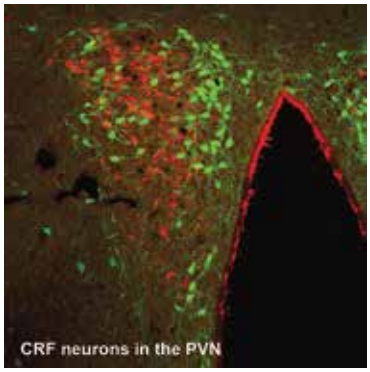
Rissman RA, Staup MA Lee AR , Justice NJ, Rice KC, Vale W, Sawchenko PE. Corticotropin-Releasing Factor Receptor-Dependent Effects of Repeated Stress on Tau Phosphorylation, Solubility and Aggregation. *Proc Natl Acad Sci*, 2012, Apr 17;109(16):6277-82.

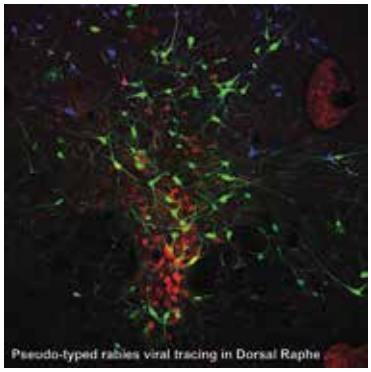
Guo Q, Zheng H, Justice NJ*. Central CRF system perturbation in an Alzheimer's disease knockin mouse model. *Neurobiol Aging*. 2012, Nov; 33(11):2678-91. (*Corresponding Author)

Guo Q, Li H, Gaddam SS, Justice NJ, Robertson CS, Zheng H. Amyloid precursor protein revisited: neuron-specific expression and highly stable nature of soluble derivatives. *J Biol Chem.* 2012 Jan 20;287(4):2437-45.

LAB MEMBERS

Graduate Student: Albert Hunt
Post-doctoral Fellows: Shiva Rajamanickam, Zhiying Jiang





Vihang Narkar, Ph.D.
Assistant Professor

Exercise mimicry in vascular, metabolic, and degenerative diseases

Exercise has long been known to have a fantastic health benefit in a range of diseases. However, universal or organ-specific molecular sensors of exercise that are responsible for exercise benefits are poorly defined. Uncovering these molecular sensors has implications for designing exercise mimetic drugs for vascular, metabolic, and degenerative diseases.

To dissect the molecular circuitry of exercise, we have focused on the skeletal muscle – one organ that is extensively used during exercise. Experimentally, we use molecular and cell biology, pharmacology, and mouse genetic engineering to discover exercise mimetic pathways in muscle. Using these techniques, we first identified that serine/threonine kinase AMPK and nuclear receptor PPARβ can mimic exercise by activating genes linked to mitochondrial biogenesis, fatty acid oxidation, and slow-twitch contractile myofibers in skeletal muscles, and improve endurance in mice, even in absence of training. Encouraged by this finding, we are currently investigating the role of estrogen receptor-related receptors (ERR) - a class of orphan nuclear receptors - in skeletal muscle. ERR's and particularly ERRγ is highly expressed in high endurance muscle fibers suggesting a role for these receptors in the regulation of aerobic metabolism. We have genetically targeted ERRγ in mice to investigate the effect of skeletal muscle-specific receptor modification on myocellular gene expression, metabolism and exercise. Furthermore, we are exploring the potential role of ERRγ in ameliorating obesity, diabetes, muscle ischemia, as well as muscular dystrophy. Our findings so far suggest that genetic ERRγ activation in the muscle can mimic exercise to increase aerobic and endurance capacity. It also prevents obesity, improving muscle vasculature to prevent ischemic, and even ameliorate pathology in orphan genetic diseases such as muscular dystrophy. One future direction is to design powerful synthetic activators (which we call exercise mimetics) for the above regulators, which will have pharmaceutical utility in various diseases.

RESEARCH PROJECTS

- ERRγ and diabetes
- ERRγ and skeletal muscle ischemic disease
- ERRγ and Duchenne Muscular Dystrophy
- Nuclear receptor co-activators in vascular diseases

KEY PUBLICATIONS

Yadav V, Matsakas A, Lorca S, Narkar VA. (2014) PGC1β activates anti-angiogenic program to repress neo-angiogenesis in muscle ischemia. *Cell Rep*. 8(3): 783-97.

Matsakas A, Yadav V, Lorca S, Narkar V. (2013) Muscle ERRγ mitigates Duchenne muscular dystrophy via metabolic and angiogenic reprogramming. *Faseb J.* 27(10): 4004-4016.

Matsakas A, Yadav V, Lorca S, Evans RM, Narkar VA (2012) Revascularization of ischemic skeletal muscle by estrogen-related receptor-γ. *Circ Res.* 110(8): 1087-96.

Narkar VA, Fan W, Downes M, Yu RT, Jonker JW, Alaynick WA, Banayo E, Karunasiri MS, Lorca S, Evans RM. (2011) Exercise and PGC-1α-Independent Synchronization of Type I Muscle Metabolism and Vasculature by ERRγ. *Cell Metabolism.* 13(3): 283-93

Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM. (2008) AMPK and PPARdelta agonists are exercise mimetics. *Cell*. 134(3): 405-15.

LAB MEMBERS

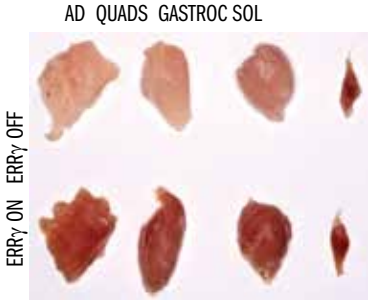
Post-doctoral fellows: Pierre-Marie Badin, Danesh Sopariwala
Technician: Sabina Lorca
Summer Students: Laura Rangel, Katha Korgaonkar
Alumni: Antonios Matsakas (Asst. Professor, Hull University, UK), Vikas Yadav (Asst. Professor, Amity University, India)

O2 + ENERGY DELIVERY
Lungs, Blood, Heart

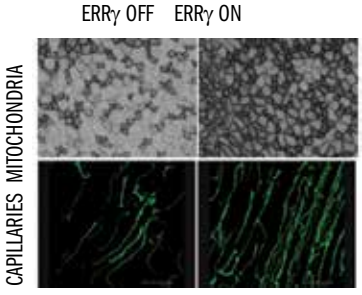
ENERGY METABOLISM
Oxidative capacity (mitochondria)
Fiber-type switch

Exercise has mega-physiological effects and health benefits. Are there landmark molecular pathways that can be pharmaceutically activated to mimic exercise? Discoveries from our laboratory are answering this question.

(A)



(B)



One of the exercise mimetic pathways we have identified is ERRγ. (A) We genetically switched-on ERRγ to make high endurance and fatigue resistant red muscle, thus mimicking exercise. Red color in muscle biopsies from ERRγ ON mice is due to increased mitochondria and blood vessels. (B) Microscopic images of muscles from ERRγ ON mice showing increase in mitochondrial density (top panel) and capillaries (bottom panel).

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

Targeting adipose tissue remodeling for obesity and Type 2 diabetes

Research in my 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 the pathophysiology of human obesity and insulin resistance.

In the past five years, I have published many paradigm-shifting findings about the dynamic process of adipose tissue expansion. Specifically, we discovered that obese fat pads are frequently hypoxic and HIF1α induction is an initial step leading to ultimate unhealthy microenvironment in adipose tissue. More importantly, we further demonstrated that the effects of modulation of angiogenic activity in white adipose tissue by VEGF-A could be dichotomous and metabolic context dependent: in healthily expanded adipose tissue, angiogenesis is beneficial by improving vascularization and inducing a "browning" of 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.

In my laboratory, we further explored the fine-tuned regulation at other levels during the pathological expansion of adipose tissue. Indeed, we found fibrosis is the hallmark in the metabolically dysfunctional adipose tissue. Interestingly, our recent research suggests that the regulation of ECM flexibility by MMPs 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 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 COL6α3 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 fibrosis and local inflammation in adipose tissue
- VEGF-A stimulated metabolic benefits during adipose tissue healthy expansion
- The effects of antiangiogenic action by blocking VEGF-A and/or its receptors in the context of preexisting adipose tissue (unhealthy expansion)
- The mechanisms by which endotrophin shapes unhealthy microenvironment in obese adipose tissue
- Reversibility of adipose tissue fibrosis by novel anti-fibrotic therapies

KEY PUBLICATIONS

Sun K, Park J, Gupta O, Holland WL, Auerbach PL, Zhang N, Marangoni RG, Nicoloro SM, Czech MP, Varga J, Ploug T, An ZQ and Scherer PE. "Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction". *Nature Commun.* 5:3485. (2014).

Sun K, Tordjman J, Clément K, and Scherer PE "Fibrosis and adipose tissue dysfunction". *Cell Metab.* 18(4):470-7. (2013).

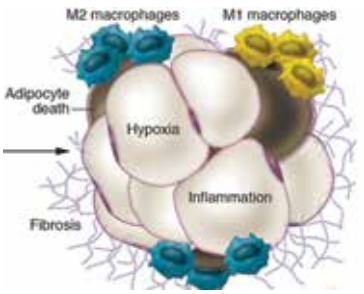
Sun K, Halberg N, Khan M, Magalang UJ, Scherer PE. "Selective inhibition of hypoxia-inducible factor 1α ameliorates adipose tissue dysfunction". *Mol Cell Biol*. 33(5):904-17. (2013)

Sun K, Wernstedt Asterholm I, Kusminski CM, Bueno AC, Wang ZV, Pollard JW, Brekken RA, Scherer PE. "Dichotomous effects of VEGF-A on adipose tissue dysfunction". *Proc Natl Acad Sci U S A*. 109(15):5874-9. (2012).

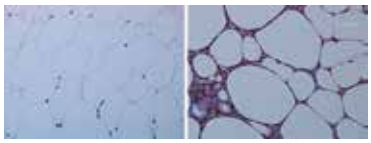
Sun K, Kusminski CM, Scherer PE. "Adipose tissue remodeling and obesity". *J Clin Invest.* 121 (6): 2094-101. (2011).

LAB MEMBERS

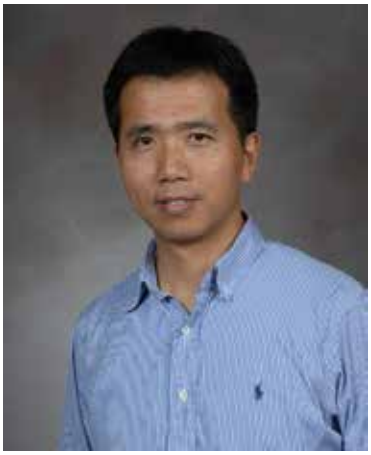
Post-doctoral Fellows: Liliang Jin, Yueshui Zhao
Research Assistant: Xue Gu



Unhealthy adipose tissue expansion is strongly associated with systemic insulin resistance. (*J Clin Invest.* 121(6):2094-101).



Healthy (left) and unhealthy (right) adipose tissue: Trichrome stain of an unhealthy adipose tissue from a high-fat diet fed mouse contains a high level of immune cell infiltration and enhanced extracellular matrix (right panel, blue areas). (*J Clin Invest*. 121(6):2094-101).



Qingchun Tong, Ph.D.
Associate Professor
Cullen Chair in Molecular Medicine

Mechanisms underlying brain control of body weight and glucose homeostasis

Obesity and diabetes are imposing a huge burden to our society, while the effective treatment is still lacking. The current obesity epidemic is due to a combination of genetic susceptibility and high-fat high caloric (HFD) environment. Thus, we aim to understand the mechanisms underlying HFD-induced obesity and its interaction with important gene functions.

Specific groups of neurons, especially those in the hypothalamus, receive and integrate nutritional status signals, and then adjust food intake and energy expenditure accordingly to maintain energy balance. To link neuron function with behavior, we specifically activate or inhibit a distinct group of neurons with various channelrhodopsins (ChRs) by light (optogenetics) or with designer receptors exclusively activated by designer drugs (DREADD). These new techniques in conjunction with our novel mouse genetic models will reveal important neurons and circuits in the brain for feeding and glucose hemostasis.

One current project in this regard is to delineate the neural pathways from lateral hypothalamus (LH) to paraventricular hypothalamus (PVH) in feeding and self-grooming behavior – a typical behavior trait in mice for obsessive compulsive diseases (OCD) in humans. The optogenetic and DREADD approaches are being used to test the hypothesis that GABAergic projections from LH to PVH promote feeding while glutamatergic projections promote self-grooming. The results will lead to important discoveries of novel neurocircuits for feeding and its relations with other behaviors, such as self-grooming.

Another ongoing project is to understand the neural pathway underlying leptin in restoring glucose to normal levels in type 1 diabetes. Identification of this pathway will offer opportunities to treat type 1 diabetes without insulin, thus avoiding hypoglycemic and lipogenic risks associated with insulin treatments.

Ultimately we try to delineate specific neural pathways underlying specific physiologic functions and provide a scientific rationale for effective therapeutic strategies against the current obesity and diabetes epidemic.

RESEARCH PROJECTS

- Brain mechanisms underlying leptin action in restoring blood glucose in type 1 diabetes
- Novel neural pathways responsible for feeding and associated behaviors
- Identification of factors that control differential diet-induced obesity

KEY PUBLICATIONS

Kim ER, Wu Z, Sun H, Xu Y, Mangieri LR, Xu Y and Tong Q. Hypothalamic Non-AgRP, Non-POMC GABAergic Neurons Are Required for Post-weaning Feeding and NPY Hyperphagia. *J. Neurosci.* 2015, 35(29): 10440-10450. PMID:26203139. Corresponding author.

Wu Z, Kim ER, Sun H, Xu Y, Mangieri LR, Li DP, Pan HL, Xu Y, Arenkiel BR and Tong Q. GABAergic Projections from Lateral Hypothalamus to Paraventricular Hypothalamic Nucleus Promote Feeding. *J. Neurosci.* 2015, 35(8): 3312-3318. PMID: 25716832. Corresponding author.

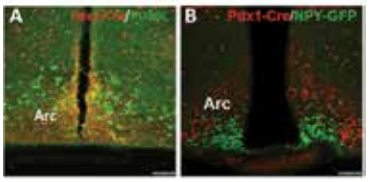
Yan C., He Y, Xu Y, Wang C, Yang Y, Saito K, Xu P, Hinton AO, Yan X, Shun G, Yu L, Wu Q, Tso P, Tong Q* and Xu Y*. Apolipoprotein A-IV inhibits food intake via the central melanocortin pathway. *Neuroendocrinology,* 2015, in press. * Co-corresponding author.

Xu Y, Kim ER, Fan S. Huang C, Xu Y and Tong Q. Profound and Rapid Reduction in Body Temperature by the Melancortin Receptor Agonist. *BBRC*, 2014, 451(2):184-189. Corresponding author.

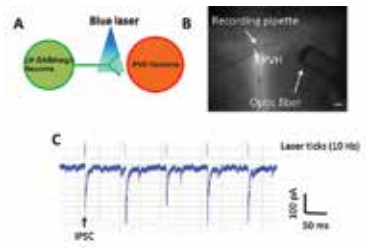
Xu Y, Wu Z, Sun H, Zhu Y, Kim ER, Arenkiel BR, Lowell BB, Xu Y and Tong Q. Glutamate Mediates the Function of MC4Rs on Sim1 Neurons in Body Weight Regulation. *Cell Metabolism.* 2013, 18 (6): 860-870. Corresponding author.

LAB MEMBERS

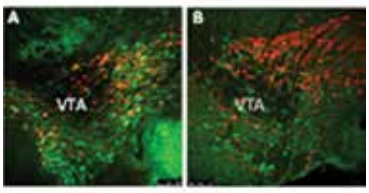
Instructor: Yuanzhong Xu
Post-doctoral Fellows: Eun Ran Kim, Shengjie Fan, Yungang Lu
Graduate student: Leandra Mangieri



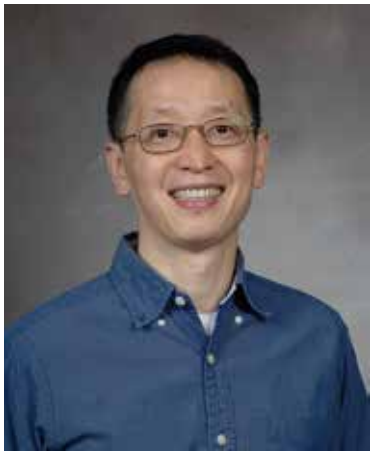
Pdx1-Cre neurons (red) are neither POMC (green in A) nor AgRP neurons (green in B).



A diagram showing an optogenetic approach for monosynaptic circuit mapping from lateral hypothalamic (LH) GABAergic neurons to paraventricular hypothalamic neurons (PVH, A)) with an actual recording setup (B) and inhibitory postsynaptic current traces (IPSCs) elicited by laser (C).



Pictures from control (A) and knockout (B) brains showing specific deletion of vesicular monoamine transporter 2 (green) in leptin receptor neurons (red).



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

Molecular mechanisms of neurodegenerative diseases

While our society is enjoying an unprecedented longer life expectancy, it is also facing a pressing threat from aging-related neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). Currently there are no effective cures or preventive approaches against these debilitating diseases, partially due to a lack of clear understanding of their etiology at molecular levels.

We are studying the underlying causes of these brain diseases by employing both mammalian systems and the invertebrate *Drosophila*, a classical genetic model organism commonly known as the fruit fly. Although smaller and simpler than mammalian systems, the fly bears many remarkable similarities to humans, including the existence in its genome of counterparts for most human disease genes. Because *Drosophila* allows fast and complicated experimental manipulations, it provides an excellent animal model for studying human diseases.

Our current research focuses on the following projects:

Protein misfolding, aggregation and cellular clearance mechanisms

The presence of abnormal aggregates (e.g., plaques and tangles) derived from misfolded proteins in the brain is a hallmark of almost all neurodegenerative diseases. We are studying how the cell's self-clearance machineries, such as chaperones and autophagy ("self-eating"), normally operate and why their functions often become compromised in affected neurons, so that we can better employ these innate self-protective mechanisms to remove misfolded proteins and fight these diseases.

Recently, we showed that Huntingtin, the HD gene, is itself an important player in a cellular clearance mechanism called *selective autophagy*, raising an intriguing possibility that the HD-causing mutation (i.e., polyglutamine expansion in Huntingtin) can interfere with this protective mechanism and affect HD pathogenesis.

Biogenesis of specialized cellular organelles

In cells, specialized organelles, such as synaptic vesicles and lysosome-related organelles (LROs), control diverse aspects of cellular functions and neuronal activities. For example, dopamine, a chemically labile neurotransmitter, is packaged inside the specialized membrane-enclosed vesicles, which are important for proper storage and regulated release of dopamine. We are studying the biogenesis and regulation of these specialized cellular organelles, as their disruption contributes to a spectrum of disorders, such as AD, PD, and schizophrenia.

RESEARCH PROJECTS

- Huntington's disease and the role of Huntingtin in selective macroautophagy
- Protein misfolding and their clearance by chaperones and autophagy
- Biogenesis of lysosome-related organelles
- Intracellular handling of dopamine in Parkinson's disease

KEY PUBLICATIONS

Cuervo AM and Zhang S. (2015) "Selective autophagy and Huntingtin: learning from disease". *Cell Cycle*;14(11):1617-8. doi: 10.1080/15384101.2015.1039365.

Xu Z, Tito A, Rui YN, and Zhang S. (2015) "Studying polyglutamine diseases in *Drosophila*". *Experimental Neurology.* doi: 10.1016/j.expneurol.2015.08.002

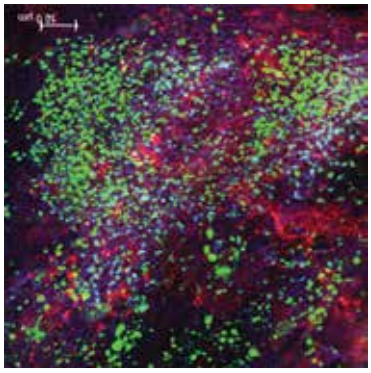
Rui YN, Xu Z, Patel B, Chen ZH, Chen DS, Tito A, David G, Sun YM, Stimming EF, Bellen H, Cuervo AM and Zhang S. (2015). "Huntingtin functions as a Scaffold for Macroautophagy". *Nature Cell Biology*, 17(3): 262 – 275. Commented in *News and Views* in the same issue.

Rui YN, Xu Z, Chen ZH, and Zhang S (2015). "The GST-BHMT Assay Reveals A Distinct Mechanism Underlying Proteasome Inhibition-Induced Macroautophagy in Mammalian Cells". *Autophagy* 11(5): 812-832.

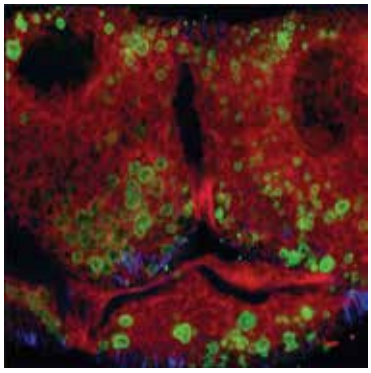
Zhang S, Xu L, Lee J, Xu T. (2002). "*Drosophila* Atrophin homolog functions as a transcriptional co-repressor in multiple developmental processes". *Cell*, 108 (1): 45-56.

LAB MEMBERS

Instructor: Dr. Zhen Xu
Post-doctoral Fellow: Dr. Yan-Ning Rui
Graduate Student: Antonio Tito
Technician: Lili Ye



Protein misfolding and their clearance by autophagy. Prominent aggregates formed by misfolded mutant Huntingtin protein (green) (green puncta) in the fly brain. These aggregates partially co-localize with autophagy markers p62/Ref(2)P (red) and ubiquitin (blue).



Biogenesis of lysosome-related organelles. The presence of many lysosome-related organelles (green color) of different sizes are detected inside two *Drosophila* cells (marked in red color).

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 that advance molecular 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 (NIR) fluorescence to enable new understandings in several disease states.

The Division of Applied Biologics focuses upon development and engineering of antibody-based diagnostics and therapeutics for high-affinity targeting of disease markers, and the Division of Next Generation Sequencing specializes in bioinformatically associating genotypes to enable discovery of disease-causing gene variants in translational studies. Biological validation of these disease-causing variants lead to the next steps of target discovery for new therapeutic and diagnostics in areas of unmet clinical need. In addition to having its own basic science and clinical research projects, the center and its divisions synergistically operate a "collaboration" center where clinicians and basic scientists from across the Texas Medical Center partner with CMI members to effectively apply diagnostics in preclinical and clinical studies.

Currently, the team effectively translates new NIRF molecular imaging technologies literally from "bench-to-bedside and back again," in efforts that embrace its division and clinical partners in the Texas Medical Center and in the Houston suburbs.

Discoveries made in the process of clinical translation require "back to the bench" studies in the CMI include:

- Biological validation of gene variants found with next generation sequencing using protein studies, cellular functional assays, and transgenic animal models;
- Identification of therapeutic targets to reverse disease phenotypes in cellular and transgenic animal models; and
- Re-engineering of imaging devices and imaging agents to improve clinical utility of diagnostics.

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

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

Molecular imaging and diagnostics

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 (NIR) fluorescence to enable new understandings in several disease states. In addition to having its own basic science and clinical research projects, the team also operates a "collaboration" center where clinicians and basic scientists from across the Texas Medical Center partner with CMI members to effectively apply diagnostics in preclinical and clinical studies. 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 research. Discoveries made from the 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 re-organization in response to surgery and radiation
- Small animal imaging and tomography

KEY PUBLICATIONS

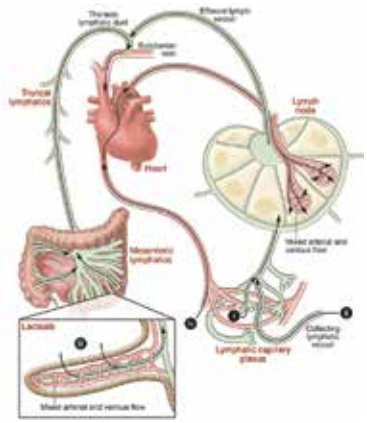
Lu, Y., Darne, C., Tian, I.C., Zhu, B., Rightmer, R., Rasmussen, J.C., and E.M. Sevick-Muraca, "Experimental comparison of continuous-wave and frequency-domain fluorescence tomography in a commercial multi-modal scanner," IEEE Trans. *Med. Imaging,* 2015, PMID: 25438307.

Agollah, G.D., Gonzalez-Garay, M.L., Rasmussen, J.C., Tan, I-C., Aldrich, M.B., Darne, C., Fife, C.E., Guilloid, R., Maus, E.A., King, P.D., and E.M. Sevick-Muraca, "Evidence for SH domain-containing 5'-inosotil phosphatase-2 (SHIP2) contributing to a lymphatic dysfunction," *Plos One,* 10;9(11):e112548, 2014. PMID: 25383712.

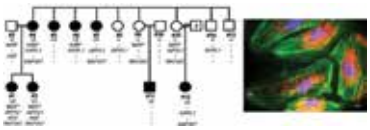
Tan, I.C., Balaguru, D., Rasmussen, J.C., Guilliod, R., Bricker, J.T., Douglas, W.I., and E.M. Sevick-Muraca, "Investigational lymphatic imaging at the bedside in a pediatric postoperative chylothorax patient," *Pediatric Cardiology,* 2014, PMID: 24972649

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

E.M. Sevick-Muraca, "Translation of near-infrared fluorescence imaging technologies: emerging clinical applications," *Ann Rev Med,* 63: 217-31, 2012, Epub 2011 Oct 27. PMID: 22034868



Routes of administration that result in delivery to the lymphatic vessels and lymph nodes. Reproduced from Sevick-Muraca, *et al.,* JCI, 2014.



(LEFT) Pedigree of family harboring inheritable non-syndromic lymphedema in which near-infrared fluorescence lymphatic imaging was used to accurately phenotype the family allowing discovery of candidate causative genetic mutations from next generation sequencing. (RIGHT) The gene candidate of INPPL1 was found from proximity ligation assay to associate with VEGFR3 a known growth factor expressed in lymphatic endothelial cells (INPPL1/VEGRF3 complex denoted in red) further evidencing biological role of INPPL1 mutation in the inheritable lymphatic condition.

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

Imaging in immunology

I bring a combination of expertise in translational science and immunology to lead the program of imaging of the lymphatics, the circulatory system which is critical to immune surveillance and response. Near-infrared fluorescence (NIRF) imaging delivers high-resolution, low-cost images of lymphatic vessel architecture and pumping. In disease states such as lymphedema, manifested by severe limb swelling, NIRF imaging can provide information for diagnosis and evaluation of treatment efficacy. Our translational team's study of NIRF images of breast cancer-related lymphedema arms revealed that the severity of the disease worsens over time not only in the "affected" arms (that received surgical and/or radiological treatment associated with breast cancer treatment), but also in the contralateral ("unaffected") arms. This work added evidence to other studies suggesting that lymphedema is a systemic, not just local, disease. Our lab has also worked in NIRF imaging studies of primary, or genetic, lymphedema and rare fat-associated genetic disorders with lymphatic abnormalities. We recently identified several previously unknown causative mutations for lymphedema in families with members suffering from the disease.

Reimbursement from Medicare and medical insurance companies for many clinical procedures is under scrutiny, as the health care system tries to make the best use of health care dollars. We recently surveyed different types of lymphedema patients, using NIRF imaging, to provide visual proof that one such procedure, pneumatic compression therapy, is effective for moving stagnant lymph. Similar NIRF studies by our group have now shown that this therapy is also efficacious for treating venous insufficiency and chronic wounds.

I am formally and practically trained in translation requirements. Understanding concepts such as validation of imaging devices and batch release of imaging agents enables researchers to discern which types of laboratory tests are necessary for moving a medical device or drug into the clinic. Working with research groups from several other institutions, I served as the leader of the NCI Network for Translational Research Validation and Clinical Studies Core that authored a consensus paper and a book chapter describing some of the translation efforts needed for validation of optical imaging devices and molecular imaging agents. This group was part of an effort by NCI to promote sharing and dissemination of translation practices amongst researchers. In addition, I published work describing processes for assessing parameters for which there was previously no FDA guidance available.

