

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

IMM pact Report

FISCAL YEAR 2016



ABOUT THE COVER

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

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

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Editor

Darla Brown, Director, Office of Communications

Contributors:

Darla Brown
Rob Cahill
IMM Faculty

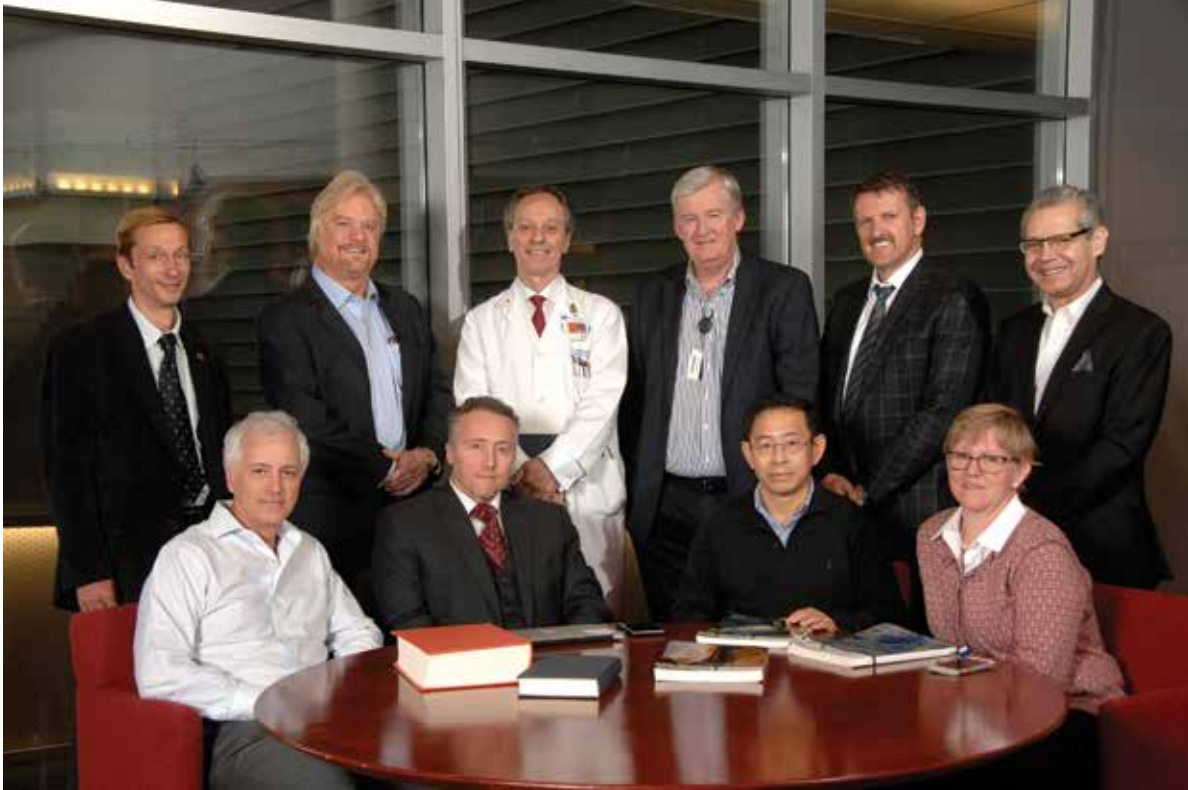
Design:

Roy Prichard

Photography:

Dwight Andrews

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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Runnels dedicated
to scientific
discovery

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 latest annual IMM² report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). The IMM is a stand-alone research institute that is embedded within McGovern Medical School. 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. Inside the report you will find in-depth feature articles on some of our faculty and donors plus an account from each IMM faculty member describing their exciting research programs. I trust that you will find the report interesting and informative.

This year the IMM has continued to grow as we have recruited additional outstanding new faculty, who bring with them with exciting research ideas and innovative technologies, details of which are described in the pages that follow. I am also pleased to report that despite an environment for scientific research funding that continues to be extremely challenging, especially from the NIH, IMM faculty have again excelled. Over the financial year just ended, our new grants and contracts were up again over the preceding last year, which in turn had seen a considerable increase over FY14. It is a testament to the remarkable quality and creativity of our scientists that the IMM remains so successful in attracting research funds from what is an ever-diminishing national pool. That said, full implementation of our mission remains heavily dependent on attracting support from alternative sources, including research charities and foundations, industry collaborations, and, most importantly, the continuing generosity of our friends and donors.

In addition to advancing science and medicine, we therefore wish to 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, which plays a key role in the continued growth and development of the IMM. This year saw a leadership transition, with Mr. Dudley Oldham stepping down after being in the chair for nearly 5 years, Dudley will continue to serve on our council having handed over the reigns to Mr. John McDonald.

If you would like to investigate how you can also be involved, I would be delighted to talk with you personally. In addition I would be delighted to see you at our annual IMM symposium. Last year 170 guests listened to three talks in the auditorium and attended a reception in the main IMM building. Next year the symposium will be held on May 3, 2017 and will follow the same format; full details are in this report. Please mark the date in your calendar because it is a great opportunity to visit the IMM, to hear exciting research stories directly from our faculty, 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

IMMImpact Symposium

*Wednesday
May 3, 2017
4-6 p.m.*

*Fayez S. Sarofim Research Building
1825 Pressler Street*

Genetics of the Aging Brain

Myriam Fornage, Ph.D.
*Professor, Center for Human Genetics
Laurence and Johanna Favrot
Distinguished Professor in Cardiology*

Obeying the Body's Clock: Healthier Aging and Disease Prevention

Kristin Mahan, Ph.D.
*Assistant Professor, Center for Metabolic
and Degenerative Diseases*

Targeting Fat in Age-related Diseases

Kai Sun, M.D., Ph.D.
*Assistant Professor, Center for Metabolic &
Degenerative Diseases*

SAVE THE DATE

CRACKING THE CODE OF STROKE - ONE GENE AT A TIME

The fifth cause of death and a leading cause of disability in the United States, stroke is a disease whose risk doubles every decade after age 55.

While age is a known risk factor for stroke, stroke may occur at any age, and there is still much scientists are uncovering about who gets stroke and why.

Myriam Fornage, Ph.D., professor in the Center for Human Genetics, specializes in research on lacunar stroke, a subtype of stroke that blocks the flow to one of the small arteries deep in the brain.

“The brain has one of the body’s richest network of blood vessels and is especially vulnerable to conditions that block or reduce blood flow to the brain, depriving brain cells of vital oxygen and nutrients,” says Dr. Fornage, the Laurence and Johanna Favrot Distinguished Professor in Cardiology. “In older people, especially those with diabetes or high blood pressure, the small blood vessels in the brain can become damaged, causing impairment in cognitive function, mood, and movement, and eventually dementia and stroke.”

Through an international collaboration, Dr. Fornage and her lab recently identified a new gene linked to the risk of stroke due small vessel disease of the brain. Called FOXF2, the gene was discovered as the result

of a population-based study, reviewing the genetic data of nearly 85,000 people – 4,300 of whom had had a stroke at the time of the data collection, from 1948 to 2013. The team reviewed DNA markers of the data, identifying seven known genes associated with stroke in addition to the new discovery.

Findings were confirmed in an independent study with Stroke Genetics Network, which included more than 70,000 people – 19,000 of whom suffered stroke.

The scientists also collaborated with researchers with animal models – a mouse and a zebrafish – to unravel the mechanism by which FOXF2 influences stroke. These findings point to a possible link to Alzheimer’s disease. The research was published in the June 2016 issue of *The Lancet*.

Dr. Fornage received her Ph.D. from the UT Graduate School of Biomedical Sciences and completed postdoctoral training at Case Western Reserve University. She joined the IMM in 1998 as a research fellow and found her niche studying stroke-prone rats.

“Stroke is a hard field to crack,” she says.

Large population-based studies following patients over many years have helped to make progress in stroke research.

“But not everyone who has a stroke has the same kind of

stroke,” she explains. “There are specific subtypes of stroke.”

Finding the genes that put a person at risk for small vessel stroke, which make up a quarter of all strokes, would allow drug targets to be designed. Currently there are few treatments for stroke.

“Identifying the genes also allows us to understand the biology of disease and would help with screening,” Dr. Fornage explains.

Neurologists have to do a lot of imaging of a stroke patient to determine the type of stroke and its effects.

“What if someone walks in the door with a stroke, and we could use their genetic information to tell the patient what kind of disease they have?” Dr. Fornage asks.

For the short-term, Dr. Fornage and her team are focused on stroke gene discoveries. “We are addressing rare variants in a subset of patients, sequencing analysis, whole exome sequencing, and working on the whole genome next year,” she says.

Future studies have the research looking beyond DNA sequencing to include environmental exposure – a risk factor of stroke.

“We are trying to understand epigenetics in complex traits to understand the biology, putting the pieces together and see the big picture of what’s happening.”



Dr. Myriam Fornage uses population studies to discover genetic markers of stroke.

BREAKING THE FAT-CANCER CONNECTION

When some people hear weight-loss drugs, they envision a magic pill to help them slim down to look great for vacation or to return the weight of their glory days.

But for researcher Mikhail Kolonin, Ph.D., director of the IMM's Center for Metabolic and Degenerative Diseases, creating an obesity drug is not just about helping people lose weight, it's about preventing cancer and other deadly diseases.

"We know that there is good fat and bad fat, and that the bad fat plays a key role in cancer, cardiovascular disease, and diabetes," Dr. Kolonin says. "This bad fat tissue actually fuels cancer and other diseases through mechanisms we are studying."

The cells of bad fat, called white adipose tissue, are the nourishment for many common cancers. Dr. Kolonin has pioneered groundbreaking work demonstrating the interactions between cancer and adipose tissue and the benefits of breaking those interactions.

"Some tumors rely on fat to grow aggressively," Dr. Kolonin says. "We've discovered a molecular network without which fat no longer promotes tumor growth."

Dr. Kolonin, who joined the IMM in 2007, had received

his Ph.D. from Wayne State University and completed postdoctoral fellowship work at The University of Texas M.D. Anderson Cancer Center.

Dr. Kolonin and his team have focused on prostate cancer, which claims more than 26,000 lives in the United States each year. Other obesity-associated malignancies include breast and colorectal cancer.

In the United States, more than a third of adults are obese and this year an estimated 1.6 million new cases of cancer will be diagnosed, according to reports by the Centers for Disease Control and Prevention and the National Cancer Institute, respectively.

"As the prevalence of obesity is rising, insights into the mechanisms underlying its link with cancer aggressiveness are urgently needed to develop new strategies for reducing prostate cancer morbidity and mortality," says Dr. Kolonin, the Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research.

In order to address this growing public health concern, Dr. Kolonin and his team are looking to take their studies beyond prostate cancer.

"Our *Nature Communication* paper published in 2016 illustrates the importance of this phenomenon for survival

of prostate cancer patients," he says. "We are now collaborating with clinical oncologists to develop therapies targeting adipose stem cells in breast cancer."

Dr. Kolonin and his team have already tested an investigational obesity drug in animal models of cancer with promising results. Mice treated with the obesity drug that destroys adipose stem cells had their tumor growth suppressed.

Dr. Kolonin's team was among the first to establish that tumors emit signals that attract adipose stem cells from fat tissue. Adipose stem cells in tumors support the vasculature, suppress anti-tumor immune response, and promote cancer cell survival.

"We propose that drugs targeting adipose stem cells can be developed as a combination therapy complementing conventional cancer treatments," wrote Dr. Kolonin and his colleagues in a paper that appeared in an issue of *Molecular Therapy*, a Nature Publishing Group journal last year.

Looking to the future, Dr. Kolonin hopes to find a way to offer his research to patients who need it most.

"We are only limited by funding to develop this drug for human clinical trials," Dr. Kolonin says. "That is our next step."

“We know that there is good fat and bad fat, and that the bad fat plays a key role in cancer, cardiovascular disease, and diabetes.” — Dr. Mikhail Kolonin



Dr. Mikhail Kolonin targets cancer by targeting fat.



The dream team tackles macular degeneration.

AN EYE FOR STEM CELL THERAPEUTICS

For some careers, reinvention is part of the job – former NFL quarterback Troy Aikman is now a commentator; Madonna changes her look every couple of years.

Scientists usually don't have that problem – or luxury. Once they choose a disease, or a discipline, it becomes a focus for a lifetime.

Rick Wetsel, Ph.D., professor and director of the IMM's Hans J. Mueller-Eberhard & Irma Gigli Research Center for Immunology and Autoimmune Diseases, has had the rare opportunity to change the direction of his research mid-career.

"It took me somewhat by surprise," he says. "I had done a sabbatical at the Basal Research Institute for Immunology in Switzerland in the early 90s, where I learned how to derive mouse embryonic stem cells. I did not anticipate at that time that I would have the opportunity to further develop this expertise into human stem cell-based therapeutics. However, when in 2004 Clive and Nancy Runnells provided my laboratory the support to initiate this new area of research, I had to, in part, reinvent myself at mid-career."

At first, most of Dr. Wetsel's effort was in developing stem cell-based therapeutics for lung disease. In the last year, he has embarked upon a major effort to develop stem cell therapies for macular degeneration. As before, this new direction was made possible by the generous support of Clive and Nancy Runnells (see p. 15).

Concentrating on a new

project – the degenerative diseases of the retina – are Dr. Wetsel and a hand-selected team of basic scientists and clinicians.

The new research project led by Dr. Wetsel, Hans J. Muller-Eberhard and Irma Gigli Distinguished Chair in Immunology, and Eva Zsigmond, Ph.D., associate professor of the Center for Immunology and Autoimmune Disease, is focused on treating age-related macular degeneration (AMD) with stem cells. The collaborative team includes John Mazzilli, M.D., postdoctoral fellow; Ken Simmons, Ph.D., research scientist; Aleksey Domozhirev, M.S., senior research associate; Stacey Mueller-Ortiz, Ph.D., senior research scientist; and Charles Garcia, M.D., clinical professor of ophthalmology and the Bernice Weingarten Chair in Ophthalmology.

AMD is the leading cause of blindness in those over age 65, affecting nearly 2 million Americans. As our population grays, AMD threatens to become a greater public health problem with no current treatment.

"The aim of our study is to develop a pipeline capable of producing high-quality replacement retinal pigment epithelium cells from stem cells to implant in the subretinal space where the old retinal cells have been damaged," Dr. Wetsel explains.

One major obstacle with any stem cell therapy is the threat of immune rejection of new cells transplanted into the body.

"Our intention is to prevent the rejection of the transplanted cells by genetically engineer-

ing the cells so that they will be unrecognizable to the immune system," he explains. "Deriving 'universal donor' cells that have minimal immunogenicity will remove, or reduce, the need for immunosuppressive drugs in patients receiving cell therapy."

To make the stem cells a "one-size-fits-all" for donors, Dr. Wetsel and his colleagues are employing the powerful CRISPR/Cas9 genome editing technology.

CRISPR/Cas9 allows scientists to very specifically target and edit the genes in the cell – modifying them to precise detail. This technique will be used to inactivate genes that elicit an immune response that causes the rejection of the transplanted cells.

"We are currently performing animal studies to test that our cells are safe, effective, and ready to progress to human clinical trials," Dr. Zsigmond says, adding that the goal is to get to clinical trials in two years.

The team's expertise in genetic engineering with CRISPR, combined with Dr. Wetsel's experience in immunology and Dr. Garcia's clinical ophthalmology skills, makes this a "perfect research collaboration," to achieve this goal, according to Dr. Zsigmond.

"Our ultimate aim is to restore the sight of patients suffering from degenerative retinal disease, a condition which is currently irreversible. We believe that stem cell-based treatments will be a life-changing therapy for these patients," she says.

RISING STAR: DR. MOMOKO YOSHIMOTO

On Aug. 22, 2016, a STAR landed at the IMM. Stem cell researcher Momoko Yoshimoto, M.D., Ph.D., the recipient of a UT System Rising STARs grant, joined the Center for Stem Cell and Regenerative Medicine as an associate professor.

The UT System Board of Regents created the Faculty Science and Technology Acquisition and Retention (STARs) Program in 2004 to help UT institutions attract and retain outstanding faculty. Awards, which can be used to purchase equipment and renovate facilities, require institutional support and are available to support the recruitment of tenure-track faculty members at any rank.

Her first visit to Houston and Texas, Dr. Yoshimoto joins UTHealth from Indiana University School of Medicine Department of Pediatrics, where she was an assistant research professor.

Earning her medical degree from Mie University School of Medicine in Japan, Dr. Yoshimoto practiced pediatric hematology/oncology in Japan for six years, treating children with leukemia with chemotherapy, radiation, and performing stem cell transplants in some of those patients.

She earned her Ph.D. at Kyoto University, developing expertise in developmental hematology, mouse embryonic stem cell culture, and biology

of hematopoietic stem cells in adult and embryonic mice. She completed a postdoctoral fellowship in Dr. Mervin C. Yoder's lab at Indiana University School of Medicine.

Deciding to pursue her own research project, Dr. Yoshimoto applied for a National Institutes of Health Research Project Grant and began her job hunt. Within a short timespan she both received a good score on her grant and earned a job interview from Brian Davis, Ph.D., director of the Center for Stem Cell and Regenerative Medicine and holder of the C. Harold and Lorine G. Wallace Distinguished University Chair.

"People here are happy and nice, and there are better Japanese restaurants here than in Indiana," Dr. Yoshimoto says of Houston with a smile. "It's a wonderful place to do research with all of the institutions so close."

Dr. Yoshimoto says her primary research goal is to understand how the first blood stem cells are generated in the mouse embryo. Blood stem cells produce all kinds of blood cells throughout life and are utilized for transplantation therapy.

"We look for donors of blood stem cells to cure leukemia patients, but sometimes it is difficult to find donors with matched HLA (types of blood cells)," Dr. Yoshimoto explains. "The knowledge how the blood stem cells are produced in the

mouse embryo will be utilized to produce blood stem cells *in vitro* from patient-derived pluripotent stem cells in the future. We are trying to understand very basic mechanisms to make it possible."

Dr. Yoshimoto's research also aims to understand special B lymphoid cell production in the mouse embryo that are related to autoimmune diseases and immune deficiencies post-transplantation.

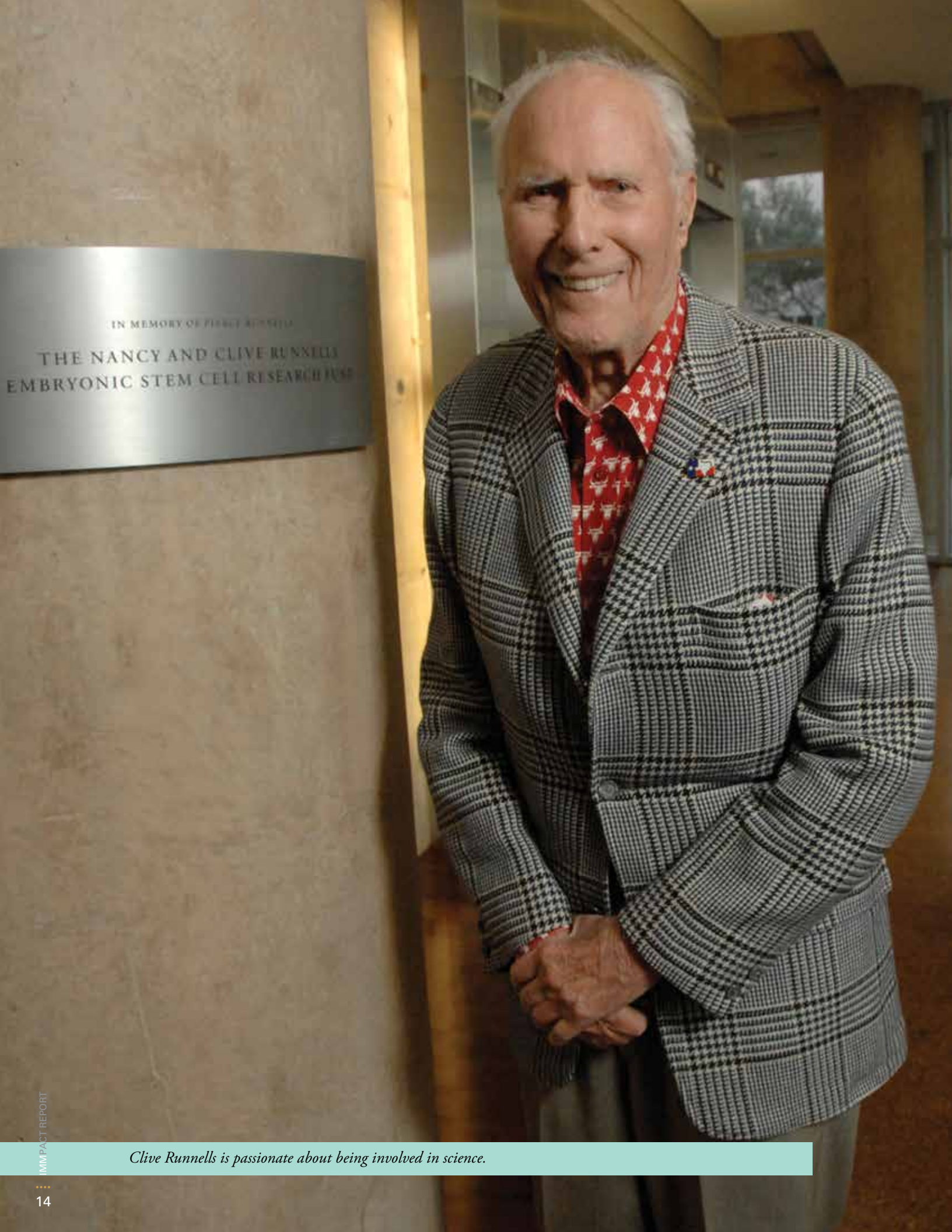
"Natural antibodies may protect us from autoimmune disease and other diseases, such as arteriosclerosis. We have found that special B lymphoid cells that produce these antibodies are generated only in the embryonic period in mice, and we are hoping to identify human counterpart of this population and eventually develop innovative therapies in the future," she says.

In her home country of Japan, Dr. Yoshimoto was a clinician, treating pediatric cancer patients. "I do miss the patient interaction, but in Japan I was so attracted by a basic scientist's talk about blood stem cells that I switched my focus to research – I thought, 'I have to do something in this field.'"

Dr. Yoshimoto knows it will take time to translate her research into patient care but says, "Important things take time to develop – we must be patient and keep working on this to move things forward."



Dr. Momoko Yoshimoto joins the IMM to focus on blood stem cells.



IN MEMORY OF FIDELITY RUNNELLS

THE NANCY AND CLIVE RUNNELLS
EMBRYONIC STEM CELL RESEARCH FUND

Clive Runnells is passionate about being involved in science.

RUNNELLS DEDICATED TO SCIENTIFIC DISCOVERY

When Clive Runnells calls, you listen. Rick Wetsel, Ph.D., learned that back in 2004 when he weathered a snowy Houston Christmas Eve for his first business meeting with Runnells.

That initial meeting 12 years ago has turned into a friendship founded on a mutual passion for science that can make a difference in the health of patients.

Runnells and his wife of 47 years, Nancy (who died earlier this year from stroke), have generously supported Dr. Wetsel's stem cell research, allowing the scientist and his team to develop their own stem cell lines for clinical applications.

The Runnells' dedication to scientific discovery is personal. Their son, Pierce, suffered a debilitating back injury due to a skiing accident and died before the promise of stem cell therapy could be realized.

"Pierce's death was a great blow to us," Runnells says. "He was kind, and he loved his parents dearly. My wife encouraged me to do something to help with stem cells."

Runnells called upon Dr. Wetsel, a stem cell immunologist with a focus on pulmonary diseases, several months ago to ask if he would target his stem cell research in a new area – degenerative eye diseases.

"My attorney in Bay City has macular degeneration. I didn't realize how hard it was, but you are blind – it's a fate worse than death," Runnells says.

Dr. Wetsel, professor and director of the IMM's Hans J. Mueller-Eberhard & Irma Gigli Research Center for Immunology and Autoimmune Diseases, quickly assembled a UTHealth dream team to focus on age-related macular degeneration

Led by Dr. Wetsel and Eva Zsigmond, Ph.D., associate professor of the Center for Immunology and Autoimmune Disease, the collaborative team is centered on treating age-related macular degeneration with stem cells and includes John Mazzilli, M.D., postdoctoral fellow; Ken Simmons, Ph.D., research scientist; Aleksey Domozhirev, M.S., senior research associate; Stacey Mueller-Ortiz, Ph.D., senior research scientist; and

Charles Garcia, M.D., clinical professor of ophthalmology and the Bernice Weingarten Chair in Ophthalmology.

"Rick and Eva are two outstanding people, and Charlie has got a lot of enthusiasm," Runnells says. "I enjoy being involved. If you're not involved, you don't do much."

The Runnells pledged \$1.25 million toward the project, and other donors, including Richard Ruiz, M.D., John S. Dunn Distinguished University Chair in Ophthalmology, and Dr. Charles Garcia also contributed.

"Clive turned 91 in January, and we want to bring this stem cell therapy to treat degenerative retinal diseases to the clinics in his lifetime," Dr. Wetsel says.

The team is on their way. The stem cell therapy (see p. 11) is being tested in animal models and the next step is FDA approval so that human clinical trials may begin within two years.

"I think we're on the right track," Runnells says. "We've got breakthroughs coming up that will be bell ringers."

“Pierce’s death was a great blow to us. He was kind, and he loved his parents dearly. My wife encouraged me to do something to help with stem cells.” — Clive Runnells

The IMM Center for Cardiovascular Genetics, established in 2006, 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. The Center also has a Research Clinic, which is utilized for clinical research activities, including NIH- and industry-sponsored clinical trials.

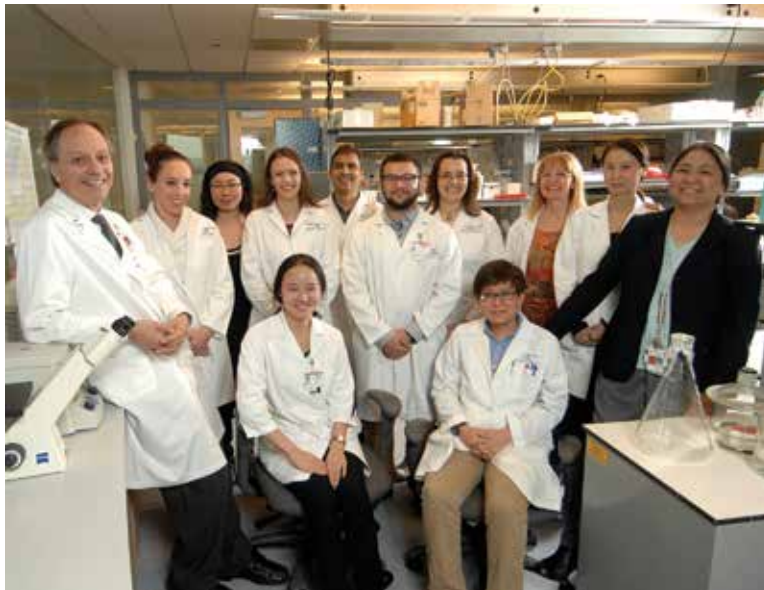
Mission: To prevent cardiovascular diseases in humans through elucidating their molecular genetic causes.

General theme of the research programs: The research programs at the center entail human molecular genetic studies through recruitment of the probands and family members, phenotypic characterization, molecular genetic and genomic studies, and targeting of the pathways involved in the pathogenesis of the disease. The main objective 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 regulation of gene expression. The findings are then applied in cell culture systems and animal models in order to delineate the molecular pathways involved in the pathogenesis of the disease. Upon identification of the perturbed pathways, they are targeted through genetic and pharmacological interventions to correct the pathways in order to prevent and reverse the phenotype. The initial intervention studies in cell and animal models are extended to humans through pilot randomized placebo-control trials.

Research Programs:

The research programs encompass four categories;

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 hypertrophic cardiomyopathy, dilated cardiomyopathy, and arrhythmogenic cardiomyopathy. The studies entail recruitment and clinical characterization of patients, genetic testing, genomic studies to delineate epigenetic changes that influence gene expression, and bioinformatics studies to support causal role of the genetic variants.

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

III. Experimental therapies: Specific pathways responsible for induction of the phenotype are targeted pharmacologically and genetically *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 patients with hereditary cardiovascular diseases. Genetic information garnered through the above studies is 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

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 (ClinicalTrials.org NCT01537926)
- LIBERTY-HCM: An industry-sponsored clinical trial to improve symptoms and exercise tolerance in patients with hypertrophic

cardiomyopathy

KEY PUBLICATIONS

Knock Down of Plakophilin 2 Downregulates miR-184 Through CpG Hypermethylation and Suppression of the E2F1 Pathway and Leads to Enhanced Adipogenesis In Vitro. Gurha P, Chen X, Lombardi R, Willerson JT, Marian, AJ. *Circulation Research*. 2016 Sept 2; 119 (6) 731-750. PMID: 27470638

Cardiac Fibro-Adipocyte Progenitors Express Desmosome Proteins and Preferentially Differentiate to Adipocytes Upon Deletion of the Desmoplakin Gene, Lombardi R, Chen SN, Ruggiero R, Gurha P, Czernuszewicz GZ, Willerson JT, Marian AJ. *Circulation Research*. 2016 Jun 24;119(1):41-54 PMID: 27121621

Arrhythmogenic Cardiomyopathy in a Patient With a Rare Loss-of-Function KCNQ1 Mutation, Xiong Q, Cao Q, Zhou Q, Xie J, Shen Y, Wan R, Yu J, Yan S, Marian AJ, Hong K., *J Am Heart Assoc*. 2015 Jan 23;4(1):e001526. PMID: 25616976

LAB MEMBERS

Post-doctoral Fellows: Gaele Auguste, Ph.D.; Jennifer Karmouch, Ph.D.; Suet Nee Chen, Ph.D.; Lili Li, Ph.D.
Research Associate: Grace Czernuszewicz, M.S.
Research Assistant: Tyrone Garnett, B.S.
Faculty Assistant Professors: Priyatansh Gurha, Ph.D.; Raffaella Lombardi, M.D., Ph.D.

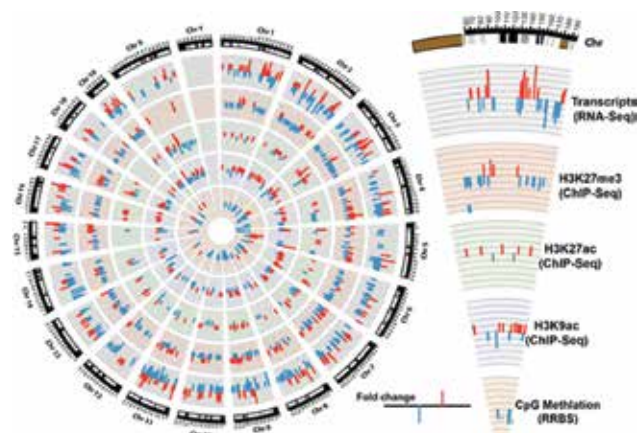
Our long-standing research objectives have been to delineate the molecular genetics and pathogenesis of hereditary cardiomyopathies in humans and apply the discoveries to prevent the evolving and reverse the established phenotypes of heart failure and sudden cardiac death. We have active research programs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



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 broad goal of my research is to better understand the molecular basis of heart failure. It is now clear that non-coding RNAs not only play a role in proper heart function but they also are important in heart failure. Recently, I identified a pathological role of miR-22, one of the most abundant miRNA in the heart. We demonstrated that miR-22 is a key regulator of cardiac hypertrophy and fibrosis. With the goal of identifying mechanisms by which miR-22 causes cardiac dysfunction we identified several miR-22 regulated candidate genes that play a role in stress-induced cardiac hypertrophy and fibrosis.

In a related project, we have discovered that several miRNAs are differentially expressed in Arrhythmogenic Cardiomyopathy (AC), a primary disease of the myocardium that clinically manifests with cardiac arrhythmias, heart failure, and sudden death. One of the key pathogenic features of AC is gradual replacement of myocytes by fibro-adipocytes. By integrating RNA-seq data with miRNA expression data we identified miR-184 as the most down-regulated miRNA (~10-fold) in AC. Given that AC represents a unique phenotype of excess myocardial adipocytes, we hypothesized that down regulation of miR-184 is pathogenic and suppression of miR-184 will lead to enhanced adipogenesis in AC. We first demonstrated that miR-184 is developmentally down regulated in mice heart and was predominantly expressed in cardiac mesenchymal progenitor cells. We also showed that in AC an epigenetic network encompassing E2F1 pathway and CpG DNA methylation transcriptionally suppress miR-184 expression in heart. We showed that suppression of miR-184 have a phenotypic consequences as in knock-down miR-184 in mesenchymal progenitor cells induced cellular differentiation and enhanced adipogenesis. Conversely, its overexpression attenuated adipogenesis in AC. Thus we identified a novel role of miR-184 in the pathogenesis of AC.

Currently using wide array of genomic approaches we are 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 molecular mechanisms and functions of long non-coding RNAs and in cardiomyopathies and heart failure.

KEY PUBLICATIONS

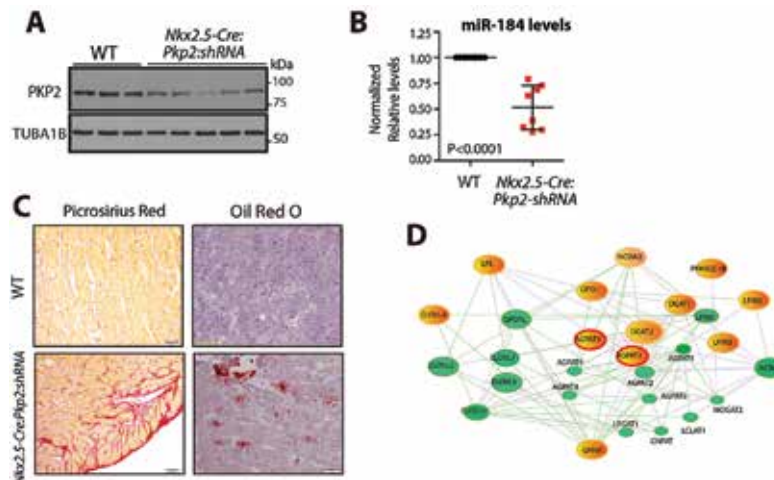
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Panel A-B: Novel mouse model of AC. ShRNA mediated cardiac specific PKP2 knockdown induces cardiac fibrosis and adipogenesis. Panel C-D: Knockdown of PKP2 leads to reduced miR-184 levels. Reduced miR-184 contributes to adipogenesis through induction of genes that are part of adipogenic network.



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

Molecular genetics and pathogenesis of hereditary cardiomyopathies

My research focus is the identification of the molecular mechanisms implicated into the pathogenesis of hereditary cardiomyopathies, which are the leading causes of sudden cardiac death in the young.

When I first started my research on genetic cardiomyopathies, during my clinical training in Italy, I studied the impact of cardiac interstitial fibrosis on diastolic function in patients with Hypertrophic Cardiomyopathy (HCM). When I moved to the United States for my postdoctoral training, I continued to work on this cardiomyopathy for several years: I contributed to the identification of 2 novel causal genes (MYOZ-2 and TRIM63), and I tested the treatment with the antioxidant drug N-acetylcysteine (NAC) in both, a mouse model and a rabbit model of HCM. The treatment has been effective in successfully reducing cardiac fibrosis and hypertrophy in both models and NAC is currently being tested in human patients with HCM, in a NIH-sponsored clinical trial.

Lately my research has focused on delineating the molecular pathogenesis of Arrhythmogenic Cardiomyopathy (AC), another familial cardiomyopathy caused by mutations in desmosomal genes. AC is characterized clinically by cardiac arrhythmias, heart failure and sudden cardiac death, and pathologically by the progressive replacement of cardiac myocytes by fibrosis and fat cells (adiposis). Although significant progresses have been made 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. Our group has developed several cell culture as well as genetically modified animal models of AC, which have been used to identify the molecular pathways affected by the presence of the mutant desmosome proteins. We have implicated suppression of the canonical Wnt signaling and activation of the Hippo kinase cascade, in the pathogenesis of AC. Furthermore, 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.

More recently, I have specifically focused on elucidating the molecular pathogenesis of the unique phenotype of fibro-fatty replacement of cardiac myocytes and the cellular origin of excess adipocytes in AC. I have shown that cardiac progenitor cells (CPCs), identified by expression of the KIT protein, are a source of adipocytes in AC. As CPCs are rare in the heart and account for only a small fraction of the adipocytes in AC, I hypothesized that cardiac cells other than CPCs might also express desmosome proteins and differentiate to adipocytes. On this line of research, I have recently implicated a novel cell type expressing the platelet-derived growth factor receptor-alpha protein (PDGFRA+), also known as fibroadipocyte progenitors (FAPs), as a source of adipocytes in AC. I have shown that FAPs express the main desmosome proteins and differentiate to adipocyte in AC through a Wnt-dependent mechanism. I also have shown that FAPs are bipotential as the majority expresses COL1A1, a marker of fibrogenesis and a smaller subset expresses predominantly CEBPA, a marker of adipogenesis. I have shown that desmoplakin is predominantly expressed in the adipogenic but not in the fibrogenic subset; hence, it is plausible that the adipogenic cells are the ones which are directly affected by the presence of the mutant desmosomal protein. The *in vitro* studies have been complemented with genetic fate-mapping experiments *in vivo*, which have confirmed that deletion of Dsp specifically in FAPs leads to increased fibro-adipogenesis in the heart and that the adipocytes infiltrating the myocardium originate from PDGFRA+ cells.

These findings expand the cellular spectrum of AC, commonly recognized as a disease of cardiac myocytes, to include non-myocyte cells in the heart. Moreover, the discoveries 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.

RESEARCH PROJECTS

- Identification of PDGFRA expressing progenitors as a cellular origin of adipocytes in arrhythmogenic cardiomyopathy
- Heterogeneous developmental origin of cardiac myocytes
- Effects of deletion of desmosomal proteins in epicardial progenitor cells

KEY PUBLICATIONS

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

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LAB MEMBERS

Assistant Professor: Priyatanish Gurha, Ph.D.
Post-doctoral Fellows: Suet Nee Chen, Ph.D., Karmouch Jennifer, Ph.D., Auguste Gaelle, Ph.D., Lili Li, Ph.D.
Sr. Research Assistant; Grazyna Z Czernuszewicz, M.S.
Research Assistant II: Tyrone Garnett, B.S.



Cardiac myocytes are costained for the structural protein alpha actinin (ACTN2 in red), EYFP (in green), and DAPI for the nuclei (in blue). While all the cardiac myocytes stain for alpha actinin, only some of them are positive for EYFP, indicating transcriptional activity of the *Pdgfra* locus at a certain time point during cardiac development only in this subset of cardiac myocytes.



Cardiovascular diseases such as stroke, heart, and kidney disease 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. Our center works to identify genes that pass cardiovascular diseases risk from one generation to the next. Discovery of these genes allows us to identify the biological pathways by which disease emerges. This, in turn, can open new avenues for disease prevention and treatment.

An emerging concept developing in our laboratories is that an important element of chronic disease of the cardiovascular system involve a chronic state of inflammation. We need a better understanding of these processes of “sterile inflammation” in which our immune systems become activated in response to the emergence of damage to cardiovascular tissues. We need greater understanding of the genetic variants that determine whether these inflammatory responses subside or remain active or even advance. This is a difficult task because immune systems have been evolving in competition with the pathogens we encounter in the world around us. These pathogens evolve rapidly. One counter strategy embedded in our immune response is selection of genetic variation that enhances the adaptability of our immune response. Such adaptation can protect us from pathogens but can also create risk of disease later in life. Nature has enriched our genomes with such variants. As our

living standards have increased and our lives have lengthened, the advantages provided earlier in life can turn into threats to our health by increasing our risk of chronic cardiovascular disease.

Progress in the laboratories of our investigators has provided important new understanding of susceptibility to atherosclerosis, coronary artery disease, neurodegenerative disease, progressive kidney disease, stroke, and high blood pressure. We have a major new initiative to identify additional genetic variation contributing to Alzheimer’s disease and age-related neurodegeneration, 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 direct treatments toward the underlying cause of disease in each affected individual. 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.

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



Peter A. Doris, Ph.D.

Professor & Director

Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research

Immune genes, genetic variation and the pathway to loss of kidney function

Our kidneys occupy a rough neighborhood in our bodies. Although they receive a sizeable fraction (25%) 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 is this process 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 and persistence 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

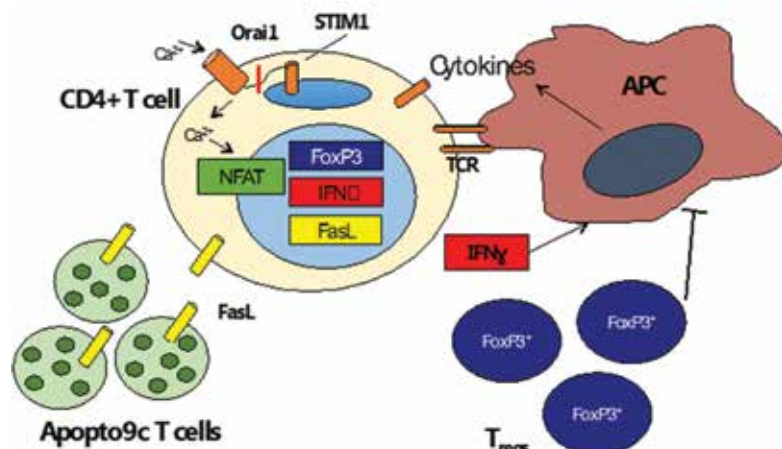
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COLLABORATORS

Manuel Gonzalez-Garay (University of Arizona)
 Michael Braun (Baylor College of Medicine)
 Scott Wenderfer (Baylor College of Medicine)
 M. John Hicks (Baylor College of Medicine)
 Roland Buelow (Ligand Pharmaceuticals)
 Technicians: Yaming Zhu, Sterling C. Kneeder
 Post-doctoral Fellow: Isha Dhande, Ph.D.
 Clinical Fellow: Xiaoyan Wu, M.D., Ph.D.



We identified a mutation in Stim1, a key protein involved in both immune cell signaling and development. Stim1 links signals arriving at the cell membrane of T cells to the control of gene expression involved in immune responses (e.g. cytokine production) and development. Effects of defective Stim1 function in CD4+ T cells include reduction in FasL expression that may increase T cell numbers by prevention of apoptosis of T cells. Another effect is to reduce FoxP3 expression. This impedes the development of T regulatory cells, disrupting their important function to provide control over immune responses.



Myriam Fornage, Ph.D.

Professor

The Laurence and Johanna Favrot Distinguished Professorship in Cardiology

Genetic and epigenetic basis of brain vascular disease and brain aging

The elderly represents the fastest growing segment of the U.S. population, and diseases of the aging brain are exacting increasing demands on patients, caregivers, and healthcare resources, making these conditions among the most significant public health problems of our time. There is growing evidence that disorders of the aging brain, such as stroke and dementia, begin years, if not over decades, before the diagnosis of clinical disease. Decline in cognitive function, including memory, executive function, and processing speed is evident beginning in the 40s. Brain abnormalities, readily detectable by neuroimaging techniques such as magnetic resonance imaging (MRI), are most common in the elderly brain but begin to appear in middle age. Although they typically do not produce symptoms, they cannot be considered benign because they increase a person's risk of stroke, cognitive and functional impairment, dementia, and death.

My research program investigates the genetics and genomics of vascular and neurodegenerative disease of the brain, both in its clinical and pre-clinical forms, in well characterized populations from young adulthood to old age. I use powerful genome-wide association studies and the latest genome technologies to discover novel genes influencing the risk for stroke, Alzheimer's disease, and brain MRI abnormalities. In collaboration with researchers in the United States and Europe, we apply genome sequencing technologies to identify DNA variants, either common or rare, which influence risk for these disorders. For example, while ischemia-related pathways have long been implicated in the pathology of white matter lesions on MRI, our recent genetic studies provided new evidence for a role of glial proliferative pathways and neuroinflammation.

We also study the links between DNA methylation and these diseases. DNA methylation is an epigenetic mechanism used by cells to control gene expression. Unlike DNA sequence variants, DNA methylation marks are not fixed at birth. Some of them can change throughout

the lifetime, and in response to environmental influences.

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
- Discovering novel epigenetic variants that influence risk for brain small vessel disease and its related neurocognitive outcomes
- 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
- Discovering common and rare genetic loci influencing cardiovascular traits in diverse ethnic groups as part of the NHGRI Population Architecture and Genomic Epidemiology (PAGE) consortium
- Discovering novel genetic loci for cardiovascular traits using gene-lifestyle interactions and pathway analysis
- Discovering novel genetic variants influencing

cognitive function and decline in middle-aged adults of European, African, and Hispanic ancestry

KEY PUBLICATIONS

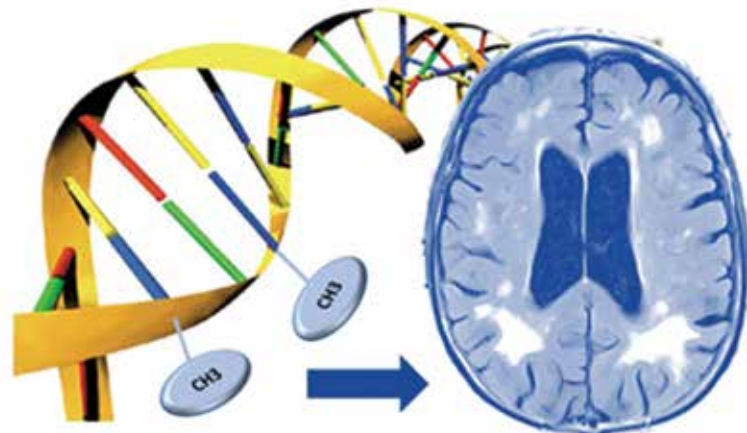
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LAB MEMBERS

Post-doctoral Fellows: Xueqiu Jian, Ph.D.; Melissa Richard, Ph.D.; Xiaoping Zhao, Ph.D.
 MS Student: Daokun Sun, B.S.
 Biostatistician: Rui Xia, Ph.D.
 Research Associate: Ping Wang, Ph.D.



DNA Methylation & Brain Vascular Disease and Aging

Brain magnetic resonance image showing subcortical white matter hyperintensity, atrophy of gray matter, and enlarged ventricles. Our studies identify links between DNA methylation and these brain abnormalities.



Ba-Bie Teng, Ph.D., FAHA

Professor

The Jerry and Maury Rubenstein Foundation

Distinguished Professorship in Heart Disease Research

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

Atherosclerosis is an inflammatory disease in the aorta that increases 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 genes of LDL receptor and an RNA editing enzyme (Ldb; *Ldlr*^{-/-}*ApoBec1*^{-/-}). These mice develop atherosclerosis as they age. Feeding on a Western high-fat diet accelerates their atherosclerosis development. Moreover, male mice develop atherosclerosis faster and more severe than females.

We deleted a causative gene for hyperlipidemia (proprotein convertase subtilisin/kexin type 9; PCSK9) from Ldb mice to generate a triple knockout mouse model (Ltp; *Ldlr*^{-/-}*ApoBec1*^{-/-}*Pcsk9*^{-/-}). We demonstrated a delay of atherosclerosis development in Ltp mice (Figure 2, triple knockout mice had significant less lesions than Ldb mice). Importantly, absence of PCSK9 modulates apolipoprotein B production via regulating autophagy pathway (Figure 3). This process produces less atherogenic LDL and reduces pro-inflammatory response to vascular endothelial cells.

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 basis to develop efficient therapeutic approaches to combat progression of diseases.

RESEARCH PROJECTS

- The role of PCSK9 in autophagy, inflammation, and atherosclerosis.
- Using RNASeq to identify genes associated with atherogenesis.
- Developing viral vectors targeting endothelial cells for therapeutic in the treatment of

vascular diseases.

- Using CRISPR/Cas9 technique to generate IL-17 RC knockout mice in the background of hyperlipidemia mice.
- Investigating the action of novel Ribozyme molecules in regulating the production of apolipoprotein B and lipoprotein-associated phospholipase A2 (Lp-PLA2) mRNAs.

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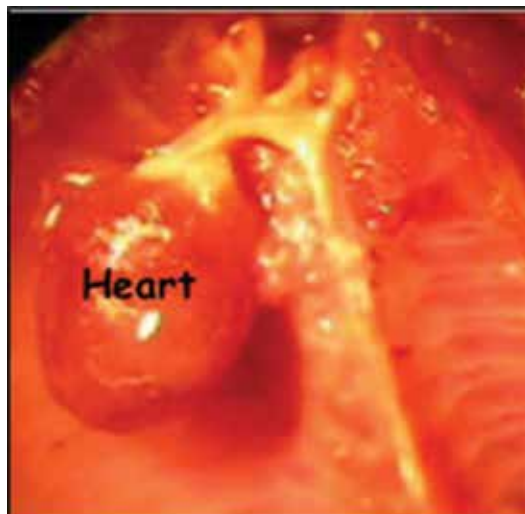
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LAB MEMBERS

Research Scientist: Hua Sun, Ph.D.

Summer Interns: David Heredia, McGovern Medical School Student Year-1, Vishnu Narayana, Debakey High School Senior, Christine Huang, UT Austin Year-1

Research Internship: Naomi Williams, University of Houston Year-2



The severe atherosclerotic lesions blocking the aorta (white deposits) 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.



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

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

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

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

erythematosus.

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

Research interests include:

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

Rick Wetsel, Ph.D.

Center Director & Professor

Hans J. 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 anaphylatoxins 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

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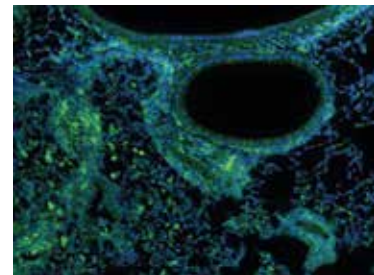
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LAB MEMBERS

- 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

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

Adenosine signaling and the regulation of chronic lung disease

- Novel regulation of mRNA polyA tails in the regulation of pulmonary fibrosis and chronic obstructive pulmonary disease
- Examination of the hypoxia as an amplifier of chronic lung disease
- Understanding novel mechanistic roles for IL-6 signaling in pulmonary fibrosis
- Systems biology approaches to understand the progression of chronic lung disease

KEY PUBLICATIONS

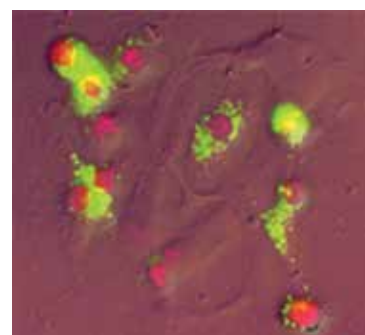
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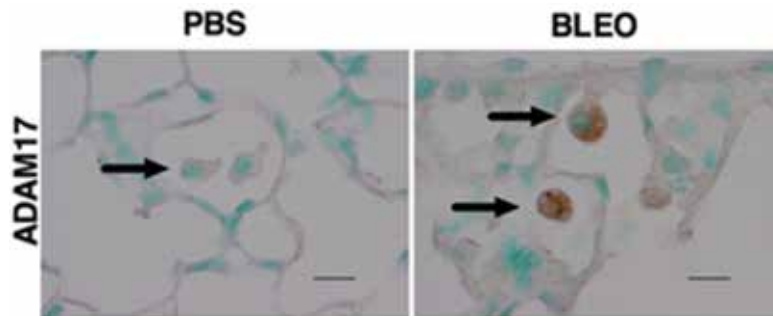
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LAB MEMBERS

Assistant Professor: Tingting Weng, Ph.D.
 Senior Research Scientist: Kelly Volcik, Ph.D.
 Post-doctoral Fellow: Frank Lou, Ph.D.
 Student: Kemly Philip, M.D./Ph.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 two groups defined by the absence or presence of nasal polyps (see image). 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.

Epithelial cells

Respiratory epithelial cells represent the first line of defense against the environment

for sinonasal mucosal. Recent studies have shown that epithelial cells serve an active role through regulation of cytokines and release of anti-microbials. Three identified epithelial cell derived cytokines, thymic stromal lymphopoeitin, 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 as a means of endotyping CRS
- Molecular signaling through respiratory epithelial cells of fungi alone and with other environmental triggers responsible for initiating and/or maintaining the characteristic Th2 immune response
- Clinical characterization and identification of biomarkers for CRS subtypes

KEY PUBLICATIONS

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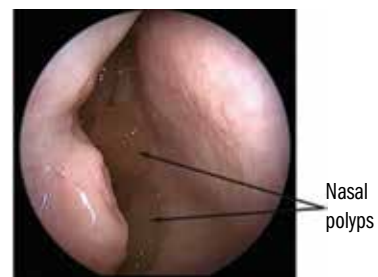
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LAB MEMBERS

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



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



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, COPD, 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 for developing of 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 of lung injuries. 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 therapeutic strategy for lung tissue regeneration; 3) to identify and characterize factors or regulatory pathways that control distal lung stem/progenitor cell fate during the disease processes for developing a novel strategy for targeted activation of endogenous stem/progenitor cells for lung tissue repair; and 4) to explore lung cancer stem cell-derived exosome miRNA pathways.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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Co-corresponding author

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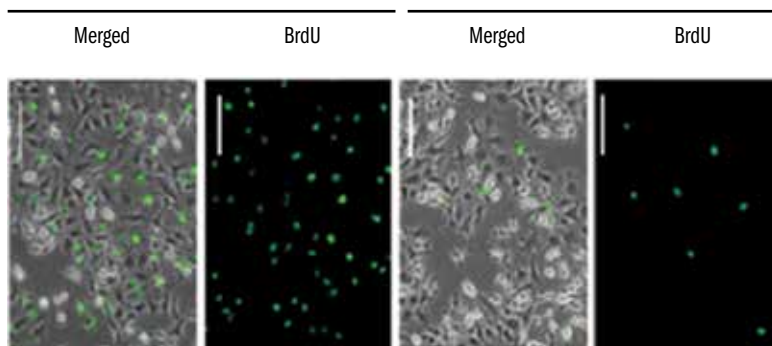
LAB MEMBERS

Research Associate: Dr. Yuan Quan

Lung cancer cell-derived miR-371b-5-p promotes lung cell proliferation

miR-371b-5-p mimic

Negative control





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

KEY PUBLICATIONS

Recent Thymus Emigrant CD4+ T Cells Predict HIV Disease Progression in Patients With Perinatally Acquired HIV. *Clin Infect Dis.* 2016 Apr 15;62(8):1029-35.

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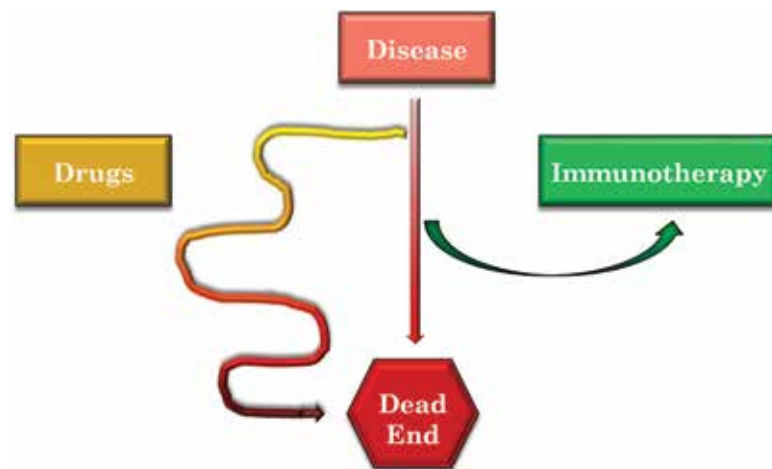
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LAB MEMBERS

Post-doctoral Fellow: Hisataka Ogawa, M.D., Ph.D.
 Research Technicians: Rachel Song, B.S. and Colby Hofferek, B.S.

The immune system is the military in our bodies that constantly protects us from the ever changing pathogens. It also functions as the police force to eradicate harmful cells in our bodies that can become cancerous. With such enormous and diverse responsibilities, it is susceptible to unintentional mistakes manifested as autoimmunity and allergy. Harnessing the immune system to optimize its defense and mitigate its errors has significant therapeutic applications. My research focuses on regulatory T cells (Tregs), which are the peacekeepers, to develop innovative immunotherapies for autoimmunity, allergy, infections and cancer. I have three expeditions in search of better curative treatments. In the area of autoimmunity, I am developing LAP+/GARP+ Tregs using my patented method for future clinical trials to prevent and treat graft-versus-host disease. For cancer treatment, I am developing novel strategies to optimize the potency of cancer-specific CAR T cell immunotherapy. For allergy, particularly food allergy, I am establishing a clinical trial using oral allergen immunotherapy to provide a unique opportunity for these patients to no longer have to avoid specific foods for the rest of their lives. We are all dreamers. The difference is when we wake up. Those who change dreams into reality move the world. Continuous support from our communities is essential for us to develop curative treatments that offer a U-turn away from the dead end paths of devastating diseases.



The need for better curative treatments of chronic diseases. Most treatments for chronic diseases prolong lives but do not cure the illnesses. Novel immune and cellular based therapies can provide a U-turn away from the dead end paths of devastating diseases.

RESEARCH PROJECTS

- Defining the role of regulatory T cells in hematopoietic stem cell transplant and graft-versus-host disease
- Develop regulatory T cell immunotherapy for the prevention and treatment of graft-versus-host disease and autoimmunity
- Optimizing the efficacy and potency of CAR T cell cancer immunotherapy



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 more than 750 new transgenic, knock-out and knock-in animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions.

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

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

RESEARCH SERVICES

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

Accomplishments:

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

RESEARCH PROJECTS

- Stem Cell Therapy for Age-Related Macular Degeneration
- Patient-derived tumor xenograft implantation models

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.

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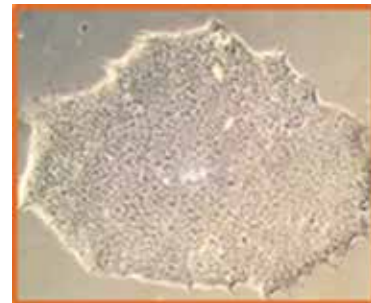
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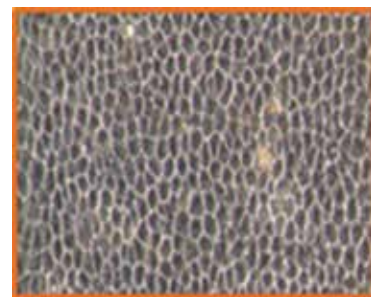
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LAB MEMBERS

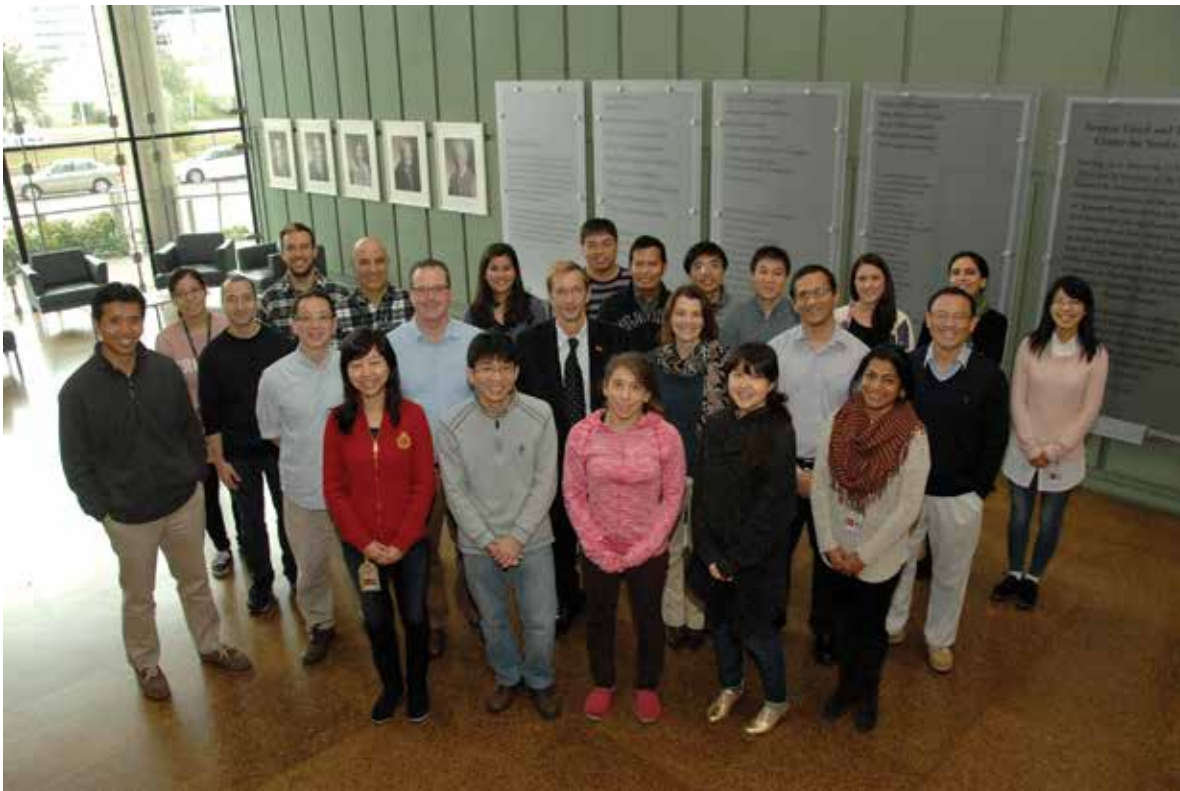
Manager: Aleksey Domozhrov
 Post-doctoral Fellow: John Mazzilli, M.D.