In addition to translational work, I am active in basic science investigations that employ the technologies I work to translate. I have investigated the effects of inflammation on lymphatic function in mice, and found that cytokines act as systemic mediators of lymphatic pumping through iNOS-associated mechanisms. Work by other groups has shown that inflammatory cytokines affect lymphatic function, but this study was the first to show that the effects are systemic, and defines a role for inflammation in some lymphatic diseases. We are now extending this work to study the role of lymphatic pumping dysfunction in rheumatoid arthritis and other inflammatory diseases.

RESEARCH PROJECTS

- Clinical studies of NIRF imaging of lymphatic architecture and function in health and disease
- Validation in the context of translation
- Inflammatory cytokine effects on systemic lymphatic function

KEY PUBLICATIONS

Aldrich MB, Sevick-Muraca EM. Cytokines are systemic effectors of lymphatic function in inflammation. 2013. *Cytokine* 64:362-9.

Agollah GD, Gonzales-Garay ML, Rasmussen JC, Tan IC, Aldrich MB, Darne C, Fife CE, Guilliod R, Maus EA, King PD, Sevick-Muraca EM. Evidence for SH2 domain-containing 5' inositol phosphatase-2 (SHIP2) contributing to a lymphatic dysfunction. 2014. *PLoS One* 9:e112548.

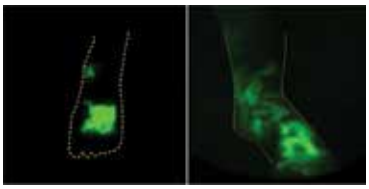
Aldrich MB, Guilliod RG, Fife CE, Maus EA, Smith L, Rasmussen JC, Sevick-Muraca EM. Lymphatic abnormalities in the normal contralateral arms of subjects with breast cancer-related lymphedema as assessed by near-infrared fluorescent imaging. 2012. *Biomedical Optics Express* 3:1256-65.

Aldrich MB, Marshall MV, Sevick-Muraca EM, Lanza G, Kotyk J, Culver J, Wang LV, Uddin J, Crews BC, Marnett LJ, Liao JC, Contag C, Crawford JM, Wang K, Reisdorph B, Appelman H, Turgeon KD, Meyer C, Wang T. Seeing it through: translational validation of new medical imaging modalities. 2012. *Biomedical Optics Express* 3(4):764-776.

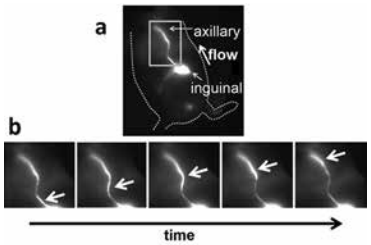
Aldrich MB, Sanders D, Lapteva N, Huang, XF, and Chen SY. SOCS1 downregulation in dendritic cells promotes memory T cell responses. 2008. *Vaccine* 26(8):1128-35.

LAB MEMBERS

Graduate students: co-advised Cynthia Davies-Venn, Pier-Anne Lachance



Foot lymphatic vessel usage in a lymphedema patient before (left) and after (right) pneumatic compression therapy



(a) lateral NIRF image of mouse lymphatics, (b) pulsative movement of lymph bolus through lymphatic collector

Ali Azhdarinia, Ph.D.
Assistant Professor

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 new bone formation techniques. Common to each project is our focus on rapid translation of discoveries and technologies into the clinic to improve human health.

RESEARCH PROJECTS

- Application of a novel chemistry platform for imaging and therapy
- Development of contrast agents for intraoperative imaging
- Tumor-specific chemotherapy
- Characterization of imaging probes targeting cancer and other diseases

KEY PUBLICATIONS

Hall, M.A., Pinkston, K.L., Wilganowski, N., Robinson, H., Ghosh, P., Azhdarinia, A., Vasquez-Arreguin, K., Kolonin, A.M., Chan, W., Harvey, B.R., and Sevick-Muraca, E.M. Comparison of mAbs targeting EpCAM for detection of prostate cancer lymph node metastases with multimodal contrast: NIRF imaging and quantitative μPET/CT. *J Nucl Med.*53(9):1427-37, 2012. PMID:22872743.

Ghosh, S.C., Ghosh, P., Wilganowski, N., Robinson, H., Hall, M.A., Dickinson, G., Harvey, B., Sevick-Muraca, E.M., and Azhdarinia, A. A Multimodal Chelation Platform for Near-infrared Fluorescence/Nuclear Imaging. *J Med Chem.* 56(2):406-16, 2013. PMID:23214723.

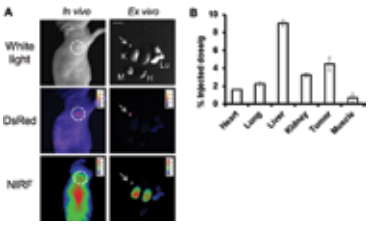
Azhdarinia, A., Daquinag, A.C., Tseng, C., Ghosh, S.C., Ghosh, P., Amaya-Manzanares F., Sevick-Muraca, E.M., Kolonin, M.G. Probes for targeted brown adipose tissue imaging. *Nat Commun.* 4:2472, 2013. PMID: 24045463

Cisneros, B.T., Matson, M.L., Law, J.L., Azhdarinia, A., Sevick-Muraca, E.M., Wilson, L.J. Stable confinement of PET & MR agents within carbon nanotube capsules for in vivo bimodal imaging. *Nanomedicine* (Lond). 9(16):2499-509, 2014. PMID:24628687.

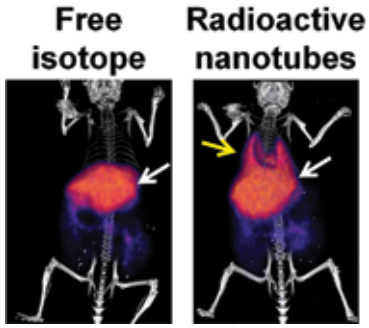
Ghosh, S.C., Pinkston, K.L., Robinson, H., Harvey, B.R., Wilganowski, N., Gore, K., Sevick-Muraca, E.M., Azhdarinia, A. Comparison of DOTA and NODAGA as Chelators for ^{64}Cu-labeled Immunoconjugates. *Nuc Med Biol.* 42(2):177-83, 2015. PMID:25457653.

LAB MEMBERS

Research Scientist: Sukhen Ghosh



Mice were implanted with prostate tumor cells and used to study the effectiveness of a contrast agent possessing radioactive and near-infrared fluorescent labels. After injection of the tumor-specific imaging agent, we observed excellent tumor visualization via whole-body imaging (A) and found that quantification of excised tissues (B) correlated with imaging findings. Arrow indicates excised tumor. K = kidney, Lu = lung, H = heart, M = muscle. (from Ghosh, S.C. et al., *J Med Chem,* 56(2):406-16, 2013).



Images of normal mice injected free isotope (^{64}Cu) and radiolabeled carbon nanotubes. Liver uptake (white arrow) is observed in both images, whereas lung uptake is only apparent after encapsulation of isotope within the carbon nanotubes. (adapted from Cisneros et al., *Nanomedicine,* 9(16):2499-509, 2014).

Barrett Rowland Harvey, Ph.D.
Assistant Professor

Therapeutic and diagnostic antibody development

Technological achievements in antibody engineering have made antibody drug development one of the fastest growing areas of the pharmaceutical industry. Successful design of antibody-based therapeutics or diagnostics requires both the ability to optimize the antibody and a clear understanding of the biology of the target antigen. To this end, our laboratory has two main focuses: 1) To identify and build a functional understanding of novel molecular targets, often utilizing custom antibodies as powerful tools to expedite the research and 2) to develop high throughput strategies and engineering methods to modify the affinity, specificity, epitope site recognition and Fc function of antibodies for therapeutic, diagnostic, and basic research use. Utilizing molecular imaging techniques, antibody agent development can be monitored using *in vivo* models to predict efficacy, specificity, and to validate targets prior to the clinic. This line of research allows our laboratory to venture into a number of diverse biological fields, with ongoing projects currently focused in oncology and infectious disease.

RESEARCH PROJECTS

- Generation of surrogate antibodies for metastatic cancer models
- Molecular imaging for cancer staging
- Virulence factor regulation governing enterococcal infection
- Passive protection from hospital acquired bacterial infection

KEY PUBLICATIONS

Gao P, Pinkston KL, Wilganowski N, Robinson H ,Azhdarinia A, Zhu B, Sevick EM, Harvey BR."Deglycosylation of mAb by EndoS for improved molecular imaging. Molecular Imaging and Biology." *Mol Imaging Biol*. 2015 Apr;17(2):195-203. PMID: 25135058

Pinkston, KL, Singh KV, Gao P, Wilganowski N, Robinson H, Ghosh SC, Azhdarinia A, Sevick-Muraca EM, Murray BE, Harvey, BR. "Targeting Pili in Enterococcal Pathogenesis" *Infection and Immunity,* 2014 Apr;82(4):1540-7. PMID: 24452680. (Featured article on April 2014 Cover).

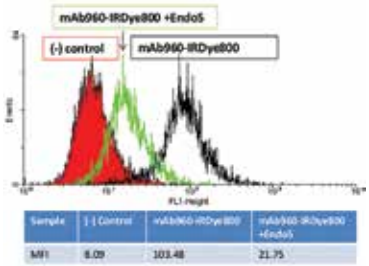
Gao P, Pinkston KL, Bourgogne A, Cruz MR, Garsin DA, Murray BE, Harvey BR. "Library Screen identifies Enterococcus faecalis CcpA, the Catabolite Control Protein A, as an Effector of Ace, A Collagen Adhesion Protein Linked to Virulence" *Journal of Bacteriology* 2013, Oct;195(20):4761-8. PMID: 23974022.

Hall, M.A.*, Pinkston, K.L.*, Wilganowski, N., Robinson, H., Ghosh, P., Azhdarinia, A., Vazquez-Arreguin, K., Kolonin, A.M., Harvey, B.R.*, Sevick-Muraca, E.M.*, "Comparison of mAbs targeting EpCAM for detection of prostate cancer lymph node metastases with multimodal contrast: NIRF imaging and quantitative μPET/CT," *J Nuc Med,* 2012. Sep;53(9):1427-37 PMID: 22872743

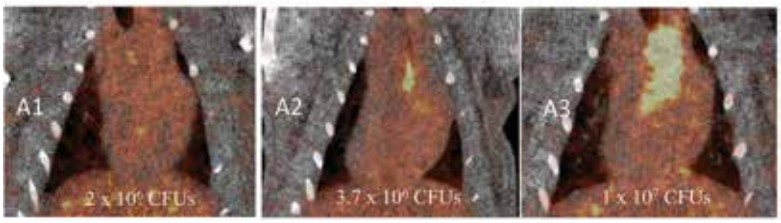
Pinkston KL*, Gao P*, Diaz-Garcia D, Sillanpää J, Nallapareddy SR, Murray BE, and Harvey BR. "The Fsr quorum-sensing system of Enterococcus faecalis modulates surface display of the collagen-binding MSCRAMM Ace through regulation of gelE." *Journal of Bacteriology,* Sep;193(17):4317-25 2011. PMID: 21705589

LAB MEMBERS

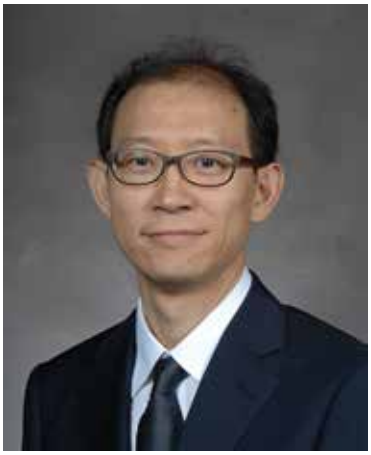
Research Instructor: Dr. Peng Gao
Research Coordinator II: Kenneth L. Pinkston
Graduate Student: Emily Stinemetz



Modification of monoclonal antibody to decrease unwanted interactions with immune cells. EndoS treatment (deglycosylation) of mAb960 inhibits binding to macrophage as analyzed by flow cytometry analysis. Preventing unwanted antibody interactions decreases background labeling when antibodies are utilized as imaging agents (as in Figure 1).



Molecularly targeted live animal imaging of heart infection for diagnostic use. PET/CT image of enterococcal endocarditis (bacterial infection of the heart valve or inner lining) in live rats (A1-A3) imaged at 72 h post infection using Cu64-DOTA labeled mAb targeting the bacterial surface. Signal increased (yellow intensity) as infection grew in size (as measured by colony forming units (CFUs)).



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 in unique animal models of vascular disease, with an emerging emphasis of gastrointestinal disease. My main research interest focuses on investigating the microcirculatory movement of fluid and macromolecules, particularly in the lymphatic system using fluorescence optical imaging techniques. The lymphatic system plays an important role in edema prevention, immune surveillance, and cancer metastasis. Although the importance of the lymphatic system in physiological and pathophysiological conditions has been well recognized, non-invasive imaging of lymphatic function has significant difficulties, due to the lack of diagnostic imaging approaches. Recently, we have developed non-invasive, dynamic near-infrared fluorescence (NIRF) imaging methods for imaging and quantifying lymphatic function in health and disease. Therefore, non-invasive NIRF imaging can be used to image changes of lymphatic function and architecture in disease and potentially to provide diagnostics and information in response to therapy.

Other directions of my scientific interests revolve around multi-modality molecular imaging. The Center for Molecular Imaging is developing and translating imaging agents, which are dual-labeled with a PET/SPECT radiotracer and a NIR fluorescent dye. We are currently conducting molecular imaging of cancer and LN metastasis and inflammation in different animal models of disease.

RESEARCH PROJECTS

- Non-invasive characterization of lymphatic function and drainage patterns in mice with lymphedema-like phenotypes, hypertension, cancer, and inflammation and tracking response to therapeutic agents
- Non-invasive imaging of gastrointestinal motility using a fluorescence optical imaging technique
- Multi-modal molecular imaging

KEY PUBLICATIONS

P.E. Burrows, M. L. Gonzalez-Garay, J.C. Rasmussen, M. B. Aldrich R. Guilliod, E. A .Maus, C. E. Fife, S. Kwon, P. E. Lapinski, P. D. King, and E. M. Sevick-Muraca, "Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man," *Proceedings of the National Academy of Sciences*, 110; 8621-8626, 2013.

E. M. Sevick-Muraca, S. Kwon, and J. Rasmussen, "Emerging Lymphatic Imaging Technologies for Mouse and Man," *Journal of Clinical Investigation.* 124; 905-914, 2013.

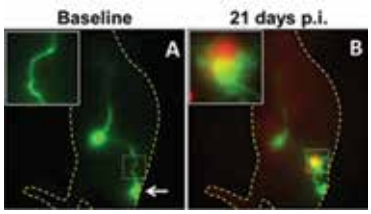
S. Kwon, D. A. Germaine, G. Wu, and E. M. Sevick-Muraca, "Spatio-temporal changes of lymphatic contractility and drainage patterns following lymphadenectomy in mice," *PLOS One.* 9; e106034, 2014.

D. A. Germaine, G. Wu, E. M. Sevick-Muraca, and S. Kwon, "In vivo lymphatic imaging of a human inflammatory breast cancer model," *Journal of Cancer.* 5; 774-783, 2014.

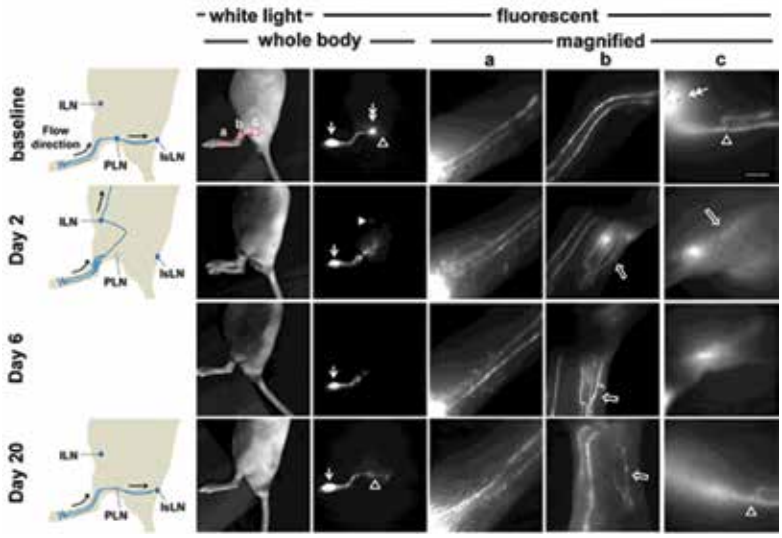
D. A. Germaine, G. Wu, H. Peng, and S. Kwon, "Dextran sulfate sodium-induced acute colitis impairs dermal lymphatic function in mice," *World Journal of Gastroenterology.* Accepted for publication, 2015.

LAB MEMBERS

Research Coordinator: Grace Wu



Images of the effect of inflammatory breast carcinoma (IBC) on lymphatic structure. (A) A NIRF image of the lymphatics prior to inoculation of IBC cells transfected with the iRFP gene reporter. (B) An overlay of a NIRF image (green) of the lymphatics over a non-invasive iRFP image of IBC (red).



At 2 days after PLN removal, alternate drainage pathways to the ILN were detected due to increased flow resistance. However, once lymphatic continuity was restored, lymphatic vessels to the ischial LN (IsLN) through the site of PLN removal were visualized at day 20.

John Rasmussen, Ph.D.
Assistant Professor

Device translation for lymphatic imaging

I am the faculty lead of the instrumentation for translational fluorescence imaging. Traditional clinical imaging modalities, such as scintigraphy, X-ray, MRI, and ultrasound, lack the spatial and/or temporal resolutions needed to resolve fine lymphatic architecture and contractile function and/or require quantities of contrast agent not easily introduced into the lymphatics. Over the past few years, my research interest 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 fluorescent contrast agent.

My work focuses upon the development of NIRF imaging methodologies and its application to answer new biological and clinical questions not addressed by other technologies. Specifically, our program focuses upon using NIRF imaging in translational clinical studies with partners across the Houston area to (i) study the growth and reorganization of the lymphatics, termed lymphangiogenesis, (ii) elucidate the role of the lymphatics in the development of lymphovascular diseases, such as lymphedema and cancer metastasis as well as in rare adipose disorders and chronic peripheral vascular diseases that may have a lymphovascular component, and (iii) identifying the lymphatic phenotype of genetic mutations that contribute to lymphatic disorders. My expertise involves the application of NIRF imaging instrumentation and development of software for clinical applications. Specific projects focus on the development of analytical tools to facilitate lymphatic image processing and analysis.

RESEARCH PROJECTS

- Nodal staging of cancer using noninvasive NIRF imaging
- Etiology of cancer-related lymphedema
- Lymphatic involvement in peripheral vascular disease
- Identification of genetic causes for lymphovascular diseases
- Development of automated NIRF image analytical algorithms
- Application driven enhancement of NIRF imaging systems

KEY PUBLICATIONS

Rasmussen, J.C., Aldrich, M.B., Tan, I-C., Darne, C.D., Zhu, B., O'Donnell, T.F., Fife, C.E., and Sevick-Muraca, E.M., "Lymphatic transport in patients with chronic venous insufficiency and venous stasis leg ulcers following sequential pneumatic compression," *Journal of Vascular Surgery: Venous and Lymphatic Disorders,* In Press, http://dx.doi.org/10.1016/j.jvsv.2015.06.001; epub 7/16/15.

Shaitelman, S.F., Cromwell, K.D., Rasmussen, J.C., Stout, N.L., Armer, J.M., Lasinski, B.B., and Cormier, J.N., "Recent Progress in Cancer-Related Lymphedema Treatment and Prevention," *CA: A Cancer Journal for Clinicians,* 65(1):55-81, 2015.

Rasmussen, J.C., Herbst, K.L., Aldrich, M.B., Darne, C.D., Tan, I-C., Zhu, B., Guilliod, R., Fife, C.E., Maus, E.A., Sevick-Muraca, E.M., "An abnormal lymphatic phenotype is associated with subcutaneous adipose tissue deposits in Dercum's disease," *Obesity,* 22(10): 2186-2192, 2014.

Rasmussen, J.C., Burrows, P.E., Gonzalez-Garay, M.L., Aldrich, M.B., Guilliod, R., Maus, E.A., Fife, C.E., Kwon, S., Lapinski, P.E., King, P.D., and E.M. Sevick-Muraca, "Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man," *Proceedings of the National Academy of Sciences,* 110(21):8621-8626, 2013.

Rasmussen, J.C., Tan, I., Marshall, M.V., Adams, K.A., Kwon, S., Fife, C.E., Maus, E.A., Smith, L., Covington, K.R., and E.M. Sevick-Muraca, "Human lymphatic architecture and (dys)function imaged using NIR fluorescence," *Translational Oncology,* 3(6):362-372, 2010.

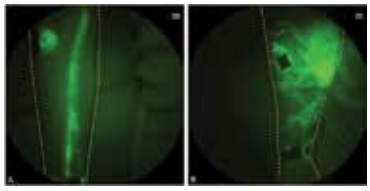
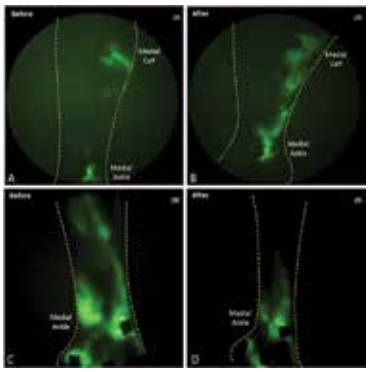


Image of (A) the well-defined, linear lymphatics in the arm of a healthy subject and (B) the disorganized, dermal lymphatics in the arm of a subject with lymphedema.



Images illustrating the effect of a single session of sequential pneumatic compression (SPC) therapy on the ulcer draining lymphatics in subjects with chronic venous insufficiency. In some subjects, NIRF images acquired (A) before and (B) after treatment indicated that additional lymphatics had been recruited to aid lymphatic drainage while, in other subjects, NIRF images acquired (C) before and (D) after treatment illustrated the emptying of wound draining lymphatic vessels following SPC treatment.

Banghe Zhu, Ph.D.
Assistant Professor

Optical imaging, instrumentation and working standards

My research program focuses upon the development of optical imaging instrumentation for preclinical and clinical studies and the establishment of traceable working standards with SI units to accelerate the translation of optical imaging into the clinic. Specifically, we are using far-red fluorescence gene reporter, iRFP, (i) to longitudinally and non-invasively track the *in vivo* process of lymphatic metastases from an orthotopic site of mammary implantation through lymphatic vessels and to draining lymph nodes, and (ii) to evaluate the therapeutic for bone formation by injection of the osteoprogenitor cells that are transduced with the inducible caspase 9 system and an Ad5-iRFP and Ad5-BMP-2 vector. In addition, we are developing and deploying a methodology to calibrate a stable, a solid phantom for fluorescent irradiance for use in charactering the measurement sensitivity of fluorescence molecular imaging devices.

RESEARCH PROJECTS

- Developing optical imaging instrumentation for medical applications
- Tracking cancer metastasis using iRFP gene reporter
- Evaluating cell-based gene therapy for targeted bone formation
- Collaborating with NIST to develop working standards for optical medical imaging

KEY PUBLICATIONS

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)

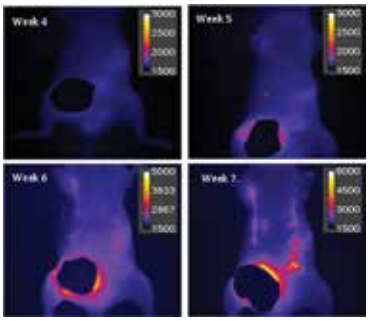
Zhu, B., Rasmussen, J. C., and Sevick-Muraca, E. M., "A matter of collection and detection for intraoperative and non-invasive near-infrared fluorescence molecular imaging: to see or not to see?", *Medical Physics,* 41, 022105 (2014).

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

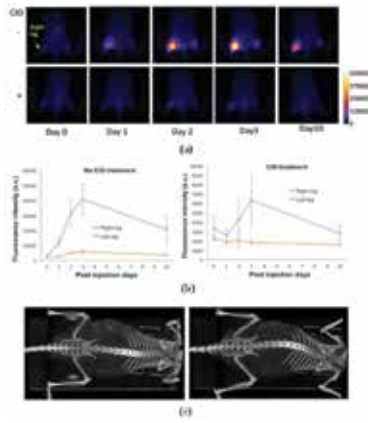
Zhu, B., Wu, G., Robinson, H., Wilganowski, N., Hall, M. A., Ghosh, S. C., Pinkston, K. L., Azhdarinia, A., Harvey, B. R., and Sevick-Muraca, E. M., "Tumor Margin Detection using Quantitative, NIRF Molecular Imaging Targeting EpCAM Validated by Far-Red Gene Reporter iRFP", *Molecular Imaging and Biology,* 15:560-568, (2013).

LAB MEMBERS

Co-advised: Grace Wu, Julie Voss



Longitudinal iRFP fluorescence imaging of cancer metastasis in preclinical models, indicating the right and left lymphatic channels (arrows) draining cells from the right fourth mammary gland by weeks 6 and 7.



(a) iRFP fluorescence imaging of bone formation over time, (b) Mean iRFP fluorescence intensity, and (c) Radiographical analysis in mice treated with a chemical inducer of dimerization (CID) or not.

Biology connects campus-wide and state-wide research efforts in systems biology, clinical and translational sciences, nanomedicine, protein chemistry, genomics, proteomics, and bioinformatics by bringing people together to promote intellectual exchange in these key fields.

While genomics can successfully catalog genetic variants, nearly all drugs on the market today target their functional protein products. Gene sequences give us starting points, but most cellular proteins are extensively processed and modified. To understand cellular regulation and disease mechanisms, or to identify drug targets, we need detailed characterization of proteins that now are achievable through mass spectrometry and other proteomic technologies.

One mission of the Center for Proteomics and Systems Biology (CPSB) is to develop the experimental and analytical technologies that will make this a reality. The CPSB will not only develop new technologies but also will provide a coordinated group of centers and programs for collaborative and service work for the UTHeath community in cutting-edge proteomics, protein chemistry, nanomedicine and systems biology research.

The Mass Spectrometry Facility is located in the IMM and houses seven state-of-the-art mass spectrometers that allow the identification and quantification of peptides, proteins, and small molecule drugs for in-depth proteomic and metabolomics analysis of cells, tissues, or biological fluids. These proteins then serve as targets for drug development and nanomedicine therapeutics and imaging agents, including next-generation X-aptamer reagents.

Hubs of Research Collaboration with the Center include:

- Protein Chemistry
- Proteomics and Therapeutic Drug Monitoring
- Systems Biology and UTHealth Bioinformatics Core Laboratory
- Clinical and Translational Proteomics Core Laboratory
- CLIA ProteoPath Molecular Diagnostics Laboratory
- NCI Programs in Cancer Computational Biology and Nanomedicine
- UT System-wide Proteomics Core Facility Network
- UTHealth / MDACC Clinical and Translational Center for Translational Technologies

David Gorenstein, Ph.D.
Professor, Center Director, & Deputy Director
James T. Willerson Distinguished Chair in Cardiovascular Research in Tribute from the Ewing Halsell Foundation

David Gorenstein, Ph.D.

Associate Dean for Research
Chair, Department of NanoMedicine and Biomedical Engineering
Professor and Director of the Center for Proteomics and Systems Biology
James T. Willerson Distinguished Chair in Cardiovascular Research in Tribute from the Ewing Halsell Foundation

NanoMedicine and proteomics in cancer and cardiovasculature disease

We have developed novel, next-generation modified DNA oligonucleotide aptamers selected from large combinatorial libraries to target a number of proteins for proteomics and nanomedicine. We have developed both *in vitro* enzymatic combinatorial selection and split-synthesis chemical combinatorial methods to identify phosphorothioate-modified oligonucleotide "thioaptamers" and next-gen "X"-aptamers to a number of different protein targets. The X-aptamers also include a large range of chemical (X) modifications to the 5-X-dU position and thus represent a hybrid of aptamer backbone, protein amino acid-like sidechains, and small molecule leads in a self-folding scaffold that can be readily identified by oligonucleotide sequencing. Compared to conventional aptamers, this approach dramatically expands the chemical diversity that can be incorporated to select X-aptamers with high affinity for diverse molecular biomarkers. Large bead-based combinatorial libraries of these aptamers can be rapidly selected. These X-aptamers and thioaptamers are being used as antibody substitutes in nanomedicine therapeutics and biomarker identification to tumor cells and tumor vasculature and in various microfluidics and mass spec chips for proteomics and diagnostics. Examples of application of the bead-based thioaptamer and X-aptamer selection are demonstrated for targeting cancer tissue and cells expressing CD44, Annexin A2 and E-Selectin.

RESEARCH PROJECTS

- Next-generation aptamer development for drug development
- Proteomics and molecular diagnostics
- Nanomedicine targeting in cancer and cardiovascular disease
- Development of novel X-aptamer targeting nanoparticles for imaging and therapeutics

KEY PUBLICATIONS

Xianbin Yang, Li Na, David G. Gorenstein, Strategies for the discovery of therapeutic aptamers, Expert Opinion in Drug Discovery, Volume 6, Number 1, January 2011, pp. 75-87(13). PMID: 21359096; *PMCID:* PMC3045091. doi: 10.1517/17460441.2011.537321

Weiguo He, Miguel-Angel Elizondo-Riojas, Xin Li, Ganesh Lakshmana Rao Lokesh, Anoma Somasunderam, Varatharasa Thiviyanathan, David E. Volk, Ross H. Durland, Johnnie Englehardt, Claudio N. Cavasotto, and David G. Gorenstein "X-Aptamers: A bead-based selection method for random incorporation of drug-like moieties onto next-generation aptamers for enhanced binding" *Biochemistry,* 2012 DOI:10.1021/bi300471d. (Front page cover).

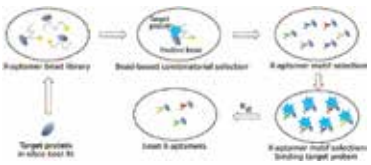
Mai J, Huang Y, Mu C, Zhang G, Xu R, Guo X, Xia X, Volk DE, Lokesh GL, Thiviyanathan V, Gorenstein DG, Liu X, Ferrari, M., and Shen, H. Bone marrow endothelium-targeted therapeutics for metastatic breast cancer. *J Control Release* 187, 22-29 (2014). doi: 10.1016/j.jconrel.2014.04.057. Epub 2014 May 10. PubMed PMID: 24818768; PubMed Central PMCID: PMC4109393.

Shin-Ae Kang, Nafis Hasan, Aman P. Mann, Wei Zheng, Lichao Zhao, Daniel Zhao, K. Stephen Suh, David G. Gorenstein, Massimo Cristofanilli, Hallgeir Rui, Takemi Tanaka, Blocking the Pre-metastatic Niche for the Prevention of Breast Cancer Metastasis, *Molecular Therapy,* 28 April 2015; doi: 10.1038/mt.2015.45

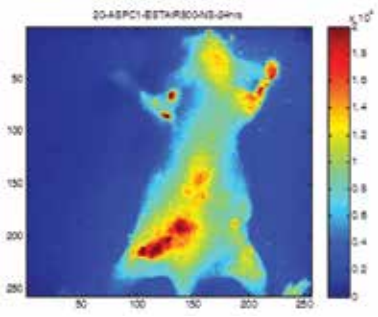
Levani Zandarashvili, Dan Nguyen, Kurtis M. Anderson, Mark A. White, David G. Gorenstein, and Junji Iwahara, "Entropic enhancement of protein-DNA affinity by oxygen-to-sulfur substitution of DNA phosphate," *Biophysical Journal,* 108, 1026-37 (2015).

LAB MEMBERS

Research Scientists: Lokesh Rao, PhD, Li Li, PhD
Research Associate: Xin Li, MS



Schematic for selection of Next-generation X-aptamers in which small molecule hits are randomly covalently bound to a combinatorial aptamer bead library.



Targeting of gold nanoshell nanoparticles with a Near-Infrared-Imaging-dye labeled thioaptamer to E-selectiin. This is a pancreatic tumor xenograft mouse model.

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 mineralization and composition, which are important determinants of bone strength. 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 method is in development to test on animal model and patients based on the above findings.

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

- Noninvasive optical diagnosis *in situ* (IBD, cancer, etc)
- Development of noninvasive transcutaneous Raman measurement (SORS)
- Assessment of metastasis and disease caused bone quality deterioration
- Biomarkers and circulating tumor cells detection
- Raman imaging for pathogenesis

KEY PUBLICATIONS

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 Jul 20, In press (2015)

Z. Wang, H. Ding, G. Lu, and X. Bi., Reverse-time migration based optical imaging, 2015, IEEE *Transactions on Medical Imaging,* 2015 Aug 18, In press (2015).

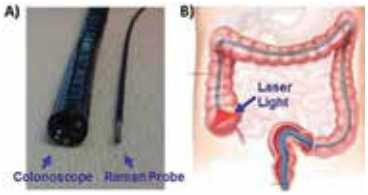
H. Ding, J.S. Nyman, J.A. Sterling, D.S. Perrien, A. Mahadevan-Jasen, and X. Bi, Development of Raman Spectral Markers to Assess Metastatic Bone in Breast Cancer, *Journal of Biomedical Optics,* 19(11): 111606 (2014)

Z. Wang, H. Ding, G. Lu, and X. Bi, Use of a mechanical iris based fiber optic probe for the spatially offset Raman spectroscopy, *Optics Letter,* 39(13):3790-3 (2014)

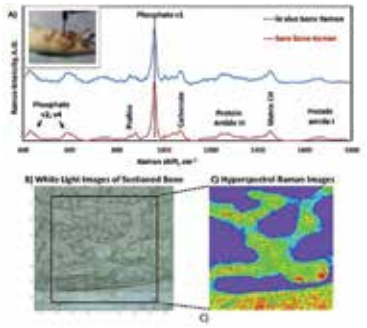
X. Bi, J.A. Sterling, A.R. Merkel, D.S. Perrien, J.S. Nyman, A. Mahadevan-Jansen, Prostate cancer metastases alter bone mineral and matrix composition independent of effects on bone architecture in mice – A quantitative study using microCT and Raman spectroscopy. *Bone,* 56(2):454-60 (2013)

LAB MEMBERS

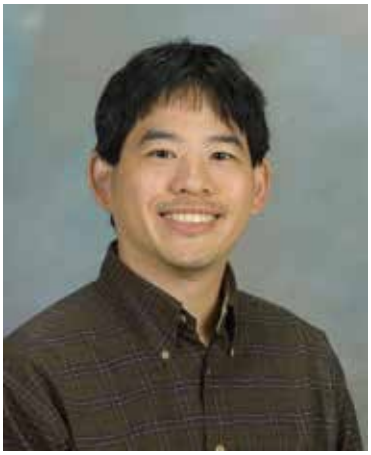
Post-doctoral Fellow: Hao Ding
Technician: Guijin Lu



A) The end of the Raman fiber optic probe (right) that can be inserted through the biopsy channel of a clinical colonoscope (left) for non-invasive detection of tissue composition. B) A scheme of the Raman spectroscopy integrated colonoscopy.



A) Bone spectrum from in vivo transcutaneous measurement (blue) is in great agreement with that from excised bone (red), proving the potential of *in vivo* Raman for noninvasive bone quality evaluation. Raman imaging (C) on sectioned bone specimen provides spatial information of biochemical components in bone (mineral and collagen), and correlates with the white light images (B).



Jeffrey Chang, Ph.D.
Assistant Professor

Genomic approaches for cancer therapies

Our lab deciphers the complexity of the cancer phenotype using genomics. Briefly, receptors in the cell membrane initiate cascades of reactions (pathways) that ultimately change the expression of genes. While cellular pathways are often thought of as independent and linear entities, the reality is that there is significant crosstalk among them. Indeed, the dense interconnections among signaling molecules exhibit a network structure. The complexity of the cell signaling network provides it the capacity to produce organisms like ourselves (a good thing) as well as diseases that are difficult to manage (a bad thing). Therefore, a challenge is to explain how the network operates in normal circumstances, and how it is rewired in disease. Specifically, we wish to understand how the signaling programs become altered in cancer and drive uncontrolled cell proliferation and metastasis.

Our research program can be grouped into three areas of focus:

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. Growth signaling networks. We are dissecting the structure of signaling cascades, focusing on the Ras network. Ras controls numerous tumorigenic processes through multiple downstream effectors. To better understand the structure of Ras signaling, we are developing strategies to dissect Ras activities into discrete sub-components called modules, represented by gene expression profiles. We have previously shown that these modules link to disease. We now wish to identify the genes that drive each module, and investigate how they may form the basis of a rational strategy for selecting clinical treatments.

3. Computational tools for genomic analysis. Lastly, we are developing infrastructure to distribute our computational algorithms. Each of our projects contains a computational component, and an important aspect of our work is to make our methods available. We have previously developed the GATHER website for analysis of gene sets, and are now developing a platform SIGNATURE for the analysis of oncogenic pathways.

Across our investigations, we use genomics to reveal the simple fundamental units that constitute complex biological phenotypes (such as the workings of a cancer cell). We use human cell culture as a model and leverage a range of techniques including bioinformatics, molecular biology, and biochemistry.

RESEARCH PROJECTS

- Cancer metastasis, cancer stem cells, and the epithelial-to-mesenchymal transition
- Genetic perturbations of Ras signaling
- Transcriptional regulatory programs of E2F1-driven apoptosis
- Intelligent computational pipelines for bioinformatic analysis

KEY PUBLICATIONS

Soundararajan R, Paranjape AN, Barsan V, Chang JT*, and Mani SA*. A novel embryonic plasticity gene signature that predicts metastatic competence and clinical outcome. *Scientific Reports*, 2015. * Co-Corresponding Authors

Chang JT* and Mani SA*. Sheep, Wolf, or Werewolf: Cancer Stem Cells and the Epithelial-to-Mesenchymal Transition. *Cancer Letters*, 2013. * Co-Corresponding Authors

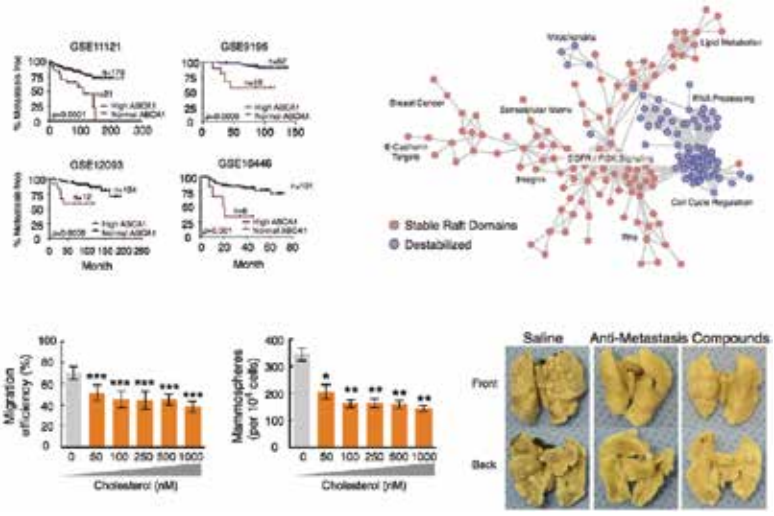
Chang JT. Deriving transcriptional programs and functional processes from gene expression databases. *Bioinformatics* 28(8), 2012.

Chang JT, Carvalho C, Mori S, Bild AH, Gatza M, Wang Q, Lucas J, Potti A, Febbo P, West M, and Nevins JR. A Genomic Strategy to Elucidate Modules of Oncogenic Pathway Signaling Networks. *Molecular Cell* 34(1): 104-114, 2009.

Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, Olson JA, Marks JR, Dressman HK, West M, and Nevins JR. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 439(7074): 353-357, 2005.

LAB MEMBERS

Post-doctoral Fellows: Weina Zhao, Ph.D., Sarah Prjic, Ph.D.,
Graduate Student: Kevin Zhu



We have discovered that cholesterol plays a key role in cancer metastasis. We have identified compounds that can alter cholesterol in cancer cells and have found in preclinical studies that they can inhibit metastasis in mice.

Vittorio Cristini, Ph.D.
Professor

Translational modeling of cancer treatment

Our group primarily focuses on developing mechanistic biophysical models for predicting tumor response to various treatment methods 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.

Multiscale methods. 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."

Coupled 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
- Upscaling and downscaling framework (i.e., functionally linking biological behaviors at different scales)
- Spatiotemporal drug pharmacokinetics and pharmacodynamics (PKPD) models

KEY PUBLICATIONS

V. Cristini, E.J. Koay, Z. Wang. Taking cancer out of the equation. *International Innovation.* 191:38-40.

Z. Wang, J.D. Butner, V. Cristini, T.S. Deisboeck. 2015. Integrated PK-PD and agent-based modeling in oncology. *Journal of pharmacokinetics and pharmacodynamics.* 42:179-189.

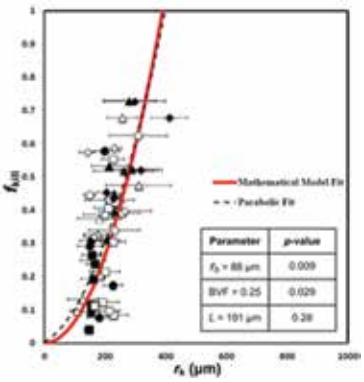
H.B. Frieboes, B.R. Smith, Z. Wang, M. Kotsuma, K. Ito, A. Day, B. Cahill, C. Flinders, S.M. Mumenthaler, P. Mallick, E. Simbawa, A.S. Al-Fhaid, S.R. Mahmoud, S.S. Gambhir, V. Cristini. 2015. Predictive Modeling of Drug Response in Non-Hodgkin's Lymphoma. *PloS one.* 10:e0129433.

Wang, Z., J.D. Butner, R. Kerketta, V. Cristini, T.S. Deisboeck. 2015. Simulating cancer growth with multiscale agent-based modeling. *Seminars in cancer biology.* 30:70-78.

E.J. Koay, F.E. Baio, A. Ondari, M.J. Truty, V. Cristini, R.M. Thomas, R. Chen, D. Chatterjee, Y. Kang, J. Zhang, L. Court, P.R. Bhosale, E.P. Tamm, A. Qayyum, C.H. Crane, M. Javle, M.H. Katz, V.N. Gottumukkala, M.A. Rozner, H. Shen, J.E. Lee, H. Wang, Y. Chen, W. Plunkett, J.L. Abbruzzese, R.A. Wolff, A. Maitra, M. Ferrari, G.R. Varadhachary, and J.B. Fleming. 2014. Intra-tumoral heterogeneity of gemcitabine delivery and mass transport in human pancreatic cancer. *Physical biology.* 11:065002.

LAB MEMBERS

Post-doctoral Fellow: Huaming Yan
Students: Joseph Butner (jointly with Dr. Wang), Prashant Dogra (jointly with Dr. Wang), Terisse Brocato (jointly with Dr. Wang)



A mechanistic model of cancer cell kill from chemotherapy (red line) describes heterogeneous response within human cancer (symbols, corresponding to 50 data points from 8 patients with colorectal cancer metastatic to liver). The model predicts the fraction of tumor f_{kill} killed by chemotherapy (i.e., pathologic response) as a function of parameters r_b (mean of the tumor vessel radii), BVF (blood volume fraction, i.e., perfusion), and L (diffusion penetration distance), which depend on cell biology. The x-axis represents a pathological surrogate for L. (Pascal *et al.*, PNAS 110 (35): 14266-71).

Philip Foster, M.D., Ph.D.
Assistant Professor

Innovative approach of the biology of oxygen (space-microgravity, cognition, nanomedicine, nucleic acids, neural and cancer stem cells)

One of our extraordinary scientific achievements from basic research to innovative human application was the success in hand-made assembly of the International Space Station in the most hostile environment that man ever had to face (spatial void, absence of gravity, extreme temperatures). During the preparation (O_2 prebreathe) for extra-vehicular activities (EVAs), the decompression from sea-level pressure to its third may lead to the presence of bona fide nano-, micronuclei of gases or microbubbles in blood, brain or other tissues forming and growing *in situ* by cavitation or tribonucleation. This extensive collaborative effort between NASA and several North American institutions led to products and procedures that were delivered to NASA, such as the decrease from 24-72 hrs EVA preparation down to two hours. Members of the team received several awards from NASA for those achievements. Non-invasive near infrared spectroscopy (NIRS) allowed observation of instantaneous variations of total, oxygenated and deoxygenated hemoglobin/myoglobin concentrations in microcirculatory networks of active limbs during the dynamic exercise that was used to for the successful two-hour O_2-prebreathe. Our conclusion was that besides N_2 tissue washout another unknown exercise-induced effect may have further enhanced the protection possibly mediated via the anti -inflammatory effect of exercise, gas micronuclei reduction, NO pathways, or other molecular mechanisms. Experience of microbubbles also leads to other applications. NanoMedicine, in a non-detrimental way, uses encapsulated gas microbubbles as drug-loaded liposomes to target tissues (tumors, ...); cavitation-induced of encapsulated microbubbles has an effect on drug release. New challenges will be to study the role of gases (O_2, CO_2) potentially generating stress on neuronal oxygen consumption (effects on cerebral circulation and vigilance), using functional MRI (fMRI), fNIRS, evaluate the individual susceptibility gene variants to anxiety and characterize some molecular mechanisms that regulate O_2-induced neurogenesis and their applications in neurodegenerative diseases (e.g. Alzheimer's). We are also looking at cerebral CO_2 levels and their influence on the relationship to cerebral-ophthalmic circulation by observing the on the cerebral hemodynamic response function using custom-made breathing systems, NIR scanning, and ophthalmic artery-transcranial Doppler. Another challenge is to determine specific X-*aptamers* specifically targeting cancer cells (ependymoma and lung cancer) with the goal to design specific treatments. An extension of the previous goal is also to study the O_2-dependent molecular mechanisms and other physical variables that regulate cancer cells such as the effects of hypoxia on tumorigenic cells.

RESEARCH PROJECTS

- Spatial environment (basic and operational research)
- Therapeutic targets for ependymoma and lung cancer
- Cognitive plasticity

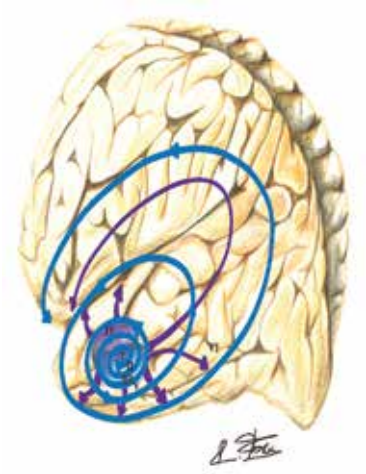
KEY PUBLICATIONS

Foster PP. Role of physical and mental training in brain network configuration. *Front Aging Neurosci.* 2015.

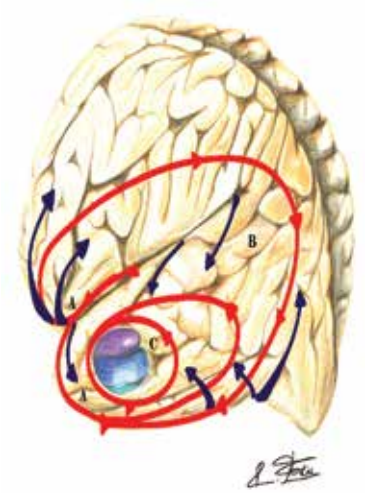
Foster PP. Mild traumatic brain injury early in life and reduced neural activity for memory processing later in midlife. *Front Aging Neurosci*. 2015.

Brobey R., ..., Foster PP, Makoto Kuro-o, and Rosenblatt, K.P. Klotho regulates 14-3-3zeta monomerizing and binding to ASK1 signaling complex in response to oxidative stress. *PlosOne.* 2015.

Brobey R., ..., Foster PP, Makoto Kuro-o, and Rosenblatt, K.P. Klotho protects dopaminergic neurons in vivo via ASK1 and p38 MAPK signaling pathways. *PlosOne.* 2015.



In Alzheimer's disease (AD), the outward progression of neurofibrillary lesions spreads from the entorhinal cortex toward the cortex (blue-purple spirals and arrows). Those lesions are crossing and blocking the path of long-distance white matter tracts.



The inward cortical myelination maturation of long-range white matter tracts is directed toward the entorhinal cortex (red spiral and purple arrows) and replicates in reversed order the neurofibrillary lesions of AD.

David Volk, Ph.D.
Assistant Professor

Targeting cancer with X-aptamers and nanoparticle conjugates

The focus of my lab is to develop novel cancer targeting agents using combinatorial, pseudo-random X-aptamer reagents that combine drugs or protein side-chains with DNA aptamers. These targeted reagents can provide directed delivery of anti-cancer medications to tumors while avoiding damage to other tissues. By conjugating them with nanoparticles, they offer the ability to provide for the slow release of anti-cancer drugs at the tumor, thereby further reducing unwanted collateral damage to remote tissue. We have developed several X-aptamers targeting E-selectin, CD44, and annexin A2, proteins that are over-expressed on the surface of tumors or tumor associated vasculature. In a recent publication (Mai et al. 2014), we showed that as part of a multistage vector ESTA1, our aptamer targeting E-selectin, directed anti-cancer siRNA to the bone marrow for the treatment of breast cancer metastasis, leading to significantly increased survival rates.

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 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. We also provide bioinformatics services through the UTHealth Bioinformatics Service Center for the analysis of biological data related to proteomics, metabonomics, and genetics.

RESEARCH PROJECTS

- Breast, ovarian and pancreatic tumor imaging and directed drug deliver
- Develop next-generation X-aptamers (DNA) for cancer targeting
- Develop novel software for the analysis of large data sets
- Provide biostatics and programming assistance

KEY PUBLICATIONS

Blocking the Adhesion Cascade at the Pre-metastatic Niche for Prevention of Breast Cancer Metastasis, Tanaka, T., Kang, S.-A., Hasan, N., Mann, A.P., Zheng, W., Zhao, L., Zhao, D., Suh, K.S., Volk, D., Gorenstein, D.G., Cristofanilli, M., and Rui, H., *Molecular Therapy,* 23(6), 1044-1054, 2015. DOI: 10.1038/mt.2015.45.

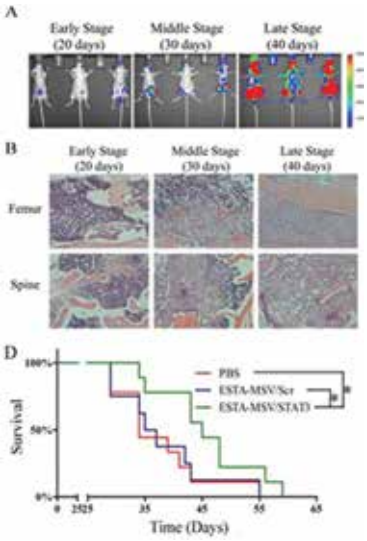
Bone marrow endothelium-targeted therapeutics for metastatic breast cancer, J. Mai, Y. Huang, C. Mu, G. Zhang, R. Xu, X. Guo, X. Xia, D.E. Volk, G. L. Lokesh, V. Thiviyanathan, D.G. Gorenstein, X. Liu, M. Ferraria, and H. Shen, *J. Controlled Release,* 2014, 187:22-29.

Aptaligner: Automated Software for Aligning Pseudorandom DNA X-Aptamers from Next-Generation Sequencing Data. E. Lu, M.-A. Elizondo-Riojas, J. T. Chang and D. E. Volk, *Biochemistry,* 2014, 53(22):3523-3525.

X-Aptamers: A Bead-Based Selection Method for Random Incorporation of Druglike Moieties onto Next-Generation Aptamers for Enhanced Binding. W. He, X. Li, M.-A. Elizondo-Riojas, G. Lokesh, A. Somasunderam, V. Thiviyanathan, D.E. Volk, R. Durland, J. Englehardt, C. Cavasotto, and D.G. Gorenstein, *Biochemistry* 2012, 51(42):8321-8323.

LAB MEMBERS

Research Scientist: Lokesh Rao, Ph.D.,
Research Associates: Xin Li, M.S., Li Li, Ph.D.
Post-doctoral Fellow: Ana Maria Zaske, Ph.D.
Medical Student: Linda Nguyen



A. Murine models of human breast cancer bone metastasis. Growth of MDA-MB-231 bone metastatic human breast cancers monitored by bioluminescence (A) and histology (B). Aptamer-targeted STAT3 siRNA delivery (D) can reduce the cancer burden and prolong life.

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

Multiscale modeling and drug target discovery

Our group 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.

Translation 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 Dr. Cristini and other 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 a dynamic molecular target identification method with multiscale modeling
- Predictive modeling of cancer treatment

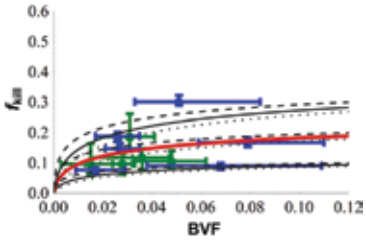
KEY PUBLICATIONS

V. Cristini, E.J. Koay, Z. Wang. Taking cancer out of the equation. *International Innovation.* 191:38-40.

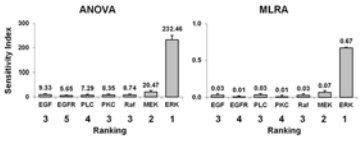
Z. Wang, J.D. Butner, V. Cristini, and T.S. Deisboeck. 2015. Integrated PK-PD and agent-based modeling in oncology. *Journal of pharmacokinetics and pharmacodynamics.* 42:179-189.

H.B. Frieboes, B.R. Smith, Z. Wang, M. Kotsuma, K. Ito, A. Day, B. Cahill, C. Flinders, S.M. Mumenthaler, P. Mallick, E. Simbawa, A.S. Al-Fhaid, S.R. Mahmoud, S.S. Gambhir, V. Cristini. 2015. Predictive Modeling of Drug Response in Non-Hodgkin's Lymphoma. *PloS one.* 10:e0129433.

Z. Wang, J.D. Butner, R. Kerketta, V. Cristini, T.S. Deisboeck. 2015. Simulating cancer growth with multiscale agent-based modeling. *Seminars in cancer biology.* 30:70-78.



Model predictions change with parameter values for a mathematical model of drug response in lymphoma. The results of the fraction of tumor killed (f_{kill}) from chemotherapy as a function of blood volume fraction (BVF) predicted by the model are in agreement with those measured from the histopathological samples of drug-sensitive (green symbols) and drug-resistant (blue symbols) tumors *in vivo*. (Frieboes *et al.,* PNAS 10(6):e0129433).



Parameter ranking of molecular targets from global parameter perturbation analysis of a multiscale cancer model. Targeting ERK was determined to be more promising in inhibiting tumor growth. ANOVA: Analysis of variance. MLRA: Multivariate linear regression analysis. (Wang *et al.,* IET Syst Biol 8 (5): 191-7).

Derek Lamont West, M.D., M.S.
Assistant Professor

Interventional oncology research

One treatment that my research centers on is electroporation. Electroporation is the application of electrical fields across cells to produce nanopores. Electrochemotherapy is a procedure that combines electroporation and systemic chemptherapy for the treatment of malignant neoplasia. In this treatment, administration of a chemotherapeutic drug is followed by local application of electroporation pulses. Electroporation (at low field strength) transiently permeabilizes tumor cell membranes, thus enabling diffusion of a chemotherapeutic drug into the cells and increasing its cytotoxicity. My laboratory research involves optimizing electroporation and electrochemotherapy for the treatment of pancreatic adenocarcinoma.

Another treatment that my research is focused is on radiogenomics in hepatocellular carcinoma. Radiogenomics is the correlation of imaging phenotypes to genomic genotypes. Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most frequent cause of cancer-related death. The majority of HCC patients are diagnosed at the advanced tumor stages and are treated with minimally invasive treatments, such as transarterial chemoembolization, ablation (radiofrequency, microwave, cryoablation or electroporation), or yttrium 90 brachytherapy. While these treatments are effective, many patients have tumoral recurrence. It has been suggested that one reason for failure is that post-treatment recurrent tumors are genetically different than the primary tumors and are more resistant treatment. To examine this, the laboratory examines tumor genetic and metabolic expression. As beginning and end-points in cellular processes, this examination can help determine if specific tumoral genetic expressions lead to specific metabolic expressions, in hopes of better understanding the genetic and metabolite changes associated with TACE and microwave ablation (MWA) protocols and their prognostic implications.