CR-4 human ES cells



Human ES cell-derived retinal pigment epithelial cells



The Center for Metabolic and Degenerative Diseases takes an integrative approach to tackle pressing aging-associated health challenges: obesity, diabetes, and cancer, as well as muscle wasting and neurodegenerative diseases. These health conditions involve defects in multiple related cell signaling pathways and physiological processes. The center's faculty investigate various aspects of energy metabolism, cell signaling and cell fate determination. Key questions being addressed include the following:

- How do progenitor cells in adipose tissue commit to white and brown adipogenesis?
- How do adipose stromal cells promote progression of cancer and other diseases?
- What is the clinical value of adipose cells targeting in obesity and disease?
- 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 clock

control the body's energy balance?

- How does the brain control glucose homeostasis in type 1 and type 2 diabetes?
- What are the functions of the genes mutated in neurodegenerative diseases?
- How does abnormal processing of proteins cause neuronal degeneration?
- How does stress impact Alzheimer's disease pathogenesis?

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 members collaborate with pathologists, epidemiologists and clinicians to translate their discoveries for the benefit of patients with metabolic and degenerative diseases.

Mikhail Kolonin, Ph.D.

Center Director & Associate Professor

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

Studies in my laboratory converge on progenitor cells, cancer, and obesity, a condition resulting from imbalance between white and brown fat tissue. Specifically, the focus of our research is on the role of adipose tissue-derived progenitor cells in metabolic disease and cancer. Based on the analysis of clinical specimens and mouse models, we have discovered the phenomenon of adipose cell mobilization in obesity and cancer. We demonstrated trafficking of adipose stromal cells to tumors where they shape tumor microenvironment and stimulate angiogenesis and cancer progression. These findings have provided new insights on the association between increased adiposity and cancer. We have also developed compounds that target white adipocyte progenitors. We have reported that these experimental drugs prevent obesity and cancer progression in mouse models and licensed them 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 and brown 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

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.

Targeted Pro-apoptotic Peptides Depleting Adipose Stromal Cells Inhibit Tumor Growth. Daquinag AC, Tseng C, et al. and Kolonin MG. *Molecular Therapy*, 2016.

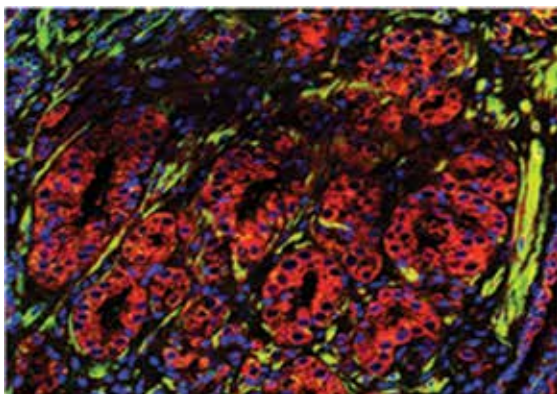
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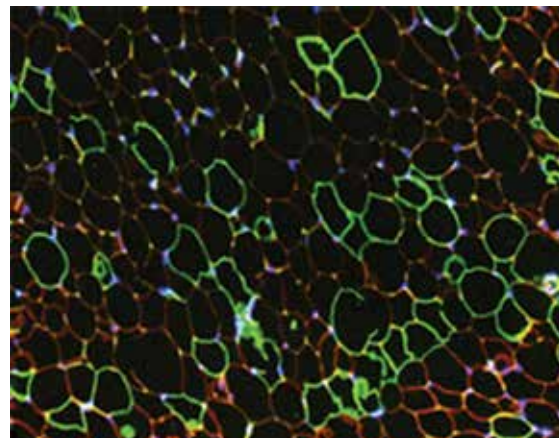
Porter C. et al., Kolonin M.G. et al., and Sidossis L.S. Human supraclavicular brown adipose tissue mitochondria have comparable UCP1 function to those found in murine intrascapular brown adipose tissue, *Cell Metabolism*, 2016.

LAB MEMBERS

Sr. Research Scientists: Alexis Daquinag, Zhanguo Gao
 Research Scientist: Fei Su
 Post-doctoral Fellow: Celine Pompeia
 Sr. Research Assistant: Ali Dadbin



Expression of CXCL1 (red) in epithelial cells and of CXCR1 (green) in stromal cells of a prostate tumor from an obese patient. Blue: nuclei.



Two types of adipocytes: white (labeled green) and brown (labeled red) identified in mouse fat tissue through lineage tracing of progenitor cells. Blue: nuclei.



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 United States, 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 obesity, SIK1 expression is highly induced in skeletal muscle. Surprisingly, SIK1-deficient mice are strongly protected from insulin resistance when fed a high fat diet, even though the mice became just as obese as control animals. SIK1 inhibits the actions of insulin and lowers metabolic rate. This makes SIK1 a very promising target for therapeutic development. We are currently investigating how this enzyme inhibits insulin action with a focus on cellular energy utilization.

Identification of novel pathways that stimulate muscle stem cell division. One aspect of aging is loss of skeletal muscle mass and strength, which impacts metabolic health as well ability to perform normal daily activities. Our laboratory has undertaken a multi-faceted approach to identify pathways that could be tar-

geted with drugs to help maintain muscle mass through activation of stem cells. We previously found that the hormone-regulated transcription factor CREB stimulates proliferation of muscle stem cells. However, it is unknown how CREB becomes activated after muscle injury or how CREB activates muscle stem cell function. We are characterizing factors released by muscle that activate the CREB pathway in muscle stem cells and studying the impact of these pathways on muscle stem cell proliferation and ultimately muscle regeneration. In addition, we created mice in which we can mimic hormonal pathways that stimulate muscle stem cell division. Using these mice and isolated muscle stem cells, we are identifying a pathway that stimulates genes associated with muscle stem cell proliferation. Ultimately we expect to uncover new pathways that could be targeted to promote muscle strength and regenerative capacity in aging individuals.

RESEARCH PROJECTS

- Contribution of cell signaling pathways to development and severity of type 2 diabetes
- Regulation of muscle stem cell proliferation and response to muscle injury

KEY PUBLICATIONS

Nixon M, Stewart-Fitzgibbon R, Fu J, Akhmedov D, Rajendran K, Mendoza-Rodriguez MG, Rivera-Molina M, Gibson M, Berglund ED, Justice NJ and Berdeaux R. (2016) Skeletal muscle salt inducible kinase 1 promotes insulin resistance in obesity. *Mol Metab*, 5: 34-46.

Akhmedov D, Rajendran K, Mendoza-Rodriguez M, and Berdeaux R. (2016) Knock-in reporter mice for monitoring CREB activity in vivo. *PLoS ONE*, 11(6): e0158274. PMC4940169.
Akhmedov D and Berdeaux R. (2013) The effects of obesity on skeletal muscle regeneration. *Front. Physiol.*, 4: 371.

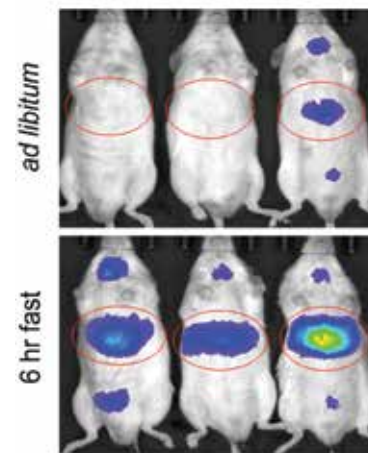
Berdeaux R and Stewart R. (2012) cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism and regeneration. *Am J Physiol: Endo and Metab.* 303(1): E1-17. (Cover illustration)

LAB MEMBERS

AHA Post-doctoral Fellow: Dmitry Akhmedov, Ph.D.

Candidate; 2016 President’s Research Scholarship Award Recipient; CCTS Pre-doctoral Fellow: Randi Fitzgibbon, B.S., Ph.D.

Senior Research Assistant: Maria Mendoza, M.S.



Detection of light from livers of CREB reporter mice in the fed state (top) or after fasting (bottom). CREB stimulates liver glucose production during fasting.



Kristin Eckel Mahan, Ph.D.
Assistant Professor

The circadian clock in health and disease

Our internal circadian clock entrains to the 24-hour environment and functions as a critical regulator of our physiology. Examples of circadian oscillations include the sleep/wake cycle, food seeking and intake, and hormone production and release. Data suggests that when our circadian clock is chronically perturbed genetically or alternatively environmentally, (such as occurs during night or rotating shift work, alternate time zone travel, under conditions of severe sleep restriction, etc.), several deleterious outcomes result, including cancer, obesity, changes in insulin sensitivity, and even accelerated aging. Our research goal is to understand why circadian disruption produces these effects.

Diseases associated with clock disruption are diverse in their phenotype, as the circadian clock is present in all tissues and cells of the body. While light strongly entrains the “central pacemaker” in our brain, the suprachiasmatic nucleus (SCN), other cues help drive the clock in peripheral organs. One such *zeitgeber* (literally translated, “time-giver”) is food. Some of our recent work provides evidence that even simply dietary deviations that produce nutrient stress can profoundly affect the circadian clock in various organs, reprogramming circadian gene expression and metabolism in a manner which disturbs circadian synchrony across the body. We are trying to reveal the mechanisms underlying nutrient sensing by the clock and how specific *zeitgebers* (such as food) drive tissue-specific clock function in a manner that maintains circadian synchrony with the central pacemaker via the circulation.

Additional projects in the lab include understanding how the circadian clock gets altered in the context of liver tumor cells, where robust oscillations of a circadian nature are present where they should not be (such as at genes controlling cell proliferation and tumor invasion). We are trying to understand whether such circadian reorganization in the tumor environment might provide novel targeting strategies and therapies.

Finally, while it is recognized that the sleep impairments and night eating habits so prominent in very obese individuals can sometimes be alleviated following significant weight loss, little is understood regarding the relationship between sleep, obesity, and chronotype. Recent studies suggest that specific chronotypes are associated with successful weight loss maintenance but the extent to which factors such as chronotype, social jetlag, and amount of weight loss contribute to sleep changes during significant weight reduction over time is not known. We have recently embarked on studies to better understand the complex relationships between obesity, chronotype, and sleep patterns in individuals before and after significant weight loss surgery, using actigraphy and chronotyping analysis.

RESEARCH PROJECTS

- Mechanisms underlying nutritional regulation of peripheral vs. central clocks
- Circadian mechanisms underlying night eating syndrome (NES)
- Mechanisms of circadian reprogramming in hepatocellular carcinoma
- Understanding relationships between human obesity, sleep quality, and chronotype using actigraphy

KEY PUBLICATIONS

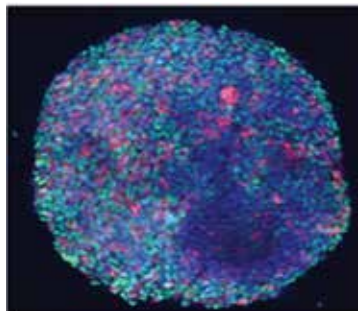
Ribas-Latre A and Eckel-Mahan, KL. Interdependence of Nutrient Metabolism and the Circadian Clock System: Importance for Metabolic Health, *Mol Metab.* 2016 Jan 14;5(3):133-52

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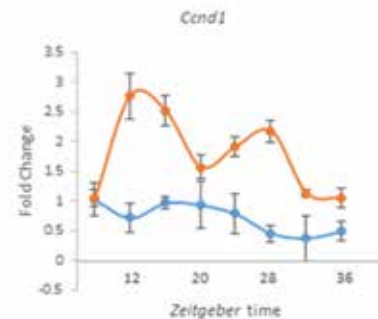
Eckel-Mahan KL, Patel VR, de Mateo S, Ceglia NJ, Sahar SS, Dilag S, Dyar KA, Orozco-Solis R, Baldi P, and Sassone-Corsi P. Reprogramming of the Circadian Clock by Nutritional Challenge *Cell*, 2013 December 19, 155(7), 1464-1478

LAB MEMBERS

Post-doctoral Fellows: Aleix Ribas Latre, Baharan Fekry



Hepatocellular carcinoma organoid stained with markers for cell cycle stage. Robust circadian expression of a gene that promotes cell proliferation and migration in tumor cells (orange) after circadian synchronization of the cells in culture.





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

RESEARCH PROJECTS

- 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.

KEY PUBLICATIONS

CRF Neurons Synapse in the External Globus Pallidus to Influence Anxiety State. Albert J. Hunt, Jr.^{1,4}, Rajan Dasgupta^{2,4}, Shivakumar Rajamanickam¹, Zhiying Jiang¹, Michael Beierlein^{2,4}, C. Savio Chan³, Nicholas J. Justice. *Cell Reports* (in revision).

A Glucocorticoid-responsive population of hypothalamic CRFR1 neurons is essential for HPA axis regulation following chronic stress. Assaf Ramot^{1,2}, Zhiying Jiang³, Jin-Bin Tian⁴, Nicholas Justice^{3*} and Alon Chen^{*}. (* co-senior authors). *Nature Neuroscience* (in press).

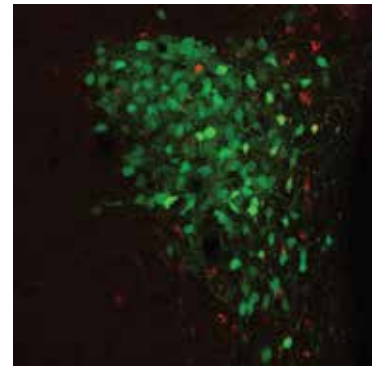
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Sex differences in NMDA GluN1 plasticity in rostral ventrolateral medulla neurons containing corticotropin-releasing factor type 1 receptor following slow-pressor angiotensin II hypertension. Van Kempen TA, Dodos M, Woods C, Marques-Lopes J, Justice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

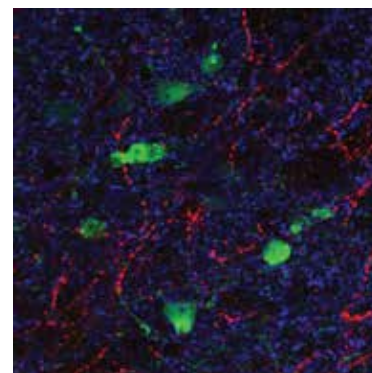
Parvalbumin+ Neurons and Npas1+ Neurons Are Distinct Neuron Classes in the Mouse External Globus Pallidus. Hernández VM, Hegeman DJ, Cui Q, Kelper DA, Fiske MP, Glajch KE, Pitt JE, Huang TY, Justice NJ, Chan CS. *J Neurosci*. 2015 Aug 26;35(34):11830-47.

LAB MEMBERS

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



Projections from CRF neurons (red) in the Globus Pallidus. CRF is released and signals to pallidal neurons that express the CRF receptor, CRFR1 (Green). Synapses are labeled in blue.



Trans-synaptic labeling of interconnected neurons in the Paraventricular nucleus of the Hypothalamus. Helper virus (red puncta) allows for infection by pseudo-typed rabies virus (green). Double labeled cells are "starter cells". The rabies virus packages in starter cells and transmits one retrograde synapse to infect monosynaptically connected neurons.



Vihang Narkar, Ph.D.
Assistant Professor

Exercise mimicry in vascular, metabolic, and degenerative diseases

Our laboratory investigates how nuclear receptor super-family and its co-regulators control various aspects of muscle function and health including: metabolic remodeling, vascular remodeling, exercise tolerance and adaptation, as well as muscle regeneration in dystrophy. To do so we use cell biology, transgenic mice, and rodent models of muscle diseases. Our ongoing research is on the following topics.

I-Exercise mimetic role of AMPK and PPAR δ . Molecular basis of endurance exercise has been a popular research area due to the potential for synthetically targeting exercise pathways to harness the beneficial effects of exercise in diseases such as diabetes and obesity. We identified that serine-threonine kinase AMPK and nuclear receptor PPAR δ agonists exert exercise-like effects in the skeletal muscle to increase high endurance type I myofibers, mitochondrial biogenesis, and overall oxidative capacity leading to increased exercise tolerance. In this work, we also demonstrated that AMPK physically interacts with PPAR δ to qualitatively and quantitatively influence PPAR δ endurance gene signature in the skeletal muscle, providing insight into how different pathways may interact to impart exercise adaptations. This work has implications in various diseases including diabetes, obesity and muscular dystrophies where it might be medically beneficial to improve aerobic capacity and have exercise-like effects in the skeletal muscle.

II-Paracrine regulation of skeletal muscle vasculature. While vascular remodeling is as important as metabolic and fiber type remodeling to skeletal muscle health, how muscle regulates its own vasculature in paracrine fashion is unclear. Vascular regression is a common pathology in various muscle diseases including diabetic myopathy, peripheral vascular disease (PVD) and Duchenne's muscular dystrophy (DMD). Our work has led to an increased understanding of paracrine pathways in the myocytes that control muscle vascularization. We showed that nuclear receptor ERR γ in the muscles cells activates an angiogenic gene program, increasing the secre-

tion of angiogenic factors such as Vegfa, which promotes muscle vascularization. On the other hand, nuclear receptor co-activator PGC1 β activates an anti-angiogenic gene program resulting in impaired muscle neo-angiogenesis in skeletal muscle ischemia, potentially by activating nuclear receptor COUP-TFI. Overall, discovery of these paracrine angiogenic pathways in the muscle offers potential targets for aforementioned diseases. In this regard, we recently showed that activation of ERR γ in the skeletal muscles is therapeutically beneficial in animal models of PVD and DMD.

RESEARCH PROJECTS

- ERR γ and diabetes
- ERR γ and skeletal muscle ischemic disease
- ERR γ and Duchenne Muscular Dystrophy
- Nuclear receptor co-activators in vascular diseases
- Nuclear receptors and co-activators in muscle wasting.

KEY PUBLICATIONS

Gallardo-Montejano VI, Saxena G, Kusminski CM, Yang C, McAfee JL, Hahner L, Hoch K, Dubin-

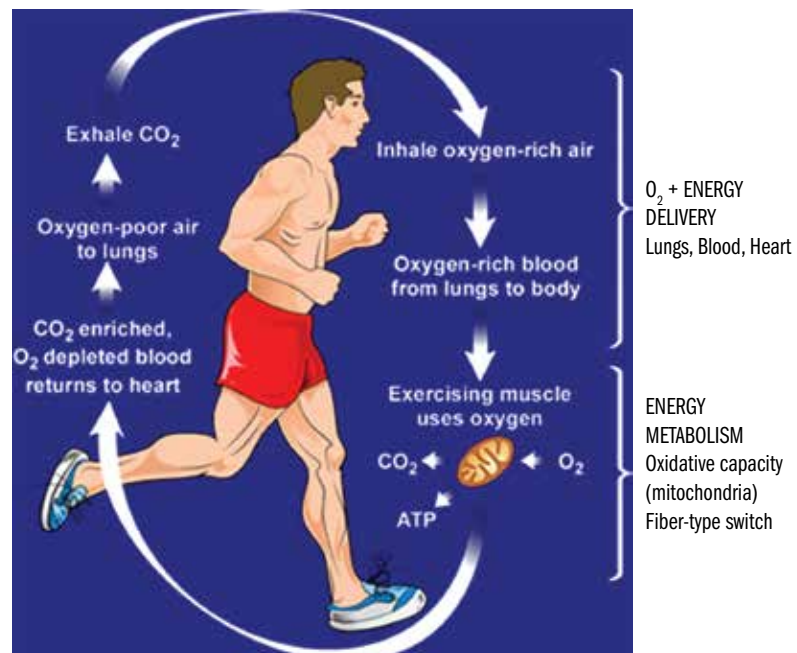
sky W, Narkar VA, Bickel PE. Nuclear Perilipin 5 integrates lipid droplet lipolysis with PGC-1 α /SIRT1-dependent transcriptional regulation of mitochondrial function. *Nat Commun.* 2016 Aug 24;7:12723.

Badin PM, Vila IK, Sopariwala DH, Yadav V, Lorca S, Louche K, Kim ER, Tong Q, Song MS, Moro C, Narkar VA. Exercise-like effects by Estrogen-related receptor-gamma in muscle do not prevent insulin resistance in db/db mice. *Sci Rep.* 2016 May 25;6:26442.

Yadav V, Matsakas A, Lorca S, Narkar VA. PGC1 β activates an antiangiogenic program to repress neoangiogenesis in muscle ischemia. *Cell Rep.* 2014 Aug 7;8(3):783-97.

LAB MEMBERS

Post-doctoral Fellows: Pierre-Marie Badin, Danesh Sopariwala, Neah Likhite
Technician: Sabina Lorca
Summer Students: Megha Seth
Alumni: Antonios Matsakas (Asst. Professor, Hull University, UK), (2) Vikas Yadav (Asst. Professor, Amity University, India)



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.



Kai Sun, M.D., Ph.D.

Assistant Professor

Targeting adipose tissue remodeling for treatment of 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 human obesity and insulin resistance.

In the past five years, I have published many paradigm-shifting findings about the tight connections between adipose tissue remodeling and obesity development. Specifically, we discovered that obese fat pads are frequently hypoxic and HIF1 α induction is the initial step which ultimately leads to local fibrosis and inflammation 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: at the early stage of obesity development, angiogenesis is metabolically beneficial by improving vascularization and inducing a “browning” phenotype in white adipocytes; In contrast, in pathologically expanded adipose tissue, antiangiogenic action leads to improvements in metabolism by ablating dysfunctional adipocytes. Our findings suggest that targeting HIF1 and VEGF-A in adipose tissue may offer the great opportunity for a novel therapeutic approach to prevent and treat the progression of obesity-related metabolic disorders.

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

collagen 6 α 3 and produces endotrophin which accelerates fibrosis and inflammation, ultimately leading a highly unfavorable microenvironment to sustain metabolic flexibility.

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 block-

ing 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

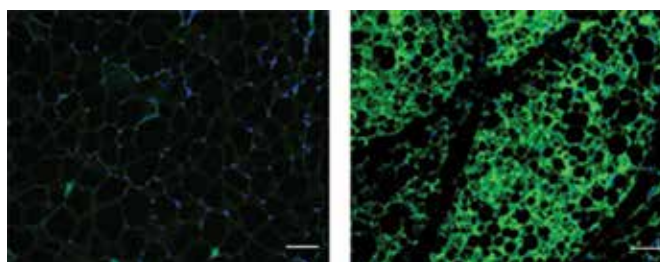
KEY PUBLICATIONS

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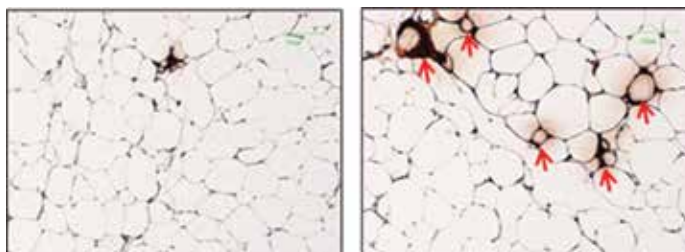
Sun K, Park J, Gupta O, Holland WL, Auerbach PL, Zhang N, Marangoni RG, Nicoloso SM, Czech MP, Varga J, Ploug T, An ZQ and Scherer PE. “Endotrophin triggers adipose tissue fibrosis and metabolic dysfunction”. *Nature Commun.* 5:3485. (2014).

LAB MEMBERS

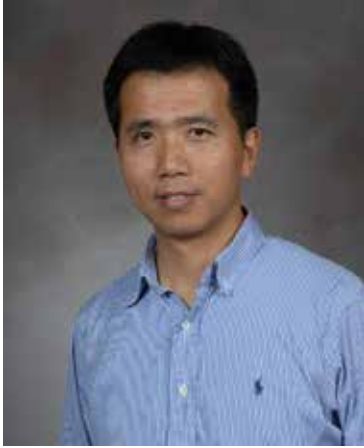
Post-doctoral Fellow: Yueshui Zhao
Sr. Research Assistant: Xue Gu



Adipose Tissue-derived VEGF-A Induces Browning of White Adipocytes: Immunofluorescent (IF) staining by anti-UCP₁ antibody (the marker of brown adipocytes) in subcutaneous adipose tissue of VEGF-A transgenic mice (right) and their littermate controls (left) (Scale bars, 50 μ m).



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



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

Brain mechanisms for obesity and diabetes

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

Euglycemia Restoration by Central Leptin in Type 1 Diabetes Requires STAT3 Signaling but Not Fast-Acting Neurotransmitter Release. Xu Y, Chang JT, Myers MG Jr, Xu Y, Tong Q. *Diabetes*. 2016, 65(4):1040-9.

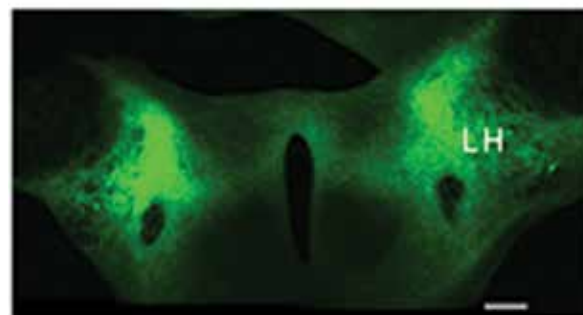
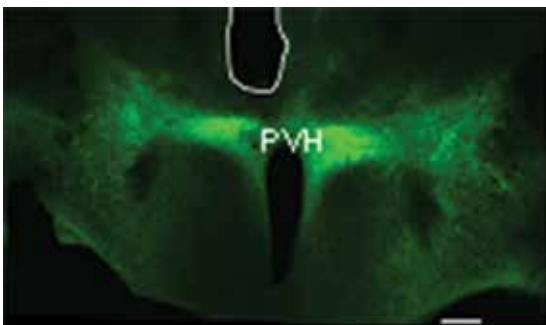
A cholinergic basal forebrain feeding circuit modulates appetite suppression. Herman AM, Ortiz-Guzman J, Kochukov M,

Herman I, Quast KB, Patel JM, Tepe B, Carlson JC, Ung K, Selever J, Tong Q, Arenkiel BR. *Nature*. 2016, 3;538(7624):253-256.

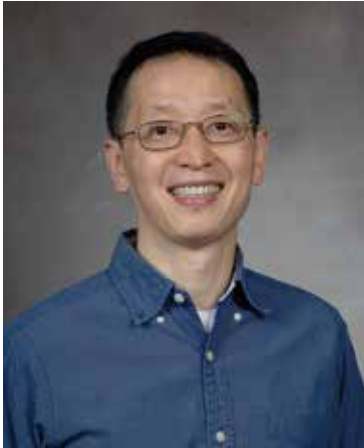
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LAB MEMBERS

Instructor: Yuanzhong Xu
Post-doctoral Fellows: Eun Ran Kim, Shengjie Fan, Yungang Lu
Graduate Students: Leandra Mangieri, Ryan Cassidy, Nick Karagas (w/ Dr. Kartik Venkatachalam)



This figure illustrates the basic strategy of adapting the optogenetic method in our studies. Pictures showing intense fibers expressing ArchT3.0 in the PVH (A) projecting from LH of Pdx1-Cre mice receiving injections of Cre-dependent AAV vectors expressing ArchT3.0 to bilateral LH (B).



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) and amyotrophic lateral sclerosis (ALS), debilitating maladies that currently have no cures or effective preventive avenues.

One challenge quite unique to neurons is that they should be long-lived, as once they are born and become mature, they can no longer regenerate and be replaced. Neurons rely on robust and reliable self-maintenance machineries in the cell to stay health and ward off internal crisis and external insults for decades to come. Two critical components of a cell's self-maintenance system are *chaperones*, which help other proteins in a cell to stay in shape, and *autophagy* (meaning "self-eating" in greek), which removes and recycles unnecessary, damaged or toxic cellular materials. Defects in chaperone and autophagy systems have been linked to many diseases including diabetes and neurodegeneration.

Using biochemical, cell biology and genetic tools in both mammalian cells and model organism *Drosophila*, we are studying how these self-maintenance machineries normally operate, with a goal of eventually utilizing these innate protective mechanisms to fight against ageing-related diseases.

Protein misfolding, aggregation and cellular clearance mechanisms

The accumulation of misfolded proteins (e.g., plaques and tangles) in the brain is a hallmark of almost all neurodegenerative diseases. We are studying how chaperones and autophagy machineries are employed by the cell to recognize and efficiently clear the misfolded proteins while spare the normal cellular constituents.