RESEARCH PROJECTS

- Prospective evaluation of hepatocellular carcinoma genetic and metabolomic tumor response to minimally invasive therapies
- An investigation on the therapeutic efficacy of coupling local tumor electroporation with TACE on patients with hepatocellular carcinoma
- Evaluation of safety and efficacy of electrochemotherapy in the treatment of pancreatic adenocarcinoma
- Use of magnetic resonance spectroscopy in the radiogenomic evaluation of childhood neuroblastoma
- Correlation of diffusion weighted MRI to cellular membrane pore formation after electroporation in pancreatic adenocarcinoma
- Evaluation of effects of electroporation and gemcitabine nanoparticle formulation on tumoral response in a pancreatic adenocarcinoma nude mouse model
- Optimization of catheter directed therapy in Rabbit VX2 rabbit animal model using vascular normalization

KEY PUBLICATIONS

West DL, White SB, Zhang Z, Larson AC, Omary RA. Optimization of Catheter Directed Therapy in Rabbit VX2 Rabbit Animal Model. *Int J Nanomedicine.* 2014; 9: 4169–4176.

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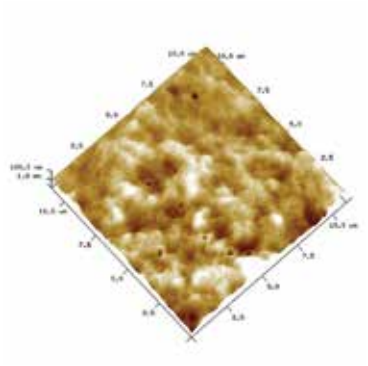
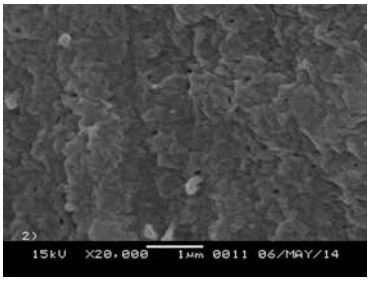
Rajesh P. Shah, James T. Bui, Derek L. West, Jose Oberholzer, Betul A. Hatipoglu, Joan N. Martellotto, Charles A. Owens. A Case of Pancreatic Islet Cell Transplantation in a Patient with Situs Ambiguous: Anatomical and Radiological Considerations. *Semin Intervent Radiol.* 2007 March; 24(1): 43–46.



Atomic force microscopy image of the surface of a panc-1 cell after electroporation demonstrating several nanopores in the cell membrane.



Scanning electron microscopic image of panc-1 cell after electroporation demonstrating several nanopores in the cell membrane.

A major focus of contemporary medicine is the development of effective therapies for the restoration of human tissues and organs lost to disease (e.g. inherited genetic diseases of the blood, such as sickle cell anemia, or immune deficiencies), trauma (e.g. spinal cord injury), or aging (e.g. degeneration of the joints). Regenerative Medicine has as its goal the replacement or regeneration of human tissues and/or organs to restore or establish normal function. Implicit in the successful design, implementation, and application of regenerative medicine approaches to the repair of a damaged tissue and/or organ is the reliance on the unique biological properties of specialized cells: stem cells.

Our focus within the Center for Stem Cell and Regenerative Medicine is to study the fundamental properties of stem cells and to translate their unique biological properties into novel cellular therapies for tissue regeneration for currently intractable disorders. It is essential that such an endeavor have at its foundation an excellence in fundamental stem cell research, coupled with a clear focus on development of tools and methodologies necessary for clinical translation. The Center has successfully recruited and retained a multidisciplinary faculty with the appropriate breadth of expertise and scientific rigor in the disciplines of stem cell biology and tissue engineering to promote the excellence and innovation of research within the Center, as well as the quality and appropriateness of stem cell based translational research initiatives emanating from the Center. By interfacing effectively with other programs and institutions within UTHealth, the Center also serves to stimulate the development and implementation of novel cellular therapies for a wide range of diseases and disorders. At present, Center faculty with primary appointments in the IMM, Neurosurgery, and Pediatric Surgery are pursuing research for therapeutic application targeting the following disease areas: Spinal Cord Injury; Stroke; Traumatic Brain Injury; Diaphragmatic Hernia; Blood Diseases; Cancer; Musculo-Skeletal Diseases; and Lung Diseases. We are currently recruiting additional outstanding basic research and translational Center faculty in order to significantly increase the breadth and depth of our research activities. Our Center also serves as the academic and administrative home for the Senator Lloyd and B.A. Bentsen Center for Stroke Research.

Brian R. Davis, Ph.D.
Associate Professor and Center Director
The G. 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 derived from patients with inherited disorders affecting the lung or blood system, with the ultimate goal of developing stem/progenitor cell-based therapeutic approaches. We have utilized Zinc Finger Nuclease-mediated Homology Directed Repair to correct the most common genetic mutations in iPS cell lines derived from patients with Cystic Fibrosis or Surfactant Protein B Deficiency – with the objective of demonstrating genotypic/phenotypic correction in lung epithelial cells derived from these corrected iPS cells. The second project in the laboratory focuses on the site-specific correction of gene mutations responsible for inherited blood disorders (e.g. Wiskott-Aldrich Syndrome) in patient-specific iPS cells – with subsequent differentiation to blood stem cells for transplantation. 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 and lung differentiation of iPS cells from inherited lung diseases (cystic fibrosis, surfactant protein-B deficiency)
- Correction and blood differentiation of iPS cells and blood stem cells from inherited blood disorders (Wiskott-Aldrich Syndrome, Pyruvate Kinase Deficiency)
- Characterization of spontaneous gene mutation resulting in correction of inherited Wiskott-Aldrich Syndrome defects

KEY PUBLICATIONS

A.M. Crane, P. Kramer, J.H. Bui, W.J. Chung, X.S. Li, M Gonzales-Garay, F. Hawkins, W. Liao, D. Mora, J. Wang, H.C. Sun, D.E. Paschon, D.Y. Guschin, P.D. Gregory, D.H. Kotton, M. Holmes, E.J. Sorscher, B.R. Davis. Targeted Correction and Restored Function of the CFTR Gene in Cystic Fibrosis Induced Pluripotent Stem Cells. *Stem Cell Reports* 4:569-577, 2015.

Garate Z, Quintana-Bustamante O, Crane AM, Oliver E, Poirot L, Galetto R, Kosinski P, Kung C, Bazinet J, Aguirre X, Orman I, Cerrato L, Alberquilla O, Rodriguez-Fornes F, Fusaki N, Garcia-Sanchez F, Maia T, Ribero M, Sevilla J, Prosper F, Jin S, Mountford J, Guenechea G, Gouble A, Bueren JA, Davis BR, Segovia JC. Generation of Healthy Erythroid cells by TALEN mediated Knock-in Gene edited Pyruvate Kinase Deficiency patient-specific Induced Pluripotent Stem Cells (*Stem Cell Reports*, 2015, in press)

Umeda K, Oda H, Yan Q, Matthias N, Zhao J, Davis BR, Nakayama N. Long-Term Expandable SOX9+ Chondrogenic Ectomesenchymal Cells from Human Pluripotent Stem Cells. *Stem Cell Reports* 4:712-726, 2015.

Z. Garate, B.R. Davis, O. Quintana-Bustamante and J.C. Segovia. New Frontier in Regenerative Medicine: Site-Specific Gene Correction in Patient-Specific Induced Pluripotent Stem Cells. *Human Gene Therapy* 24:571-583, 2013

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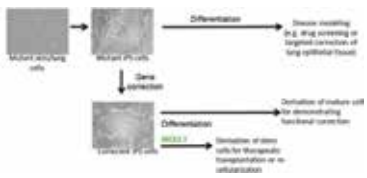
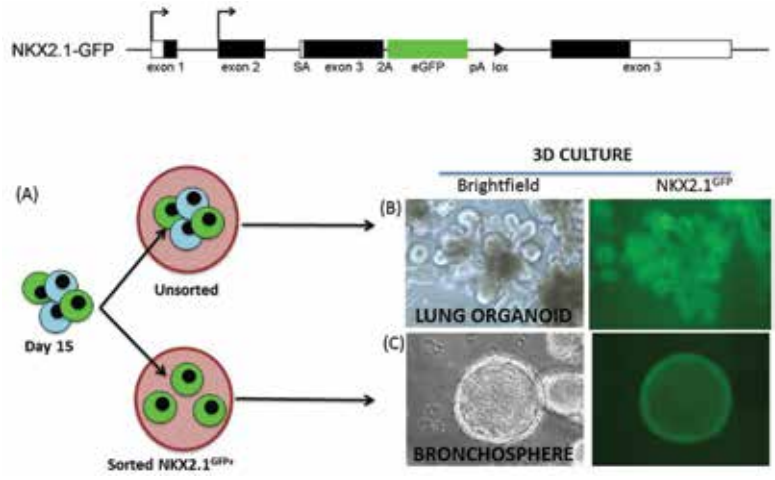


Applications of Cystic Fibrosis iPS Cells

LAB MEMBERS

Research Staff: Dr. Ana M. Crane, Dr. Nadine Matthias
Post-doctoral Fellow: Dr. Leila Rouhigharabaei



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

Development of hybrid tissue engineering scaffolds for use in the central nervous system

The research in my laboratory focuses on developing tissue engineering approaches toward clinical treatments for spinal cord injury, traumatic brain injury, and cartilage defects. 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 scaffolding systems. These scaffolding systems maximize the advantages of both synthetic (consistency in fabrication and cellular response) and natural (natural 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 scaffolds can begin to emulate the native tissue microenvironment and support tissue development far better than traditional scaffolds. Preliminary studies have focused on optimizing the concentration of bioactive laminin fragments for the differentiation of stem cells to neurons and the development of novel synthetic polymers capable of displaying multiple bioactive signaling peptides at independent concentrations.

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

- Development of multi-component scaffolds to facilitate tissue regeneration through better replication of the native extracellular matrix
- 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
- Synthesis of novel biomaterials for spinal cord, brain, and vertebral disc repair

KEY PUBLICATIONS

Yang Y-H, Kahn Z, Ma C, Lim HJ, Smith Callahan LA. Optimization of adhesive conditions for neural differentiation of murine embryonic stem cells using hydrogels functionalized with continuous Ile-Lys-Val-Ala-Val concentration gradients. *Acta Biomaterialia*. 21: 55-62, 2015.

Smith Callahan LA, Xie S, Barker IA, Zheng J, Dove AP, Becker ML. Directed Differentiation and Neurite Extension of mouse Embryonic Stem Cell on Aligned Poly(lactide) Nanofibers Functionalized with YIGSR Peptide. *Biomaterials*. 34(36): 9089-9095, 2013.

Smith Callahan LA, Ma Y, Stafford CM, Becker ML. Concentration Dependent Neural Differentiation and Neurite Extension of mouse ESC on Primary Amine-derivatized Surfaces. *Biomaterials Science.* 1(5):537-544, 2013.

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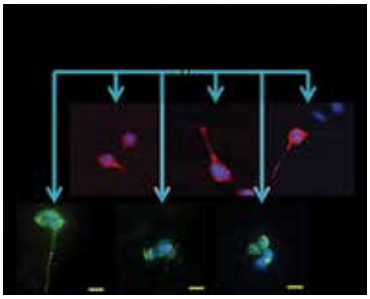
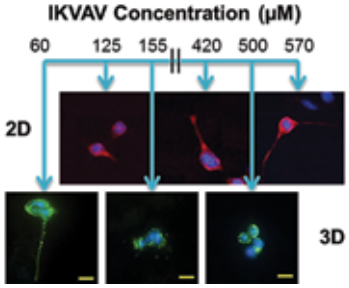
Smith LA, Liu X, Hu J, Ma PX. The Enhancement of Human Embryonic Stem Cell Osteogenic Differentiation with Nano-fibrous Scaffolding. *Biomaterials* 31(21): 5526-5539, 2010.

LAB MEMBERS

Post-doctoral Fellows: Hyun Ju Lim, Thuduwage Hiran Perera and Thomas Wilems
Medical Student: Alexander Aria
Undergraduate Students (Rice University): Matthew Mosley and Zara Khan



Optimization of the concentration of laminin derived peptide IKVAV for mouse embryonic stem cell neurite extension in both 2D (surface) and 3D (encapsulated) culture using polyethylene glycol hydrogels containing a continuous gradient of IKVAV. Peptide concentration plays a significant role in cellular response to artificial extracellular matrices and must be optimized for appropriate cell types in order for matrix supported stem cell therapy to be successful.



Qi Lin Cao, M.D.
Associate Professor

Stem cells for neurological diseases

Transplantation of neural stem cells (NSCs) is 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

- The long-term therapeutic efficacy and safety of hiPSC-derived neural stem or precursor cells for spinal cord injury and stroke
- Identification and characterization of key regulators for oligodendrocyte differentiation and remyelination after spinal cord injury
- The molecular mechanisms to regulate astrogliosis and the functions of astrogliosis after spinal cord injury, traumatic brain injury, or stroke using conditioned knockout mice models
- *In situ* reprogramming of astrocytes into functional neurons
- Screening and identification of novel neuroprotection agents for spinal cord injury

KEY PUBLICATIONS

Cao QL, He Q, Wang YP, Cheng XX, Howard RM, Zhang YP, DeVries WH, Shields CB, Magnuson DSK, Xu XM, Kim DH and Whittemore SR (2010) Transplantation of CNTF-expressing adult oligodendrocyte precursor cells promotes remyelination and functional recovery after spinal cord injury. *J Neurosci* 30: 2989-3001.

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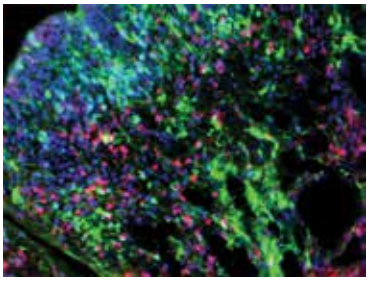
Chen KN, Deng SY, Lu HZ, Zheng YY, Yang GD, Kim DH, Cao QL* and Wu JQ* (2013). RNA-Seq characterization of spinal cord injury transcriptome in acute/subacute phases: a resource for understanding the pathology at the systems level. *PLoS One* (in Press). * co-corresponding authors.

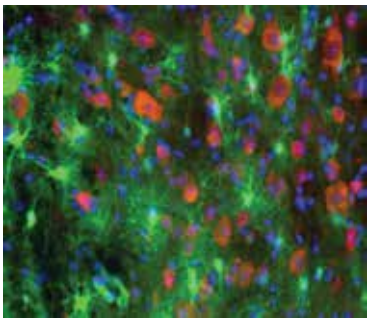
Fan CL, Wang H, Chen D, Cheng XX, Xiong K, Luo XG and Cao QL (2014) Effect of type-2 astrocytes on the viability of dorsal root ganglion neurons and length of neuronal processes. *Neural Regen. Res.* 9: 119-128.

LAB MEMBERS

Post-doctoral Fellow Research Associate:
Michelle Wang, Yiyan Zheng
Senior Research Assistant: Jun Li
Student: Chrystine Gallegos



Survival and neuronal differentiation of grafted neural stem cells derived from human induced pluripotent stem cells after SCI.



Specific expression of green fluorescence protein in astrocytes using AAV8-Flex-GFP injection in GFAP-cre mice.

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

Cox CS, Baumgartner JE, Harting MT, Worth L, Walker PA, Shah SK, Ewing-Cobbs L, Hasen K, Day MC, Lee D, Jimenez F, Gee A. 2010. Phase 1 clinical trial of autologous bone marrow mono nuclear cells for severe traumatic brain injury in children. *Neurosurgery* 68: 588-600, 2011.

Walker PA, Shah SK, Jimenez F, Gerber MH, Xue H, Cutrone R, Hamilton JA, Mays RW, Deans RA, Pati S, Dash PK, Cox CS. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: Preserving the blood-brain barrier via interaction with splenocytes. *Exp Neural* 225:341-352, 2010.

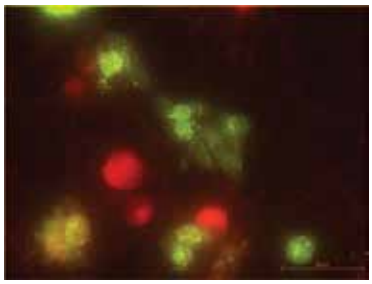
Bedi SS, Hetz R, Thomas C, Olsen A, Williams S, Smith P, Xue H, Aroom K, Uray K, Hamilton T, Mays RW, Cox CS. Intravenous MAPC therapy improves spatial learning after TBI. *Stem Cells/ Translational Medicine.* 2:953-960, 2013.

Menge T, Zhao Y, Zhao J, Wataha X, Gerber M, Zhang J, LeToumeau P, Redell J, Shen L, Wang J, Peng Z, Xue H, Kozar R, Cox CS, Khakoo A, Holcomb JH, Dash PK, Pati S. Mesenchymal stem cells regulate Blood Brain Barrier integrity in traumatic brain injury through productions of the soluble factor TIMP-3. *Science/Transl Med* 4: 161ra150, 2012. PMID: 23175708

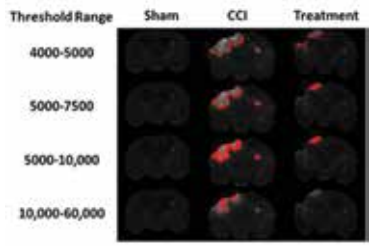
Walker PA, Bedi SS, Shah SK, Jimenez F, Xue H, Hamilton JA, Smith P. Thomas CP, Mays RW, Pati S, Cox CS. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: Modulation of microglia/macrophages . *J Neuroinflammation* 9: 228-240, 2012. PMID: 23020860

LAB MEMBERS

Instructor: Supinder Bedi, Ph.D.
Assistant Professor: Karen Uray, Ph.D.
Glassell Foundation Fellow: Ben Aertker, M.D.
NIH T32 Fellow: Margaret Jackson, M.D.
Post-doctoral Fellow: Suchit Sahal, Ph.D.
Flow Cytometry Technician: Phillipa Smith, M.S.
Medical Student: Henry Caplan, B.S.
Research Scientist: Hasan Xue, M.D.
GMP Center Director: Fabio Triolo, Ph.D.
GMP-QA Director: Sufira Kiran



Electrospun PLGA nanofiber scaffold seeded with MAPCs and NSCs as a composite graft for implantation into focal cavitary neurological injury sites.



The use of a range of stem and progenitor cells demonstrate improvement in blood-brain barrier permeability after traumatic brain injury in a rodent model. These data coincide with clinical observations of a reduced cerebral inflammatory mediated edema after cell therapy in patients. (Liao, *et al.* Peds Crit Care Med, 2015)

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

Disease modeling and skeletal muscle regeneration using human ES/iPS 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 mouse/human embryonic stem cells (ES cells) and induced pluripotent cells (iPS cells) for cell therapy in mice models for different types of muscular dystrophies (MDs).

Here at IMM, the lab focuses on the approaches to use stem cell therapies for skeletal muscle regeneration. Currently our lab uses cutting-edge gene editing technologies (CRISPR/Cas9) for generation of knock-in reporter lines in human ES/iPS cells 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 these cells for future iPS cell based therapies.

Using high throughput screening (HTS) to identify important inducers of myogenesis in iPS cells and evaluation of *in vivo* regeneration potential of the cells in mice models for MDs and muscle mass injuries are other major aims of the lab.

Our lab research also includes derivation of iPS cells from MD 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.

My lab is currently funded by a research grant award from Muscular Dystrophy Association (MDA) over a period of three years to develop methods using stem cells to regenerate skeletal muscle tissue in a mouse model of Duchenne muscular dystrophy (DMD).

RESEARCH PROJECTS

- Generation of knock-in human ES/iPS cell lines for early myogenic genes (PAX7, MYF5)
- Gene correction of MD iPS cells using CRISPR/Cas9 system
- Study the role of local tissue perfusion on survival and engraftment of human ES/ iPS derived myogenic progenitors in skeletal muscle
- Generation of integration-free/safe myogenic progenitors from human ES/ iPS cells
- Systemic/arterial cell delivery approaches for cell therapy in muscular dystrophies
- Using bio-scaffolds for cell delivery in mice models for skeletal mass loss injuries

KEY PUBLICATIONS

Darabi R, Gehlbach K, Bachoo MR, Kamath S, Osawa M, Kam KE, Kyba M, Perlingeiro RCR. Functional skeletal muscle regeneration from differentiating embryonic stem cells. *Nature Medicine,* 2008; 14 (2): 134-143.

Ramos AL, Darabi R (equal contribution), Akbarloo N, Borges L, Catanese J, Dineen SP, Brekken RA, Perlingeiro RC. Clonal Analysis Reveals a Common Progenitor for Endothelial, Myeloid, and Lymphoid Precursors in Umbilical Cord Blood. *Circulation Research,* 2010 Dec 10; 107(12):1460-9.

Darabi R, Santos FN, Filareto A, Pan W, Koene R, Rudnicki MA, Kyba M, Perlingeiro RC. Assessment of the myogenic stem cell compartment following transplantation of pax3/pax7-induced embryonic stem cell-derived progenitors. *Stem Cells,* 2011 May;29(5):777-90.

Darabi R, Arpke RW, Irion S, Dimos JT, Grskovic M, Kyba M, Perlingeiro RC. Human ES- and iPS-Derived Myogenic Progenitors Restore Dystrophin and Improve Contractility upon Transplantation in Dystrophic Mice. *Cell Stem Cell,* 2012 May; 10 (5), 610-619.

Filareto A, Parker S, Darabi R, Borges L, Iacovino M, Schaaf T, Mayerhofer T, Chamberlain J, Ervasti J, Scott McIvor R, Kyba M, Perlingeiro RCR. An ex vivo Gene Therapy Approach to Treat Muscular Dystrophy Using iPS cells. *Nature Communications,* 2013; 4:1549.

Arpke RW, Darabi R, Mader TL, Zhang Y, Toyama A, Lontree CL, Nash N, Lowe DA, Perlingeiro RC, Kyba M. A New Immuno- Dystrophin- Deficient Model, The NSG-mdx4cv Mouse, Provides Evidence for Functional Improvement Following Allogenic Satellite Cell Transplantation. *Stem Cells,* 2013 Aug; 31(8):1611-20.

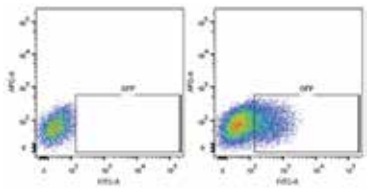
Skoglund G, Lainé J, Darabi R, Fournier E, Perlingeiro R, Tabti N. Physiological and ultrastructural features of human induced pluripotent and embryonic stem cell-derived skeletal myocytes in vitro. *Proc Natl Acad Sci* USA. 2014 Jun 3; 111(22):8275-80.

Darabi R, Perlingeiro RC. Derivation of Skeletal Myogenic Precursors from Human Pluripotent Stem Cells Using Conditional Expression of PAX7. *Methods Mol Biol.* 2014 Nov 18.

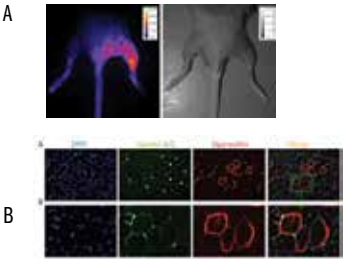
Matthias N, Hunt SD, Wu J, Darabi R. Skeletal muscle perfusion and stem cell delivery in muscle disorders using intra-femoral artery canulation in mice. *Exp Cell Res.* 2015 Sep 1.

LAB MEMBERS

Post-doctoral Fellows: Jianbo Wu, Nadine Matthias
Research Technician: Samuel D. Hunt



Human PAX7 reporter iPS cells show the expression of the GFP reporter following PAX7 activation using a dead Cas9 VP160 mediated PAX7 gene activator.



A. NIRF image demonstrates high efficiency hindlimb perfusion following intra-arterial (intra-femoral artery) canulation/perfusion in mouse. B. Human iPS-derived myogenic progenitors engraft and restore the missing protein/dystrophin (red) in a mouse model of Duchenne muscular dystrophy (DMD) following intra-arterial perfusion. Asterisks and arrows mark the presence of human nuclei (green) in perfused region.

Pramod Dash, Ph.D.
Professor
Nina and Michael Zilkha Distinguished Chair, Neurodegenerative Disease Research

Cellular and molecular mechanisms of memory and its dysfunction

Clinical and experimental evidence indicates that traumatic brain injury (TBI), especially repetitive mild traumatic brain injury (rmTBI or repeated concussion), is a risk factor for the development of neurodegenerative diseases such as Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE). Both AD and CTE are characterized by the deposition of the neuronal proteins microtubule-associated protein TAU and amyloid-beta (Aß). However, the cellular and molecular mechanisms that trigger TAU and Aß aggregation after rmTBI are largely unknown. One of the protein kinases that phosphorylates TAU is glycogen synthase kinase 3 (GSK3), and its dysregulation can lead to TAU hyperphosphorylation and aggregation. Using the pharmacological inhibitor of GSK3 lithium, we have found that post-injury treatment reduces experimental TBI pathology and improves learning and memory. We are currently exploring the possibility that targeting this pathway can reduce TAU phosphorylation, and attenuate neurodegeneration.

Another focus of our laboratory is to identify the signaling cascade(s) that are critical for memory formation and determine if dysregulation of these cascades contributes to learning and memory impairments and/or neurodegeneration after TBI. We have shown that mammalian target of rapamycin (mTOR) plays an obligatory role in memory formation, and that memory enhancers such as glucose act, in part, through this pathway. mTOR activity is negatively regulated by the tuberous sclerosis complex, the protein components of which are encoded by the *TSC1* and *TSC2* genes. Mutations in these genes cause mTOR over-activation and tuberous sclerosis, a disease characterized by the formation of brain tumors, learning and memory impairments and autism spectrum disorder (ASD). In collaboration with Dr. Michael Gambello of Emory University School of Medicine, we have shown that loss of *TSC2* in radial glia causes abnormal neuronal migration and learning and memory dysfunction that can be partially corrected by rapamycin.

Interestingly, loss of *TSC2* in cerebellar Purkinje cells causes neurodegeneration and the development of autistic-like behaviors. Based on mTOR's role in memory formation, we have been examining if this cascade contributes to TBI-elicited learning and memory impairments.

RESEARCH PROJECTS

- Role of systemic inflammation in TBI pathology and outcome
- Energy utilization in the injured brain and strategies to mitigate energy crisis
- Development of strategies to reduce protein aggregation in the brain and attenuate neurodegeneration.

KEY PUBLICATIONS

Hoskison, M.M., Moore, A.N., Hu, B., Orsi, S.A., Kobori, N. and Dash, P.K. Persistent working memory dysfunction following traumatic brain injury: evidence for a time-dependent mechanism. *Neuroscience*, 159:483-491, 2009

Dash PK, Johnson D, Clark J, Orsi SA, Zhang M, Zhao J, Grill RJ, Moore AN, Pati S. Involvement of the Glycogen Synthase Kinase-3 Signaling Pathway in TBI Pathology and Neurocognitive Outcome. *PLoS One.* 6(9):e24648, 2011.

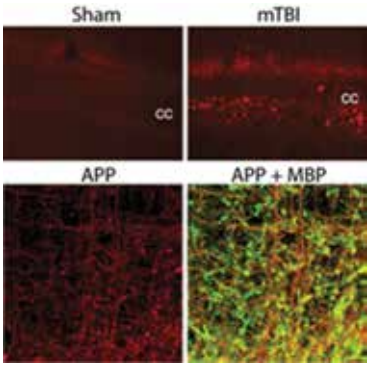
Hylin MJ, Orsi SA, Rozas NS, Hill JK, Zhao J, Redell JB, Moore AN and Dash PK. Repeated mild closed head injury impairs short-term visuospatial memory and complex learning. *J Neurotrauma* 30:716-726, 2013.

Dash PK, Hylin MJ, Hood KN, Orsi A, Zhao J, Redell JB, Tsvetkov AS and Moore AN. Inhibition of eIF2α phosphatase reduces tissue damage and improves learning and memory following traumatic brain injury. *J Neurotrauma*, (April 4 epub) 2015.

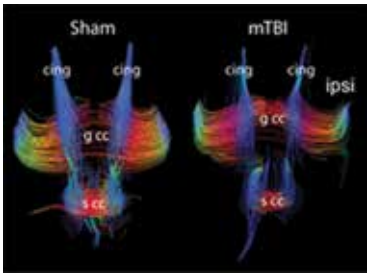
Rozas NS, Redell JB, Pita-Almenara J, McKenna J, Moore AN, Gambello MJ and Dash PK. Intrahippocampal glutamine administration inhibits mTORC1 signaling and impairs long-term memory. *Learning and Memory* 22:239-246, 2015.