Chaperone Hsp110 is a main protein component in the brain. Hsp110 together with two other chaperones Hsp70 and Hsp40 form a potent molecular machine capable of

dismantling large tightly packed aggregates. We are exploring how to manipulate the activities of Hsp110 as well as the effect of such manipulation on neuronal survival.

Huntingtin is the causative gene for another brain degenerative disorder called Huntington's disease (HD). Recently we find that Huntingtin is itself an important player in a subtype of autophagy called *selective autophagy*, raising an intriguing possibility that the HD-causing mutation (i.e., polyglutamine expansion in Huntingtin) can interfere with this cellular protective mechanism and contribute to HD pathogenesis.

Biogenesis of specialized cellular organelles and their dysfunction in diseases

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

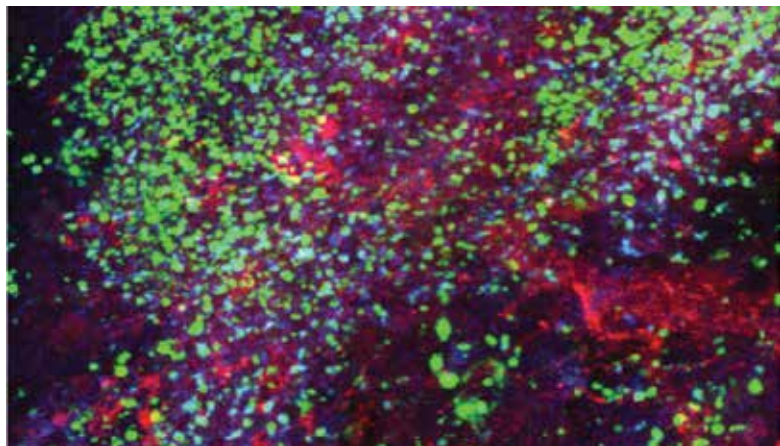
Rui YN, Xu Z, Patel B, Chen ZH, Chen DS, Tito A, David G, Sun YM, Stimming EF, Bellen H, Cuervo AM and Zhang S. (2015). "Huntingtin functions as a Scaffold for Macroautophagy". *Nature Cell Biology*, 17(3): 262 - 275.

LAB MEMBERS

Post-doctoral Fellow: Dr. Xiao Sun, Ph.D.

Student: Antonio Tito

Technician: Lili Ye, Research Assistant I



Protein misfolding and their clearance by autophagy

The accumulation of protein aggregates formed by misfolded mutant Huntingtin (green puncta) in *Drosophila* brain. These aggregates partially co-localize with autophagy markers p62/Ref(2)P (red) and ubiquitin (blue).



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 team has experts in instrumentation, imaging agent development, animal models of human disease, and translational science that effectively moves inventions and discoveries, “bench to bedside”.

A unique entity within the CMI is the Division of Applied Biologics, which focuses upon development and engineering of antibody-based diagnostics and therapeutics for high-affinity targeting of disease markers for cancer, bacterial infection, and other prevalent diseases. This team innovates antibody-based imaging agents for preclinical and translational science.

Another unique division includes a clinical

translational team, which engages in FDA-approved clinical studies in the areas of radiation oncology, cardiovascular disease, autoimmune disease, and cancer survivorship at 7 different clinical sites involving 10 clinical protocols across 14 academic clinicians. Currently, the team effectively translates new NIRF molecular imaging technologies literally from “bench-to bedside and back again,” in efforts that embrace its clinical partners in the Texas Medical Center and in the Houston suburbs.

In addition to having an assembly of faculty-driven independent 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.

*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 fluorescence (NIRF) to enable new understandings in several disease states. Between 2005-2007, my team was the first to dual label imaging agents with nuclear and NIR fluorescence agents to demonstrate the potential for pre-surgical PET imaging and intraoperative NIRF imaging for reduced tumor burden in surgical oncology. In addition my team adapted NIRF tomography within the CT gantry of a PET/CT device to demonstrate hybrid small animal PET/CT/NIRF tomography. Finally, we translated into the clinical setting, unique NIRF instrumentation and trace dose of NIRF imaging agent to demonstrate for the first time to visualize the active “pumping” of the lymphatic vasculature, which mediates immune response and maintains fluid homeostasis (Figures 1&2). 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.

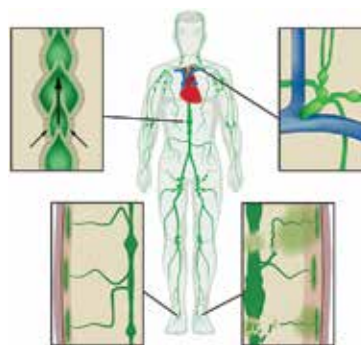
- Lymphatic delivery of therapeutics targeting the immune system.
- Imaging cancer-positive lymph nodes with a cancer-specific near-infrared fluorescent molecular imaging agent to guide intraoperative lymph node dissection.
- Imaging lymphatic responses to progressive rheumatoid arthritis and its treatment with biologics.
- Imaging lymphatic responses to radiation and surgery in head and neck cancer.
- Small animal imaging and tomography.

KEY PUBLICATIONS

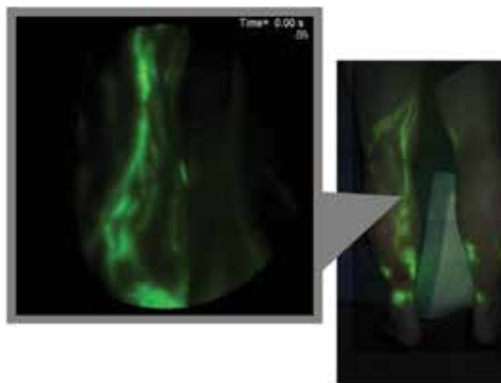
O'Donnell, T.F., Rasmussen, J.C., and E.M. Sevick-Muraca, “New diagnostic modalities in the evaluation of lymphedema,” accepted for publication, *Journal of Vascular Surgery*, 2016.

Gonzalez-Garay, M.L., Aldrich, M.B., Rasmussen, J.C., Guilliod, R., King, P.D., and E.M. Sevick-Muraca, “A novel mutation in CELSR1 is pathogenic for a new phenotype of hereditary lymphedema,” *Vascular Cell*, 8:1, 2016 PMID: 26855770.

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



Schematic of the lymphatic system throughout the body (center). The initial lymphatic capillaries, such as those located beneath the epidermis (lower left), drain to collecting and conducting lymphatics consisting of series of lymphangions, or lymph ‘hearts.’ Lymphangions (upper left) are bounded by endothelial cells and valves and sequentially contract to propel lymph to regional lymph node basins (center, green circles). From the lymph node basins, lymphangions propel lymph through the thoracic duct and its tributaries to ultimately drain into the venous system at the subclavian vein (upper right). When there is an upstream blockage, vessel deterioration, or insufficient lymphatic pump, lymph can flow backwards into the initial lymphatic capillaries and into the extravascular space (lower right).



NIRF lymphatic imaging of the posterior legs of a patients with peripheral vascular disease and mixed arterial-venous wound. Insert is expansion on a single image within a dynamic movie showing lymphatic reflux.



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

Imaging in immunology

I bring a combination of expertise in translational science and basic science to lead the immunology 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.

We recently received notice of funding for a five-year study of breast cancer-related lymphedema. We intend to prospectively survey changes in lymphatic anatomy and peripheral immune status as patients proceed through surgery and radiation treatments for cancer, identifying crucial molecular and cellular triggers for lymphedema development.

Reimbursement from Medicare and medical insurance companies for many clinical procedures is under scrutiny, as the health care funders try 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.

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. This study was the first to show that inflammatory effects on lymphatics can be systemic, and suggests a role for inflammation in some lymphatic diseases. We recently found that lymphatic pulsing in a rat model of rheumatoid arthritis, in which inflammatory cytokines are elevated, is arrested in the disease. We have now extended this work to show that nanotopographic delivery of a therapeutic, Enbrel, directly to lymphatics and lymph nodes is superior to traditional subcutaneous or intravenous administration for rheumatoid arthritis.

RESEARCH PROJECTS

- Five-year surveillance of breast cancer patients, investigating causes of treatment-related lymphedema

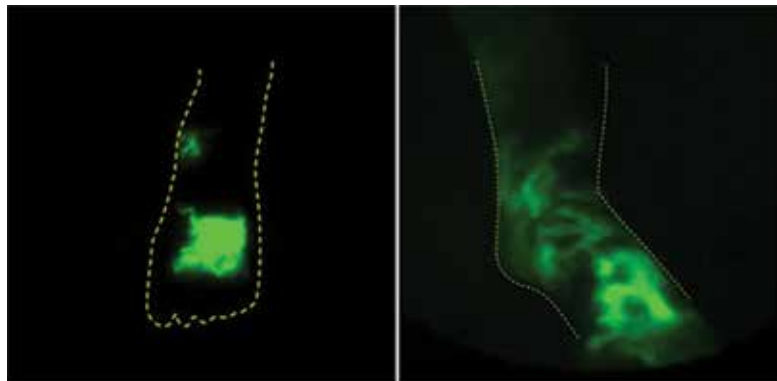
- Clinical studies of NIRF imaging of lymphatic architecture and function in health and disease
- Preclinical and clinical studies of inflammatory cytokine effects on lymphatic function in rheumatoid arthritis

KEY PUBLICATIONS

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Gonzalez-Garay ML, Aldrich MB, Rasmussen JC, Guillod R, Lapinski PE, King PD, Sevic-Muraca EM. A novel mutation in CELSR1 is associated with hereditary lymphedema. *Vasc Cell* 8:1, 2016

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



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



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 emerging antibody-based cancer treatments. Common to each project is our focus on translation of discoveries and technologies into the clinic to improve human health.

RESEARCH PROJECTS

- 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

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

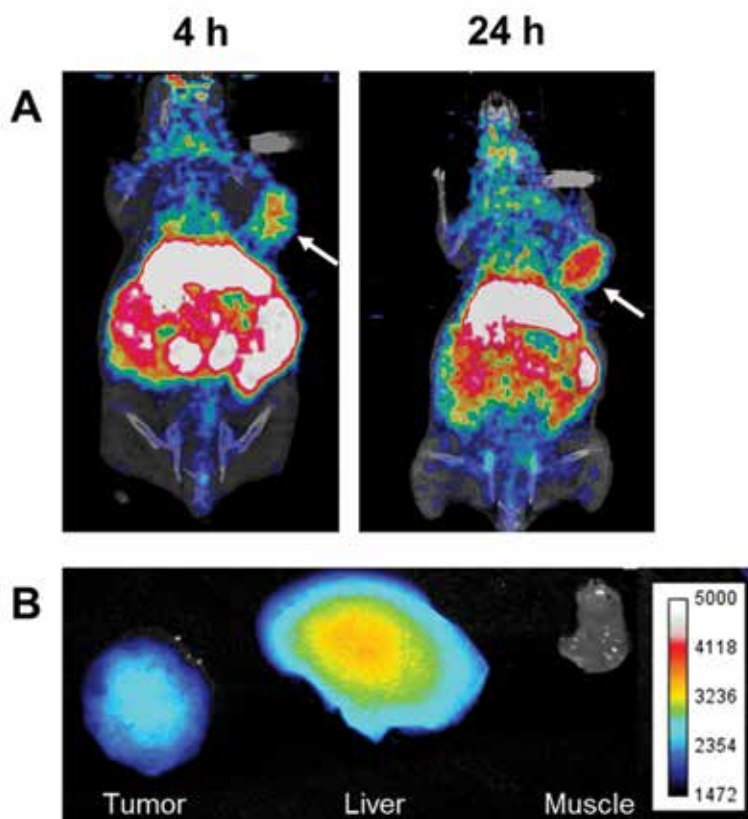
Ghosh, S.C., Pinkston, K.L., Robinson, H., Harvey, B.R., Wilganowski, N., Gore, K., Sevick-Muraca, E.M., Azhdarinia, A. Comparison of DOTA and NODAGA as Chelators for 64Cu-labeled Immunoconjugates. *Nuc Med Biol.* 42(2):177-83, 2015. PMID:25457653.

Ghosh, S.C and Azhdarinia, A. Advances in the Development of Multimodal Imaging Agents for Nuclear/Near-infrared Fluorescence Imaging. *Curr Med Chem.* 22(29):3390-404, 2015. PMID:26337105.

Gong, X., Azhdarinia, A., Ghosh, S.C., Xiong, W., An, Z., Liu, Q., Carmon, K.S. LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol Cancer Ther.* 15(7):1580-90, 2016. PMID:27207778.

LAB MEMBERS

Research Scientist: Sukhen Ghosh, Ph.D.
 Research Coordinator: Julie Voss
 Graduate Student: Servando Hernandez Vargas



Mice were implanted with pancreatic 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 in the live mouse (A) and in resected tissues (B). This study demonstrated correlation between both imaging techniques and supports further evaluation for pre-operative and real-time surgical imaging using a single agent. Arrow indicates tumor.



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 agent 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 expedited through *in vivo* models to monitor efficacy, specificity and to validate targets prior to clinical use. 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

- Molecular imaging for cancer staging
- Targeting established 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).

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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 μ PET/CT," *J Nuc Med*, 2012. Sep;53(9):1427-37 PMID: 22872743

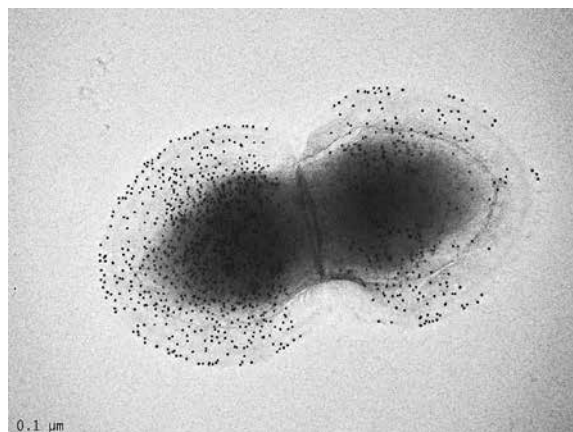
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the collagen-binding MSCRAMM Ace through regulation of gelE." *Journal of Bacteriology*, Sep;193(17):4317-25 2011. PMID: 21705589

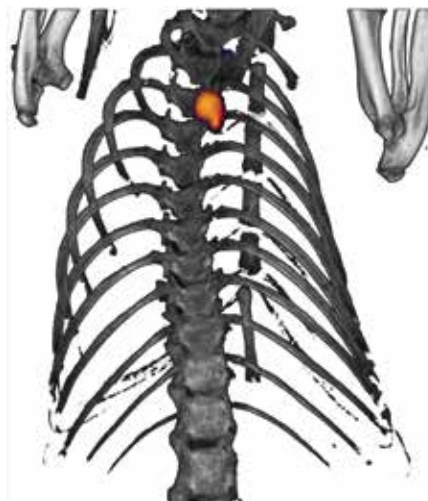
Gao P, Pinkston KL, Nallapareddy SR, van Hoof A, Murray BE, Harvey BR. "The *Enterococcus faecalis rnjB* is required for pili gene expression and biofilm formation." *Journal of Bacteriology*, 192(20): 5489-98, 2010. PMID: 20729365

LAB MEMBERS

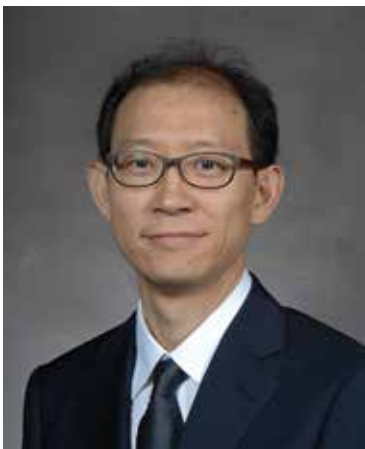
Research Instructor: Dr. Peng Gao
Graduate Student: Emily Stinemetz



Electron microscopy image of antibody gold bead conjugated agent targeting major surface antigen of *Enterococcus faecalis* using in-house generated high affinity antibody.



Molecularly targeted live animal imaging of bacterial infection. PET/CT image of enterococcal endocarditis in a live rat imaged 72 h post infection using Cu64-DOTA labeled mAb targeting antigen on the bacterial target.



Sun Kuk Kwon, Ph.D.

Assistant Professor

Functional lymphatic imaging in animal models of lymphovascular disorders

My lab leads the development and application of small animal imaging techniques to address biological questions. Our 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 fluid homeostasis, 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. Recently, I have developed non-invasive, dynamic near-infrared fluorescence (NIRF) imaging methods for imaging and quantifying lymphatic function in health and disease. Using this novel technique, I showed abnormal lymphatic function and drainage patterns in animal models of lymph node metastasis, hypertension, and inflammation. Recently, I also demonstrated that when lymphatic drainage is obstructed due to surgical disruption, collateral lymphatic circulation is normally established and used to overcome pathways rendered nonfunctional. However, these activated collateral draining pathways also can provide a route for metastatic dissemination of cancer, possibly negating the use of prophylactic lymphadenectomies to disrupt metastatic pathways.

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

the head and neck can be used as a diagnostic target for predicting onset, progression, and response to AD pharmacological intervention.

Taken together, 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 his 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. I am 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, Alzheimer disease, and inflammation and tracking response to therapeutic agents.
- Imaging cancer therapy induced lymphatic remodeling and immune responses.
- Multi-modal molecular imaging.

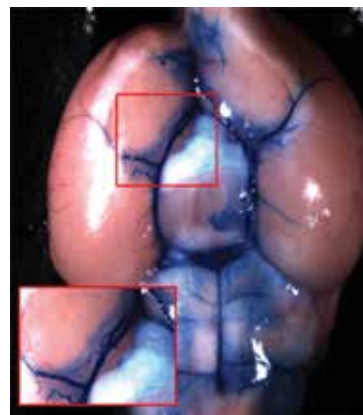
KEY PUBLICATIONS

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.

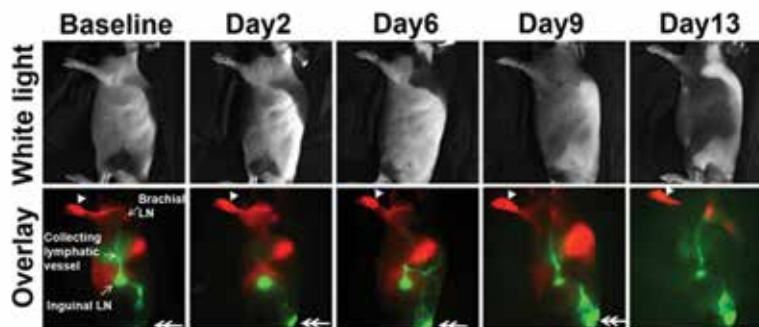
D. A. Germaine, G. Wu, E. M. Sevick-Muraca, and S. Kwon, “In vivo lymphatic imaging of a human

inflammatory breast cancer model,” *Journal of Cancer*. 5; 774-783, 2014.

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



Typical Evan’s blue dye (EBD) filling of Virchow-Robin spaces (i.e., glymphatics) in the ventral view of an extracted brain from a mouse harvested after injection of mixture of EBD and ICG in cisterna magna.



Abnormal, but transient changes of lymphatic drainage pathways in response to axillary lymphadenectomy after injection of Alexa680-BSA (arrow head) and ICG (double arrows).



John Rasmussen, Ph.D.

Assistant Professor

Device translation for lymphatic imaging

- vascular 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*, 4(1):9-17, 2016.

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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.

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

- Lymphatic involvement in peripheral vascular disease
- Etiology of cancer-related lymphedema
- Nodal staging of cancer using noninvasive NIRF imaging
- Identification of genetic causes for lympho-

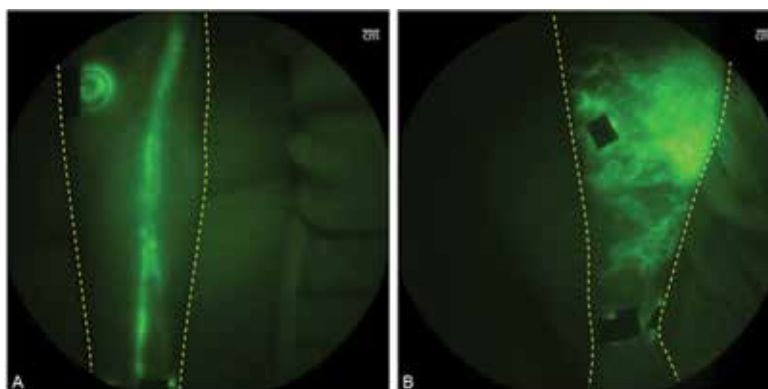


Image of (A) the well-defined, linear lymphatics in the arm of a healthy subject and (B) the disorganized, dermal lymphatics in the arm of a subject with lymphedema. Injection sites are covered by round bandages and/or black vinyl tape. Reproduced from Shaitelman, et al., *CA: A Cancer Journal for Clinicians*, 65(1):55-81, 2015.



Banghe Zhu, Ph.D.

Assistant Professor

Development of optical imaging systems for preclinical and clinical applications

My research program focuses on the development of high-resolution optical imaging system for functional brain mapping and the establishment of working standards for accelerating the translation of optical medical imaging into the clinic.

Although functional MRI (fMRI) can be used to diagnose brain network dysfunction by using the blood oxygen level dependent (BOLD) MRI signal, a proxy for brain glucose metabolism, the complexities and general anesthesia sedation needs to obtain motion-free BOLD MRI data limit its practical utility in children. Specifically, functional brain networks are best assessed with BOLD MRI on awake patients and this cannot be obtained in most pediatric brain tumor patients due to sickness and lack of cooperation. Hence, the chemotherapy and radiation treatments are made on a general basis and cannot be individualized for optimal management of these patients. Functional near infrared spectroscopy and diffuse optical tomography (fNIRS-DOT), which allows a similar assessment to the BOLD MRI signal and is particularly suitable for children since NIR light can easily pass the whole skull of children than adult, allowing whole brain imaging and the device can be mounted in a comfortable cap on an awake child. However, current fNIRS-DOT imaging systems are limited by their spatial resolutions due to the limited number of detectors. We are developing a high-resolution fNIRS-DOT imaging system to acquire hundreds of measurements simultaneously with high sensitivity for imaging of chemotherapeutic effects on pediatric brain networks.

To date, several near-infrared fluorescence (NIRF) imaging devices have emerged for clinical applications that include intraoperative surgical guidance with cancer-targeting probes. However, the performance of these NIRF imaging devices needs to be validated on working standards with traceable, International Systems of Units (SI units) of radiance ($mW \cdot sr^{-1} \cdot cm^{-2}$) that enable comparison or quantitative quality assurance. Quantifying device performance

at relevant radiance levels is critical for translational NIRF molecular imaging using molecularly-targeting moieties conjugated to NIRF dyes. Without a standardized measure of device performance, it will be difficult to distinguish whether a cancer-targeting probe fails to detect cancer, or if the device is too insensitive to detect it. We are developing and deploying a methodology to calibrate a stable, a solid phantom for fluorescent irradiance for use in characterizing the measurement sensitivity of fluorescence molecular imaging devices.

In addition, we are collaborating to use far-red fluorescence gene reporter, iRFP, for assessing the gene therapies in bone formation and heart diseases.

RESEARCH PROJECTS

- Develop high-resolution optical imaging system for functional brain mapping.
- Develop working standards for optical medical imaging.
- Assess the gene therapy using iRFP gene reporter.

KEY PUBLICATIONS

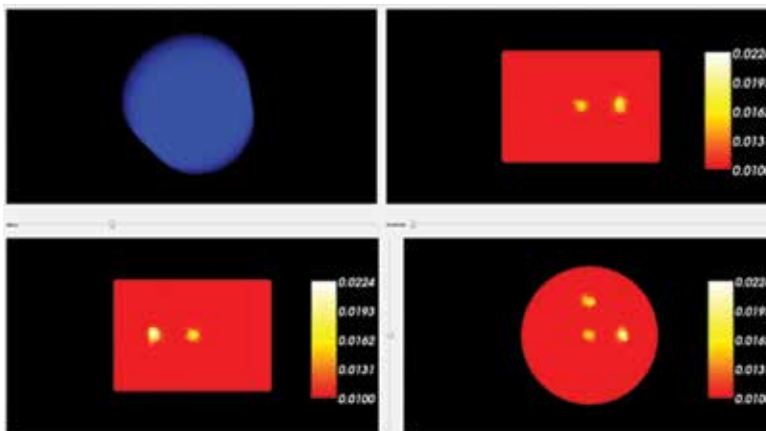
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Zhu, B., Robinson, H., Zhang, S., Wu, G., and Sevick-Muraca, E. M., "Longitudinal far red gene-reporter imaging of cancer metastasis in preclinical models: a tool for accelerating drug discovery", *Biomedical Optics Express*, 6:3346-3351 (2015).

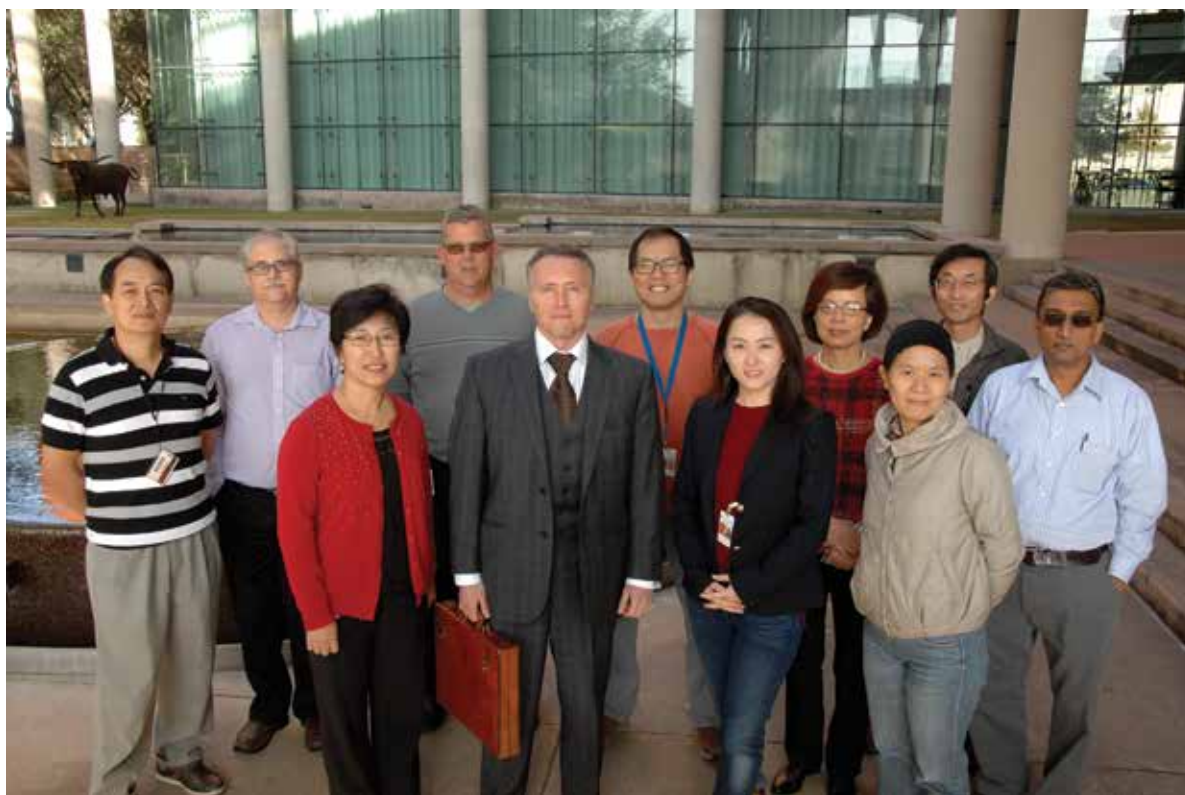
Zhu, B., and Sevick-Muraca, E.M., "Update on clinical near-infrared fluorescence imaging: A review of devices, performance, and applications," *British Journal of Radiology*, 88:20140547(2015).

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).



3-D, Sagittal, Coronal and Axial cross-sections of the reconstructed images on the size of children's brain, demonstrating high-spatial resolution.



The Center for Precision Biomedicine focuses on developing the mathematical, experimental and analytical technologies that will deliver precise medication to the correct tissues. This is accomplished by understanding the underlying physiological problems, the proteomic and genomic biomarkers indicative of disease, selective targeting of tissues or toxins, the use of targeted nanoparticles, and mathematical models of tissue and vasculature to predict and overcome biological barriers to tissue penetration. These efforts connect us with collaborators across UTHealth, the Texas Medical Center, including Baylor, Methodist and MD Anderson, and state-wide through interactions with the Center for Clinical and Translational Science, nanomedicine researchers, and faculty studying proteomics, genomics, and bioinformatics.

At the IMM, we have state-of-the art mass spectrometers , providing in-depth proteomic analysis of cells, tissues or biological fluids, leading to novel targets for drug development and nanomedicine therapeutics and imaging agents, including next-generation X-aptamer reagents. We also have large-scale, multi-color, high-resolution 3D printers for both fast prototypes and finished production level models of new

surgical tools and instruments. We provide state-of-the-art mathematical and computational modeling to aid current prospective clinical trials focusing on understanding drug penetration barriers in tumors and improving tumor response and patient outcome.

Hubs of Research Collaboration with the Center include:

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

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



Vittorio Cristini, Ph.D.

Professor

Translational modeling of cancer treatment

Our group 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. Towards 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 modeling. Biological processes can occur across physical time and space scales, forming a complex system with multiple feedback and feed-forward loops. Advanced multiscale methods are therefore needed to simulate and predict the behavior of complex biological systems. We are developing methods to address a significant challenge in multiscale modeling, i.e., bridging the gaps between different modeling methods and between models at different scales, from the molecular,

to the cellular and tissue scales, based on “dynamic density functional theory,” a technique implemented in the physical sciences.