LAB MEMBERS

Research Assistant Professor: John B. Redell, Ph.D.
Sr. Research Scientist: Jing Zhao, M.D., Ph.D.
Research Scientist: Nobuhide Kobori, M.D., Ph.D.
Post-doctoral Fellow: Karthikeyan Tangavelou, Ph.D.
Research Coordinator: Anthony N. Moore, B.S.
Research Assistants: Kimberly Hood, M.A., Jacalyn S. MacGowan



mTBI causes axonal injury in the corpus callosum (cc) as indicated by increased amyloid precursor protein (APP) immunoreactivity compared to sham-operated controls. APP fibers (red) colocalized with myelin basic protein (MBP; green) indicating damage to myelinated axons. Areas of overlap appear yellow in the merged image.



Tract tracings obtained from diffusion tensor imaging (DTI) demonstrating the axonal fibers passing through the area of the cingulum from a sham and a mTBI rat. An apparent shortening of the fibers in the ipsilateral (ipsi) cingulum (cing) can be observed, suggesting axonal disruptions.

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

Advancing the field of neuroscience

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

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

Named to the US News and World Report's Top 1% Doctors, and America's Top Surgeons, I have received 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

Kim DH, Mohapatra G, Bollen A, Waldman FM, Feuerstein BG: Chromosomal abnormalities in glioblastoma multiforme and glioma cell lines detected by comparative genomic hybridization. *Int J Cancer.* 60:812-815, 1995.

Mohapatra G, Kim DH, Feuerstein BG: Detection of multiple gain and losses in ten glioma cell lines by comparative genomic hybridization. Genes, Chrom, *Cancer.* 13:86-93, 1995.

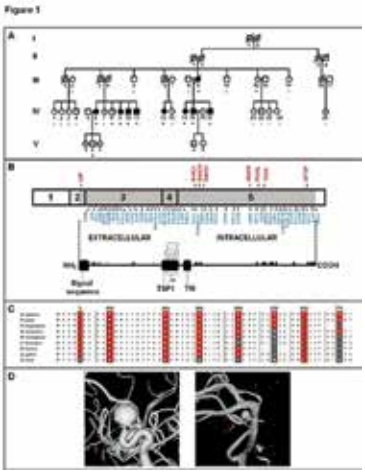
Kim DH, Van Ginhoven G, Milewicz DM: The incidence of familial intracranial aneurysms in 200 patients: comparison between Caucasian, African-American, and Hispanic populations. *Neurosurgery.* 53(2):303-8, 2003.

Kim DH, Van Ginhoven G, Milewicz DM: Familial aggregation of both aortic and cerebral aneurysms: evidence for a common genetic basis in a subset of families. *J Neurosurg.* 56:655-61, 2005.

Santiago-Sim T, Depalma SR, Ju KL, McDonough B, Seidman CE, Seidman JG, Kim DH: Genome-wide linkage in a large Caucasian family maps a new locus for intracranial aneurysms to chromosome 13q. *Stroke.* 40[suppl 1] S57-S60, 2009.

Cao Q, He Q, Wang, Yaping, Cheng, Xiaoxin, Howard, Russell M., Zhang, Yiping, DeVries, William H., Shields CB, Magnuson DSK, Xu X, Kim D, Whittemore SR: Transplantation of Ciliary Neurotrophic Factor-Expressing Adult Oligodendrocyte Precursor Cells Promotes Remyelination and Functional Recovery after Spinal Cord Injury. *J Neuroscience.* 30 (8):2989-3001, 2010.

Santiago-Sim T, Fang X, Hennessy M, Nalbach S, DePalma S, Lee MS, Greenway S, McDonough B, Hergenroeder G, Patek K, Colosimo S, Qualmann K, Milewicz D, MacRae C, Dymecki S, Seidman C, Seidman JG. Kim DH: Mutations in *THSD1* as a Cause of Intracranial Aneurysm. (submitted) : 2015.



Identification of the *THSD1* R450X Mutation in Large Family with IA and the Spectrum of *THSD1* Rare Variants.
Panel A shows the pedigree of family IA001. Squares represent males, circles females, black symbols affected persons, white symbols unaffected persons, gray symbols unknown phenotype, plus symbols R450X mutation carriers, and negative symbols non-carriers. Slashes denote deceased individuals. Panel B shows the spectrum of *THSD1* Rare Variants in unrelated IA patients (red)and in control individuals (blue) in relation to exons (numbered), and domain organization. *THSD1* control variants presented here are from the NHLBI GO Exome Sequencing Project. Panel C shows a protein sequence alignment displaying evolutionary conservation of substituted amino acids in *THSD1* orthologs. Panel D shows images of aneurysms in patients with a *THSD1* mutation.

Yong Li, M.D., Ph.D.
Associate Professor

Pluripotent stem cell and regenerative medicine

This research team has developed several novel techniques for molecular, cellular, and animal-based studies to focus on few major areas of study: 1) exploring the properties of the injury induced pluripotent stem cells (iMuSCs) and their natural role during tissue healing, including vascular and neuromuscular junction (NMJ) reconstruction; 2) studying the mechanism behind aging processes in the musculoskeletal system and detecting candidate genes for aging prevention; and 3) use of bioengineering tissues to repair wound defects with scarless healing, which include repair of children's diaphragm hernia (CDH). The laboratory is also interested in translational study and clinical application of stem cells and engineered tissue for treating congenital diseases and traumatic injuries. We have set up a classic tissue/organ regeneration model, e.g. a newt model that can rebuild most missing body parts (such as limbs, liver, lens and heart) after injury. However, injured mammalian tissue, including that of humans, is usually replaced with fibrotic scar tissue at the end of the healing process. Our aim is to determine the mechanism(s) behind the regenerative process in the newts, and ascertain the relationship(s) to human tissue regeneration. Currently, we are using murine digit as the amputation model to accelerate regeneration by duplicating the processes of newt limb regrowth. Our expectation is to transfer our learning from newt regenerative models to regenerative medicine applications.

RESEARCH PROJECTS

- Children's Regenerative Medicine: The project will use various cell sources combined with bioengineering scaffolds to build functional tissues for repair of pediatric defects, such as children's diaphragmatic hernia (CDH). We are also building 3D printer by using natural proteins and cells to create a functional tissue compound for wound tissue repair
- Injured Tissue Derived Stem Cells: The project aims to identify the characteristics including the pluripotency of injured tissues derived stem cells, to clarify their essential role during tissue injury and repair
- Fibrosis and Prevention Studies: Investigate the mechanism behind the fibrosis process after injuries and diseases, and seek methods for prevention and treatment of fibrous scar tissue formation
- Aging study: With our specific murine aging model, we will identify the anti-aging genes and determine the specific molecular mechanisms and biomarkers for aging repression by screening genome-wide transcriptome and protein profiling within the model system
- Neuromuscular junction: We have built a model of human neuromuscular junction (NMJ) in dish, which can be used as several studies, such as neuromuscular diseases, muscle atrophy, drug screening

KEY PUBLICATIONS

Pan HY, Vojnits K, Meng FW, Liu T, Yang L, Wang YG, Huard J, Cox C, Lally KP, Li Y. MMP1 gene expression enhances myoblast migration and engraftment following implanting into mdx/SCID mice. *Cell Adhesion & Migration* 2015;9(4):283-292.

Vojnits K, Pan HY, Mu XD, Li Y. Characterization of an Injury Induced Population of Muscle-Derived Stem Cell-Like Cells. *Scientific Reports* (2015, accepted)

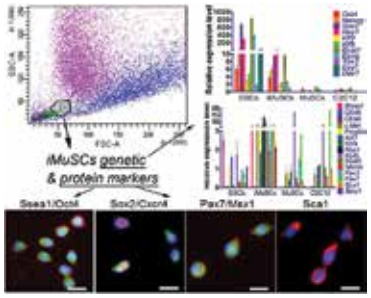
Chatterjee S, Yin HS, Nam D, Li Y, Ma K. Brain and muscle Arnt-like 1 promotes skeletal muscle regeneration through satellite cell expansion. *Experimental Cell Research* 2015;331(1):200-210.

Choi YH, Cox CS, Lally KP, Li Y. The strategy and method in modulating finger regeneration. *Regenerative Medicine* 2014; 9(2):231-242

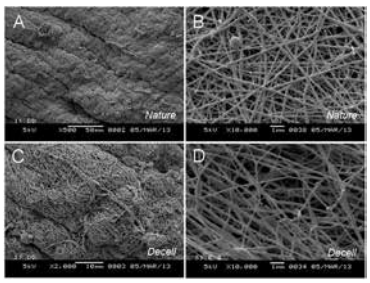
Vojnits K, Zhan M, Cox CS, Li Y. Small embryonic stem cell, a new type of stem cell. *Journal of Stem Cell* 2014; 9(1):1-16.

LAB MEMBERS

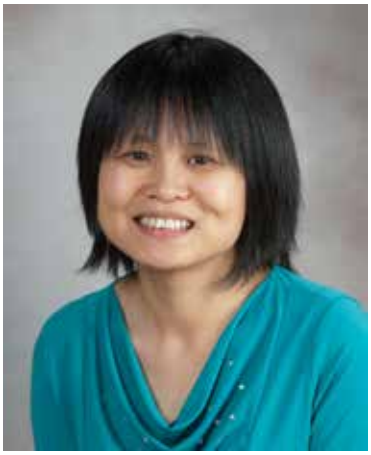
Administrator: Stephanie Baca
Lab senior technician/manager: Haiying Pan
Postdoc research fellows: Dr. Kinga Vojnits; Dr. Fanwei Meng.
Medical students: Chen Fu, Parsha Forouzan, Justin Pham



The characteristics of injured muscle derived stem cell-like cells (iMuSCs).



Scanning Electron Microscope (SEM) visualizes an extracellular matrix (ECM) from decellularization of rat diaphragms.



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 injury and neurodegenerative 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
- Creation of neural lineage hiPSC reporters by CRISPR/Cas9 mediated gene targeting
- Identification of optimal neural lineage progenitors for cell-based therapy in spinal cord injury and stroke
- Characterization of the role of OLIG genes in Down syndrome using patient derived iPSCs and neural populations

KEY PUBLICATIONS

Liu, Y. and Deng W. (2015) Reverse Engineering Human Neurodegenerative Disease Using Pluripotent Stem Cell Technology. *Brain Research,* in press.

Xue H, Wu J, Li S, Rao MS, Liu Y. (2014) Genetic modification in human pluripotent stem cells by homologous recombination and CRISPR/Cas9 System. *Methods Mol Biol.* 2014 Mar 11. [Epub ahead of print] PMID:24615461

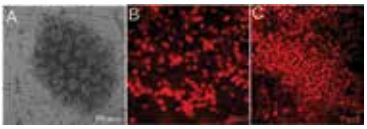
Chen, C., Jiang, P., Xue, H., Peterson, S., Tran, H.T., McCann, A., Parast, M., Li, S., Pleasure, D.E., Laurent, L.C., Loring, J. F., Liu, Y.*, and Deng, W*. (2014) Role of astroglia in Down Syndrome revealed by patient-derived human induced pluripotent stem cells. *Nat Commun.* Jul 18;5:4430. doi: 10.1038/ncomms5430 (*Corresponding authors)

Li, S., Xue, H., Long, B., Sun, L., Truong, T., and Liu, Y. (2014) Efficient generation of hiPSC neural lineage specific knockin reporters using the CRISPR/Cas9 and Cas9 double nickase system. *J Vis Exp.* e52539, doi:10.3791/52539.

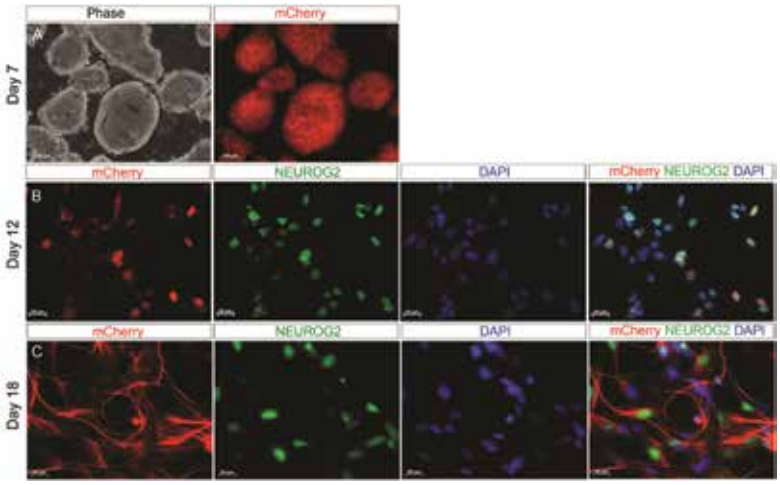
Liu, Y.*, Jiang, P., and Deng, W.* (2011) Olig gene targeting in human pluripotent stem cells for motor neuron and oligodendrocyte differentiation. *Nat Prot.* 6, 640-655. (*Corresponding authors)

LAB MEMBERS

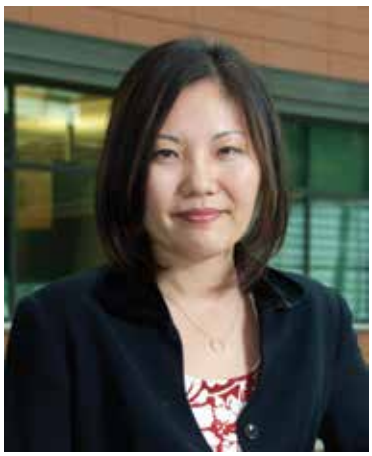
Post-doctoral Fellow: Shenglan Li
Research Associate: Haipeng Xue
Visiting Scientists: Seung H Yang, Bo Long, Li Sun, Lihua Luo



Directed neural differentiation of human induced pluripotent stem cells (hiPSCs). Neural rosettes (A) express neural stem cell markers Sox1 (B) and Pax6 (C)



A Neurogenin 2 knockin hiPSC reporter line made using the CRISPR/Cas9 system. NEUROG2-mCherry hiPSC 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 molecular and cellular mechanisms of pathogenesis and drug resistance in human lymphoma and multiple myeloma

The major goals of my research program are to decipher molecular pathways that confer selective growth and survival advantages to malignant B cells. We are interested in understanding how transcription factors that determine normal B cell lineage differentiation are involved in malignant B cell initiation and progression. One of those factors is paired box 5 (PAX5), a determinant of normal B cell lineage development. We discovered that PAX5 silencing in MCL leads to increased tumor formation *in vitro* and in xenograft mice, indicating that PAX5 is a potential tumor suppressor. We also 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 MCL have direct translational applications. Downstream of PAX5 signaling is BACH2 (BTB and CNC homology), which is another transcription factor that is involved in drug responses in MCL. We discovered that BACH2 nucleo/cytoplasmic shuttling influences resistance to drugs that generate reactive oxygen species (ROS). We also have developed a new line of research studying in multiple myeloma (MM) and their interaction with microenvironment. MM is heterogeneous disease due to their manifestation in the bone marrow compartment. These cells were also drug resistance and contributed to increased tumor formation in the secondary xenograft mice. We conducted gene profiling analyses for the quiescent PKH+ populations and the characterization of PKH+ cells and their interaction with microenvironment is underway.

RESEARCH PROJECTS

- MM cells and microenvironment niche: We conducted microarray analyses to identify genes expressed in quiescent MM cells from osteoblastic, vascular and spleen niches. We will continue to characterize functions of these genes in the MM interaction with bone marrow microenvironment
- Development of small molecule inhibitors for targeting advanced lymphomas: We have conducted high throughput chemical screening to identify the compounds that selectively target MCL cells that home to the bone marrow compartment. We will further develop and test these compounds in animal models for pre-clinical studies and plan to test its efficacies in the patients
- Delineating transcription factor networks on drug resistant lymphomas: We will continue to address roles for PAX5 signaling in MCL pathogenesis. We also will closely work with collaborators at MDACC to determine whether BACH2 sub-cellular localization in the cell determine drug resistance outcome and patient survival

KEY PUBLICATIONS

Jung, H-J., Zheng, C., Wang, M., Fayad, L., Romaguera, J., Kwak, L.W., and McCarty, N. Calcium blockers decrease the bortezomib resistance in mantle cell lymphoma (MCL) via manipulation of tissue transglutaminase activities. *Blood* 119:2568-2578, 2012.

Jung, H-J., Chen, Z., and McCarty, N. Synergistic antiproliferative effects of arsenic trioxide (ATO) with bortezomib in mantle cell lymphoma (MCL). *American Journal of Hematology* 87:1057-1064, 2012.

Chen, Z., Romaguera, J., Wang, M., Fayad, L., Kwak, L.W., and McCarty, N. Verapamil synergistically enhances cytotoxicity of bortezomib in mantle cell lymphoma via induction of reactive oxygen species production. *British Journal of Hematology* 159:243-246, 2012.

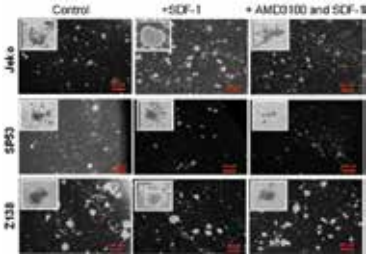
Chen, Z., Pittman, E.F., Romaguera, J., Fayad, L., Wang, M., Neelapu, S.S., McLaughlin, P., Kwak, L., and McCarty, N. Nuclear translocation of B-cell-specific transcription factor, BACH2, modulates ROS mediated cytotoxic responses in mantle cell lymphoma. *PLOS one* 8(8):e69126. doi:10.1371/journal.pone.0069126, 2013.

Chen, Z., Orlowski, R.Z., Wang, M., Kwak, L., and McCarty, N. Osteoblastic niche supports the growth of quiescent multiple myeloma cells. *Blood* 123: 2204-2208, 2014.

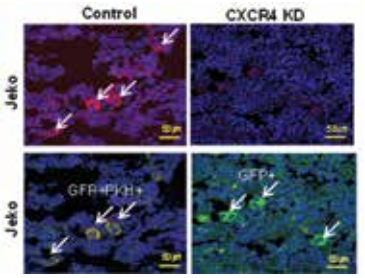
Teo, A.E., Chen, Z., Miranda, R.N., McDonnell, T., Medeiros, L.J., and McCarty, N. Differential PAX5 levels promote malignant B-cell infiltration, progression and drug resistance, and predict a poor prognosis in MCL patients independent of CCND1. *Leukemia* 15. doi: 10.1038/leu.2015.140. [Epub ahead of print], 2015

LAB MEMBERS

Senior Research Associate: Judy Chen
Post-doctoral Fellows: Jennifer Han, Jimmy Lin



Jeko, SP53 and Z138 mantle cell lymphoma (MCL) cells (5x103) were cultured in PHA-LCM (phytohemagglutinin human leukocyte conditioned medium) methylcellulose medium.



Decreased quiescent PKH-positive cell recovery in vivo upon CXCR4 silencing. GFP-positive CXCR4 shRNA MCL cells (CXCR4 KD) or control shRNA cells (control, 5x105) were labeled using the fluorescent dye PKH26 and were injected into NOD/SCID mice to evaluate engraftment.

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 mesenchymal stromal cells (MSCs) face the problems of low yield of cells and their tendency to yield unsuitable and/or unstable cartilage after expansion. Joint is formed during embryogenesis. Therefore, we hypothesize that the embryonic cell-type responsible for limb and vertebral joint formation: i.e. joint progenitor, the common precursor of synovial joint components including articular and meniscal chondrocytes and ligaments, 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 somatic cell-type in culture through processes that mimic embryogenesis *in vivo*, making human (h)PSCs a promising source of embryonic cells for regenerative medicine. The major challenges have been to direct their differentiation toward the cell type of interest (i.e. to obtain progeny of the right quality), and to isolate them in large quantities without introducing transgenes and mutations.

Permanent cartilage formation – proper signaling and right cell type: We have previously developed and purified from hPSCs paraxial mesoderm and neural crest progeny, two of the three embryonic origins of chondrocytes, with the capacity to expand and differentiate into sclerotomal and ectomesenchymal chondroprogenitors, respectively. We have recently established a condition to generate chondrogenic lateral plate mesoderm, the third embryonic origin of chondrocytes, from hPSCs, too. All these progeny give rise to hyaline-like cartilage particles in culture under our standard condition. However, most of them are unstable *in vivo* and are mineralized and turned into bone when ectopically transplanted into immunocompromised mice. We have discovered a way to selectively generate and to a limited extent, expand joint progenitor-like cells that express syndetomal (ligament precursor) markers from the paraxial mesoderm progeny. Furthermore, we have recently discovered a way to generate cartilage particles from the hPSC-derived chondroprogenitors, that show no to very limited mineralization/bone formation after transplantation. We are currently focusing both on the characterization of joint progenitor-like cells and on the elucidation of critical signaling mechanism for stable cartilage formation, aiming to establish cellular and humoral environment suitable for reproducibly generating joint-type stable cartilage.

We have established culture conditions that maintain and expand the sclerotomal and ectomesenchymal chondroprogenitors for an extended period of time, without loss of their chondrogenicity. Such stable expansion of chondrogenic activity is currently very hard to achieve with adult MSCs. We are also focusing on genome-wide molecular search (e.g. transcriptome, proteome, epigenome analyses) in these expandable chondroprogenitors, aiming to understand the mechanistic basis that may be applied to improve the expansion culture method for adult MSCs and the hPSC-derived join progenitor-like cells in future.

RESEARCH PROJECTS

- Specification, prospective isolation and expansion of three embryonic chondroprogenitors (sclerotome, limb mesenchyme and ectomesenchyme) from hPSCs
- Elucidation of the molecular basis of long-term expansion without loss of chondrogenic activity of the hPSC-derived chondroprogenitors
- Generation, detection, isolation and expansion of joint progenitors from hPSCs using fluorescence reporter PSC lines
- Defining the process of chondrogenesis from the hPSC-derived chondroprogenitors and joint progenitors to elucidate the molecular basis of joint chondrogenesis
- Establishment of an orthotopic xenotransplantation model for cell-based articular cartilage repair

KEY PUBLICATIONS

Umeda, K., Oda, H., Matthias, N., et al. (2015) "Long-term expandable SOX9+ chondrogenic ectomesenchymal cells from human pluripotent stem cells" *Stem Cell Reports* 4:712-726.

Yokoyama, K., Ikeya, M., Umeda, K., et al. (2015) "Enhanced chondrogenesis of iPS cells from neonatal-onset multisystem inflammatory disease occurs via the caspase-1-independent cAMP/PKA/CREB pathway" *Arthritis Rheumatol.* 67: 302-314.

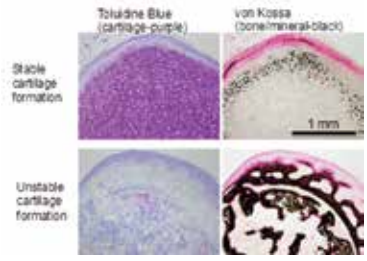
Zhao, J., Li, S., Trilok, S., Tanaka, M., et al. (2014) "Small molecule-directed specification of sclerotome-like chondroprogenitors and induction of a somitic chondrogenesis program from embryonic stem cells" *Development,* 141: 3848-3858.

Mae, S., Shono, A., Shioda, F., et al. (2013) "Monitoring and robust induction of nephrogenic intermediate mesoderm from human pluripotent stem cells" *Nat Commun*, 4:1367

Umeda, K., Zhao, J., Simmons, P, et al. (2012) "Human chondrogenic paraxial mesoderm, directed specification and prospective isolation from pluripotent stem cells" *Sci Rep*, 2:455.

LAB MEMBERS

Research Assistant: John Lee, BA.
Senior Research Associate and Animal Specialist: Nadine Matthias, DVM



Eight weeks after transplantation of cartilage particles generated in vitro from hiPSC-derived ectomesenchymal cells under two different culture conditions: Stable cartilage-forming condition (upper panels) and growth plate-like unstable cartilage-forming condition (lower panels).

Pamela Wenzel, Ph.D.
Assistant Professor

Biomechanical force regulates cell potential and behavior

Members of our lab study 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 blood development during embryogenesis and how we might use this information in the laboratory to expand improved sources of hematopoietic stem cells for clinical use. A number of genetic and biochemical pathways are currently under investigation as key players mediating this signaling cascade, and we employ various approaches to evaluate their role in blood development, including microfluidics, pharmacology, mouse genetics, and transplantation assays.

Mesenchymal stromal cells have attracted a great deal of attention as potent therapeutics for regenerative medicine. These stem-like cells can be found in a vast array of tissues throughout the body, including the bone marrow, umbilical cord, and fat. Current research suggests that mesenchymal stromal cells reduce inflammatory signaling and innate immune response which can accompany traumatic injury and chronic states of immune dysfunction. Consequently, our second area of interest is to determine how mechanical force alters the biology of mesenchymal stem cells, including their ability to modulate inflammation and vascular permeability. We utilize culture-based assays and animal models of traumatic brain injury as readouts of stem cell response to mechanical stimuli.

Finally, fluid flow and hydrostatic pressure 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 microenvironmental cancer biology, we hope to identify new treatment options for patients affected by cancer.

RESEARCH PROJECTS

- Mechanobiology of blood development
- Modulation of anti-inflammatory programs in mesenchymal stem cells
- Fluid flow in initiation of metastasis

KEY PUBLICATIONS

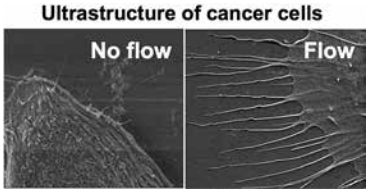
Diaz, M.F., Li, N., Lee, H.J., Adamo, L., Evans, S.M., Willey, H.E., Arora, N., Torisawa, Y., Vickers, D.A., Morris, S.A., Naveiras, O., Murthy, S.K., Ingber, D.E., Daley, G.Q., García-Cardeña, G., and Wenzel, P.L. (2015). Biomechanical Forces Promote Blood Development through Prostaglandin E2 and the cAMP-PKA Signaling Axis. *J Exp Med*, 212: 665-680. Featured article and cover art.

Li, N., Diaz, M.F., Wenzel, P.L. (2015). Application of Fluid Mechanical Force to Embryonic Sources of Hemogenic Endothelium and Hematopoietic Stem Cells. *Methods Mol Biol,* 1212: 183-193.

J
ang, I.-H., Lu, Y.-F., Zhao, L., Wenzel, P.L., Kume, T., Datta, S.M., Arora, N., Guiu, J., Lagha, M., Kim, P.G., Schlaeger, T.M., Zon, L.I., Bigas, A., Burns, C.E., and Daley, G.Q. (2015). Notch1 acts via Foxc2 to induce specification of hemogenic endothelial cells during mouse and zebrafish embryo development. *Blood,* 125: 1418-1426.

Arora, N., Wenzel, P.L., McKinney-Freeman, S.L., Ross, S.J., Kim, P.G., Chou, S., Yoshimoto, M., Yoder, M.C., Daley, G.Q. (2014) Neonatal engraftment defines the most nascent embryonic HSCs. *Dev Cell,* 29: 621-628.

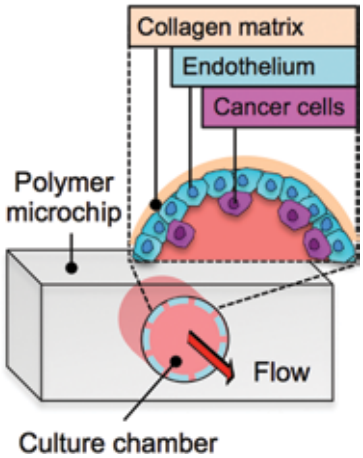
Lee, H.J., Li, N., Evans, S.E., Diaz, M.F., Wenzel, P.L. (2013) Biomechanical force in blood development: extrinsic physical cues drive pro-hematopoietic signaling. *Differentiation* 89: 92-103.



Utrastructural analysis of cancer cells by scanning electron microscopy reveals development of filopodial extensions indicative of active cell migration under fluid flow.

LAB MEMBERS

Research Associate: Miguel Diaz
Postdoctoral Fellow: Hyun Jung Lee, Ph.D.
Research Assistant: Abishek Vaidya, M.S.
Student: Katherine Price
Student: Alexander Alexander
Administrative Assistant: Stephanie Baca (Pediatric Surgery)



Elastic polymer is cast by soft lithography to create a cylindrical culture surface embedded within a small transparent device. The microchannel is treated with collagen matrix, followed by introduction of microvascular endothelial cells and cancer cells. Culture medium is pushed through the microchannel to mimic lymphatic flow. The design of this biomimetic microchip permits 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.