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

Hosoya H, Dobroff AS, Driessen WH, Cristini V, et al. 2016. Integrated nanotechnology platform for tumor-targeted multimodal imaging and therapeutic cargo release. *Proc Natl Acad Sci U S A.* 113(7):1877-82. PMC4763738

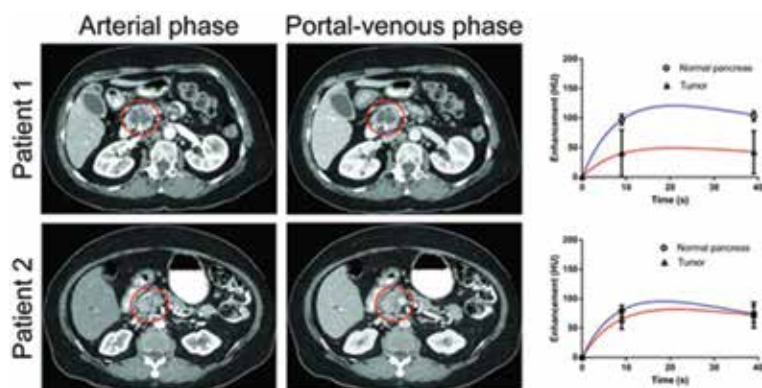
Paine I, Chauviere A, Landua J, Sreekumar A, Cristini V, Rosen J, Lewis MT. 2016. A Geometrically-Constrained Mathematical Model of Mammary Gland Ductal Elongation Reveals Novel Cellular Dynamics within the Terminal End Bud. *PLoS Comput Biol.* 12(4):e1004839. PMC4845990

Wang Z, Kerketta R, Chuang Y-L, et al., Ferrari M, Koay EJ, Cristini V. 2016. Theory and experimental validation of a spatio-temporal model of chemotherapy transport to enhance tumor cell kill. *PLoS Computational Biology* 12(6):e1004969. PMC4902302

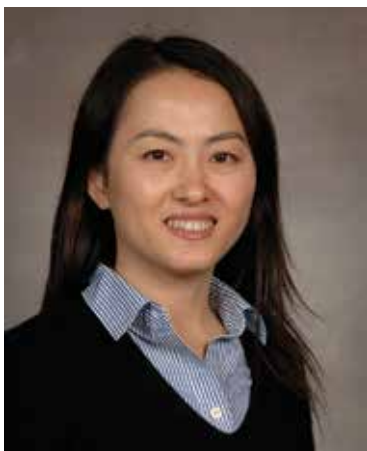
LAB MEMBERS

Post-doctoral Fellows: Huaming Yan, Greg von Winckel

Students: Joseph Butner (jointly with Dr. Wang), Prashant Dogra (jointly with Dr. Wang), Terisse Brocato (jointly with Dr. Wang)



Measuring enhancement profiles of pancreatic cancer during the timed phases of standard-of-care computed tomography scans. Patients 1 and 2 both had adenocarcinoma of the pancreas but different imaging characteristics and enhancement profiles in the tumor. (Koay et al., *J Clin Invest* 2014; PMC3973100).



Xiaohong Bi, Ph.D.

Assistant Professor

Optical spectroscopy and imaging for medicine

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

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

X. Bi, I. Grafe, H. Ding, R. Flores, E. Munivez, M.M. Jiang, B. Dawson, B. Lee, C.G. Ambrose, Correlations between bone mechanical properties and bone composition parameters in mouse models of dominant and recessive Osteogenesis Imperfecta and the response to anti-TGF- β treatment, *Journal of Bone and Mineral Research*, e-pub online September 2016

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)

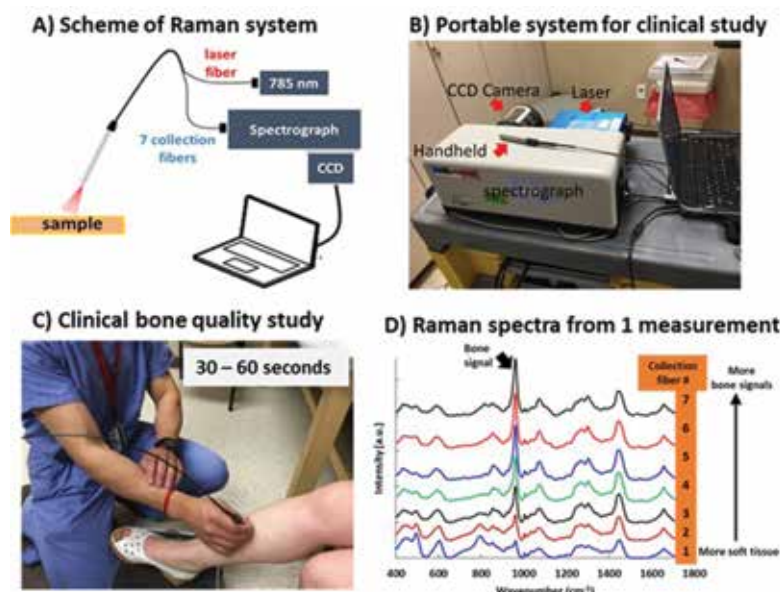
H. Ding, J.S. Nyman, J.A. Sterling, D.S. Perrien, A. Mahadevan-Jasen, and X. Bi, Development of Raman Spectral Markers to Assess Metastatic Bone in Breast Cancer, *Journal of Biomedical Optics*, 19(11): 111606 (2014)

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)

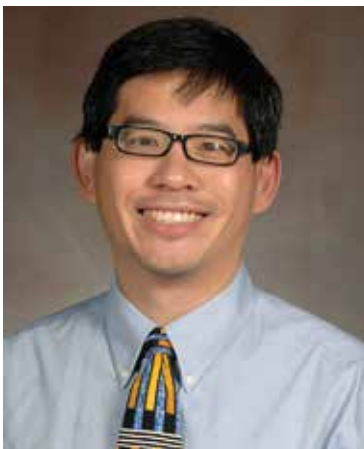
X. Bi, J.A. Sterling, A.R. Merkel, D.S. Perrien, J.S. Nyman, A. Mahadevan-Jansen, Prostate cancer metastases alter bone mineral and matrix composition independent of effects on bone architecture in mice – A quantitative study using microCT and Raman spectroscopy. *Bone*, 56(2):454-60 (2013)

LAB MEMBERS

Post-doctoral Fellow: Hao Ding
Research Assistant: Guijin Lu



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



Jeffrey Chang, Ph.D.
Assistant Professor

Genomic approaches to decipher cancer signaling programs

Our lab deciphers the complex signaling programs that underlie aspects of the cancer phenotype, including cell cycle control, stem cell behavior, and metastasis using genomics. 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 developed the GATHER website for analysis of gene sets, the SIGNATURE platform for the analysis of oncogenic pathways, and the BETSY knowledge base for planning bioinformatics analyses.

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.

- Cholesterol processing and cancer metastasis.
- Intelligent computational pipelines for bioinformatic analysis.
- Genetic perturbations of Ras signaling.

KEY PUBLICATIONS

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

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

* Co-Corresponding Authors

Chang J.: Core services: Reward bioinformaticians. *Nature* 520(7546):151-2, 2015.

Soundararajan R., Paranjape A.N., Barsan V., Chang J.T.*, and Mani S.A.*: A novel embryonic plasticity gene signature that predicts metastatic competence and clinical outcome. *Scientific Reports* 5:11766, 2015.

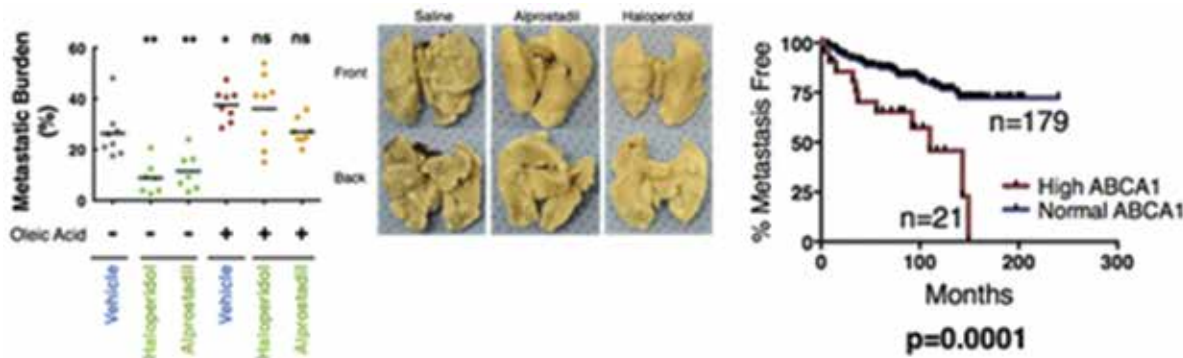
* Co-Corresponding Authors

LAB MEMBERS

Post-doctoral Fellows: Weina Zhao, Ph.D., Sarah Prijic, Ph.D.

Graduate Student: Kevin Zhu

Undergraduate Student: Aurnab Baidya



We have used genomic analysis and identified compounds that can inhibit metastatic capacity in cancer cells (left and middle panel). These compounds work by decreasing the fluidity of the plasma membrane, which reprograms the cells to a less aggressive state. In breast cancer cells, fluidity is mediated by the ABCA1 cholesterol efflux channel in cell line models, and in human breast tumors, is correlated with a significantly shorter time to distant metastasis (right panel).



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 along with backbone modifications on DNA aptamers, thioaptamers and dithioaptamers. These targeting 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 or siRNA 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. More recently (Mangala et al, 2016) we have shown that aptamer directed delivery of siRNA improves vascular maturation thereby enhancing the anti-tumor effects of chemotherapy. A number of exciting X-aptamers are in development for use in the fight against cancer and other diseases.

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

A recent project in the lab is the creation of specialty software for the analysis of the significant DNA sequence changes in the vsIE surface protein as a function of time during B. burg-

dorferi infections, the cause of Lyme disease. A poorly understood process called antigenic variation leads to large-scale changes in the bacteria's *vsE* locus, and thus the bacteria's protein surface, which leads to escape from the host's immune system. Antigenic variation is thought to be the cause of long-term Lyme Disease infection and post-infection deficits.

RESEARCH PROJECTS

- Breast, ovarian and pancreatic tumor imaging and directed drug delivery
- Developing X-aptamers targeting cancer and other diseases
- Developing novel software to analyze antigenic variation in Lyme disease

KEY PUBLICATIONS

Improving vascular maturation using non-coding RNAs increases anti-tumor effect of chemotherapy, Mangala, L.S., Wang, H., et al. , *Journal of Clinical Investigation - Insights*, 1(17):e87754, 2016. doi:10.1172/jci.insight.87754

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.

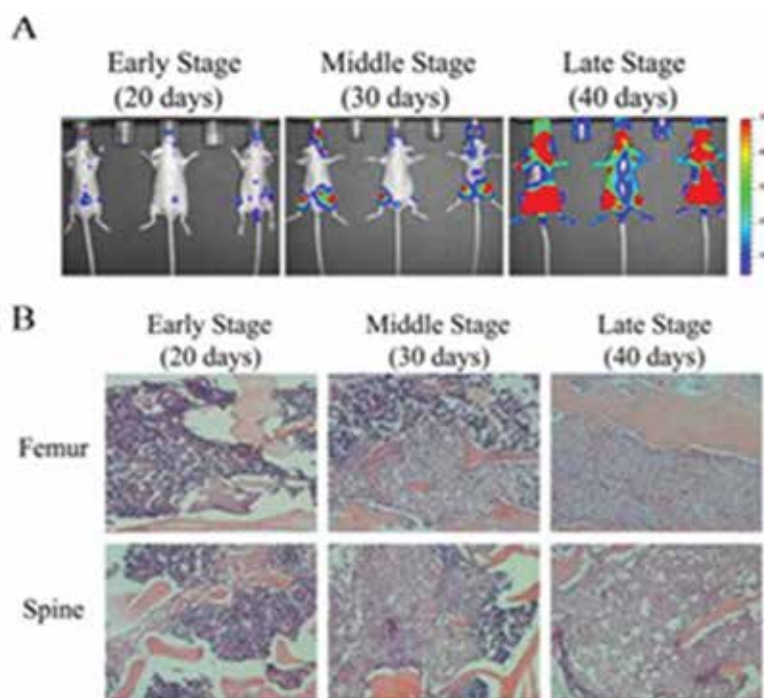
Bone marrow endothelium-targeted therapeutics for metastatic breast cancer, J. Mai, Y. Huang, C. Mu, G. Zhang, R. Xu, X. Guo, X. Xia, D.E. Volk, G. L. Lokesh, V. Thivyanathan, D.G. Gorenstein, X. Liu, M. Ferrara, and H. Shen, *J. Controlled Release*, 2014, 187:22-29.

LAB MEMBERS

Research Scientist: Lokesh Rao, Ph.D.

Research Associate: Xin Li, M.S.

Medical Students: Andrea Costello, MSII, Brenda Saucedo, MSII, James Maybery, MSIV



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 can reduce the cancer burden and prolong life.



Hongyu Wang, Ph.D.

Assistant Professor

Aptamer mediated biomarker discovery and targeted therapy

DNA aptamers represent a novel platform for identifying high-affinity synthetic ligands with desired specificity and are attractive alternatives to antibodies in targeted therapy. High affinity aptamer based biomarker discovery has the advantages of simultaneously discovering an aptamer affinity reagent and target biomarker protein. We have developed a morphologically-based tissue aptamer selection (Morph-X-Select) method that combines a thiophosphoate modified DNA aptamer library with image-directed laser microdissection to use tissue sections from individual patients and identify high affinity aptamers and their associated target proteins in a systematic and accurate way. Combining modified aptamers (X-aptamer) bead-based library with flow cytometry and mass spectrometry, we have created a

proteomics based X-aptamer selection method, which enables rapid selection of X-aptamer affinity reagents to a target biomolecule in solution. The identified thioaptamer/X-aptamer can specifically bind biomarkers on cancer cells and applied in clinical diagnosis and prognosis. The technology developed in those projects will build up a new platform for biomarker discovery and can be extended to many other cancers or other diseases for biomarker discovery, imaging and even targeted therapies.

RESEARCH PROJECTS

- Morph-X-Select tissue biomarker discovery
- Proteomics biomarker discovery
- Develop immune-checkpoint blockade X-aptamers for cancer immunotherapy

KEY PUBLICATIONS

Mangala, L.S., *Wang, H., *Jiang, D., Wu, S.Y., Somasunderam, A., Volk, D.E., Lokesh, R. L.G., Li, X., Pradeep, S., Yang, X., Rodriguez-Aguayo, C., Nagaraja A.S., Ivan C., Gharpure, K.M., McGuire, M.H., Haemmerle M., Thiviyanathan, V., Maiti,

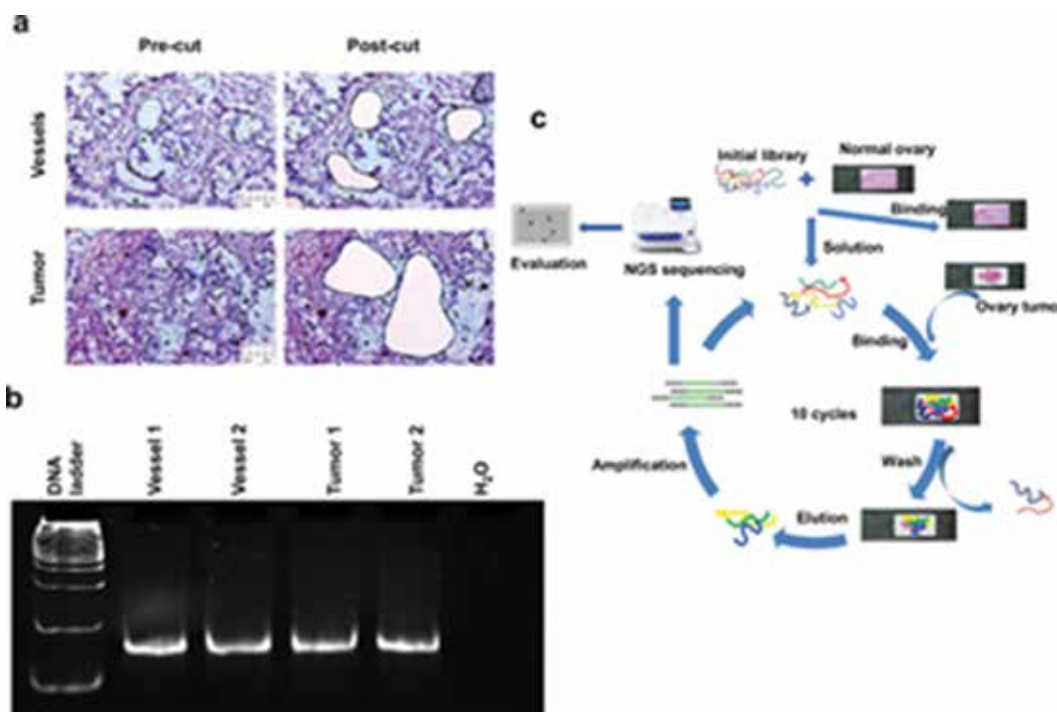
S.N., Cooper L.J.N., Bulayeva N., Choi, H.-J., Dorniak, P.L., Li, L., Rosenblatt, K.P., Tanaka, T., Lopez-Berestein, G., Gorenstein, D.G., and Sood, A.K., Improving vascular maturation using non-coding RNAs increases anti-tumor effect of chemotherapy. *JCI Insight*. 2016;1(17):e87754. (*equal contributions)

Wang, H., Li, X., Volk, D.E., Lokesh, R. L.G., Elizondo-Riojas M-A, Nick, A.M., Sood, A.K., Rosenblatt, K.P., Gorenstein, D.G. Morph-X-Select, a Morphologically-Based Aptamer Tissue Selection for Personalized Ovarian Cancer Biomarker Discovery. *BioTechniques*. In press.

Mu Q, Akshaya A, Mayank S, Li X, Wu J, Wang H. et al. Conjugate-SELEX: A high-throughput screening of aptamer-liposomal nanoparticle conjugates for targeted intracellular delivery of anticancer drugs. *Molecular therapy-Nucleic acids*. In press.

LAB MEMBERS

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



Procedures of Morph-X-Select. (a) LMD of blood vessels (pre- and post-cut, upper row), tumor cells (pre- and post-cut, lower row) of human ovarian cancer. Scale bar=40 μm. (b) PCR amplification of eluted thioaptamers from laser microdissected blood vessels and tumor cells. (c) Scheme of the tissue Morph-X-Select.



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 pow-

erful) 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

Pascal J*, Bearer EL*, Wang Z*, Koay EJ, Curley

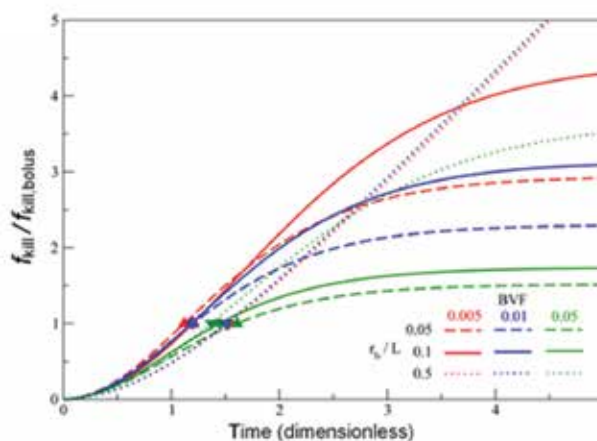
SA, Cristini V 2013. Mechanistic patient-specific predictive correlation of tumor drug response with microenvironment and perfusion measurements. *Proc. Natl. Acad. Sci. USA* 110(35):14266-71. PMC3761643. (*Equal contribution)

Pascal J*, Ashley CE*, Wang Z*, Brocato TA, Butner J, Carnes EC, Koay EJ, Brinker CJ, Cristini V. 2013. Mechanistic modeling identifies drug-uptake history as predictor of tumor drug resistance and nanocarrier-mediated response. *ACS Nano*. 7(12):11174-82. PMC3891887. (*Equal contribution)

Wang Z, Kerketta R, Chuang Y-L, Dogra P, Butner JD, Brocato TA, Day A, Xu R, Shen H, Simbawa E, AL-Fhaid AS, Mahmoud SR, Curley SA, Ferrari M, Koay EJ, Cristini V. 2016. Theory and experimental validation of a spatio-temporal model of chemotherapy transport to enhance tumor cell kill. *PLoS Computational Biology* 12(6):e1004969. PMC4902302

LAB MEMBERS

Students: Joseph Butner (jointly with Dr. Cristini), Prashant Dogra (jointly with Dr. Cristini), Terisse Brocato (jointly with Dr. Cristini)



Drug-loaded nanoparticles lead to cell-kill enhancement over conventional bolus delivery. Time-evolution curves of chemotherapeutic efficacy f_{kill} of nanoparticles releasing drug compared to the estimated efficacy (symbols) of conventional chemotherapy, for parameter values: $r_b/L = 0.05$ (dashed curves, upper triangles), 0.1 (solid curves, diamonds), and 0.5 (dotted curves, lower triangles), paired with BVF = 0.005 (red curves and symbols), 0.01 (blue curves and symbols), and 0.05 (green curves and symbols). Normalized to the corresponding bolus values of tumor kill, $f_{kill} - r_b$; r_b : blood vessel radius; L : drug diffusion penetration length; BVF: blood volume fraction. (Wang et al., PLoS Comput Biol 2016, PMC4902302.)



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 UTHHealth, 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; Blood Diseases; Cancer; Musculo-Skeletal Diseases; and Lung Diseases. Over the past several years we have successfully recruited 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 tissue-specific stem cells and/or 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 – and have demonstrated 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 blood stem cells – or 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.

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.N. Kotton, M. Holmes, E.J. Sorscher, B.R. Davis: Targeted Correction and Restored Function of CFTR Gene in Cystic Fibrosis Induced Pluripotent Stem Cells. *Stem Cell Reports*. 2015, 4:569-577.

Z. Garate, O. Quintana-Bustamante, A.M. Crane, E. Oliver, L. Poirot, R. Galetto, P. Kosinski, C. Kung, J. Bazinet, X. Aguirre, I. Orman, L. Cerrato, O. Alberquilla, F. Rodriguez-Fornes, N. Fusaki, F. Garcia-Sanchez, T. Maia, M. Ribero, J. Sevilla, F. Prosper, S. Jin, J. Mountford, G. Guenechea, A. Gouble, J.A. Bueren, B.R. Davis, J.C. Segovia: 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, 5:1053-

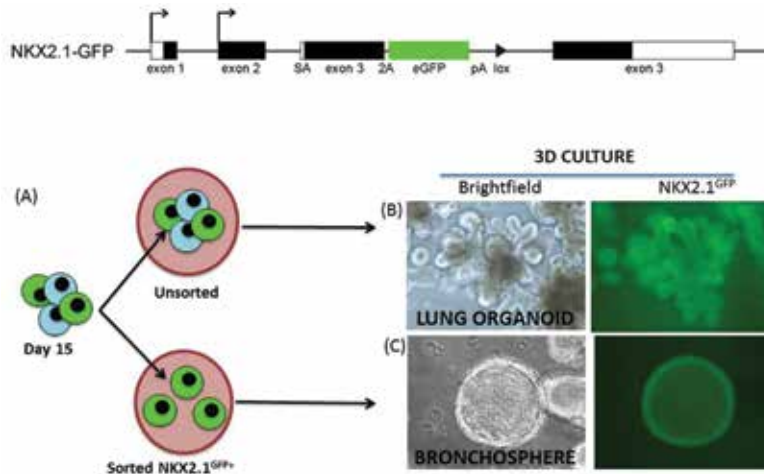
66.

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

LAB MEMBERS

Research Staff: Dr. Ana M. Crane, Dr. Nadine Matthias

Post-doctoral Fellow: Dr. Leila Rouhgharabaei



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).

RESEARCH PROJECTS

- Correction and lung differentiation of iPS cells from inherited lung diseases (Cystic Fibrosis)
- 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,



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 stroke. The laboratory uses an interdisciplinary approach involving techniques from cell, molecular, and stem cell biology, chemistry, and material science. Utilizing engineering approaches, the laboratory seeks to optimize scaffold design and the expansion of clinically relevant cell sources for use in stem cell therapy.

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

In order to advance tissue engineering to wide spread clinical use, protocols for the expansion and differentiation of clinically relevant cell sources, also, need to be optimized. Human induced pluripotent stem cells (hiPSC) offer a potentially autologous cell sources for the treatment of traumatic injuries to the central nervous system. However, the number of viable

cells for transplant produced from current differentiation protocols is extremely low. Both biochemical and mechanical properties of the cell culture surface have been shown to significantly affect cellular differentiation, but have not been studied significantly in respect to hiPSC differentiation. The laboratory seeks to extend our knowledge of three dimensional culture systems to optimize two dimensional cell culture surfaces for differentiation of neural stem cells and oligodendrocyte progenitor cells from hiPSC. Preliminary studies have focused on the covalent tethering of proteins to the surface of hydrogels with containing a Young's Modulus gradient to study the effect of mechanical properties on hiPSC lineage choice.

RESEARCH PROJECTS

- 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 disk repair.

KEY PUBLICATIONS

Lim HJ, Perera TH, Wilems T, Ghosh S, Zheng YY, Azhdarinia A, Cao Q, Smith Callahan LA. Re-

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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.

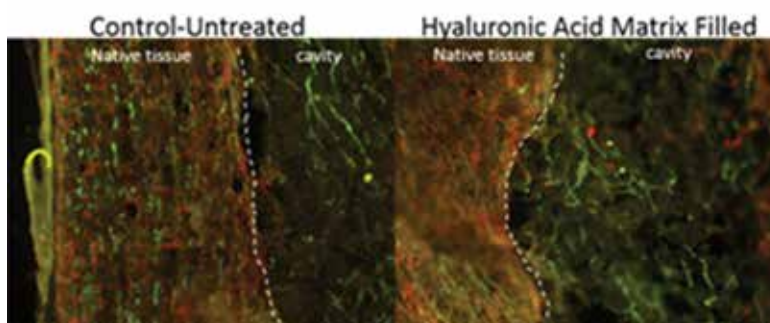
Smith Callahan LA, Xie S, Barker IA, Zheng J, Dove AP, Becker ML. Directed Differentiation and Neurite Extension of mouse Embryonic Stem Cell on Aligned Poly(lactide) Nanofibers Functionalized with YIGSR Peptide. *Biomaterials*. 34(36): 9089-9095, 2013.

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.

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: T. Hiran Perera, Xi Lu
Undergraduate Students: Zara Khan, Teja Ravi-rapuru, Sonali Mahendran, and Yuki Kurosuo



Extension of host axons (green) into untreated and di-functional hyaluronic acid matrix filled spinal cord lesion cavity in injured rats one week after scaffold injection and a total of 3 weeks after spinal cord injury. A dense grouping of astrocytes (red) marks the glial scar at the edge of the lesion cavity, which the hyaluronic acid matrix helps more axons to cross and is a first step toward functional neurological recovery after spinal cord injury.



Qi Lin Cao, M.D.
Associate Professor

Stem cells for neurological diseases

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

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

RESEARCH PROJECTS

- 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 vivo* reprogramming of reactive astrocyte and chemogenetic approach for SCI repair
- Treating neuropathic pain by *in vivo* reprogramming of astrocytes after SCI

KEY PUBLICATIONS

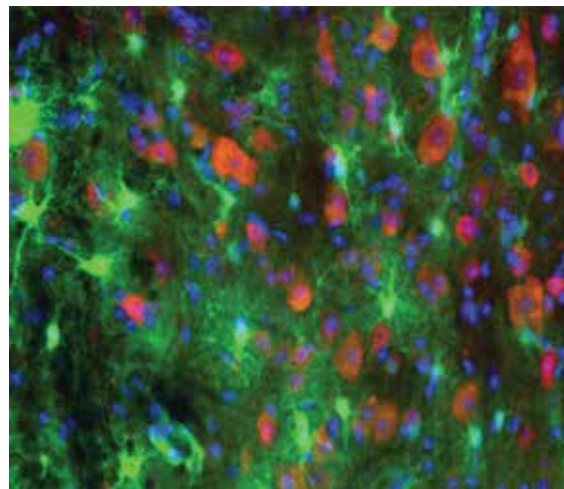
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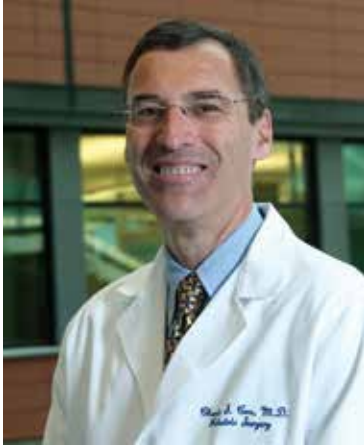
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LAB MEMBERS

Research Associate: Yiyang Zheng; Haipeng Xue
Senior Research Assistant: Jun Li
Visiting Scholar: Jinlong Shi; Xiuquan He
Student: Chrystine Gallegos



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

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.

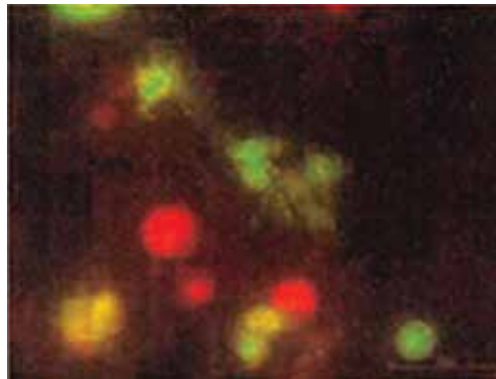
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H, Hamilton JA, Smith P, Thomas CP, Mays RW, Pati S, Cox CS. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: Modulation of microglia/macrophages. *J Neuroinflammation* 9: 228-240, 2012. PMID: 23020860

Liao G, Harting MT, Hetz RA, Walker PA, Shah SK, Corkins CJ, Jimenez F, Kosmach SC, Day MC, Tsao KT, Lee DA, Worth LL, Baumgartner JE, Cox CS. Autologous bone marrow mononuclear cells reduce therapeutic intensity for severe traumatic brain injury in children. *Pediatr Crit Care Med* 16:245-255, 2015. PMID: 25581630, PMCID: PMC4351120

LAB MEMBERS

- Instructor: Supinder Bedi, Ph.D.
- TBI-clinical and Cell Therapy: Margaret Jackson, M.D.
- Post-Doctoral Fellow: Suchit Sahal, Ph.D.
- TBI-clinical and Cell Therapy: Chris Schneider, M.D.
- MSC biologist: Amit Srivastava, M.D.
- Flow Cytometry/innate immunity: Naama Toledo-Furman, M.D.
- 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.



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

Skeletal muscle regeneration using human stem cells

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

Here at IMM, by using cutting-edge gene editing technologies (such as CRISPR/Cas9 system) our lab has successfully generated knock-in human ES/iPS reporter cell lines for early myogenic genes such as PAX7 and MYF5. This will allow studying the emergence of early myogenic progenitors from human ES/iPS cells; a crucial step to identify and isolate myogenic progenitors for future cell based therapies. Other major goals of the lab include using high throughput screening (HTS) to identify important inducers of myogenesis in human stem cells and evaluation of *in vivo* regeneration potential of these cells in mice models of muscular dystrophies and muscle mass injuries.

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

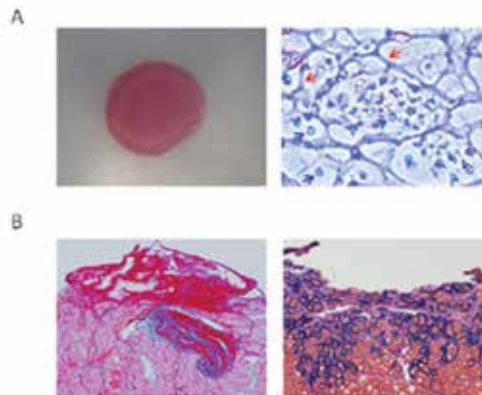
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Wu J, Hunt SD, Xue H, Liu Y, Darabi R. Generation and Validation of PAX7 Reporter Lines from Human iPS Cells Using CRISPR/Cas9 Technology. *Stem Cell Research*. 2016 Mar; 16(2):220-8.

LAB MEMBERS

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

Muscle Injury Repair Using Bio-scaffold Seeded With Stem Cells



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



Pramod Dash, Ph.D.

Professor

Nina and Michael Zilkha Distinguished Chair, Neurodegenerative Disease Research

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 (Aβ). However, the cellular and molecular mechanisms that trigger TAU and Aβ 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

Dash PK, Zhao J, Kobori N, Redell JB, Hylin MJ, Hood, KN and Moore AN. Activation of alpha7 cholinergic nicotinic receptors reduces blood-brain barrier permeability following experimental traumatic brain injury. *J. Neurosci*, 36:2109-2818, 2016.

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decreases AMP-activated protein kinase activity and pharmacological enhancement of its activity improves cognitive outcome. *J. Neurochem*, 139(1):106-19, 2016.

Zhao Y, Gibb SL, Zhao J, Moore, AN, Hylin M, Menge T, Xue H, Baimukanova G, Potter D, Johnson E, Holcomb JB, Cox Jr CS, Dash, PK and Pati S. Wnt3a, a protein secreted by mesenchymal stem cells is neuroprotective and promotes neurocognitive recovery following traumatic brain injury. *Stem Cells*, 34(5):1263-72, 2016.

LAB MEMBERS

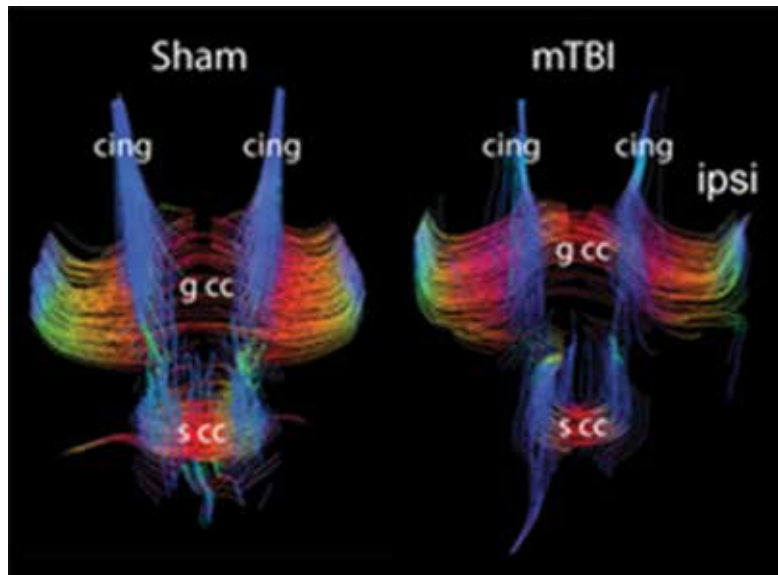
Research Assistant Professor: John B. Redell, Ph.D.

Sr. Research Scientist: Jing Zhao, M.D., Ph.D. Research Scientists: Nobuhide Kobori, M.D., Ph.D., John Broussard, Ph.D.

Post-doctoral Fellows: Karthikeyan Tangavelou, Ph.D., Mark E. Maynard, Ph.D.

Program Manager: Anthony N. Moore, B.S. Research Associate: Kimberly Hood, M.A.

Sr. Research Assistant: Jacalyn S. MacGowan Graduate Students: Georgene Hergenroeder, R.N., MPH, Tara Diane Fischer, B.S.



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

RESEARCH PROJECTS

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

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.* 2005; 56: 655-61.

KEY PUBLICATIONS

Kim DH, Mathew J, Ziadi S, Nates J, Dannenbaum M, Malkoff M: Increases in cardiac output can reverse flow deficits from vasospasm independent of blood pressure: a study using xenon computed tomographic measurement of cerebral blood flow. *Neurosurgery.* 2003 Nov; 53(5):1044-51.

Kim DH, Haney CL, Van Ginhoven G: Reduction of pulmonary edema after SAH with a Pulmonary-Artery Guided Hemodynamic Management Protocol. *Neurocrit Care.* 2005; 3(1):11-15.

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

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

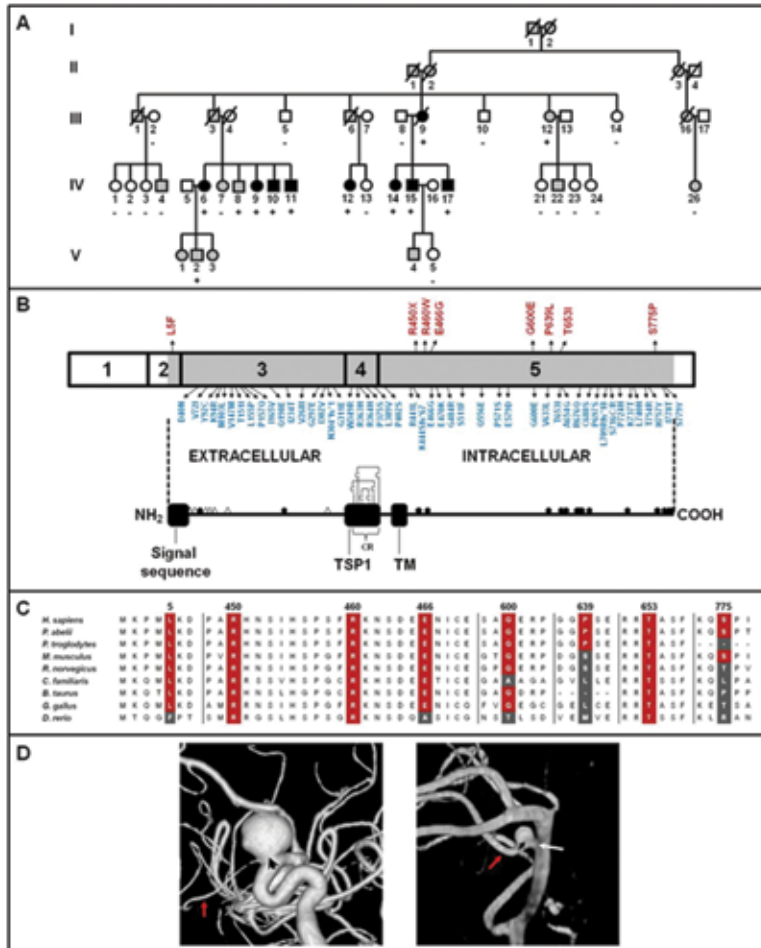
I was named to the US News and World Report's Top 1% Doctors, and America's Top Surgeons. I am the recipient of grants from the National Institutes of Health and the American Stroke Association.

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

I specialize in the following diseases:

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

Figure 1



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



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

Associate Professor

Fetal derived B-1a cell development and self-renewal

My lab focuses on how innate immune B-1a cells develop in the mouse embryo and how they are maintained in the adult peritoneal cavity without being replenished by adult bone marrow hematopoietic stem cells (HSCs).

B-1 cells are unique murine innate immune cells that are distinguished from conventional adoptive B cells (B-2 cells). B-1 cells localize in the peritoneal and pleural cavities and secrete natural antibodies without T cell help, displaying important roles in the first line of defense against various infections, atherosclerosis, and autoimmunity. It has been postulated for decades that B-1a cells are derived from fetal progenitor cells, not from adult bone marrow HSCs, based on the results of transplantation assays. We have recently reported the presence of HSC-independent B-1 progenitors in HSC-deficient embryos. Our data and others' publication showed lack of B-1a cell potential in highly purified HSCs in adult bone marrow and fetal liver, suggesting that HSC-independent B-1 progenitors are produced somewhere in the mouse embryo and contribute to producing B-1 cell pool that persists to postnatal life. Our aim is to identify the main source of HSC-independent B-1 progenitor cells and evaluate its real contribution to postnatal B-1 cell pool, utilizing various lineage tracing mouse models and transplantation assays with hematopoietic progenitors and hemogenic endothelial cells.

Another important question that has not been resolved for decades is how B-1a cells are maintained without being replenished by adult bone marrow HSCs. We hypothesize that one of the polycomb proteins, Bmi1 plays a role in self-renewal capacity of mature B-1a cells in the adult peritoneal cavity. Using B cell lineage specific Bmi1 deletion, we are evaluating the B-1a cell number and their self-renewal ability upon transplantation. Comparing RNA expression profiles between wild type and Bmi1 deleted B-1a cells, we are trying to identify target genes of Bmi1 in B-1a cell self-renewal ability. We are also evaluating fat associated lymphoid clusters (FALCs) as a niche for B-1 cells in the

peritoneal cavity if microenvironment is altered in Bmi1^{-/-} mice.

With knowledge obtained from above projects, we are developing a culture system to produce B-1 cells from mouse ES cells and human iPS cells *in vitro*. Since B-1 cells are not replenished by adult HSCs after transplantation, producing B-1 cells *in vitro* might open a path of cell therapy for immunocompromised patients after bone marrow transplantation.

RESEARCH PROJECTS

- Lineage tracing for HSC-independent B-1 cell development from embryos to adults.
- Elucidating cell intrinsic and cell extrinsic mechanisms for maintain B-1a cell self-renewal ability.
- Identifying and producing human B-1 cells.
- Understanding the multiple waves of hematopoiesis in the mouse embryo.

KEY PUBLICATIONS

Yoshimoto M. The first wave of B lymphopoiesis develops independently of stem cells in the murine embryo. *Ann N Y Acad Sci.* 2015;1362:16-22.

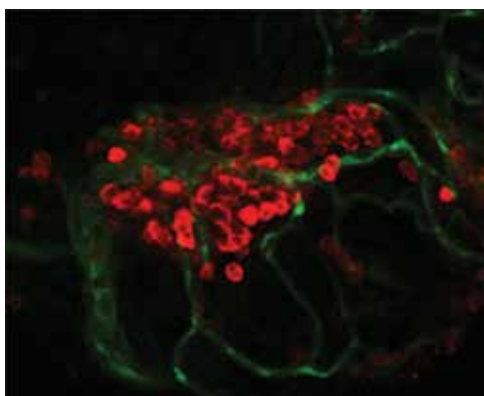
Kobayashi M, Shelley WC, Seo W, Vemula S, Lin Y, Liu Y, Kapur R, Taniuchi I, Yoshimoto M. Functional B-1 progenitor cells are present in the hematopoietic stem cell-deficient embryo and depend on Cbfbeta for their development. *Proc Natl Acad Sci U S A.* 2014;111(33):12151-12156.

Lin Y, Yoder MC, Yoshimoto M. Lymphoid progenitor emergence in the murine embryo and yolk sac precedes stem cell detection. *Stem Cells Dev.* 2014;23(11):1168-1177.

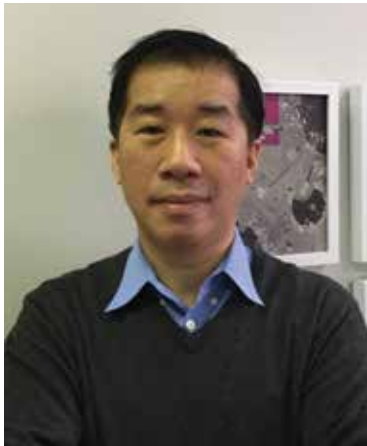
Prasain N, Lee MR, Vemula S, Meador JL, Yoshimoto M, Ferkowicz MJ, Fett A, Gupta M, Rapp BM, Saadatzadeh MR, Ginsberg M, Elemento O, Lee Y, Voytik-Harbin SL, Chung HM, Hong KS, Reid E, O'Neill CL, Medina RJ, Stitt AW, Murphy MP, Rafii S, Broxmeyer HE, Yoder MC. Differentiation of human pluripotent stem cells to cells similar to cord-blood endothelial colony-forming cells. *Nat Biotechnol.* 2014;32(11):1151-1157.

LAB MEMBERS

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



Fat Associated Lymphoid Clusters (FALCs) as a niche of B-1 cells. FALCs are seen in the omentum of wild type mouse. Red: Anti-mouse IgM, green: anti-CD31(endothelial)



Dung-Fang Lee, Ph.D.

Assistant Professor

Familial cancer syndromes in a dish

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

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

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

TALEN/CRISPR genetically engineered hESCs to illuminate cancer pathological mechanisms.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

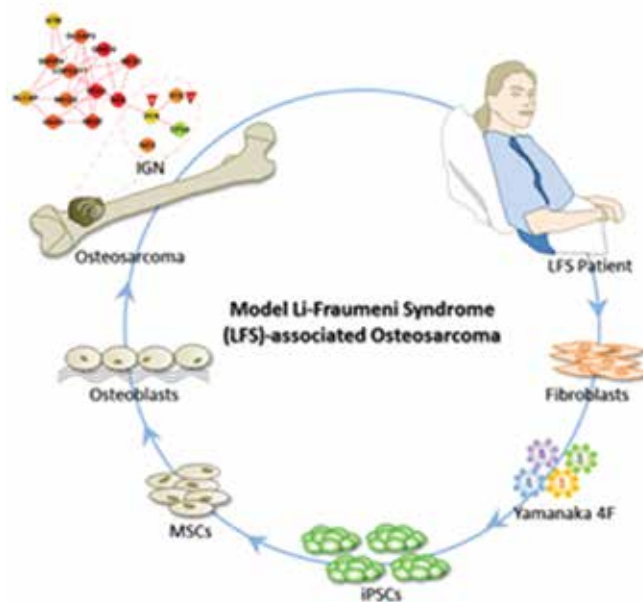
Gingold J, Zhou R, Lemischka IR, Lee DF. Modeling cancer with pluripotent stem cells. *Trends Cancer*. 2(9):485-494, 2016.

Lee DF, Walsh MJ, Aguiló F. ZNF217/ZFP217 meets chromatin and RNA. *Trends Biochem Sci*. 2016 Aug 9. [Epub ahead of print]

Lu J, Song G, Tang Q, Yin J, Zou C, Zhao Z, Xie X, Xu H, Huang G, Wang J, Lee DF, Khokha R, Yang H, Shen J. MiR-26a inhibits stem cell-like phenotype and tumor growth of osteosarcoma by targeting Jagged1. *Oncogene*. 2016 Jun 6. [Epub ahead of print]

LAB MEMBERS

Post-doctoral Fellow: An Xu
 Students: Ruoji Zhou, Brittany E Jewell
 Technicians: Yu-Hsuan Lin, Ying Liu
 Visiting Scholars: Jian Tu, Donghui Wang



Model LFS-associated osteosarcoma by patient specific iPSCs (Cell. 161(2):240-54., 2015).



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

Potency 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 and their natural role during tissue healing; 2) studying the mechanism behinds 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 basic research and translational study and engineered tissue for treating congenital diseases and traumatic injuries with stem cells. This lab has 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 amputation model to accelerate regeneration by duplicate 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, and its mechanism behinds induc-

tion, homeostasis and differentiations 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 expression and protein profile within the model system.

KEY PUBLICATIONS

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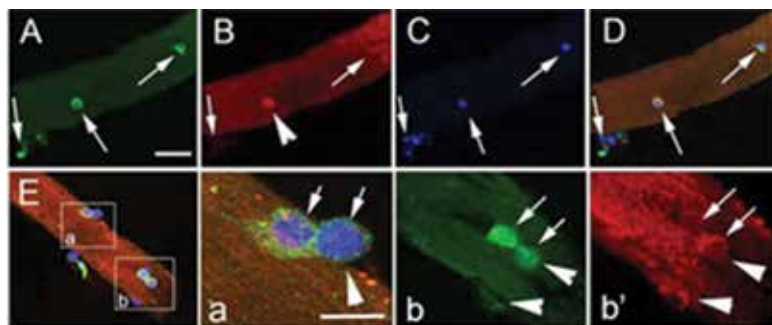
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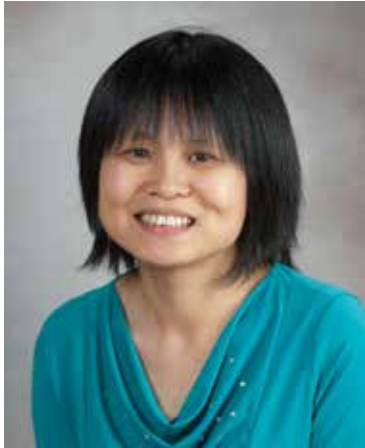
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LAB MEMBERS

Administrator: Stephanie Baca
Lab Research Assistant: Xiaojing Dai
Post-doctoral Fellows: Dr. Kinga Vojnits; Dr. Fan Yang
Resident Fellow: Robert Lugo
Medical Student: Parendi Birdie



Identify muscle stem cells within an *in vitro* culture of muscle fibers.



Ying Liu, Ph.D.
Assistant Professor

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

We have been pursuing basic and translational research in the following two areas: (i) stem cell biology and regenerative medicine, and (ii) pathogenesis of neurodegenerative disease and CNS injury. Our research entails the use of combined genetic and molecular and cellular biological approaches applied to *in vitro* and *in vivo* models. We focus on dissecting the neural developmental pathways and the corresponding pathogenesis in spinal cord injury and stroke. Our long-term goal is to identify therapeutic targets for the treatment of CNS diseases.

By transient overexpression of four transcription factors, OCT4, SOX2, KLF4 and C-MYC, somatic cells such as dermal fibroblasts, keratinocytes, and blood cells, can be reprogrammed to human induced pluripotent stem cells (iPSCs). Most critically, iPSCs provide autologous materials for patients, which theoretically omit the need for immune suppression. We have optimized the more clinically relevant, integration-free hiPSC generation protocol and performed directed differentiation of patient-specific iPSCs into neural stem cells, neuronal and glial progenitors, as well as mature cell types for disease modeling, transplantation studies, neural regeneration and repair, and drug screening and testing. Recently we have adapted the highly efficient genome editing tool CRISPR/Cas9 system in creation of neural lineage reporters and gene corrections of patient iPSCs. These neural lineage specific cells are applied to in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

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

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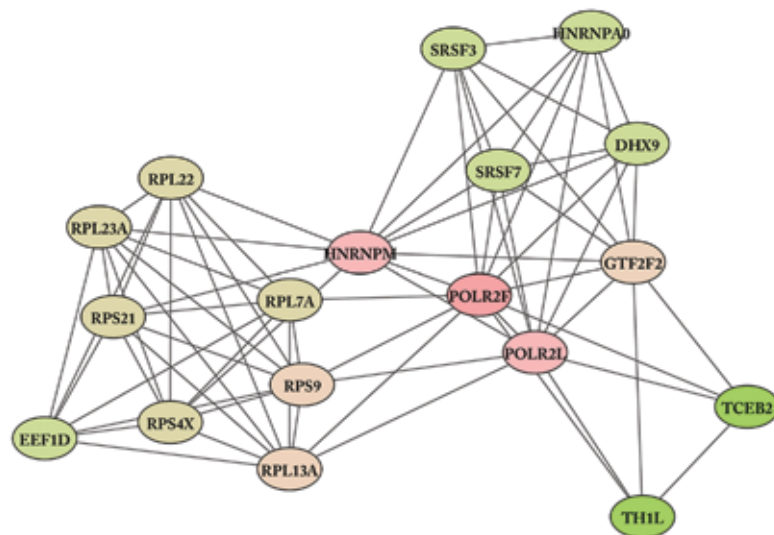
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LAB MEMBERS

Post-doctoral Fellows: Shenglan Li, Dali Li
Research Associate: Haipeng Xue, Min Zhao



An example network extracted from co-regulated genes that are altered by temozolomide and reversed by metformin treatment in glioblastoma cells.



Nami McCarty, Ph.D.

Associate Professor

Jerold B. Katz Distinguished Professorship in Stem Cell Research

Deciphering mechanisms of human cancer cell survival within the bone microenvironment.

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

We have conducted high throughput drug screening using libraries comprised of 3991 compounds of NCI oncology, custom clinical, and prestwick libraries. We discovered that select compounds target the survival pathways of PAX5 silenced cells. Given that PAX5 silenced cells are highly drug resistant, discovery of compounds that target drug resistance populations in cancer cells will have direct translational applications.

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

RESEARCH PROJECTS

- 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 will also 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

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* 30:580-93, 2016.

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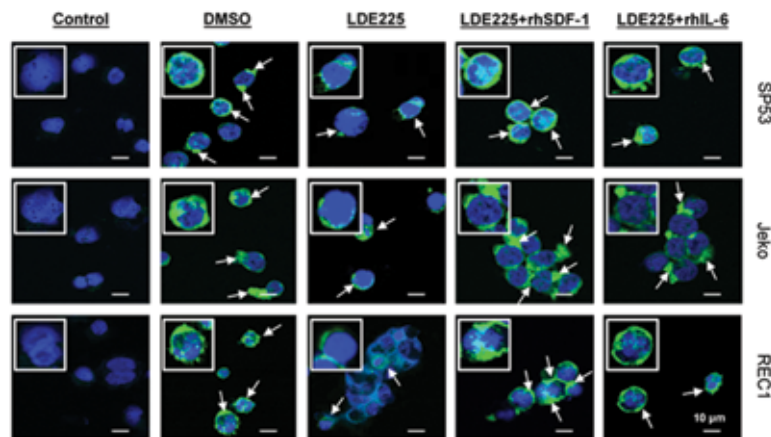
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Zhang, H and McCarty, N. CRISPR-Cas9 technology and its application in haematological disorders. *British Journal of Haematology*, Accepted. Invited review. 2016.

LAB MEMBERS

Senior Research Associate: Judy Chen, M.S.
 Postdoctoral Fellows: Jennifer Han, M.D., Ph.D., Jimmy Lin, Ph.D.
 Research Scientist: Gang Li, Ph.D.



IL-6 and SDF-1 reverse the effect on actin polymerization after LDE225 treatment. Representative confocal microscopic images of MCL cells under different treatments. To visualize F-actin and the nucleus, cells were stained with FITC-phalloidin (green) and drq5 (blue), respectively. F-actin fluorescence (pointed with arrows) in cells treated with LDE225 (30 μM) was reduced compared to DMSO-treated cells. Combination of LDE225 with either SDF-1 (100 ng/ml) or IL-6 (50 ng/ml) reversed F-actin fluorescence to a nearly normal level. Scale bar, 10 μm. Control represents drq-5 stained cells without FITC-phalloidin staining.



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 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-types responsible for joint formation: i.e. joint progenitor and embryonic chondroprogenitor, 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, making human (h)PSCs a promising source of embryonic cells for regenerative medicine.

Permanent cartilage formation - proper signaling and right cell type: We have previously developed and purified from hPSCs, lateral plate mesoderm, paraxial mesoderm and neural crest-like progeny, the three embryonic origins of chondrocytes, and demonstrated that these cells are able to expand and differentiate into corresponding chondroprogenitors. All such chondroprogenitors are capable of giving rise to hyaline-like cartilage *in vitro*. However, most of them are unstable *in vivo* and are mineralized and turned into bone when ectopically transplanted into immunocompromised mice. In order to establish methods to generate cartilage that stays as cartilage permanently after transplantation (i.e., permanent cartilage), we aim to achieve the following two goals; 1) generating the embryonic joint progenitor from hPSCs and 2) demonstrating that they allow the *in vitro*-made cartilage to be stably maintained even after transplantation. We previously discovered a way to selectively generate and to a limited extent, expand joint progenitor-like cells that express ligament precursor markers from the paraxial mesodermal progeny of hPSCs. To purify and further characterize the joint

progenitor-like cells, we have generated in collaboration hiPSC lines that carry fluorescence marker genes in the SOX9 and GDF5 gene loci. Furthermore, we have recently discovered a way to generate cartilage pellets from the hPSC-derived chondroprogenitors, which show very limited bone forming capacity after transplantation (i.e. pseudo-permanent cartilage). We are currently focusing both on the characterization of the joint progenitor-like cells and on the elucidation of critical signaling mechanism for the *in vitro* formation of permanent cartilage.

Large quantity of articular cartilage-forming cells - long-term expansion of PSC-derived human chondroprogenitors: We previously established culture conditions that maintained and expanded the hPSC-derived chondroprogenitors for an extended period of time, without loss of their chondrogenicity. Such stable expansion of chondrogenic activity is currently hard to achieve with adult MSCs. We are also on the way to elucidate the mechanistic basis, which may be applied to improve the expansion culture method for adult MSCs in future.

RESEARCH PROJECTS

- Elucidation of the molecular basis of long-term expansion without loss of chondrogenic activity of the hPSC-derived chondroprogenitors.
- 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

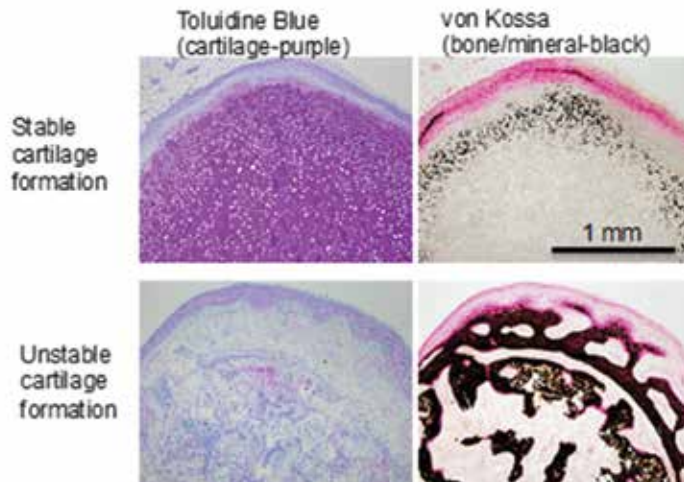
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LAB MEMBERS

Students: Azim Pothiwala
 Research Assistants: Bryan Ang, 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 stromal 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 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.

Joyce Ozuna
Administrative Assistant: Stephanie Baca
(Pediatric Surgery)

RESEARCH PROJECTS

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

KEY PUBLICATIONS

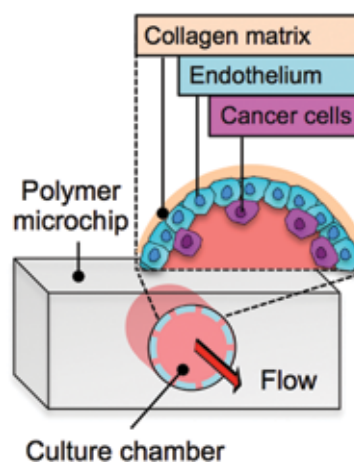
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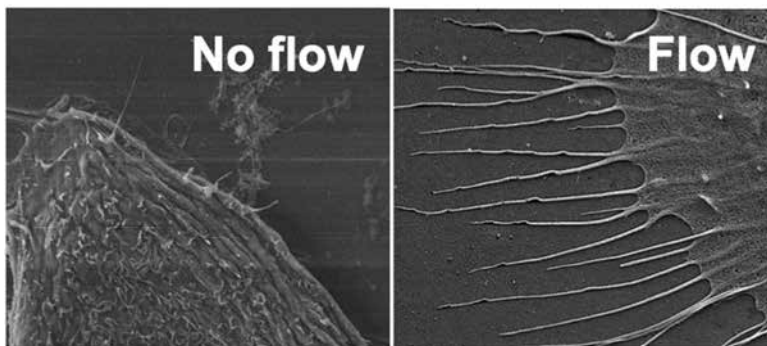
LAB MEMBERS

Research Associate: Miguel Diaz
Postdoctoral Fellow: Hyun Jung Lee, Ph.D.
Research Assistant: Abishek Vaidya, M.S.
Students: Katherine Price, Alexander Alexander,



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.

Ultrastructure of cancer cells



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



Jiaqian Wu, Ph.D.
Assistant Professor

Gene transcription and regulation of stem cell differentiation

This 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 desired neural cell types 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

- Investigate gene expression and regulatory mechanisms during stem cell differentiation
- Characterize molecular signatures and identify therapeutic targets for spinal cord injury and

- neurological diseases
- Pinpoint key transcription factors and regulatory RNAs, and modulate key regulators to steer the direction of stem cell differentiation and improve efficiency
- Determine the molecular switch of hematopoietic precursor cell self-renewal and differentiation
- Network analysis of stem cell differentiation and global network integration of multiple types of omic data

KEY PUBLICATIONS

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.