Jiaqian Wu, Ph.D.
Assistant Professor

Gene transcription and regulation of stem cell differentiation

My laboratory combines stem cell biology and systems-based approaches involving genomics, proteomics, bioinformatics, and functional assays to unravel gene transcription and regulatory mechanisms governing stem cell differentiation. One major focus of our 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, including RNA-Seq and ChIP-Seq. These studies are crucial in understanding the molecular mechanism of stem cell neural differentiation and its clinical implications. Our goal is to identify and modulate key regulators as therapeutic targets to direct the differentiation of stem cell into neural cells more efficiently and to increase transplantation safety.

The other area of our 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 TCF7, together with RUNX1 are important regulators in this process. Future study will generate 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, including reprogramming partially differentiated cells to return them to a self-renewing state.

RESEARCH PROJECTS

- Characterize molecular signatures of spinal cord injury and neurological diseases
- Investigate gene expression during stem cell neural differentiation
- Identify key transcription factors and regulatory RNAs, and modulate key regulators to improve differentiation efficiency and transplantation safety
- Identify the molecular switch of hematopoietic precursor cell self-renewal and differentiation
- Network analysis of stem cell differentiation and global network integration of genomic and proteomic data

KEY PUBLICATIONS

Wu, J. Q., Habegger, L., Noisa, P., Szekely, A., Qiu, C., Hutchison, S., Raha, D., Lin, H., Egholm, M., Weissman, S., Cui, W., Gerstein, M., and Snyder, M. (2010). Dynamic Transcriptomes during Neural Differentiation of Human Embryonic Stem Cells Revealed by Integrating Short, Long, and Paired-end Sequencing. *PNAS*. 107: 5254-5259.

Wu, J. Q., Seay, M., Schulz, V., Hariharan, M., Tuck, D., Lian, J., Du, J., Shi, M., Ye, Z. J., Gerstein, M., Snyder, M., and Weissman, S. (2012). Tcf7 is a key regulator of the self-renewal and differentiation switch in a multipotential hematopoietic cell line. *PLoS Genet* 8(3): e1002565.

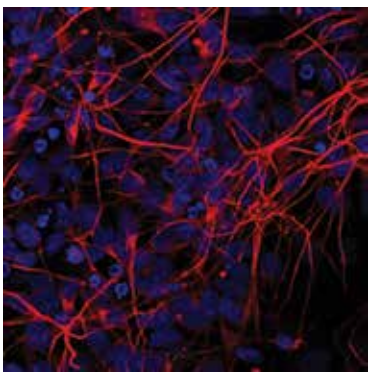
Chen, K., Deng, S., Lu, H., Zheng, Y., Yang, G., Kim, D., Cao, Q., and Wu, J.Q. (2013). RNA-Seq characterization of spinal cord injury transcriptome in acute/subacute phases: a resource for understanding the pathology at the systems level. *Plos One*. 8(8):e72567. PMC3739761

Zhang, Y., Chen, K., Sloan, S., Bennett, M., Scholze, A., O'Keeffe, S., Phatnani, H., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S., Zhang, C., Daneman, R., Maniatis, T., Barres, B., Wu, J.Q. An RNA-Seq transcriptome and splicing database of neurons, glia and vascular cells of the cerebral cortex. *J. Neurosci.*, 34(36): 11929-11947. PMID:25186741

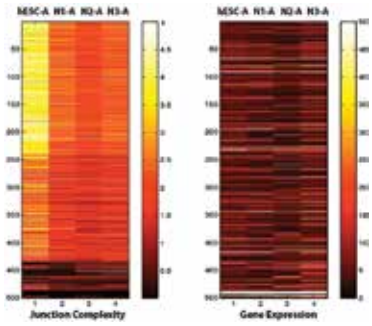
Chen, K., Dong, X., and Wu, J.Q. The Application of single-cell sequencing in dynamic transcriptomes. Single Cell Sequencing. X. Wang (ed.), *Single Cell Sequencing and Systems Immunology, Translational Bioinformatics 5.* Springer Publisher Dordrecht 2015. 41-63

LAB MEMBERS

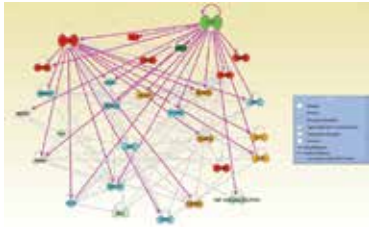
Post-doctoral Fellows: Xiaomin Dong, Ph.D., Yanan You, Ph.D., Raquel Duran, Ph.D.
Research Associate: Han Yan, Ph.D.
Undergraduate Students (Rice University): Fanny Huang, Ashkan Rohani



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.



"Isoform specialization"--Splicing diversity is the highest in hESCs and decreases when cells commit to neural differentiation.



TCF7, together with RUNX1, regulates a transcriptional regulatory network.

Wa Xian, Ph.D.
Assistant Professor
CPRIT Scholar

Personalizing stem cells from chronic diseases and malignancies

The potential of tissue-specific stem cells for modeling critical diseases and for regenerative medicine has been limited by our inability to clone these cells. This is in contrast to embryonic and induced pluripotent stem cells that, while clonogenic, are far removed from disease states. My laboratory is developing robust technologies for cloning stem cells from normal and diseased human epithelial tissues such as lung, gastrointestinal tract, pancreas, and liver. Our analyses are revealing that these cells are marked by 1) unlimited proliferative potential, 2) are multipotent, and 3) are absolutely committed to the tissues from which they were derived, all of which define them as true stem cells.

Perhaps the most remarkable features of these cloned stem cells are their ability to reveal disease mechanisms and faithfully regenerate complex epithelia. My laboratory is particularly interested in the contribution of epithelial stem cells to chronic inflammatory diseases of the airways (asthma, COPD) and of the gastrointestinal tract (inflammatory bowel diseases). Our analyses of patient-derived stem cells are revealing altered inflammatory signaling that likely drives these diseases. That these stem cells also possess the information for complex epithelial structures such as alveoli in lung and 3-D villi structures of the intestine and colon, coupled with advances in genome editing, offers new approaches to chronic and invariably lethal diseases of the lung, liver, pancreas, and kidney.

Finally, we have recently adapted our stem cell cloning technology to the problem of the evolution and lethality of epithelial cancers of the ovary, esophagus, pancreas, and lung. Our ability to clone stem cells of precursor lesions such as Barrett's esophagus, gastric intestinal metaplasia, and PanINs that often appear decades before frank cancers offers the possibility of preemptive therapies. But perhaps the most striking development is that we can efficiently clone the elusive "cancer stem cell" from highly malignant human carcinomas such that each patient's tumor is represented as a "library" of thousands of such cells. Functional and genomic analyses of these libraries is revealing remarkable features of cancer stem cell heterogeneity including subsets of cancer stem cells that possess intrinsic resistance to standard-of-care chemotherapy. This knowledge is now being employed to identify optimal patient-specific therapies to mitigate recurrent disease mounted, in large part, by this minority population of resistant cancer stem cells. We anticipate that such approaches will alter the outcome for patients with these diseases.

RESEARCH PROJECTS

- Libraries of cancer stem cells from high-grade ovarian cancer
- Cancer stem cells from upper gastrointestinal tract cancers
- Evolution of cancers via metaplastic precursors, dysplasia, adenocarcinoma
- Gastrointestinal stem cells from patients with inflammatory bowel disease
- Stem cell alterations in patients with COPD and asthma
- Models for lung stem cell transplantation in acute and chronic lung damage

KEY PUBLICATIONS

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

Zuo, W., Zhang, T., Wu, D.Z-A, Guan, SP, Liew, AA, Yamamoto, Y., Wang, X., Lim, SW, Vincent, M., Lessard, M., Crum, CP, Xian, W*, McKeon, F*. (2014). p63+/Krt5+ distal airway stem cells are essential for lung regeneration. *Nature* 517, 616-620.

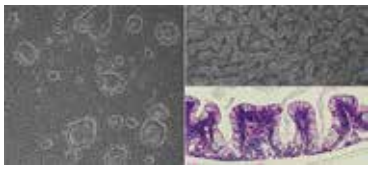
Herfs M, Yamamoto Y, Laury A, Wang X, Nucci MR, McLaughlin-Drubin ME, Münger K, Feldman S, McKeon FD, Xian W*, Crum CP*. (2012). A discrete population of squamocolumnar junction cells implicated in the pathogenesis of cervical cancer. *Proc Natl Acad Sci USA.* 109:10516-10521.

Kumar, P.A., Hu, Y., Yamamoto, Y. Neo, B.N., Wei, T.S., Mu, D., Sun, Y., Lim, S.J., Dagher, R., Zielonka, E.M., Wang, D.Y., Lim, B., Chow, V.T., Crum, C.P., Xian, W*, and McKeon, F*. (2011). Distal airway stem cells render alveoli in vitro and during lung regeneration following H1N1 influenza infection. *Cell* 147, 525-538.

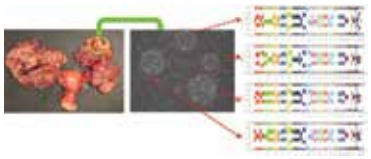
Wang X, Ouyang H, Yamamoto Y, Kumar PA, Wei TS, Dagher R, Vincent M, Lu X, Bellizzi AM, Ho KY, Crum CP, Xian W*, McKeon F*. (2011). Residual embryonic cells as precursors of a Barrett's-like metaplasia. *Cell* 145, 1023-1035. *Communicating author

LAB MEMBERS

Senior Research Scientist: Xia Wang, Ph.D.



Colonies of approximately 500-1000 colonic stem cells on irradiated 3T3 feeder cells (left). Right, Colonic epithelia derived from a single colonic stem cell pedigree following exposure to air-liquid inferface conditions. Top, view of culture en face. Bottom, H&E stained histological section.



Derivation of patient-specific cancer stem cell library of clones from high-grade ovarian cancer case (left). Four of 96 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. Understanding the molecular basis of aging and developing strategies for preventing or delaying age-associated diseases are therefore two of the most 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, 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 programs are focused on methods and techniques to ameliorate and reverse these changes.

The successful completion of this research will result in the development of novel approaches for the use of stem/progenitor cells or rejuvenating factors, derived from functional stem/progenitor cells, to extend human health and lifespsn. 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.
Center Director & Distinguished Wallace Professor
Vice Chair for Research, Department of Orthopaedic Surgery
Chief Scientific Officer & Director of Center for Regenerative Sports Medicine
Steadman Philippon Research Institute, Vail, Colorado

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

Heterogeneity of tumor microenvironment and cancer resistance mechanisms to therapeutic antibody treatment

The focus of my research program is in the areas of gene therapy, tissue engineering, and regenerative medicine applications based on the use of muscle-derived stem cells (MDSCs). My primary areas of interest are in basic stem cell biology and their translation to clinic to aid in the healing and regeneration of a variety of tissues. My team has received national and international recognition, and the technologies that we have developed, have been licensed to industry. The MDSCs that have been isolated by my team are currently undergoing clinical trials for the treatment of urinary stress incontinence and myocardial infarction. As of this date, more than 400 patients in Canada and the U.S have volunteered for this stem cell therapy. Our current major research interests include: skeletal muscle stem cell isolation and their characterization; alleviation of the muscular degeneration associated with Duchenne's muscular dystrophy (DMD) through MDSC transplantation; bone and articular cartilage regeneration through stem cell transplantation; cardiac and skeletal muscle injury repair, regeneration, and fibrosis prevention; peripheral nerve regeneration using MDSCs; the use of MDSCs as a source for paracrine factors to alleviate the phenotypic changes associated with natural aging and progeria. My research team has published over 300 peer reviewed papers, 82 book chapters, and have had 757 abstracts accepted for presentation at national and international conferences.

RESEARCH PROJECTS

- Bone abnormalities and healing defect in muscular dystrophy
- The use of coacervate technology as a new drug delivery system for musculoskeletal tissue repair
- Biomimetic coacervate delivery of muscle stem cells to improve cardiac repair
- Cell autonomous and non-autonomous mechanisms of stem defects with aging
- Development of biological approaches to improve functional recovery after compartment syndrome injury

KEY PUBLICATIONS

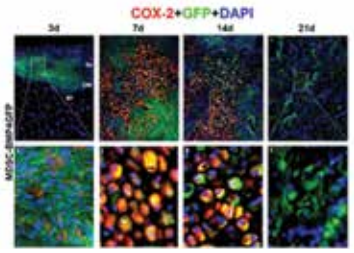
Cao B, Zheng B, Jankowski RJ, Kimura S, Ikezawa M, Deasy B, Cummins J, Epperly M, Qu-Petersen Z, Huard J. Muscle stem cells differentiate into hematopoietic lineages but retain myogenic potential. *Nat Cell Biol* 2003 Jul; 5(7):640-6.

Zheng B, Cao B, Crisan M, Sun B, Li G, Logar A, Yap S, Pollett, JB, Drowley L, Cassino T, Gharaibeh B, Deasy B, Huard J, Péault B. Prospective identification of myogenic endothelial cells in human skeletal muscle. *Nat Biotech* 2007 Sep; 25(9):1025-34. J. Huard corresponding author. PMID: 17767154

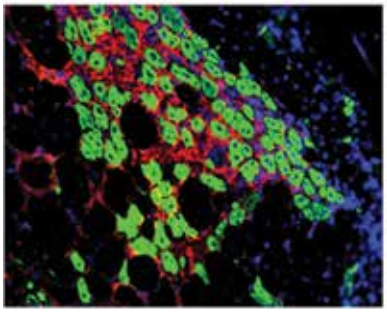
Crisan M, Park, TS Casteilla, L, Sun B, Zheng B, Yap S, Norotte C, Corselli M, Traas J, Deasy B, Andriolo G, Bühring HJ, Lazzari L, Giacobino JP, Huard J, Péault B. Perivascular origin of mesenchymal stem cells in multiple human tissues. *Cell Stem Cell,* 2008 Sep 11;3(3):301-13.

Lavasani M, Robinson A, Lu A, Song M, Feduska J, Ahani B, Tilstra J, Feldman C, Robbins P, Niedernhofer L, Huard J. Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model. *Nat Commun* 2012 Jan; 3:608. PMID: 22215083

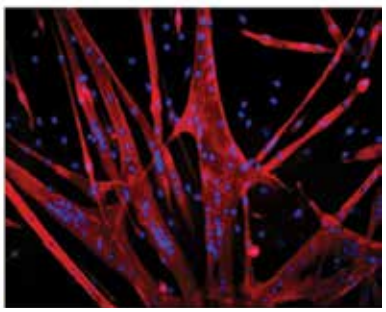
Lavasani M, Thompson SD, Pollett JB, Usas A, Lu A, Stolz DB, Clark KA, Sun B, Péault B, Huard J. "Human muscle-derived stem/progenitor cells promote functional murine peripheral nerve regeneration." *J Clin Invest*. 2014 April 1. 124(4). 1745-56. PMID: 24642464



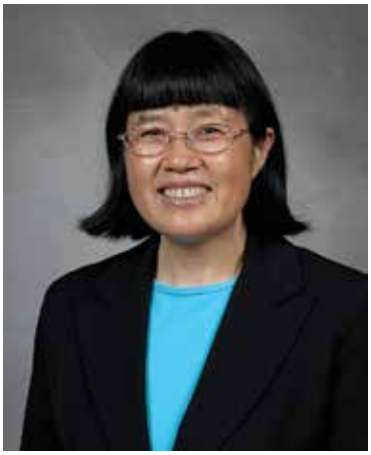
Cox-2 is important for muscle derived stem cell (MDSC) mediated bone regeneration. It is expressed highly in the chondrocyte stage (7 and 14 days post-implantation) and colicalizes with GFP (donor cells) during MDSC mediated endochondral bone formation when transplanted in a critical size cranial defect model. DE: Defect: DE, Dura mater: BR: Brain.



Embryonic myosin (green, showing newly regenerated muscle fibers) and macrophages (F4/80, red) infiltration were detected in dystrophic muscle.



Muscle derived stem cells are able to differentiated into Myosin heavy chain positive myotubes (Red) in vitro, Nuclei stained with DAPI (Blue).



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 in Dr. Huard's laboratory focuses on using muscle-derived stem cells and gene therapy for bone and cartilage repair. I am doing translational studies to use muscle-derived stem cells for the treatment of bone defect, fracture non-union, and age-related bone and cartilage conditions such as osteoporosis and osteoarthritis. I am also working on the bone biology of a disease model – muscular dystrophy.

Human muscle derived stem cells for bone regeneration

Large segmental bone defect and non-union fracture caused by traumatic injury, cancer resection represent major issues in orthopaedic clinic. Using stem cells, growth factors, and scaffold to regenerate bone tissue to replace traditional autografts and allografts to treat these disease is a new trend and has achieved a lot of progress. We are exploring new vectors, growth factors to mediate human muscle derived stem cells *ex vivo* gene therapy. We also are investigating the age of human muscle-derived stem cells and host on the human muscle-derived stem cells mediated bone repair.

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

Much progress has been made in using stem cells, including murine muscle derived stem cells for osteochondral defect or osteoarthritis repair. In this project, we will explore *ex vivo* gene therapy including using viral vector and biomaterial to deliver growth factors for cartilage repair especially osteoarthritis using both *in vitro* and *in vivo* model.

Exploring the muscle and bone interaction in a muscular dystrophy model

Muscular dystrophy is a deadly muscle disease that inflicts 1 in 3,000 boys. Patients often become wheelchair bound in their second decade of life. We have found bone abnormalites in a dystrophin /utrophin double knock out (dko) model that closely mimic the clinical manifestation of human DMD patients. We are investigating how the muscular dystrophy affects the bone and how muscle and bone interact in this mouse model. We are hoping to unveil mechanisms that can be used as a strategy to benefit DMD patients.

RESEARCH PROJECTS

- Utilizing human muscle derived stem cells and gene therapy for the bone tissue repair
- Human muscle derived stem cells for cartilage and osteoarthritis repair using *ex vivo* gene therapy and biomaterial scaffold.
- Exploring the mechanism of bone abnormalities in muscular dystrophy disease mode

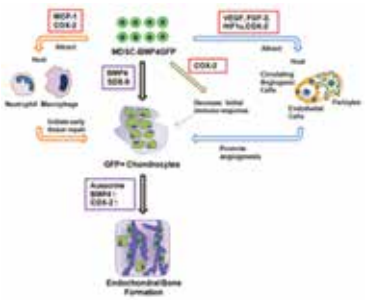
KEY PUBLICATIONS

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

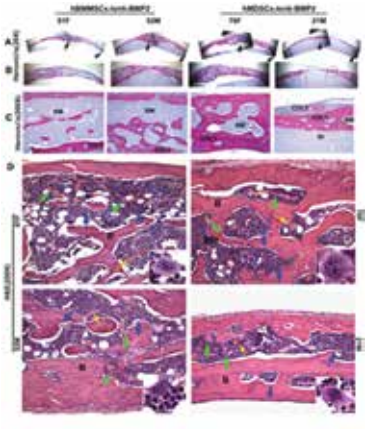
Gao X, Usas A, Proto JD, Lu A, Cummins JH, Proctor A, Chen CW, Huard J. Role of donor and host cells in muscle-derived stem cell-mediated bone repair: differentiation vs. paracrine effects. *FASEB J.* 2014 Aug;28(8):3792-809. PMID:24843069

Gao X, Usas A, Lu A, Tang Y, Wang B, Chen CW, Li H, Tebbets JC, Cummins JH, Huard J. BMP2 is superior to BMP4 for promoting human muscle-derived stem cell-mediated bone regeneration in a critical-sized calvarial defect model. *Cell Transplant.* 2013;22(12):2393-408. PMID:23244588

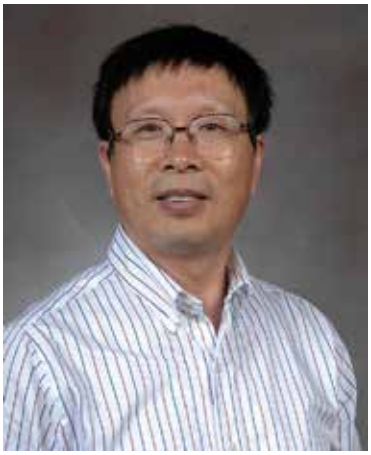
Chen CW, Okada M, Proto JD, Gao X, Sekiya N, Beckman SA, Corselli M, Crisan M, Saparov A, Tobita K, Péault B, Huard J. Human pericytes for ischemic heart repair. *Stem Cells.* 2013 Feb;31(2):305-16. PMID: 23165704



Mechanisms of murine BMP4GFP-transduced MDSCs mediated bone regeneration: differentiation and interaction with host cells via paracrine effect (*FASEB J.* 2014 Aug;28(8):3792-809.).



Human muscle-derived stem cells transduced with lenti-BMP2 regenerated functional bone that constitute bone and bone marrow with three lineages of hematopoietic stem cells in the critical size bone defect model and are as efficient as bone marrow MSCs (*Biomaterials.* 2014 Aug;35(25):6859-70.).



Ping Guo, Ph.D.
Assistant Professor

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.

1) Biomimetic coacervate delivery of MDSCs for cardiac repair and regeneration.

Cellular cardiomyoplasty (CCM) is a promising approach to repair injured myocardium and improve cardiac function. 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.

2) The effects of continuous pressure on MDSCs to enhance articular cartilage repair.

Osteoarthritis (OA) is a debilitating musculoskeletal disease for which there is currently no cure. In clinical physical therapy, joint degeneration associated with immobilization of a joint in a forced position caused from the effects of continuous compression of living articular cartilage in patients, which may prevent stem cell regeneration. Whether the stem cells can survive, proliferate, and differentiate under the high pressure microenvironment is unknown. To utilize stem cells for OA therapy, we have developed a continuous pressure culture system to mimic the *in vivo* high pressure microenvironment of the joint. The project will elucidate whether hMDSCs can tolerate a high pressure microenvironment and whether the high pressure can enhance the hMDSCs to differentiate into chondrocytes or whether the high pressure inhibits chondrogenesis.

3) MDSCs and regeneration in digestive tract organs.

The digestive system is critical for human life and, of course, for providing essential nutrients and energy for athletes. The pancreas is a vital part of the digestive system and a critical controller of blood sugar levels. Stem cells hold tremendous potential because they have the potential to become virtually any kind of cell and hence could be a source of pancreatic cells that could be placed in a Biohub, which mimics the native pancreas. Many sources of stem cells are being studied for their efficacy at improving pancreatic diseases in animals and patients; however, the use of muscle derived stem cells (MDSCs) for treating pancreatitis and diabetes have not been reported. Our lab has shown that MDSCs are capable of differentiating into nerve, bone, cartilage, muscle, and heart. Here we propose that MDSCs may have the potential capacity to differentiate into pancreatic cells, or could recruit other host cells to differentiate into pancreatic cells in order to treat pancreatitis or diabetes.

RESEARCH PROJECTS

- hMDSCs and mMDSCs plus coacervate delivery of muscle stem cells for cardiac repair and regeneration in infarction mice
- *In vitro* continuous pressure culture of hMDSCs to mimic *in vivo* high pressure microenverement for chondrogenic differentiation
- hMDSCs potential therapeutic development in type I diabetes mouse

KEY PUBLICATIONS

Xiao X, Guo P, Prasadan K, Shiota C, Peirish L, Fischbach S, Song Z, Gaffar I, Wiersch J, El-Gohary Y, Husain SZ, Gittes GK., Pancreatic cell tracing, lineage tagging and targeted genetic manipulations in multiple cell types using pancreatic ductal infusion of adeno-associated viral vectors and/or cell-tagging dyes. *Nature Protocols,* 2014, (12):2719-24.

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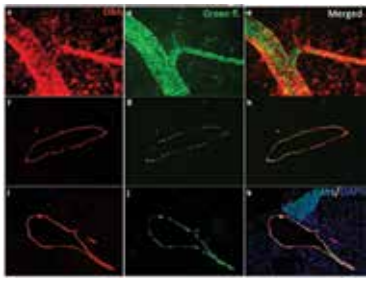
Guo P, Xiao X, El-Gohary Y, Criscimanna A, Prasadan K, Rymer C, Shiota C, Wiersch J, Gaffar I, Esni F, Gittes GK., Specific transduction and labeling of pancreatic ducts by targeted recombinant viral infusion into mouse pancreatic ducts. *Laboratory investigation,* 2013, 93(11):1241-53.

Guo P, El-Gohary Y, Prasadan K, Shiota C, Xiao X, Wiersch J, Paredes J, Tulachan S, Gittes GK., Rapid and simplified purification of recombinant adeno-associated virus. *Journal of virological methods.*2012, 183(2):139-46.

LAB MEMBERS

Post-doctoral Fellow: Dr. Ping Guo joined the Department of Orthopedic Surgery in June 2015 and is currently seeking postdoctoral fellows with experience in cell biology/molecular biology and small animal surgery.
Technicians: Andrea Liebowiz, Elizabeth Morris



GFP expression specifically in AAV6-Sox9-GFP virus-infected pancreatic ducts.

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 muscular disease and accelerate aging, including Duchenne Muscular Dystrophy (DMD) and progeria. Currently, we have two major research areas.

The Role of Muscle Progenitor Cell Exhaustion in the Rapid Disease Progression in Dystrophic Mice.

Duchenne muscular dystrophy (DMD) patients lack dystrophin from birth; however, muscle weakness only becomes apparent at 3-5 years of age, which happens to coincide with the depletion of the muscle progenitor cell (MPC) pools. We are investigating whether the progression of muscular dystrophy is a consequence of the decline in functional MPCs. We are working on different animal models. We believe that alleviating MPC depletion could represent an approach to delay the onset of the histopathologies associated with DMD patients.

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)

We have already demonstrated that muscle derived stem/progenitor cells (MDSPCs) isolated from the skeletal muscle of naturally aged and progeroid *ERCC1*- deficient mice have a reduced ability to proliferate and differentiate, and an impaired regenerative capacity compared to MDSPCs isolated from young wild type (WT) mice. Although this dysfunction is consistent with the concept that the accumulation of damage directly to the stem/progenitor cells (cell autonomous effect) contributes to the loss of tissue regeneration and homeostasis associated with aging, a potential defect in the stem cell niche or circulating factors could also affect MDSPC function in a non-autonomous manner. It remains unclear if aging-related loss of adult stem cell function is primarily driven by cell autonomous (e.g. increased DNA damage) and/or non-autonomous mechanisms (aged microenvironment or circulating factors). In the current study, we utilized a mouse model that had a tissue-specific deletion of the *ERCC* gene and investigated if increased DNA damage in the skeletal muscle niche was sufficient to induce MDSPC dysfunction. We also will use parabiosis to determine whether aging affects the stem cell niche, impacting stem cell function via a nonautonomous mechanism.

RESEARCH PROJECTS

- Muscle stem cell depletion in different animal models, including mdx/mTR and dystrophic dog for stem cell therapy for DMD
- Isolation and characterization of MDSPCs from different transgenic mice for PO1 aging projects
- Paraboisis between dystrophic mice and WT mice, for evaluate muscle and heart
- To investigate the cell- cell interaction between myogenic cells and non-myogenic cells

KEY PUBLICATIONS

Xueqin Gao, Arvydas Usas, Ying Tang, Aiping Lu, Jian Tan, Johannes Schneppendahl, Adam Kozemchak, Bing Wang, James H Cummins, Rocky S Tuan, Johnny Huard. 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.

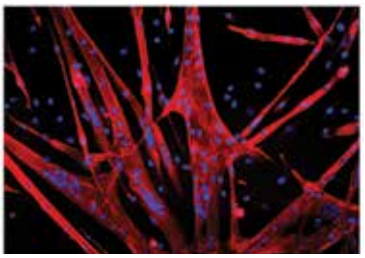
Xueqin Gao, Arvydas Usas, Jonathan D. Proto, Aiping Lu, James H Cummins, Alexander Proctor, Chien-Wen Chen and Johnny Huard. Role of donor and host cells in muscle derived stem cell mediated bone repair: differentiation vs. paracrine effects. *The FASEB Journal.* 2014 Aug; 28(8):3792-809.

Lu A, Poddar M, Tang Y, Proto JD, Sohn J, Mu X, Oyster N, Wang B, Huard J. Rapid Depletion of Muscle Progenitor Cells in Dystrophic mdx/utrophin-/- Mice. *Hum Mol Genet.* 2014 Sep 15; 23(18): 4786-800.