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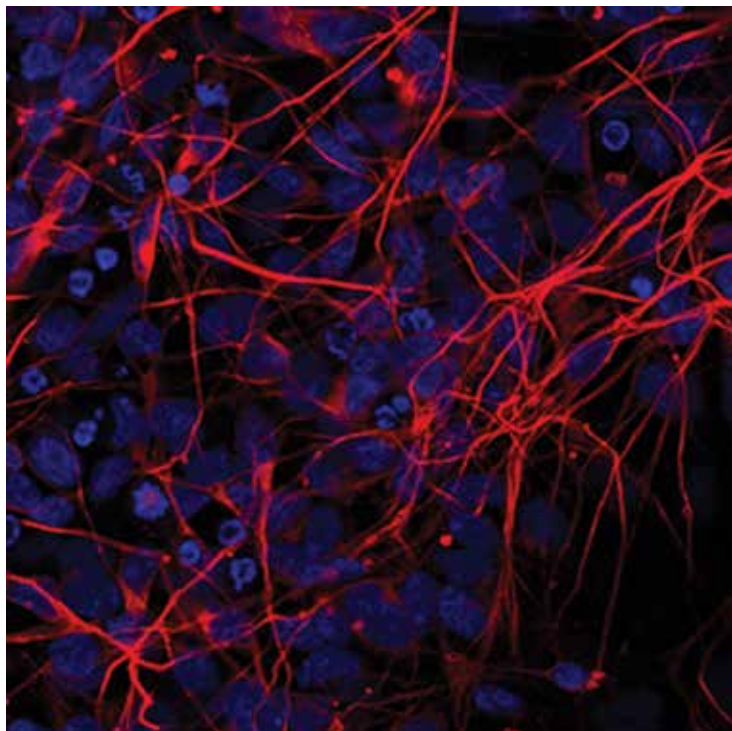
T., Barres, B., Wu, J.Q. (2014). An RNA-Seq transcriptome and splicing database of neurons, glia and vascular cells of the cerebral cortex. *J. Neurosci.*, 34(36): 11929-11947. PMID:25186741

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

LAB MEMBERS

Post-doctoral Fellows: Xiaomin Dong, Ph.D., Yanan You, Ph.D., Raquel Duran, Ph.D., Haichao Wei., Ph.D.
Research Associate: Han Yan, Ph.D.



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



Wa Xian, Ph.D.

Assistant Professor
CPRIT Scholar

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 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
- Evolution of cancers via metaplastic precursors, dysplasia, adenocarcinoma
- Gastrointestinal stem cells from patients with inflammatory bowel disease

KEY PUBLICATIONS

Yamamoto Y, Wang X, Bertrand D, Kern F, Zhang T, Hu YY, Deluba M, Srivastava S, Ming T, Khor CC, Wilson L, Blaszyk H, Rolshud D, Liu JJ, Howitt B, Crum CP, Nagarajan N, Ho KY, McKeon F*,

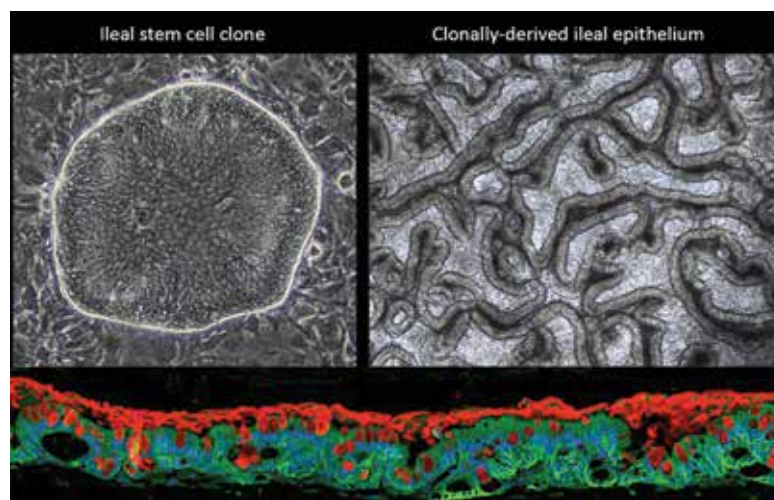
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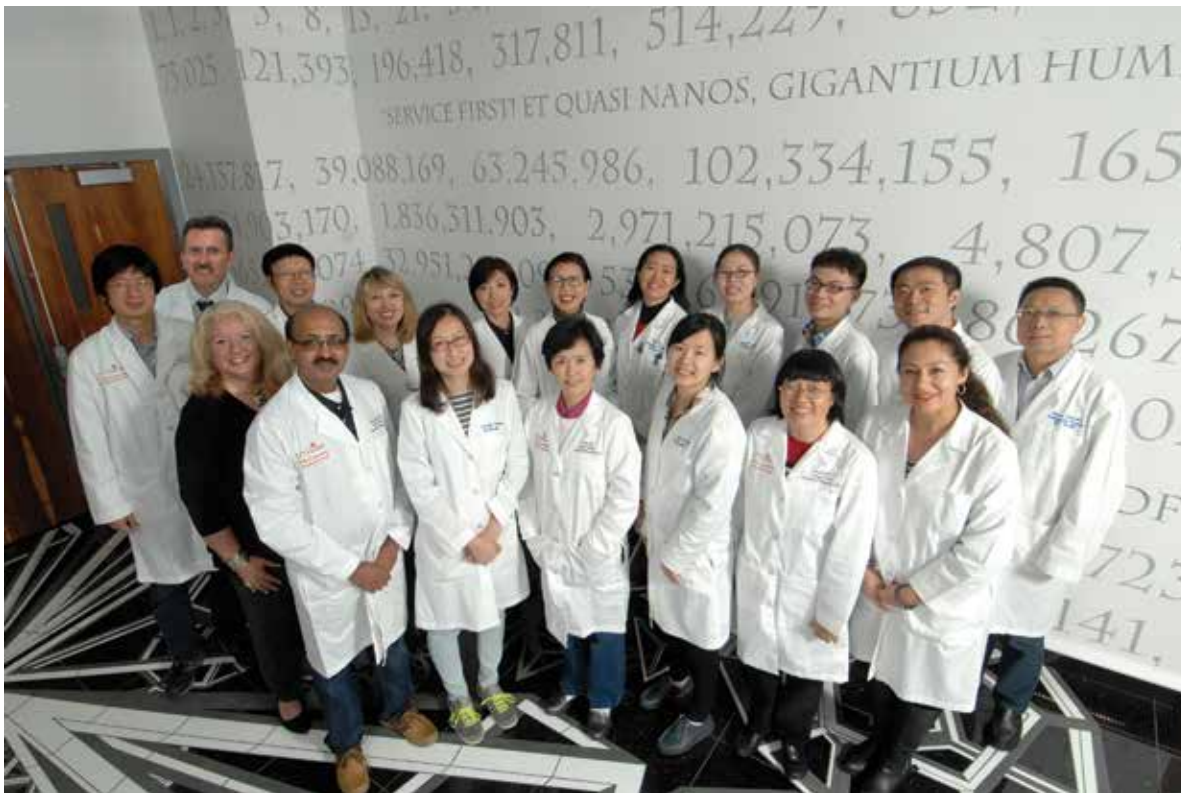
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LAB MEMBERS

Senior Research Scientist: Bailiang Wang, Ph.D.
Postdoctoral Fellow: Guoqiang Chang, Li Guan, Wei Rao



Single stem cell derived ileal clone on irradiated 3T3 feeder cells (left). Ileal epithelia derived from a single ileal stem cell pedigree following exposure to air-liquid interface conditions (right). Bottom, Immunofluorescence stain of histological section (red, Muc2; green, E-Cad; blue, nuclei).



The demographics of the American population are shifting toward an increasing elderly population, placing extraordinary demands on our health care system. Aging results in the progressive attrition of homeostasis and functional reserve of all organ systems. As a consequence, the incidence of numerous debilitating diseases, including neurodegeneration, osteoporosis, sarcopenia, sensorineural defects, cardiovascular disease, diabetes, and cancer increases with age. The major focus of the center is to understand the molecular basis of aging and to develop strategies for preventing or delaying age-associated diseases, which represent a fundamental and pressing challenge for today's medical research community. 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 due to the progressive depletion of stem cells, which leads to an impaired ability to respond to stress, and as a consequence, dramatically increases the risk of morbidity and mortality. This, and the exponentially increased incidence of

numerous degenerative diseases in the elderly, has led to the hypothesis that aging is caused, in part, by the loss of functional stem cells necessary for tissue rejuvenation.

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



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

Muscle-derived stem cell applications

The focus of my research program is in the areas of gene therapy, tissue engineering & 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.

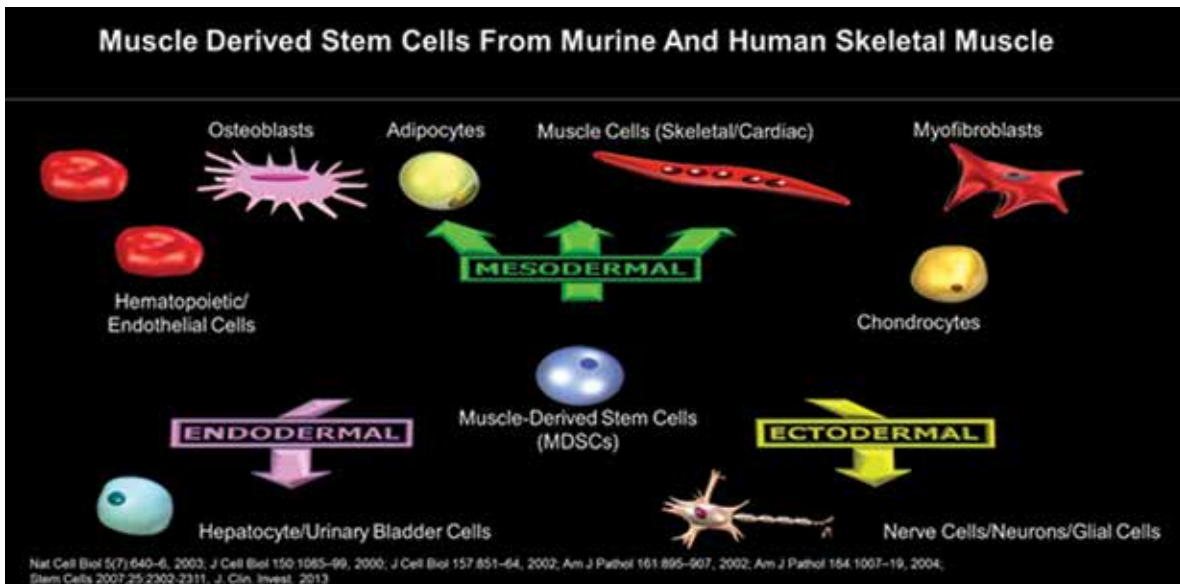
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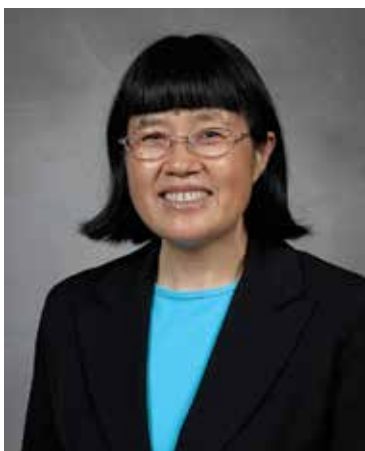
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Lavasani M, Thompson SD, Pollett JB, Usas A, Lu A, Stolz DB, Clark KA, Sun B, Péault B, Huard J. "Human muscle-derived stem/progenitor cells promote functional murine peripheral nerve regeneration." *J Clin Invest*. 2014 April 1. 124(4). 1745-56. PMID: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





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 conduct translational studies to use muscle-derived stem cells for the treatment of bone defects, fracture non-union, and age-related bone and cartilage conditions such as osteoporosis and osteoarthritis. I am also currently investigating the bone biology of a disease model: muscular dystrophy.

Human muscle-derived stem cells for bone regeneration

Large segmental bone defects and non-union fractures caused by traumatic injury or cancer resection represent major issues in clinical orthopaedics. The use of stem cells, growth factors, and a scaffold to regenerate bone tissue to replace traditional autografts and allografts to treat these diseases is a new trend and has achieved a lot of progress. We are exploring new vectors and growth factors to mediate *ex vivo* gene therapy in human muscle-derived stem cells. We also investigate the effect of the age of these human muscle-derived stem cells, and of the host, on bone repair mediated by these human muscle-derived stem cells. 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 treating osteochondral defects or in osteoarthritis repair. In this project, we explore *ex vivo* gene therapy including using viral vectors and biomaterials to deliver growth factors for cartilage repair, particularly for the treatment of osteoarthritis, using both *in vitro* and *in vivo* models.

Exploring the interaction between muscle and bone 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 abnormalities in a dystrophin/utrophin double knock out

(dco) model that closely mimics the clinical manifestation of human DMD patients. We investigate how muscular dystrophy affects the bone and how muscle and bone interact in this mouse model, in order 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 bone tissue repair
- Using human muscle-derived stem cells for cartilage and osteoarthritis repair using *ex vivo* gene therapy and biomaterial scaffold.
- Exploring mechanisms of bone abnormalities in a muscular dystrophy disease model

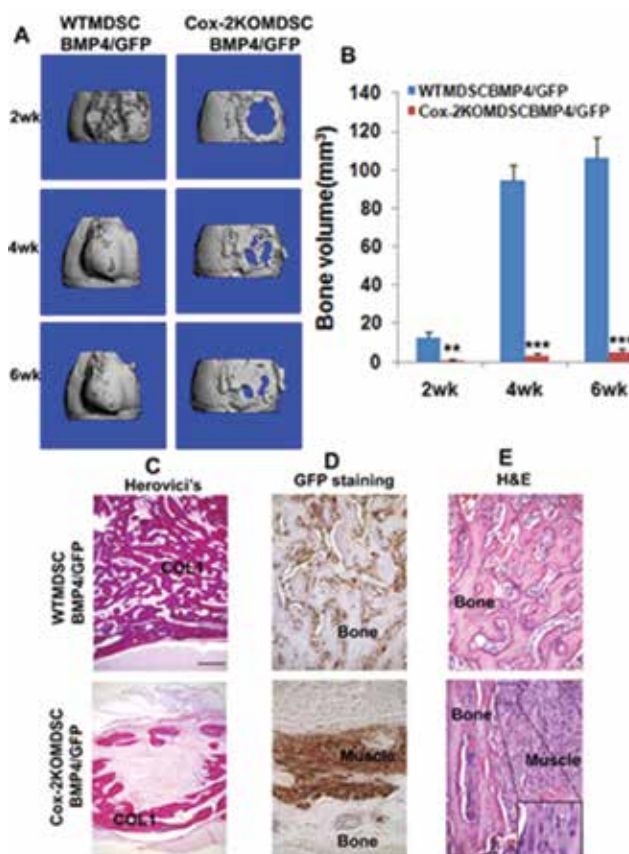
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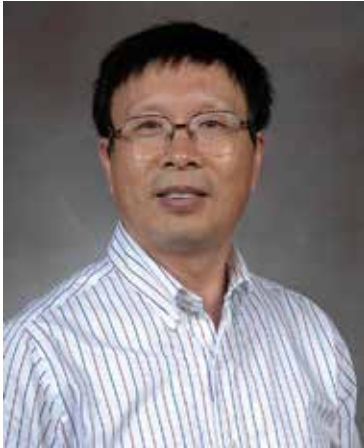
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Gao X, Usas A, Tang Y, Lu A, Tan J, Schnependahl J, Kozemchak AM, Wang B, Cummins JH, Tuan RS, Huard J. A comparison of bone regeneration with human mesenchymal stem cells and muscle-derived stem cells and the critical role of BMP. *Biomaterials.*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

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



Cox-2 -deficient MDSCBMP4/GFP-mediated bone regeneration is impaired *in vivo* using a critical-size bone defect model (Gao X, Huard J et al., Human Molecular Genetics, 2016).



Ping Guo, Ph.D.
Assistant Professor

Gene therapy and tissue engineering for treating sports-related diseases

Our lab focuses on the discovery and development of gene modification and stem cell therapy for treating sports-related diseases. We are currently involved in three major areas of research.

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

Cellular cardiomyoplasty (CCM), which involves the transplantation of exogenous cells into the heart, is a promising approach to repair injured myocardium and improve cardiac function. We have successfully expanded human muscle-derived stem cells (MDSCs), to clinically relevant numbers in culture and more importantly, human MDSCs have already entered the clinical arena for the treatment of bladder dysfunction and myocardial infarction, confirming that MDSCs represent a viable therapeutic cell source for CCM.

2) The effects of continuous pressure on muscle derived stem cells to enhance articular cartilage repair.

Osteoarthritis is a debilitating musculoskeletal disease for which there is currently no cure. While osteoarthritis is an organ-level degenerative disease that affects the entirety of the joint, it is articular cartilage degeneration and loss that most directly impairs joint function. In clini-

cal 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. The project will elucidate whether hMDSCs can tolerate a high-pressure microenvironment, as well as determine whether the effect of high pressure is to enhance hMDSCs to differentiate into chondrocytes or to inhibit chondrogenesis.

3) Stem cells 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 complicated glandular organ that is involved in hormone and digestive enzyme secretion. The pancreas is a vital part of the digestive system and a critical controller of blood sugar levels. Diabetes develops when the beta-cells of the pancreas fail to produce sufficient quantities of insulin. Here we propose that MDSCs may have the potential capacity to differentiate into insulin producing cells, or could recruit other host cells to differentiate into beta-cells in order to treat diabetes.

- Potential development of hMDSCs as therapeutics in the type I diabetic mouse.

KEY PUBLICATIONS

Xiao X, Guo P, Prasad 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.

Guo P, Preuett B, Krishna P, Xiao X, Shiota C, Wiersch J, Gaffar I, Tulachan S, El-Gohary Y, Song Z, Gittes G. Barrier function of the coelomic epithelium in the developing pancreas. *Mechanisms of development*, 2014 (134):67-79.

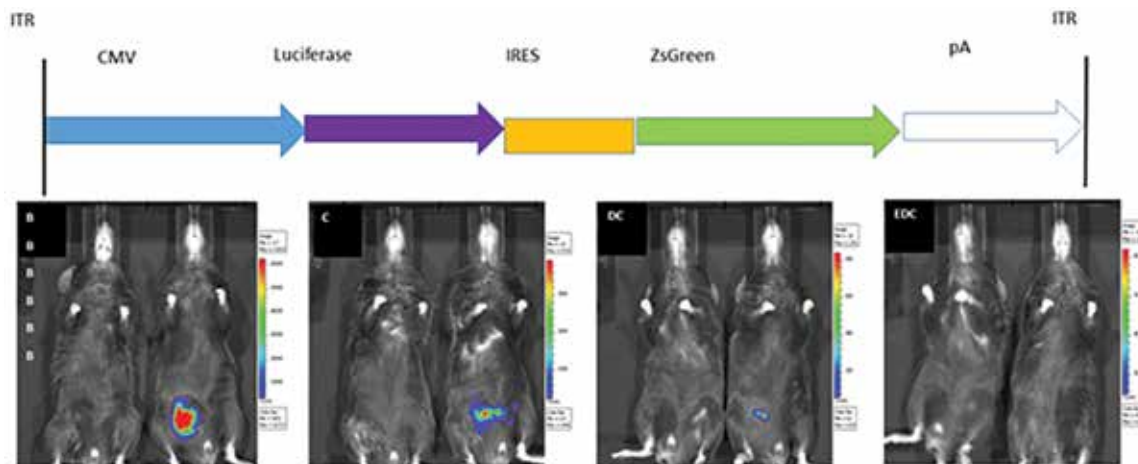
Xiao X, Gaffar I, Guo P, Wiersch J, Fischbach S, Peirish L, Song Z, El-Gohary Y, Prasad K, Shiota C, Gittes GK., M2 macrophages promote beta-cell proliferation by up-regulation of SMAD7. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, 2014, 111(13): E1211-20.

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 microenvironment for chondrogenic differentiation.

LAB MEMBERS

Post-doctoral Fellow: Shanshan "Ellen" Gao
Students: McGovern Medical School at UTHealth student, Matt Fiala
Technicians: Andrea Liebowitz, Elizabeth Morris



Luciferase luminescence tracing of MDSCs to develop a therapeutic for cardiomyopathy.



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 the identification of therapeutic muscle-derived stem cells for the treatment of muscular disease and accelerated aging conditions, including Duchenne muscular dystrophy (DMD) and progeria. Currently, we have two major research areas.

The Role of Muscle Progenitor Cell Exhaustion in 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. Working with different animal models, we are currently investigating whether the progression of muscular dystrophy is a consequence of the decline in functional MPCs. 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 cellular autonomous (e.g. increased DNA damage) and/or non-autonomous mechanisms (aged microenvironment or circulating factors). We currently utilize a mouse model containing a tissue-specific deletion of the ERCC gene, and

are investigating whether increased DNA damage in the skeletal muscle niche is sufficient to induce MDSPC dysfunction. We will also use parabiosis to determine whether aging affects the stem cell niche, impacting stem cell function via a non-autonomous mechanism.

RESEARCH PROJECTS

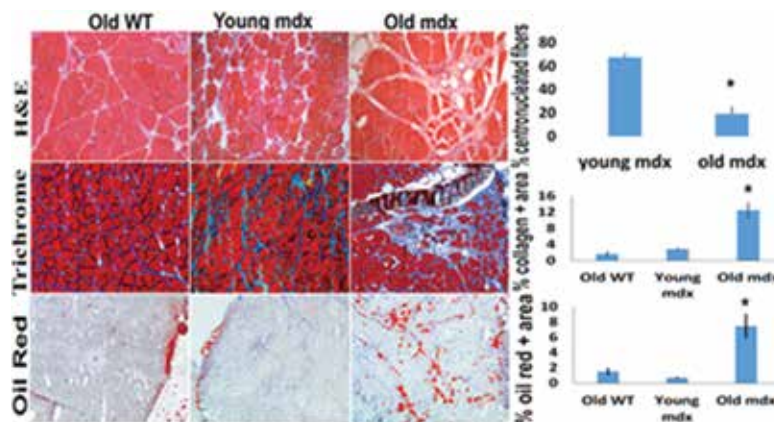
- Investigating muscle stem cell depletion in different animal models including mdx/mTR and dystrophic dog to develop stem cell therapy for DMD
- Isolation and characterization of MDSPCs from different transgenic mice for PO1 aging projects.
- Parabiosis between dystrophic mice and WT mice, for evaluating muscle and heart function.
- To investigate the cell-cell interaction between myogenic cells and non-myogenic cells.
- The role of pregnancy in muscle disease

KEY PUBLICATIONS

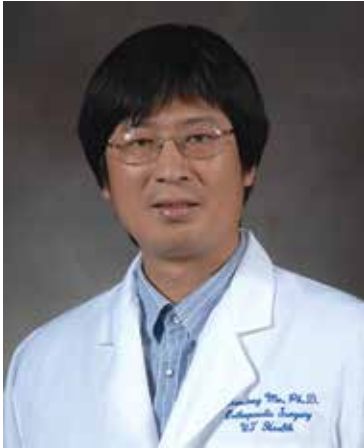
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Gao X, Usas A, Lu A, Kozemchak A, Tang Y, Poddar M, Sun X, Cummins JH, Huard J. Cyclooxygenase-2 deficiency impairs muscle-derived stem cell-mediated bone regeneration via cellular autonomous and non-autonomous mechanisms. *Hum Mol Genet.* 2016 Jun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

Alessandra Pisciotto, Massimo Riccio, Gianluca Carnevale, Aiping Lu, Sara De Biasi, Lara Gibellini, Giovanni B. La Sala, Giacomo Bruzzesi, Adriano Ferrari, Johnny Huard, and Anto De Pol. Stem cells isolated from human dental pulp and amniotic fluid improve skeletal muscle histopathology in mdx/SCID mice. *Stem Cell Res Ther.* 2015 Aug 28; 6:156.



Aged *mdx* mice exhibit a reduction in muscle regeneration, which coincides with the occurrence of fatty infiltration (FI) and fibrosis in the skeletal muscle. Hematoxylin/eosin (H&E) staining shows severe muscle damage in the gastrocnemius muscle of old *mdx* mice (2 years old), compared to age-matched WT and young *mdx* (6 weeks) skeletal muscle, similar to that seen in the muscles of DMD patients.



Xiaodong Mu, Ph.D.
Assistant Professor

Rescuing cancer cachexia-associated muscle atrophy by regulating muscle stem cells

Our “Stem Cell in Aging and Cancer” research group is a part of Dr. Johnny Huard’s research center in the Department of Orthopaedic Surgery, and our studies have involved stem cell biology, wound regeneration, fibrosis prevention, muscular dystrophy, premature aging, and cancer biology (osteosarcoma). Currently, we are particularly 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, my research is focused on the following areas:

1) Investigating the mechanism of muscle atrophy in osteosarcoma-induced cancer cachexia, and the potential effect of muscle stem cells in reducing muscle atrophy.

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

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

Accelerated exhaustion, senescence, and loss of regeneration potential of stem cells have been observed in diseased skeletal muscle, such as those affected by progeria and muscular dystrophic disease. Some key stem cell regulators – the Notch, Wnt, RhoA, and mTOR signaling pathways – have all been shown to be important regulators of tissue aging and cell senescence.

3) Improvement of tissue wound healing by

reducing fibrosis or application of stem cells.

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

RESEARCH PROJECTS

- Investigating the mechanism of muscle atrophy in osteosarcoma-induced cancer cachexia, and the potential effects of muscle stem cells in reducing muscle atrophy.
- Understanding the cellular and molecular regulatory mechanisms underlying muscle stem cell defects in diseased and aged muscles.
- Improvement of soft tissue wound healing by reducing fibrosis or by the application of stem cells.
- Investigating the role of Notch and Wnt signaling in regulating stem cell senescence and cancer stem cells in an animal model of accelerated aging, in contrast to an animal model of normal aging.

KEY PUBLICATIONS

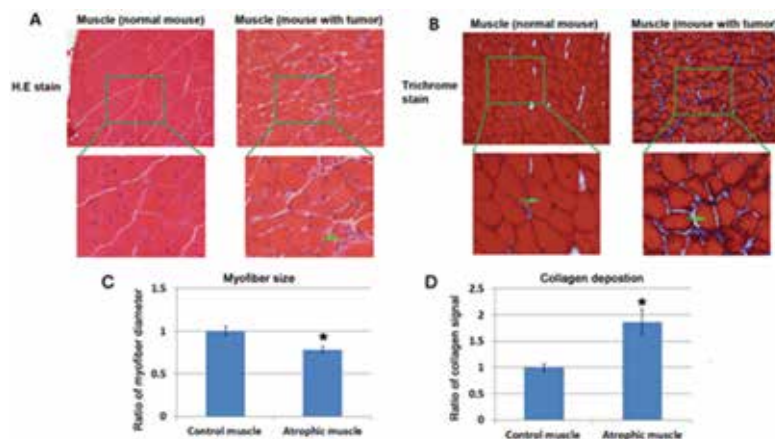
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Mu X, Patel S, Mektepbayeva D, Mahjoub A, Huard J, Weiss K. Retinal Targets ALDH Positive Cancer Stem Cell and Alters the Phenotype of Highly Metastatic Osteosarcoma Cell. *Sarcoma*. 2015.

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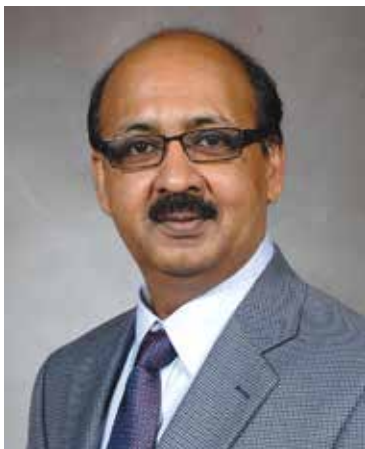
Mu X, Usas A, Tang Y, Lu A, Wang B, Weiss K, and Huard J. RhoA mediates defective stem cell function and heterotopic ossification in dystrophic muscle of mice. *FASEB J*. 2013, May.

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.



Skeletal muscle from tumor-carrying mice developed muscle atrophy.

A. H&E staining of GM muscles, revealing relative myofiber sizes and numbers of mononuclear cells (i.e., macrophages or un-differentiated muscle stem cells) in mice with and without tumors; N = 4 mice in each group. B. Trichrome staining of GM muscles, demonstrating differential myofiber size and collagen deposition; N = 4 mice in each group. C. Myofiber size in GM muscles. D. Collagen deposition in GM muscles. “*” in the bar chart indicates P < 0.05.



Krishna Sinha, Ph.D.
Assistant Professor

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

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

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

Differentiation of osteoblasts commences from the mesenchyme, with stage-specific gene activation that supports the maturation and function of osteoblasts. Using a proteomic approach, I identified a chromatin regulator NO66 as an 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 it controls the activation of a repertoire of genes in osteoblasts and osteocytes. NO66 inhibits Osterix activity through interactions with Osx and through demethylation of histones at specific euchromatin regions in osteoblasts.

We have reported the crystal structure of NO66 and its interface that is required for interactions with Osx; this provides useful structural insight for the design and development of 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 a molecular switch from gene repression to gene activation. We have shown that during the early embryonic stages of mouse development, the promoters of *Osx*, *Col1a1* and *Bsp* are hypermethylated in mesenchymal cells and are also bound to methylation-induced repressor complexes including NO66, which prevents gene activation in those cells as well as in *Osx*-null cells. In differentiating osteoblasts, these genes become hypomethylated, and are bound to Runx2, Osx and other active histone modifiers, during the activation of those genes. We then demonstrated, by conditionally inactivating NO66 in mesenchymal cells, that loss of function of NO66 in mice leads to an increased bone mass phenotype.