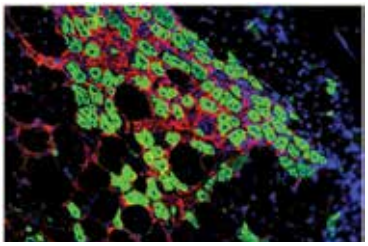
Lavasani M, Thompson SD, Pollett JB, Usas A, Lu A, Stolz DB, Clark KA, Sun B, Péault B, Huard J. Human muscle-derived stem/progenitor cells promote functional murine peripheral nerve regeneration. *J Clin Invest.* 2014 Apr 1; 124(4):1745-56

LAB MEMBERS

I started my employment with the University of Texas Health Science Center at Houston in May 2015, and I am currently seeking to hire one research technician and one postdoc with experience of cell biology.



Muscle derived stem cells are able to differentiate into myosin heavy chain positive myotubes (red) *in vitro*.



Embryonic myosin (green, showing new regenerated muscle fibers) and macrophage (F4/80, red) infiltration were detected in dystrophic muscle.

Xiaodong Mu, Ph.D.
Assistant Professor

Stem cell senescence and dysfunction in diseased muscle tissues and premature aged disease models

Our "Stem Cell in Aging and Cancer" research group is a part of Dr. Johnny Huard's research center in the Department of Orthopedic Surgery, and our studies have involved stem cell biology, wound regeneration, fibrosis prevention, muscular dystrophy, premature aging, and cancer biology (osteosarcoma). Currently we are especially interested in studying the mechanism of stem cell senescence and premature aging, and the mechanism of various musculoskeletal disorders (i.e., Duchenne Muscular Dystrophy, cancer cachexia-related muscle atrophy, heterotopic ossification, osteosarcoma, and so on).

Currently I am focusing my research in the following areas:

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

Accelerated exhaustion, senescence, and loss of regeneration potential of stem cells have been observed in the diseased skeletal muscle, such as that of progeria (accelerated aging) and Duchenne Muscular Dystrophy (DMD, a degenerative muscle disorder in boys caused by dystrophin deficiency). The key stem cell regulators: Notch, Wnt, RhoA, and mammalian Target of Rapamycin (mTOR) signaling pathways have all been shown as important regulators of tissue aging and cell senescence.

2). The cellular and molecular mechanism of heterotopic ossification (calcification) in skeletal muscle, cardiac muscle, and blood vessels, and its relevance to bone osteoporosis.

I have previously reported extensive HO in the skeletal muscle and cardiac muscle of severely affected dystrophic mice, and RhoA signaling was found as a key mediator. Question remains as to whether RhoA activation could be a mediator of HO caused by surgery or trauma to the hips and legs, and traumatic brain or spinal cord injuries, as wells as its correlation with other factors (i.e., BMPs, Notch, and NF-κB).

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

A higher ratio of matrix metalloproteinases (MMPs) to their tissue inhibitors (TIMPs) has been observed in scarless wound healing. I have 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 the healing of diseased skeletal muscle (i.e., dystrophic muscle) is usually accompanied with excessive fibrosis, I would next find out how to help the 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 defect in diseased and aged muscles
- The cellular and molecular mechanism of heterotopic ossification (calcification) in skeletal muscle, cardiac muscle, and blood vessels, and its relevance to bone osteoporosis
- Improvement of soft tissue wound healing by reducing fibrosis or application of stem cells
- Role of Notch and Wnt signaling in regulating stem cell senescence of accelerated aging animal in contrast to normal aging animal, as well as the cancer stem cells

KEY PUBLICATIONS

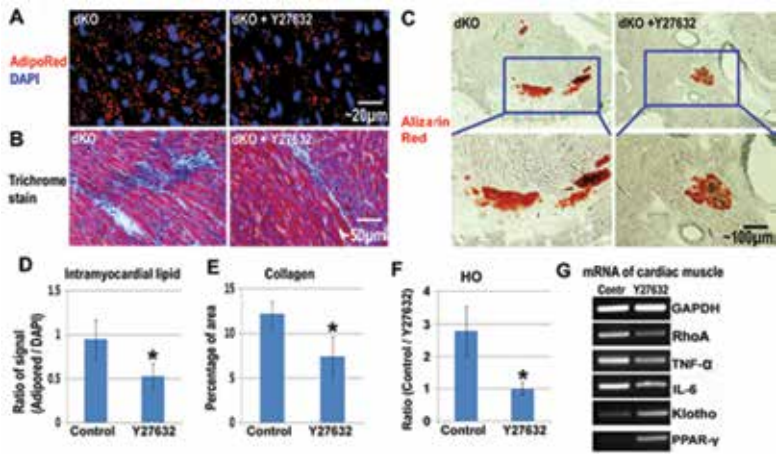
Mu X, Tang Y, Lu A, Takayama K, Usas A, Wang B, Weiss K, Huard J. The role of Notch signaling in muscle progenitor cell depletion and the rapid onset of histopathology in muscular dystrophy. *Hum Mol Genet.* 2015 Feb 12.

Mu X, Usas A, Tang Y, Lu A, Wang B, Weiss K, and Huard J. RhoA mediates defective stem cell function and heterotopic ossification in dystrophic muscle of mice. *FASEB J.* 2013, May.

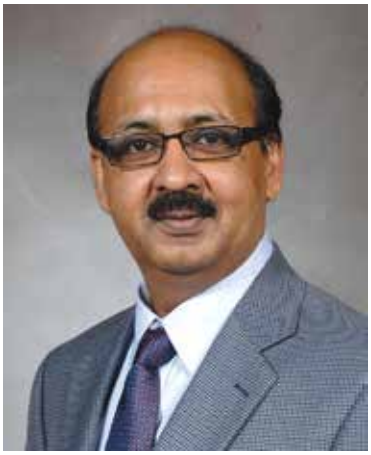
Mu X, Isaac C, Greco N, Huard J, and Weiss K. Notch Signaling Is Associated With ALDH Activity And An Aggressive Metastatic Phenotype In Murine Osteosarcoma Cells. *Frontiers in Pediatric Oncology.* 2013, May.

Mu X, Sultankulov B, Agarwal R, Mahjoub A, Schott T, Greco N, Huard J and Weiss K. Chick Embryo Extract demethylates tumor suppressor genes in osteosarcoma cells. *Clinical Orthopaedics and Related Research.* 2013, May.

Mu X, Urso M, Murray K, Fu F, and Li Y. Relaxin regulates MMP expression and promotes satellite cell mobilization during muscle healing in both young and aged mice. *American Journal of Pathology,* 2010;177:2399-2410.



Inactivation of RhoA/ROCK signaling reduced the intramyocardial lipid accumulation, fibrosis, and heterotopic ossification in the cardiac muscles of muscular dystrophic mice.



Krishna Sinha, Ph.D.
Assistant Professor

Epigenetic control of bone formation and bone metastases

My research interests are focused on epigenetic control of cell-fate determination with specifics on critical factors responsible for bone formation and bone homeostasis, as well as prostate cancer-induced skeletal metastases. The current research plan in Dr. Johnny Huard's lab is to identify and study gene function in age-related bone disorders, which provide pivotal information in considering preventive measures and treating bone disorders for better bone health and life.

Epigenetic control of bone formation by Osterix and histone demethylase NO66.

Differentiation of osteoblasts commences from mesenchymes with stage-specific gene activation, which supports maturation and function of osteoblasts. Using a proteomic approach, I identified a chromatin regulator NO66 as Osterix-interacting protein. NO66 is a JmjC-domain histone demethylase specific for lysine 4 and 36 of histone H3. Osterix (Osx) is an essential transcription factor required for bone and tooth formation and controls activation of a repertoire of genes in osteoblast and osteocytes. NO66 inhibits Osterix activity through interactions with Osx and through demethylation of histone at specific euchromatin regions in osteoblasts. Later on, we reported the crystal structure of NO66 and its interface required for interactions with Osx, which provides useful structural insights while designing or developing inhibitors against NO66. NO66 interacts with EZH2 (enhancer of zeste homolog 2) containing PRC2 (polycomb-repressive complex 2), which has histone K27 methyl transferase activity and inhibits gene activation during ES cell differentiation.

Osterix acts as the molecular switch from gene repression to gene activation.

We showed that during early embryonic stages of mouse development, the promoters of *Osx*, *Col1a1* and *Bsp* are hypermethylated in mesenchyme cells and are also bound with methylation-induced repressors complex including NO66, which prevents gene activation in those cells as well as in *Osx*-null cell. In differentiating osteoblasts, these genes become hypomethylated, and are bound with Runx2, Osx, and other active histone modifiers during their activation. We then demonstrated with conditional inactivation of *NO66* in mesenchymal cells that loss of function of NO66 in mice leads to increased bone mass phenotype.

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

The fact that bone is the most susceptible organ for metastases by nearly all types of cancer primarily by prostate and breast, skeletal metastases further lead to severe defects in bone architectures during aging. NO66 is up-regulated in lung cancer. We also found that NO66 levels are elevated in prostate cancer patient samples and xenografted samples. Our data indicates that NO66 overexpression promotes 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.

RESEARCH PROJECTS

- Use of Next-Gen sequencing approach to identify epigenetic signature marks of histone methylations and DNA methylation in the key osteoblast genes and to better study the multiple pathways/mechanisms involved in normal and malignant bone formation
- To study the post-translational modification of Osterix by lysine methylation in regulation of Osx activity during osteoblast differentiation and bone formation
- To study the *in vivo* role of NO66 in bone remodeling process using mouse model
- Oncogenic function of NO66 in PCa progression and bone metastasis

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

KEY PUBLICATIONS

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Sinha K, Yasuda H, Zhou X and deCrombrugghe B (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.

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Brien G, Gambero G, O'Connell D, Jerman E, Turner S, Egan C, Dunne E, Jurgens M, Wynne K, Piao L, Lohan A, Ferguson N, Shi X, Sinha K, Loftus J, Cagney G, and Bracken, A (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.

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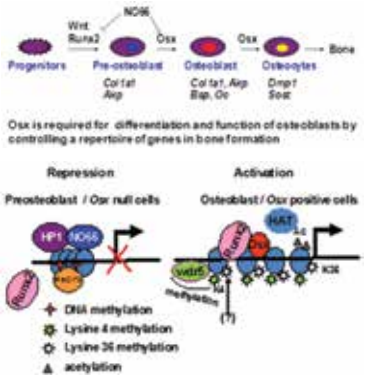


Osterix serves as molecular switch for gene activation in osteoblasts (*JBMR,* Epub 2013 Sep 23)

TEXAS THERAPEUTICS INSTITUTE

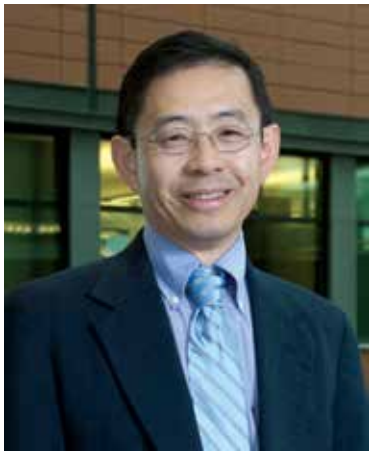
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston for the discovery, development, and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

During the past five years, TTI has grown into a comprehensive academic drug discovery center and made significant scientific discoveries in the areas of cancer biology and biologics drug development. TTI investigators have brought in significant funding from the pharmaceutical and the biotechnology industry, including Johnson & Johnson, Merck and PanaMab, the National Institutes of Health, and the Cancer Prevention and Research Institute of Texas (CPRIT). It is noteworthy that TTI investigators were awarded four major grants totaling more than $7 million from CPRIT in 2015.

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

In addition to the 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, but they also support collaborative projects with scientists from the IMM, the Texas Medical Center, and other Texas-based institutions. As an academic drug discovery center, TTI faculty have founded two UTHealth spinoff biotech companies with significant venture capital funding to translate TTI discoveries into lifesaving medicines.

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.

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 their mode of actions.

Antibodies response to experimental HIV, dengue, and CMV vaccines. Design of highly immunogenic peptide and protein based vaccines that induce neutralizing antibodies against a broad range of clinical isolates is one of the approaches in developing effective HIV and dengue vaccines. We have an ongoing project to aid the design of HIV and dengue vaccines by profiling antibody response to the experimental vaccines in rhesus. We also have a project in the discovery and development of neutralizing antibodies against the human cytomegalovirus (HCMV).

Pnemocandin biosynthesis and biocombinatorial chemistry approach for natural products drug discovery. The antifungal therapy caspofungin is a semi-synthetic derivative of pneumocandin B_0, a lipohexapeptide produced by a fungus. In collaboration with Dr. Gerald Bills' group, we are studying the pneumocandin biosynthesis pathway using a combination of genomic, genetic, and chemical approaches. Elucidation of the pneumocandin biosynthetic pathway will pave the way for designing experimental procedures to engineering analogues with improved oral availability or broader spectrum of antifungal activities.

Therapeutic monoclonal antibody drug discovery platform. Supported by a grant from the Texas Emerging Technology Fund and as part of the Texas Therapeutics Institute, our group has been building 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.

RESEARCH PROJECTS

- HER3 mediated cell signaling and the development of HER3 targeting monoclonal antibodies for cancer therapy
- Evaluation of vaccine-induced antibody responses in preclinical animal models and humans
- Biocombinatorial chemistry approach for natural products drug discovery
- Therapeutic antibody discovery and development

KEY PUBLICATIONS

Ningyan Zhang, Hui Deng, Xuejun Fan, Anneliese Gonzalez, Songlin Zhang, Randall J Brezski, Byung-Kwon Choi, Michael Rycyzyn, William R Strohl, Robert E. Jordan, and Zhiqiang An, 2015. Dysfunctional antibodies in the tumor microenvironment associate with impaired anticancer immunity. *Clinical Cancer Research* (Published OnlineFirst July 29, 2015; doi: 10.1158/1078-0432.CCR-15-1057).

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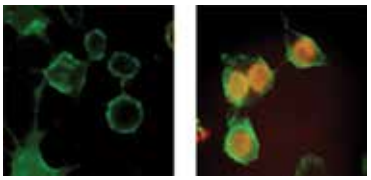
LAB MEMBERS

Post-doctoral Fellows: Ahmad S. Salameh, Weixu (Ella) Meng, Leike (Simon) Li, Xun (Mark) Gui, Hao Ching Hsiao, Robbie D. Schultz, Qun Yue (jointly with Dr. Bills), Yan Li (jointly with Dr. Bills), Li Chen (jointly with Dr. Bills)
Student: Jingnan An

Differential intensity of tumor associated macrophage (CD68+) infiltration in breast tumor tissues (Clinical Cancer Research, 2015; doi: 10.1158/1078-0432.CCR-15-1057).



Trastuzumab-mediated phagocytosis of cancer cells by macrophages. (Journal of Immunology, 2015, 194:4379-4386).

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

Technological breakthroughs in microbial genomics have led to a new view that natural product biosynthetic pathways are vastly more diverse and experimentally tractable than previously imagined. Experimental tractability has led to the functional characterization of pathways of many historically important natural products and new natural product that have been revealed through genomic mining. This view and other converging technologies, such as measuring microbial diversity from metagenomic approaches, improved culturing strategies, engineering unnatural natural products via bioengineering, improved analytical mass spectrometry (MS) and NMR, and the availability of novel disease targets are rapidly changing the approachability of natural product drug discovery.

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 characterized biosynthetic pathways responsible for the family echinocandin antifungals, including pneumocandin B_0, the starting molecule for the antifungal drug CANCIDAS. We have programmed pneumocandin biosynthesis to produce new analogues that have improved product purity, increased potency. We ultimately wish to broaden the spectrum and improve pharmacokinetics, while reducing fermentation production costs. Access to the genes of the echinocandin pathways will enable us to recombine genes from these pathways to produce hybrid natural products with improved therapeutic properties.

We are developing new genetic and physiological methods for expressing and unregulating unexpressed biosynthetic pathways using filamentous fungi as model organisms. We are in the early stages of building a microbial chemical collection focused on metabolites appropriate for intervention in cancer biology, modulation of human molecular signalling pathways, and in other human therapies. Texas is the USA's second most biodiverse state. Therefore, our collection will emphasize the vast microbial resources available from Texas and will be promoted among Texas-based screening centers resulting in new chemicals as probes in cell biology and for intervention in human diseases.

RESEARCH PROJECTS

- Biosynthesis and pathway engineering of the echinocandin lipopeptides for improved antifungals
- Understanding how echinocandin fungi resist their own antifungal metabolites
- Development of methods for reprogramming transcription of biosynthetic genes of fungi to discover new natural products useful to treat human diseases
- Development of a natural products 'chemical resource platform' for drug discovery for other investigators within the UT System, Texas, and elsewhere

KEY PUBLICATIONS

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Yue, Q., L. Chen, Y. Li, G.F. Bills, X. Zhang, M. Xiang, S. Lia, Y. Che, C. Wang, X. Niu, Z. An & X. Liu. 2015. Functional operons in secondary metabolic gene clusters in Glarea lozoyensis (Fungi, Ascomycota, Leotiomycetes). *mBio* 6:e00703-15.

Cautain, B., N. de Pedro, C. Schulz, J. Pascual, T. da Sousa, J. Martín, I. Pérez Victoria, F. Asensio, I. González, G. Bills, F. Reyes, O. Genilloud & F. Vicente. 2015. Identification of the lipodepsipeptide MDN-0066, a novel inhibitor of VHL/HIF pathway produced by a new Pseudomonas species. *PLoS One* 10:e0125221.

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Bills, G.F., J.B. Gloer & Z. An. 2013. Coprophilous fungi: Antibiotic discovery and functions in an underexplored arena of microbial defensive mutualism. *Current Opinion in Microbiology* 16:549–565. (featured cover article).

LAB MEMBERS

Research Associates: Dr. Qun Yue, Dr. Yan Li, Dr. Li Chen (all visiting from the Institute of Microbiology, Chinese Academy of Sciences).

We have made new derivatives of the pneumocandins by inactivating different pathway genes. New pneumocandins F and G were eight-fold more potent against the human pathogen, *Candida albicans*, than the parent compounds, pneumocandins A_0 and B_0.

Kendra S. Carmon, Ph.D.
Assistant Professor

Development of antibody-drug conjugates for targeting cancer stem cells

Emerging evidence has shown that within several different malignant tumors types there exists a subpopulation of cancer cells that behave like normal stem cells. These cancer stem-like cells (CSCs), or tumor-initiating cells, can renew themselves and sustain the cancer, much like normal stem cells repopulate and maintain our organs and tissues. CSCs can drive tumor growth, metastasis, and resistance to anti-cancer therapies. Since CSCs are often not entirely eliminated by conventional treatments, they can regenerate the tumor and potentially metastasize, leading to a decline in patient quality of life and survival. Thus it is essential to develop a new generation of novel therapies that can ultimately target and destroy CSCs.

The LGR5 (Leucine-rich repeat-containing, G protein-coupled receptor 5) receptor is a bona fide marker of normal adult stem cells in the intestine and multiple other epithelial tissues. As a postdoctoral trainee in Dr. Qingyun's (Jim) Liu's laboratory, I discovered 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. Since then, numerous reports have shown that LGR5 expression is significantly elevated in several major tumor types, including colon, liver, gastric, and ovarian carcinomas. Recent evidence has further demonstrated that human cancer cells with high levels of LGR5 behave like CSCs, fueling tumor growth, metastasis, and drug resistance. These findings suggest the potential of LGR5 as a promising new target for the development of CSC-based therapies.

My research is focused on the development of innovative anti-LGR5 antibody-drug conjugates (ADCs) that will "seek and destroy" LGR5-expressing tumors and CSCs, similar to guided missiles. These ADCs will incorporate a chemical toxin that is only released once it enters target cells with high levels of LGR5. My previous work has shown that LGR5 is continuously and rapidly internalized into the cell, making it an exceptional transit for fast and specific delivery of ADCs into CSCs. A series of anti-LGR5 ADCs are being generated using functionally different antibodies and distinct chemical linkers to incorporate the toxins. Using cutting-edge techniques, we are testing the ability of the ADCs to precisely bind and destroy LGR5-positive cancer cells and evaluating their anti-tumor effects in xenograft tumor mouse models. Additionally, we are further investigating the signaling mechanism(s) of LGR5 and its role in cancer and chemoresistance. This research will provide preclinical proof-of-concept for the feasibility of the future development of anti-LGR5 ADCs. A CSC-targeted ADC could be a breakthrough treatment to eradicate residual tumors and metastasis, and more importantly, prolong overall quality of life and survival for a large number of cancer patients.

RESEARCH PROJECTS

- Development of anti-LGR5 antibody-drug conjugates
- Investigation of LGR5 signaling and its role in cancer and drug resistance

KEY PUBLICATIONS

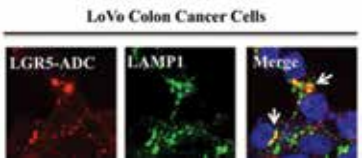
Carmon K.S., Gong X., Yi J., Thomas A., Liu Q. (2014) RSPO-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proc Natl Acad Sci* U S A. 111(13): E1221-9.

Carmon, K.S.*, Lin, Q., Gong, X., Thomas, A. Liu, Q*. (2012) LGR5 Interacts and Co-Internalizes with Wnt Receptors to Modulate Wnt/beta-catenin Signaling. *Mol Cell Biol.* 32(11): 2054-2064. (*Corresponding Authors)

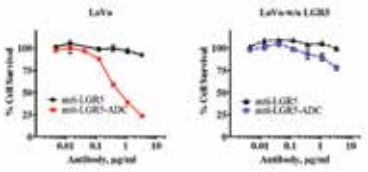
Carmon, K.S., Gong, X., Lin, Q., Thomas, A. Liu, Q. (2011) R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci* USA. 108 (28): 11452-7.

Carmon, K.S., and Loose, D.S. (2010) Development of a Bioassay for Detection of Wnt Binding Affinities for Individual Frizzled Receptors. *Anal Biochem.* 401(2): 288-94.

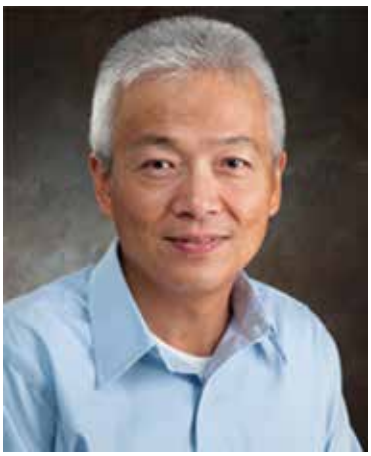
Carmon, K.S., and Loose, D.S. (2008) SFRP4 Regulates Two Wnt7a Signaling Pathways and Inhibits Proliferation in Endometrial Cancer Cells. *Mol Cancer Res.* 6 (6): 1017-1028.



The anti-LGR5-ADC is highly specific and forms a complex with the LGR5 receptor at the cell surface of colon cancer cells. The LGR5-ADC complex (red) is then internalized and transported to the lysosome compartment (LAMP1, green) where the drug can be released to destroy the cancer cells (co-localization, yellow).



Anti-LGR5-ADC treatment significantly decreases colon cancer cell survival (left) and has no effect in cells manipulated to no longer express LGR5 (right). Unconjugated anti-LGR5 antibody has no effect on cell survival.



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.

Our laboratory has 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, diabetes, chronic pain and infections.

RESEARCH PROJECTS

- Structural and functional analyses of the exchange proteins directly activated by cAMP (EPAC), funded by NIH
- Development of *in vivo* chemical probes targeting EPAC for suppressing pancreatic cancer metastasis, 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
- Development of next generation of isoform specific EPAC modulators, in collaboration with NIH Chemical Genomics Center (NCGC)
- Examine the roles of EPAC proteins in major human diseases, such as cancer, chronic pain, diabetes and obesity, using EPAC knockout mouse models

KEY PUBLICATIONS

Almahariq, M., Chao, C., Mei, F. C., Hellmich, M. R., Patrikeev, I., Motamedi, M., and Cheng, X. Pharmacological Inhibition and Genetic Knockdown of EPAC1 Reduce Pancreatic Cancer Metastasis in vivo. *Molecular Pharmacology.* 87:142-149, 2015.

Banerjee U. and Cheng, X. Exchange Protein Directly Activated by cAMP Encoded by the Mammalian rapgef3 Gene: Structure, Function and Therapeutics. *Gene.* 570:157-167, 2015.

Almahariq, M., Mei, F., and Cheng, X. cAMP Sensor EPAC Proteins and Energy Homeostasis. *Trends in Endocrinology and Metabolism.* 25:60-71, 2014.

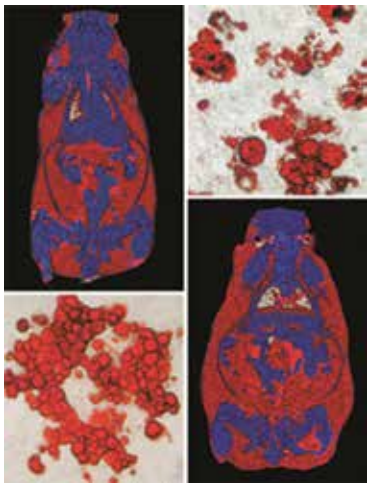
Gong, B.*, Shelite, T., Mei, F., Ha, T., Xu, G., Chang, Q., Hu, Y., Wakamiya, M., Ksiazek, T. G., Boor, P, J., Bouyer, R., Popov, V., Chen, J., Walker, D. H., and Cheng, X.* Exchange protein directly activated by cAMP plays critical role in fatal rickettsioses. *Proc. Acad. Natl. Sci.* USA. 110:19615-19620, 2013.

Almahariq, M., Tsalkova, T., Mei, F. C., Chen, H., Zhou, J., Sastry, S. K., Schwede, F., and Cheng, X. A novel EPAC-specific inhibitor suppresses pancreatic cancer cell migration and invasion. *Molecular Pharmacology*. 83:122-128, 2013.

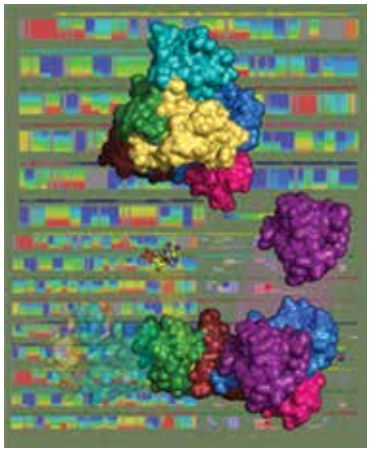
Yan, J., Mei, F. C., Cheng, H. Q., Lao, D. H., Hu, Y., Wei, J., Patrikeev, I., Hao, D., Stutz, S. J., Dineley, K. T., Motamedi, M., Hommel, J. D., Cunningham, K. A., Chen, J.*, and Cheng, X*. Enhanced leptin sensitivity, reduced adiposity and improved glucose homeostasis in mice lacking of exchange protein directly activated by cAMP isoform 1. *Molecular Cellular Biology*. 33:918-926, 2013.

LAB MEMBERS

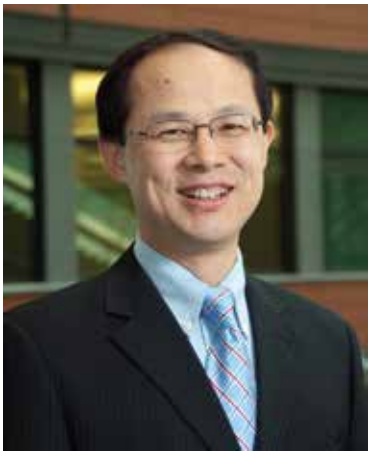
Research Assistant Professor: Fang Mei
Research Scientist: Yingmin Zhu
Post-doctoral Fellows: Upasana Banerjee, Hui Wang, William Robichaux
Student: Yaohua Hu



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



Molecular mechanism of EPAC activation revealed by amide hydrogen exchange mass spectrometry



Wenliang Li, Ph.D.
Assistant Professor

Molecular mechanisms of cancer metastasis

My research is to study novel molecular mechanisms of cancer metastasis with the goal of identifying 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, it is still poorly understood and the current approaches to prevent or treat human metastatic diseases are mostly unsuccessful. Through genomics, RNAi and cDNA functional screens, my lab has identified several critical but previously unknown regulators for cancer metastasis. Signaling pathways and molecular mechanisms of these genes are under investigation with molecular, cellular, biochemical, genomic, proteomic approaches, and mouse models. These studies will yield new insights for cancer metastasis and may facilitate the development of new therapeutics and biomarkers.

Epithelial-mesenchymal transition (EMT), a developmental process, is believed to play a key role in cancer metastasis, drug resistance, organ fibrosis, and stem cell phenotypes. Another exciting research program in the lab is involved in identifying and studying human kinases as novel regulators for EMT. Kinases play central roles in many aspects of signaling transduction, cell physiology, and diseases. They are also one of the most important gene families for cancer drug development. Our literature search indicated that the majority of >700 kinases in human genome are still poorly studied. Our lab is employing unbiased functional screens against hundreds of human kinases to identify novel regulators for EMT and linking them to stem cell phenotypes and cancer metastasis. Investigation of the molecular mechanisms of these kinases will have a significant impact in expanding our knowledge in the crossroad of exciting and critical areas, such as development, stem cell, drug resistance, and metastasis. These kinases may also become new biomarkers and cancer drug targets for the development of novel therapeutics for human cancer.

RESEARCH PROJECTS

- GRK3 mechanism in aggressive therapy-induced neuroendocrine prostate cancers
- New regulators for EMT and their mechanisms in cancer progression
- Development of precision medicine based on genetic profiles and drug sensitivities of patient samples and patient-derived-xenografts (PDXs) growing in mice

KEY PUBLICATIONS

Li L, Li W*. Epithelial-mesenchymal transition in human cancer: comprehensive reprogramming of metabolism, epigenetics and differentiation. *Pharmacology & Therapeutics* 2015 Jun; 150:33-46. *corresponding author

Li L, Liu C, Chang JT, Du G, Li W*. CDKL2 promotes epithelial-mesenchymal transition and breast cancer progression. *Oncotarget* 2014 Nov 15; 5(21):10840-53. *corresponding author

Li W*, Ai N, Wang S, Bhattacharya N, Vrbanac V, Collins M, Signoretti S, Hu Y, Boyce FM, Gravdal K, Halvorsen OJ, Nalwoga H, Akslen LA, Harlow E*, Watnick RS. GRK3 is essential for metastatic cells and promotes prostate tumor progression. *Proceedings of the National Academy of Sciences* USA (PNAS) 2014 Jan 28;111(4):1521-6. *corresponding author

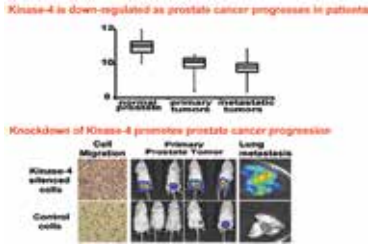
Grueneberg DA*, Li W*, Davies JE and Harlow, E. IV. shRNA screens identify kinase requirements in human cells: differential kinase requirements in cervical and renal human tumor cell lines. *Proceedings of the National Academy of Sciences* USA (PNAS). 2008 Oct 28;105(43):16490-5. *these authors contributed equally (co-first author)

Baldwin A, Li W, Grace M, Harlow E, Münger K and Grueneberg DA. II. Genetic Interaction Screens Identify Alterations in Kinase Requirements Following HPV16 E7 Expression in Cancer Cells. *Proceedings of the National Academy of Sciences* USA (PNAS). 2008 Oct 28;105(43):16478-83.

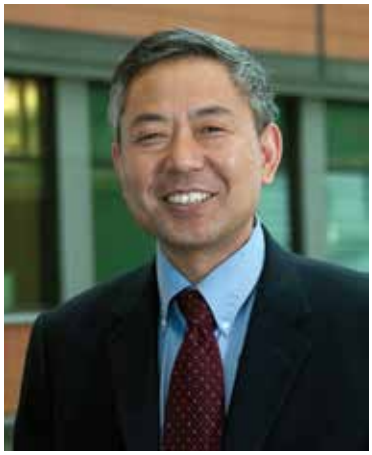
Grueneberg DA*, Degot S*, Pearlberg J*, Li W*, Davies JE*, Baldwin A*, Endege W, Doench J, Sawyer J, Hu Y, Boyce F, Xian J, Munger K, Harlow E. I. Comparing Kinase requirements across Various Cell types. *Proceedings of the National Academy of Sciences* USA (PNAS). 2008 Oct 28;105(43):16472-7. *these authors contributed equally (co-first author)

LAB MEMBERS

Post-doctoral Fellows: Zhi Li, Yan Zhang, Dayong Zheng
Ph.D. Student: Mohit Hulsurkar



We discovered that human kinase GRK3 promotes prostate primary tumor growth and lung metastasis in mouse xenografts.



Qingyun (Jim) Liu, Ph.D.
Professor and Co-Director of the Texas Therapeutics Institute
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. Most recently, we uncovered that RSPO3-LGR4 has a major role in the aggressiveness of lung adenocarcinomas. Our current efforts are focused on identifying and characterizing drug leads targeting the RSPO-LGR system as potential treatment for colon and lung cancers.

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

KEY PUBLICATIONS

Carmon KC, Lin Q, Gong X, Thomas A, and Liu Q (2012). LGR5 Interacts and Cointernalizes with Wnt Receptors To Modulate Wnt/beta-Catenin Signaling. *Mol Cell Biol* 32:2054-2064.

Gong X, Carmon KC, Lin Q, Thomas A, Yi J, and Liu Q (2012). LGR6 Is a High Affinity Receptor of R-Spondins and Potentially Functions as a Tumor Suppressor. *PLoS One* 7:e37137-e37146.

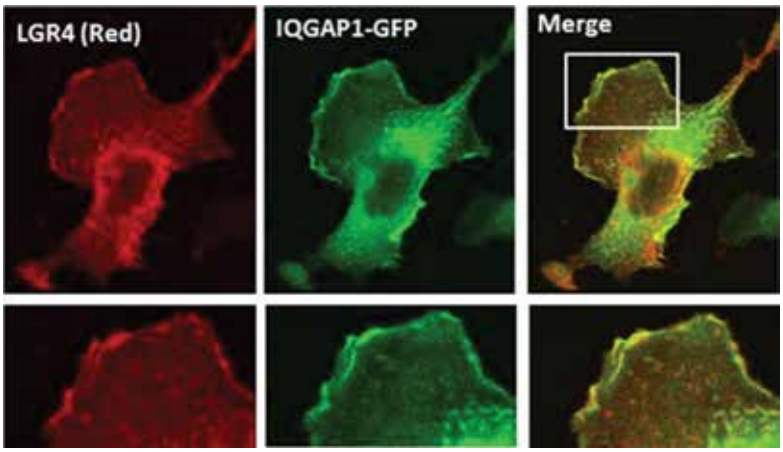
Carmon, K.S., Gong, X, Lin, Q., Thomas, A., and Liu, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci* U S A, 108:11452-11457 (2011).

Yi, J., Wei, X., Gong, X., Bellister, S., Ellis, L.M., and Liu, Q. Analysis of LGR4 Receptor Distribution in human and mouse tissues. *PloS One,* 8:e78144-e7850 (2013).

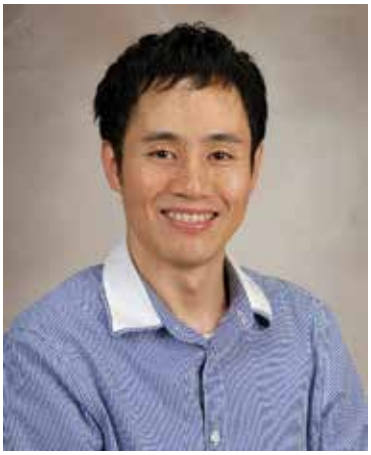
Carmon, K. S., Gong, X., Yi, J., Thomas, A., Liu, Q. RSPO-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proc Natl Acad Sci* U S A. 111: E1221-E1229 (2014).

LAB MEMBERS

Instructor: Xing Gong
Post-doctoral Fellow: Jing Yi
Technician: Anthony Thomas



LGR4 is co-localized with IQGAP1 at the leading edge of cell migration. LGR4 and IQGAP1 play critical roles in the regulation of cell migration.



Kyoji Tsuchikama, Ph.D.
Assistant Professor

Development of chemical agents, tools, and strategies for combating infectious diseases

Antibiotics are powerful agents for the treatment of infectious diseases. However, their strong pharmacological effect poses evolutionary pressure on pathogenic microbes, leading to the development of drug resistance. The emergence of drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Pseudomonas aeruginosa* is an increasingly serious problem for human health. According to the report from the Centers for Disease Control and Prevention (CDC, April 2011), antibiotic resistance in the United States costs us approximately twenty billion dollars per year in excess health care costs and more than eight million additional days spent in hospital. Therefore, alternative antimicrobial strategies based on a novel mechanism of action has been pursued to overcome this clinical challenge.

With this background in mind, my lab is working on two research projects by taking advantage of the power of chemical synthesis and chemical biology. Firstly, we design, synthesize, and evaluate untraditional antimicrobial agents that could potentially circumvent the drug resistance problem. Our molecular design stems from the concept of "delivering catastrophic agents only to target pathogenic cells." We are developing and evaluating various types of molecules consisting of "targeting" and "killing" motifs. Based on this concept, we have succeeded in synthesizing such a molecular conjugate named "RoseBengal/C11-AHL", which gratifyingly exerted >2-fold greater bactericidal activity than Rose Bengal alone. We are currently optimizing this structure to achieve greater potency and broader antibacterial spectra. Secondly, we are conducting proteomic profiling using synthetic chemical probes based on antimicrobial metal complexes. In the past decades, metal complexes have attracted increasing attention as potential drugs for the treatment of cancer, autoimmune diseases, and, more recently, infectious diseases caused by drug-resistant pathogens. However, in general, their molecular targets are still unclear. Chemical probes based on such antimicrobial metal complexes will enable us to identify their protein targets and thus provide novel insights into pharmacological mechanisms and drug design for developing innovative antimicrobial therapeutics. Throughout these projects, we hope to drive our efforts toward innovative medical cures to save people suffering from serious diseases.

RESEARCH PROJECTS

- Selective killing of drug-resistant bacteria using untraditional chemical agents
- Proteomic profiling using chemical probes based on antimicrobial metal complexes

KEY PUBLICATIONS

Collins, K. C., Tsuchikama, K., Lowery, C. A., Zhu, J., Janda, K. D. Dissecting AI-2-mediated quorum sensing through C5-analogue synthesis and biochemical analysis. *Tetrahedron* 2015, in press.

Cai, X.; Tsuchikama, K.; Janda, K. D. Modulating Cocaine Vaccine Potency Through Hapten Fluorination. *J. Am. Chem. Soc.* 2013, 135, 2971-2974.

Tsuchikama, K.; Zhu, J.; Lowery, C. A.; Kaufmann, G. F.; Janda, K. D. C4-Alkoxy-HPD: A Potent Class of Synthetic Modulators Surpassing Nature in AI-2 Quorum Sensing. *J. Am. Chem. Soc.* 2012, 134, 13562-13564.

Tsuchikama, K.; Lowery, C. A.; Janda, K. D. Probing Autoinducer-2 Based Quorum Sensing: The Biological Consequences of Molecules Unable To Traverse Equilibrium States. *J. Org. Chem.* 2011, 76, 6981-7294. Selected as a Featured Article and featured on the front cover of Volume 76, Issue 17.

Tsuchikama, K.; Hashimoto, Y.; Endo, K.; Shibata, T. Iridium-Catalyzed Selective Synthesis of 4-Substituted Benzofurans and Indoles via Directed Cyclodehydration. *Adv. Synth. Catal.* 2010, 351, 2850-2854.

Tsuchikama, K.; Kasagawa, M.; Endo, K.; Shibata, T. "Cationic Ir(I)-Catalyzed sp3 C-H Bond Alkenylation of Amides with Alkynes", *Org. Lett.* 2009, 11, 1821-1823.

Shibata, T.; Tsuchikama, K. Recent advances in enantioselective [2+2+2] cycloaddition. *Org. Biomol. Chem.* 2008, 6, 1317-1323.

Tsuchikama, K.; Kuwata, Y.; Shibata, T. Highly Enantioselective Construction of a chiral Spirocyclic Structure by the [2 + 2 + 2] Cycloaddition of Diynes and exo-Methylene Cyclic Compounds. *J. Am. Chem. Soc.* 2006, 128, 13686-13687.

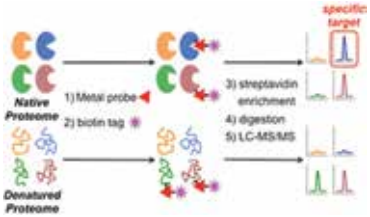
Shibata, T.; Tsuchikama, K. Ir-catalyzed almost perfect enantioselective synthesis of helical polyaryls based on an axially-chiral sequence. *Chem. Commun.* 2005, 6017-6019.

LAB MEMBERS

Post-doctoral Fellows: Upasana Banejee, Ph.D., Yasuhiro Shimamoto, Ph.D.

Dr. Tsuchikama joined TTI in July 2014 and is currently seeking postdoctoral fellows with experience in organic chemistry/medicinal chemistry.

Scheme of selective killing of pathogenic bacteria.



Scheme of target identification using antibacterial metal probes.

Ningyan Zhang, Ph.D.
Associate Professor

Heterogeneity of tumor microenvironment and cancer resistance mechanisms to therapeutic antibody treatment

Monoclonal antibodies are becoming a major drug modality for cancer treatment and have shown clinical success for treatment of various types of cancer. Human epidermal growth factor receptor (EGFR) family consists of four closely related type 1 transmembrane tyrosine kinase receptors (EGFR/HER1, HER2, HER3 and HER4) and plays important roles in cell growth and signaling. Abnormal gene amplification and overexpression of EGFR and HER2 are well documented in many types of cancer and multiple therapeutic monoclonal antibodies such as, cetuximab, panitumumab targeting EGFR and trastuzumab and pertuzumab against HER2, are currently used in the clinic for treatment of different types of cancer. However, both innate and acquired resistance to the therapeutic antibodies have been widely reported and present a significant challenge in the clinic.

My research interest is to understand the resistance mechanisms to cancer therapeutic antibodies targeting EGFR family members in the context of heterogeneous tumor microenvironment. We are using the HER2 targeting antibody trastuzumab as a model system to explore immune evasion mechanisms of cancer and their role in resistance to therapeutic antibodies. Multiple mechanisms of action of trastuzumab have been proposed, including inhibition of HER2 signaling, prevention of HER2 extracellular domain shedding, and triggering immune effector function such as antibody dependent cellular cytotoxicity (ADCC) through the antibody Fc interaction with activating Fc gamma receptors expressed on immune effector cells. Our recent work revealed a prevalent existence of impaired antibodies with compromised immunity in tumor tissues from breast cancer patients. The findings lead us to hypothesize that proteolytic cleavage of antibodies by matrix metalloproteinases (MMPs) plays an important role in cancer resistance to therapeutic antibodies. We have established cancer cells/immune cells co-culture system and mouse tumor models to investigate cancer immune evasion and resistance to antibody therapeutics. We employ a wide array of experimental approaches including *in vitro* 2D and 3D cell culture, mouse tumor models, and studies with clinical samples from cancer patients. State-of-the-art technologies are used in our studies such as high content fluorescence imaging, mass spectrometry, multi-color flow analysis and fluorescence activated cell sorting (FACS). The long-term goal of our research is to identify key molecular targets that govern the dynamic interaction between cancer cells and immune cells in tumor microenvironment and to design effective antibody therapeutic strategies for activation of immunity against cancer.

RESEARCH PROJECTS

- Role of proteolytic hinge cleavage of antibody in cancer immune evasion and resistance to antibody therapeutics
- Discovery novel cancer targets for development of antibody therapeutics.

KEY PUBLICATIONS

Zhang* NY, Deng H, Fan X, Gonzalez A, Zhang S, Brezski RJ, Choi BK, Rycyzyn M, Strohl WR, Jordan RE, and An Z (2015) Dysfunctional antibodies in the tumor microenvironment associate with impaired anticancer immunity. Clinical Cancer Research, *Clinical Cancer Research,* doi: 10.1158/1078-0432.

Fan X, Brezski RJ, Deng H, Dhupkar P, Shi Y, Gonzalez A, Zhang S, Rycyzyn M, Strohl WR, Jordan RE, Zhang* NY, and Zhiqiang An (2015) A novel therapeutic strategy to rescue the immune effector function of proteolytically-inactivated cancer therapeutic antibodies. *Molecular Cancer Therapeutics* 14(3): 681-691.

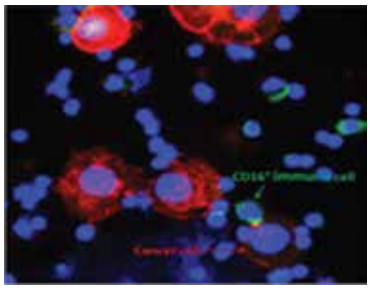
Shi Y, Fan X, Deng H, Brezski R, Rycyzyn M, Jordan R, Strohl WR, Zou Q, Zhang* NY and An Z (2015) Trastuzumab triggers phagocytic killing of high HER2 cancer cells by interaction with FcγRIV on immune cells. *Journal of Immunology,* 194:4379-4386.

Huang Z, Choi BK, Mujoo K, Fan X, Fa M, Mukherjee S, Owiti N, Zhang* NY, and An Z (2014) The E3 ubiquitin Ligase NEDD4 Negatively Regulates HER3/ErbB3 Level and Signaling. *Oncogene* doi: 10.1038/onc.2014.56.

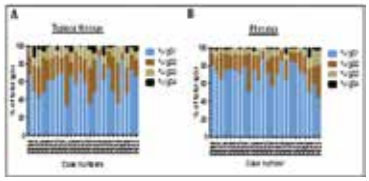
Shi Y, Fan X, Meng W, Deng H, Zhang* NY, and An Z (2014) Engagement of immune effector cells by trastuzumab induces HER2 downregulation in cancer cells through STAT1 activation. *Breast Cancer Research* 16(2):R33.

LAB MEMBERS

Hui Deng, M.S.
Xuejun Fan, M.D., Ph.D.
Wei Xiong, Ph.D.
Joined team with Dr. Zhiqiang An's laboratory, see complete list of members in Dr. An's lab page.



Immune cells/cancer cells interaction in a co-culture condition



Profiles of IgG subclasses in tumor and plasma samples. Tumor tissues (A) showed lower IgG1 subclass than that in the paired plasma (B) in breast cancer patients. Percentage of each subclass is calculated based on the sum of all IgG subclassess. (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 represent a major breakthrough in combating human diseases, including cancer. Even though the pharmaceutical and biotechnology industries are in the center stage of drug discovery and development, academic researchers are increasingly engaged in discovering new antibody drug candidates. However, advancement of some of the promising antibodies in the early stage of discovery from academic research laboratories is often hindered by the lack of access to the expertise and infrastructure required for antibody engineering and other related key technologies. Our antibody engineering and expression service center will fill the gap of the much needed expertise for early discovery of monoclonal antibodies and lead to the research and drug discovery communities. The objective of the service center is to provide technical support and services to molecular cloning and antibody expression and advance antibodies to the stage of preclinical development. 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 biological specimens for the differential expression and modification of proteins in different sample populations. Our Service Center provides state-of-the-art services to UTHealth, the Texas Medical Center, other UT community and external organizations.

The basic services provided are designed to identify and quantitate proteins and their modifications in a broad range of research specimens from simple purified protein samples to biomarker discovery and verification in complex mixtures such as cell and tissue extracts, plasma, and/or other biofluids. The service center contains the latest and most advanced instrumentation and trained personnel to provide sample preparation and analysis services of research specimens. This type of instrumentation is highly sophisticated both in terms of the mechanics of operation and maintenance as well as the extraction and interpretation of the data.

The Image Oriented Navigation Laser Microdissection Device (ION LMD) offers a unique feature of UV laser microdissection to efficiently isolate single or groups of cells from tissue sections without changing morphology or integrity of the biological content. Using LMD technology, quality material for a wide variety of DNA, RNA, and protein analyses can be obtained for sensitive and accurate molecular assays, such as Sanger sequencing, next-generation sequencing, and quantitative PCR. Beyond the optical

microscope function, our fluorescence light source enables us to microdissect any fluorescence labeled cells or tissue.

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 7 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

The Flow Cytometry Service Center is located on the sixth floor of the Fayez S. Sarofim Research Building and maintains four instruments: BD FACS Calibur, BD FACS Aria II, BCI FC500, and a Luminex 200.

These instruments are available on a fee per services charge to all research investigators from UTHealth or external organizations. These instruments allow scientist to evaluate a large number of samples in a short time frame and gather information on very rare populations of cells. The service center provides training, instrumentation, and technical expertise for both analysis and cell sorting.

Tissue Histopathology Service Center

The Tissue Histopathology Service Center provides in-house routine histology, special stain, and immunohistochemistry services in support of research projects to all research investigators from UTHealth or external organizations. With the growth of research activities that require histopathology services, the laboratory houses equipment for the preparation of thin sections; both paraffin and fresh frozen-tissue.

A full range of histopathology services is provided:

- Routine histology (process, embed, cut and stain)
- Section cut rolled and placed in micro centrifuge tub for DNA, RNA studies
- Multi-tissue embedding and sectioning
- Frozen tissue embedding and sectioning
- Blood Smear stain
- Immunohistochemistry and special stain

Microscopy Service Center

The IMM Microscopy Service Center provides assistance in confocal microscopy, wide-field fluorescence microscopy, brightfield microscopy, and image analysis. The facility is equipped with a Leica TSC SP5 upright confocal microscope with conventional and resonant scanner, a Nikon Eclipse TE2000E inverted wide-field microscope, a Zeiss Axio Scope brightfield microscope, and a computer workstation running Amira software for post-acquisition analysis of imaging data.

The Microscopy Service Center will support the research needs of all research investigators from UTHealth or external organizations on a fee-for-service basis by providing microscopy technical support, training, and consultation

Molecular Diagnostics Service Center

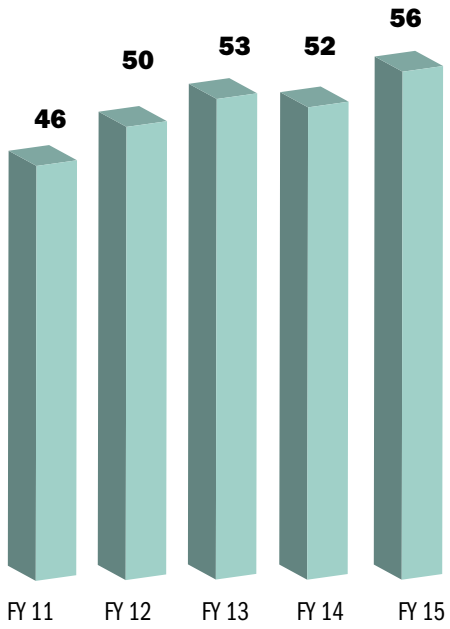
Our Molecular Diagnostic Laboratory, ProteoPath, provides diagnostic testing in a CLIA certified laboratory to all research investigators from UTHealth or external organizations on a fee-for-service basis. Major testing includes mass spectrometry based Therapeutic Drug Monitoring (TDM) program and Vitamin D Monitoring program for monitoring trends and anomalies during clinical treatment, as well as metabolites analysis for research testing. We also provide manufactured and customized ELISA assays for a variety of targets. We serve as a diagnostic technology development site for The Brown Foundation Institute of Molecular Medicine, Clinical Laboratories, physicians, and other external organizations.

Transgenic and Stem Cell Services

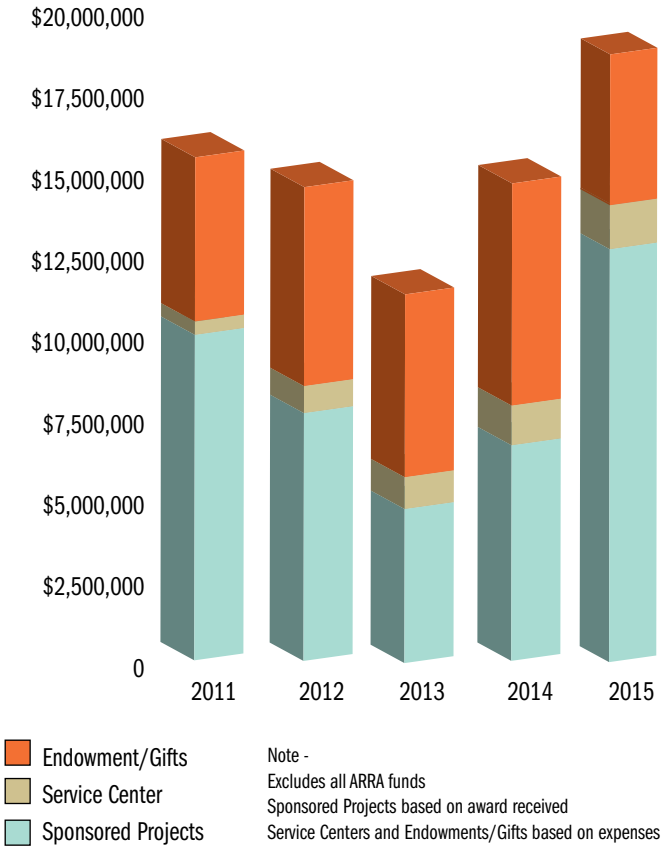
Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cells service center, which was established in 1998. It has generated over 750 new transgenic and knock-out mouse animal models for all research investigators from UTHealth and external organizations on a fee-for-service basis.

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.

Number of Faculty

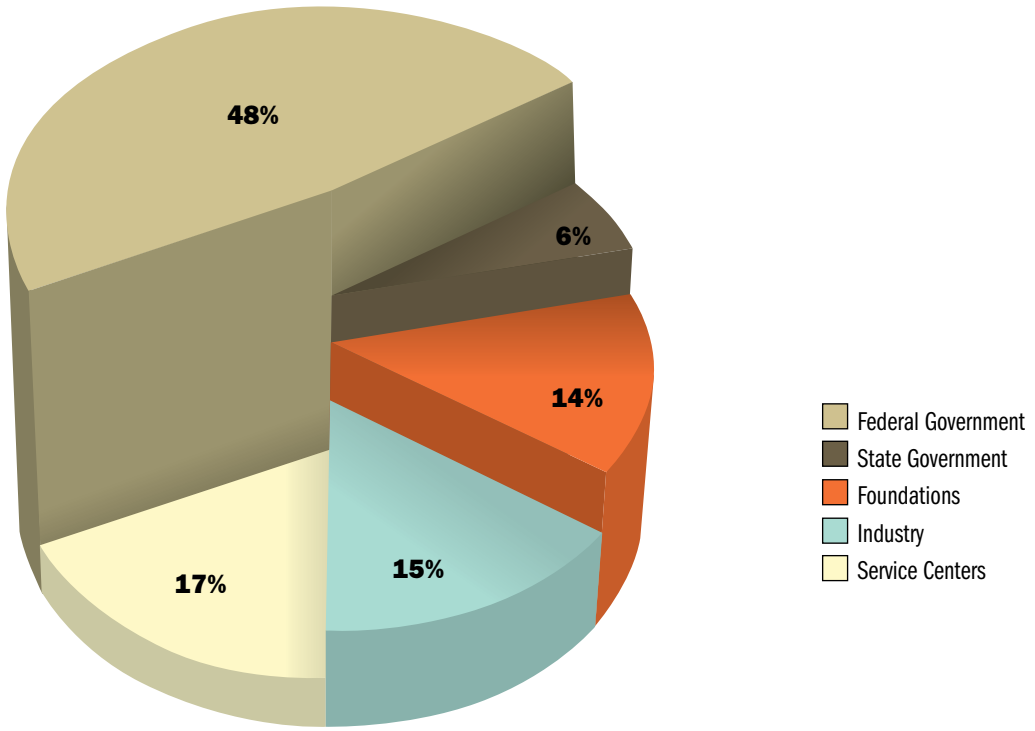


Total Funds Supporting Research



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

Total Expenses Supporting Research



Gift Report

New Gifts and Bequests Fiscal Year 2015

We are deeply grateful to UTHealth benefactors
who generously made a gift of $1,000 or greater to the
Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases
during fiscal year 2015, September 1, 2014 – August 31, 2015.

Irma Gigli, M.D.

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Estate of Marjorie Poyner

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Office of Communications
6431 Fannin Street
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