The histone demethylase NO66 has an oncogenic role in PCa and bone metastasis. Bone is the most susceptible organ for metastases by nearly all types of cancer, particularly prostate and breast cancers, and skeletal metastases lead to severe defects in bone architectures during aging. NO66 is upregulated in lung cancer. We have found that NO66 levels are elevated in prostate cancer patient samples and xenografted samples. Our data indicate that NO66 overexpression promotes the proliferation and invasion of prostate cancer cells. In xenograft studies, femurs of male SCID mice implanted with NO66-overexpressing PC3 cells have significant bone loss compared with mice

with control PC3 cells, suggesting that NO66 plays an oncogenic role in PCa progression and bone metastasis.

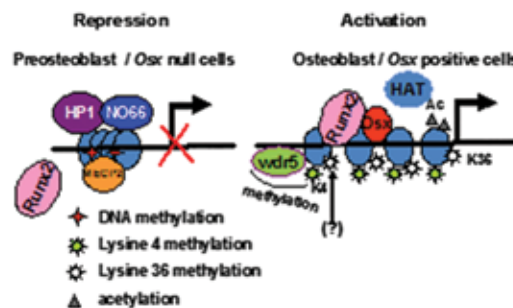
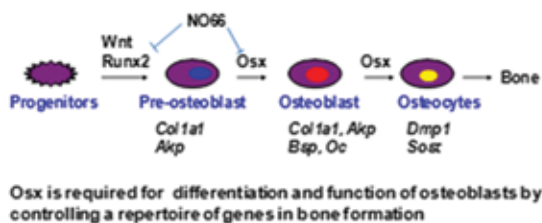
RESEARCH PROJECTS

- Use of next-gen sequencing approaches to identify epigenetic signature markers of histone methylation and DNA methylation in key osteoblast genes, and to better study the multiple pathways/mechanisms involved in normal and malignant bone formation.
- To study post-translational modification of Osterix by lysine methylation in differentiation of skeletal muscle stem cells into osteoblasts and bone formation.
- To study mechanisms of self-renewal and depletion of skeletal muscle-derived stem cells, and in the repair of musculoskeletal tissues using these multipotent stem cells.
- To investigate the oncogenic function of NO66 in PCa progression and bone metastasis, and develop stem cell therapy to inhibit the development of PCa in bone.

KEY PUBLICATIONS

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

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



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



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

TTI-IMM investigators have brought in significant funding from the pharmaceutical and the biotechnology industry, including Johnson & Johnson, Merck, and Cidara, and the National Institutes of Health, the Cancer Prevention and Research Institute of Texas, and the Department of Energy, and have made significant scientific discoveries in the areas of cancer biology, fungal natural products and biologics drug development.

Current research activities at TTI-IMM include: 1) signaling mechanisms of receptors and enzymes that have critical roles in human diseases;

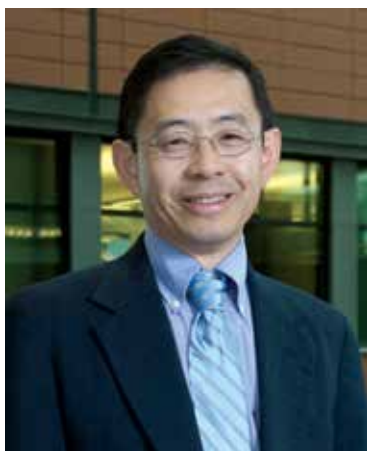
2) discovery of biologics, natural products, and synthetic small molecules that modulate the activity of these targets as potential lead molecules for drug discovery; and 3) characterization of antibodies from animals and humans in response to experimental vaccines. On the translational side, research from TTI has resulted in the establishment of two UT-spin off biotech companies.

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.

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 vaccine. 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).

Pneumocandin biosynthesis and biocombinatorial chemistry approach for natural products drug discovery. The antifungal therapy caspofungin is a semi-synthetic derivative of pneumocandin B0, 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 engineer-

ing 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 the Cancer Prevention and Research Institute of Texas, 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. Currently, we have multiple collaborative antibody drug discovery projects targeting various cancers.

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

Shu Zhang, Seema Mukherjee, Xuejun Fan, Ahmad Salameh, Zhao Huang, Kalpana Mujoo, Georgina Salazar, Ningyan Zhang, and Zhiqiang

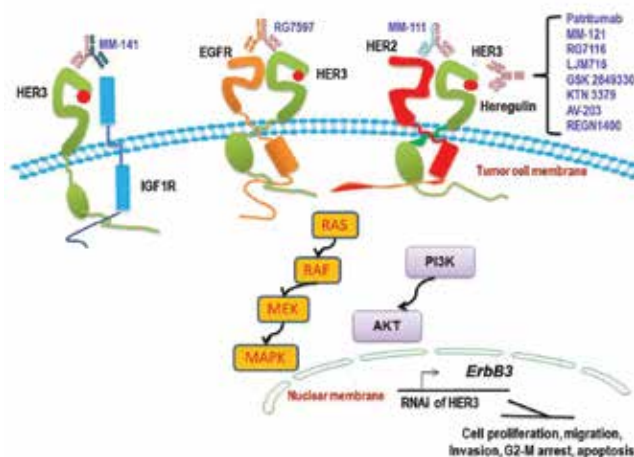
An. 2016. Novel Association of DJ-1 with HER3 potentiates HER3 Activation and Signaling in Cancer. *Oncotarget* DOI: 10.18632/oncotarget.11613

Ningyan Zhang, Hui Deng, Xuejun Fan, Anneliese Gonzalez, Songlin Zhang, Randall J Brezski, Byung-Kwon Choi, Michael Ryczyn, 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).

Xuejun Fan, Randall J. Brezski, Hui Deng, Pooja Dhupkar, Yun Shi, Anneliese Gonzalez, Songlin Zhang, Michael Ryczyn, William R. Strohl, Dr. Robert E. Jordan, Ningyan Zhang, 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

LAB MEMBERS

Post-doctoral Fellows: Ahmad S. Salameh, Leike (Simon) Li, Xun (Mark) Gui, Hao Ching Hsiao, Robbie D. Schultz, Haotai (Martin) Chen, Qun Yue (jointly with Dr. Bills), and Yan Li (jointly with Dr. Bills).
Students: Jingnan (Anna) An and Yuanzhi (Nate) Chen



Key HER3 hetero-dimerization partners, signaling pathways, and anti-HER3 monoclonal antibodies in clinical development for the treatment of various solid cancers. (Zhang, et al., 2016. *Acta Biochim B*



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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Li, Y., Q. Yue, N. M. Krausert, Z. An, J.B. Gloer & G.F. Bills. 2016. Emestrins: anti-Cryptococcus epipolythiodioxopiperazines from *Podospora australis*. 2016. *Journal of Natural Products* 79:2357-2363.

Chen, L., Y. Li, Q. Yue, A. Lokszejn, K. Yokoyama, E.A. Felix, X. Liu, N. Zhang, Z. An & G.F. Bills. 2016. Engineering of new pneumocandin side-chain analogues from *Glaea lozoyensis* by mutasynthesis and evaluation of their antifungal

activity. *ACS Chemical Biology* DOI: 10.1021/acscchembio.6b00604.

Pascual, J. M. García-López, G.F. Bills & O. Geniloud. 2016. *Longimicrobium terrae* gen. nov., sp. nov., a novel oligotrophic bacterium of the underrepresented phylum Gemmatimonadetes isolated through a system of miniaturized diffusion chambers. *International Journal of Systematic and Evolutionary Microbiology* 66:1976-1985.

LAB MEMBERS

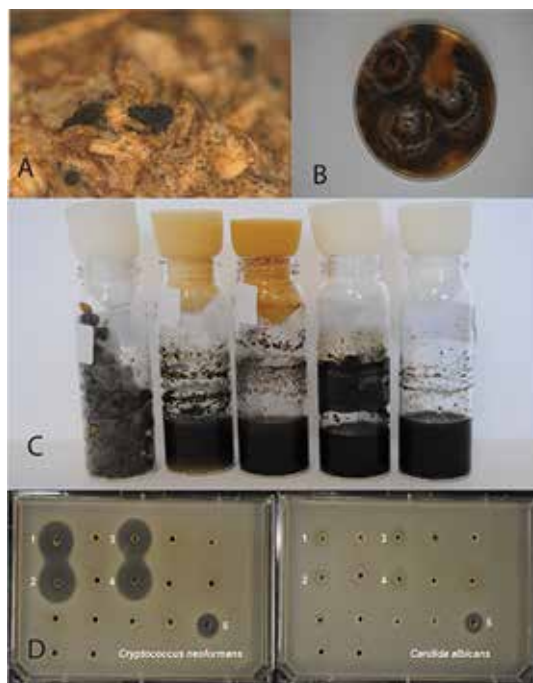
Post-doctoral Fellows: Dr. Qun Yue, Dr. Yan Li, Dr. Nan Lan (visiting from the Institute of Microbiology, Chinese Academy of Sciences), Dr. Eric Kuhnert (visiting from the Helmholtz Center for Infection Research).

Visiting Professor: Prof. Lijian Xu (from Heilongjiang University).

Microorganisms have produced many of our most important drugs. Our research involves testing microbial natural products for therapeutic applications, making natural products through fermentation to support medicinal chemistry synthesis and elucidating biosynthetic pathways of bioactive natural products.

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

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



The steps leading to the discovery that emestrin-type epipolythiodioxopiperazines specifically inhibit the growth of the meningitis pathogen, *Cryptococcus neoformans*. A. The fungus producing emestrins is *Podospora australis* growing in its natural habitat on rabbit pellets, Kinney County, Texas. B. *Podospora australis* growing in agar culture. C. *Podospora australis* fermented in five different metabolite-inducing cultures. D. Testing of fermentation extracts in agar inhibition assays. Note the strong inhibition zones of extracts (1-4) against *Cryptococcus neoformans*, but not the human pathogen *Candida albicans*.



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

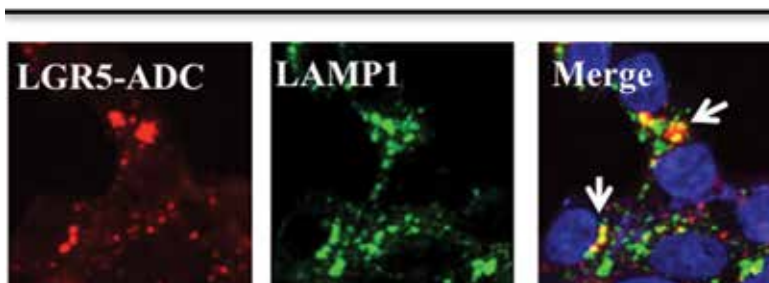
- Development of anti-LGR5 antibody-drug conjugates
- Investigation of LGR5 signaling and its role in cancer and drug resistance

KEY PUBLICATIONS

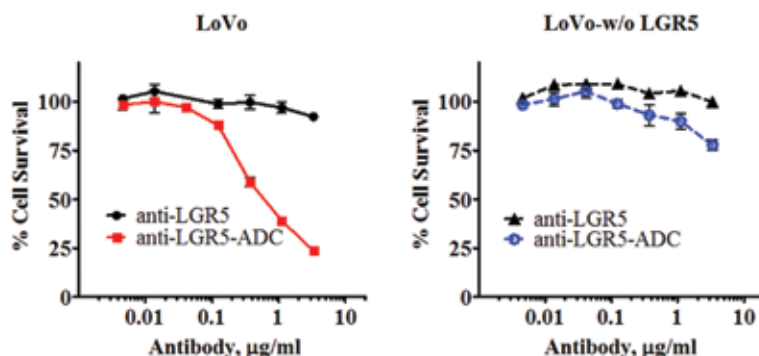
Gong, X., Azhdarinia, A., Ghosh, S.C., Xiong, W., An, Z., Liu, Q., and Carmon, K.S.* (2016) LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence, *Mol. Cancer Ther.* 15(7):1580-90.

Carmon K.S., Gong X., Yi J., Thomas A., Liu Q. (2014) RSP0-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proc Natl Acad Sci U S A.* 111(13): E1221-9.

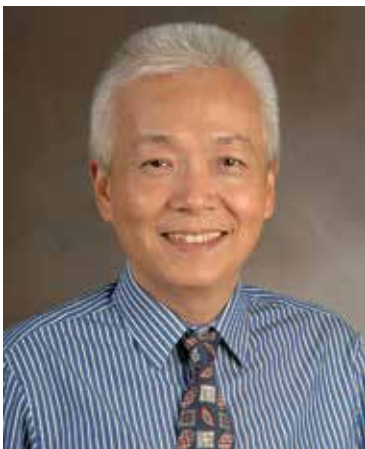
LoVo Colon Cancer Cells



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.



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 and pharmacological inhibitors.

KEY PUBLICATIONS

Hu, Y., Robichaux, W. G., Kim, E. R., Mei, F. C., Wang, H., Tong, Q., Xu, M., Chen, J., and Cheng, X. Role of exchange protein directly activated by cAMP isoform 1 in energy homeostasis: regulation of leptin expression and secretion in white adipose tissue. *Molecular Cellular Biology*. 36:2440-1250, 2016.

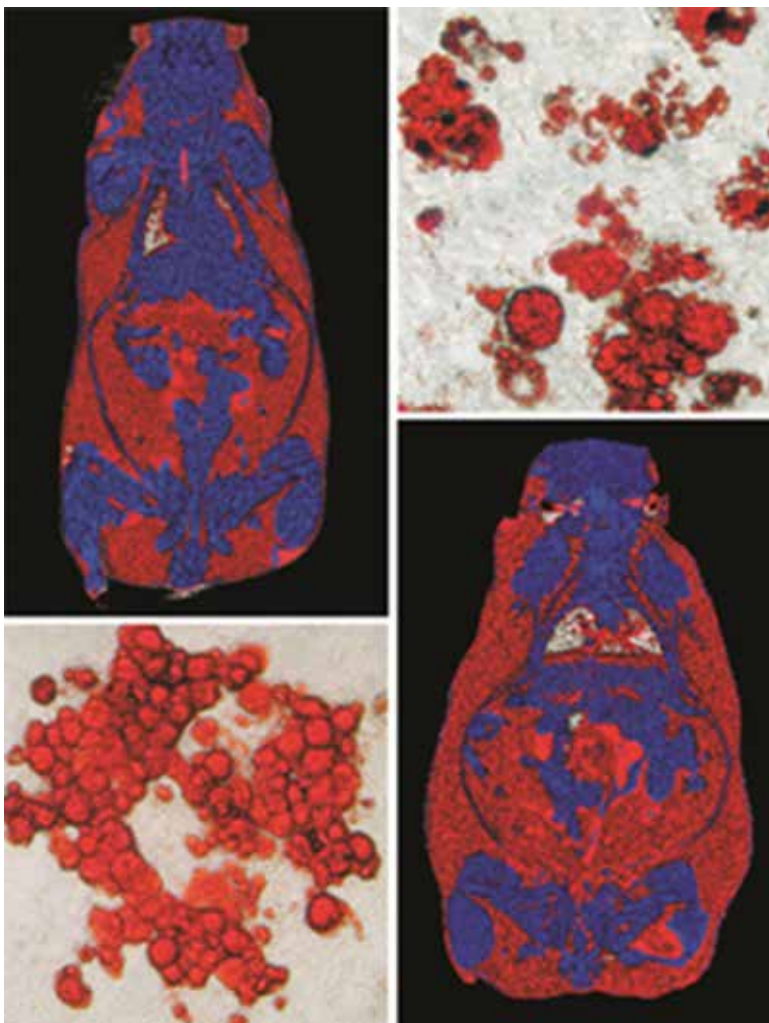
Almahariq, M., Chao, C., Mei, F. C., Hellmich, M. R., Patrikeev, I., Motamedi, M., and Cheng, X. Pharmacological Inhibition and Genetic Knockdown of EPAC1 Reduce Pancreatic Cancer

Metastasis *in vivo*. *Molecular Pharmacology*. 87:142-149, 2015.

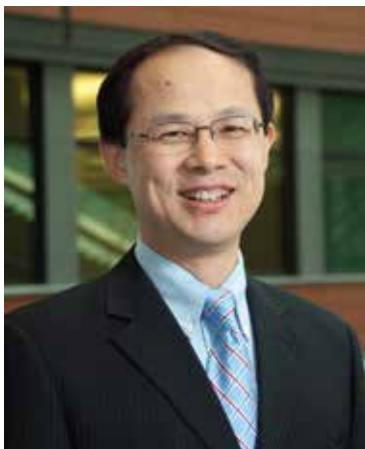
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LAB MEMBERS

Research Assistant Professor: Fang Mei
 Research Scientist: Yingmin Zhu, Wenli Yang
 Post-doctoral Fellow: William Robichaux
 Technician: Pei Luo



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



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, Our 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

- Targeting GRK3 to inhibit aggressive neuroendocrine prostate cancers.
- New regulators for EMT and their mechanisms in cancer progression.
- Mechanisms of chronic biobehavior stress in promoting cancer progression.
- Development of precision medicine based on genetic profiles and drug sensitivities of patient samples and patient-derived-xenografts (PDXs) in mice.

KEY PUBLICATIONS

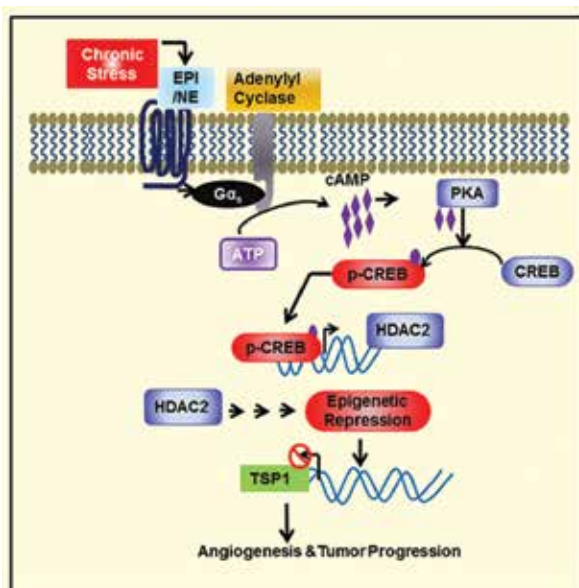
Hulsurkar M, Li Z, Li X, Zhang Y, Zheng D, Li W*. Beta-adrenergic signaling promotes tumor angiogenesis and prostate cancer progression through HDAC2-mediated suppression of thrombospondin-1. *Oncogene* (in press). *corresponding author

Sang M, Hulsurkar M, Zhang X, Song H, Zheng D, Zhang Y, Li M, Zhang S, Xu J, Ittmann M, Li W*. GRK3 is a direct target of CREB activation and regulates neuroendocrine differentiation of prostate cancer cells. *Oncotarget* 2016, May 14. *corresponding author

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

LAB MEMBERS

Post-doctoral Fellows: Yan Zhang, Dayong Zheng
Ph.D. student: Mohit Hulsurkar



Summary model for our findings that chronic behavioral stress and stress hormones induce tumor growth in mouse xenograft, through HDAC2-mediated epigenetic repression of angiogenesis inhibitor thrombospondin (TSP1).



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. In particular, we uncovered that RSPO3-LGR4 has a major role in the aggressiveness of lung adenocarcinomas. Most recently, we showed that drug conjugates of ant-LGR5 antibodies showed excellent anti-tumor efficacy in preclinical models of colon cancer. 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, 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).

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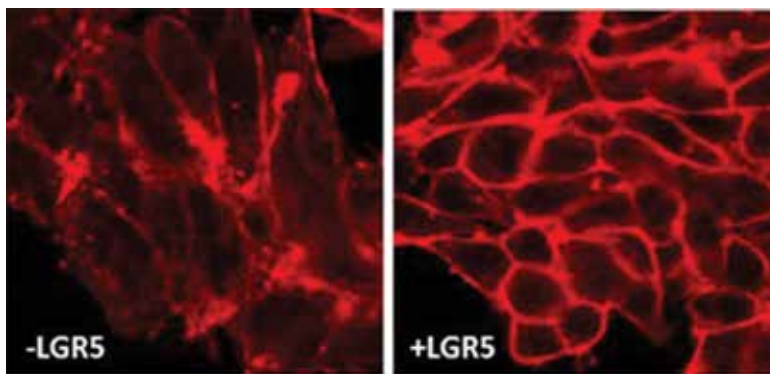
Carmon, K. S., Gong, X., Yi, J., Thomas, A., Liu, Q. RSPO-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proc Natl Acad Sci U S A*. 111: E1221-E1229 (2014).

Gong, X., Yi, J., Carmon, K.S., Crumbley, C.A., Xiong, W., Thomas, A., Fan., X., Guo, S., An, A., Chang, J.T., and Liu, Q. Aberrant RSPO3-LGR4 signaling in Keap1-deficient lung adenocarcinomas promotes. *Oncogene*. 34:4692-701 (2015).

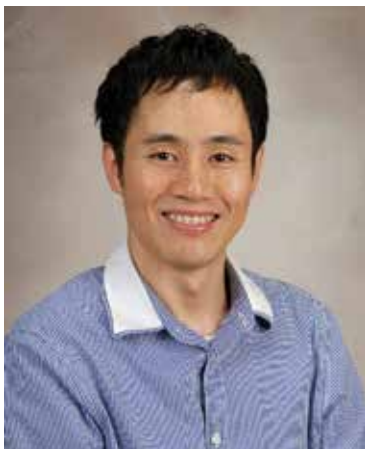
Gong, X., Azhdarinia, A., Ghosh, S., Xiong, W., An, Z., Liu, Q., Carmon, K.S. (2016). LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol Cancer Ther*. 15:1580-1590 (2016).

LAB MEMBERS

Assistant Professor: Kendra Carmon
Research Scientist: Xing Gong
Research Associate: Wangsheng Alice Yu



LGR5 controls cytoskeletal structure. Colon cancer cell LoVo cells without (left panel) and with (right panel) expression of LGR5 stained for F-actin.



Kyoji Tsuchikama, Ph.D.

Assistant Professor

Development of chemical agents, tools, and strategies for combating drug-resistant bacteria

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 a serious clinical problem for human health. According to the report from the Centers for Disease Control and Prevention (CDC, April 2013), antibiotic resistance in the United States costs us approximately 20 billion dollars per year in excess health care costs and more than 8 million additional days spent in hospital. Therefore, alternative antimicrobial strategies based on a novel mechanism of action is of critical importance to overcome this clinical challenge.

My laboratory is working on two research projects by taking advantage of the power of organic chemistry and chemical biology. Firstly, we design, synthesize, and evaluate non-traditional antimicrobial agents that could potentially circumvent the drug resistance problem. Specifically, we are committed to develop novel antibacterial agents relying on a unique pinpoint drug delivery system. Based on this concept, we have synthesized a peptide-based molecular conjugate named "I₂-BODIPY-AIP", which gratifyingly exerts >16-fold greater bactericidal activity against MRSA than the parent antibacterial agent alone. We are currently optimizing the chemical structure to achieve greater potency, stability under physiological conditions, and target specificity. Secondly, we are focusing on the development of novel chemical tools for precise detection of MRSA. By taking advantage of the findings obtained from the above-mentioned success, we have developed a fluorescent probe that can selectively label MRSA cells. We are currently assessing the potential of this novel probe in *in-vivo* imaging. Throughout these projects, we hope to provide useful chemical tools to better understand biochemical and pathophysiological

mechanisms critically involved in MRSA infections, and novel drug design for anti-MRSA therapies.

RESEARCH PROJECTS

- Selective killing of drug-resistant bacteria using non-traditional chemical agents
- Development of novel chemical detection tools toward precise imaging and diagnosis

KEY PUBLICATIONS

Tsuchikama, K.*, An, Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. *Protein Cell*, in press. doi:10.1007/s13238-016-0323-0

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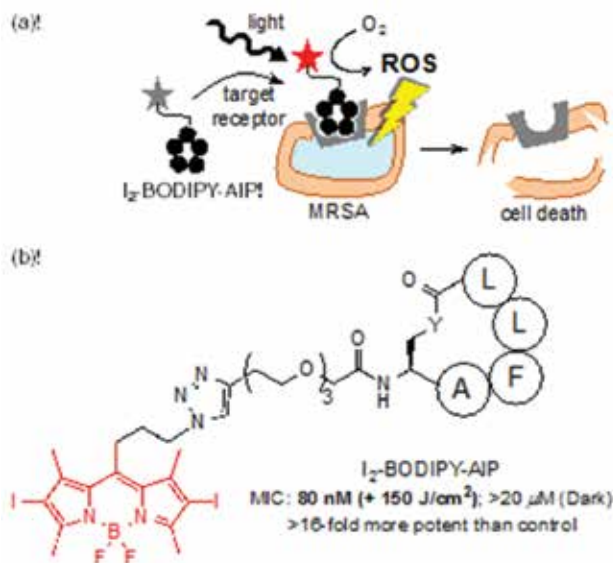
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Cai, X.; Tsuchikama, K.; Janda, K. D. Modulating Cocaine Vaccine Potency Through Hapten Fluorination. *J. Am. Chem. Soc.* 2013, 135, 2971-2974.

LAB MEMBERS

Post-doctoral Fellows: Yasuhiro Shimamoto, Ph.D., Yasuaki Anami, Ph.D., Mohammad Younus Wani, Ph.D.

Dr. Tsuchikama is currently seeking postdoctoral fellows with experience in organic chemistry, medicinal chemistry, and/or pharmacology.



(a) a proposed mechanism of photoinactivation using I₂-BODIPY-AIP, (b) MIC in a cell-based photoinactivation assay. ROS, reactive oxygen species; MIC, minimum inhibitory concentration.



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 these therapeutic antibodies are widely reported and present a significant challenge in the clinic.

Our recent work showed a sensitivity of antibody IgG hinge to proteolytic cleavages and the therapeutic antibody trastuzumab with a single hinge cleavage had a reduced antitumor activity in *in vitro* and *in vivo* using a mouse tumor model. More significantly, we revealed a prevalent existence of hinge impaired antibodies with a compromised anticancer immunity in tumor tissues from cancer patients. These findings lead us to hypothesize that antibodies recognizing cancer neoepitopes may suffer from a proteolytic hinge cleavage in the proteinase-rich tumor microenvironment, and the proteolytic impairment of those antibodies by matrix metalloproteinases (MMPs) contributes to the cancer immune evasion. 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, fluorescence activated cell sorting (FACS), and single cell cloning of

antibodies. 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.

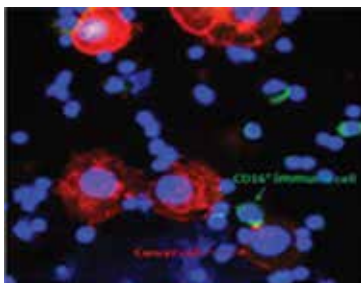
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Zhang S, Mukherjee S., Fan X, Salameh A, Huang Z, Mujoo K, Salazar G, Zhang* NY, and An* Z. (2016) Novel Association of DJ-1 with HER3 potentiates HER3 Activation and Signaling in Cancer. *Oncotarget*, doi: 10.18632/11613.

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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.



Immune cells/cancer cells interaction in a co-culture condition

LAB MEMBERS

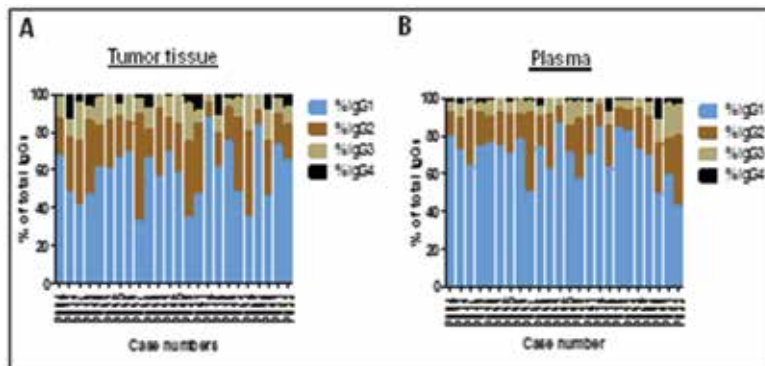
Hui Deng, M.S.

Xuejun Fan, M.D., Ph.D.

Georgina Salazar, 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



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 subclasses. (Clinical Cancer Research, 2015; doi: 10.1158/1078-0432.CCR-15-1057)

IMM SERVICE CENTERS

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

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

ANTIBODY ENGINEERING AND EXPRESSION SERVICE CENTER

Antibody therapeutics represents a major breakthrough in combating human diseases, including cancer. Even though the pharmaceutical and biotechnology industries are in the center stage of drug discovery and development, academic researchers are increasingly engaged in discovering new antibody drug candidates. However, advancement of some the promising antibodies in the early stage of discovery from academic research laboratories is often hindered by the lack of access to the expertise and infrastructure required for antibody engineering and other related key technologies. Our antibody engineering and expression service center will fill the gap of the much needed expertise for early discovery of monoclonal antibodies and lead optimization to the research and drug discovery communities. The objective of the service center is to provide technical support and services to molecular cloning, 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 the entire UTHealth, 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 group 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 seven engineering and basic science faculty members whose research focuses on different aspects of molecular imaging including new instrumentation, design and chemistry of targeted probes, innovative algorithms, and pioneering translation of new imaging technologies into clinical trials. The newly formed Molecular Imaging “collaboration” center utilizes this existing expertise to interact with clinicians, clinician-scientists, as well as academic and industry researchers across the nation on translational projects in cancer, drug discovery, autoimmune disorders, gastrointestinal disorders, nanotechnology, chronic wound care, peripheral vascular disease, and others. Facilities include a Siemens hybrid PET/CT small animal scanner with custom fluorescence tomography capabilities and an array of custom bioluminescence and fluorescence instrumentation that is paired with unique imaging agents/gene reporter systems. Generalized protocols are available to investigators to maximize benefit from the latest developments in molecular imaging.

FLOW CYTOMETRY SERVICE CENTER

Flow cytometry is a technique used to analyze the characteristics of particles in a fluid. Typically a variety of cellular components are fluorescently labelled and then passed in front of lasers of varying wavelengths. The fluorescence emission can be then be measured to determine properties of individual cells such as relative size, complexity and cell type.

Thousands of cells can be analyzed per second as they pass through the liquid in front of the lasers. 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. These instruments are available on a fee per services charge to all research investigators from UTHealth or external organizations.

TRANSGENIC AND STEM CELL SERVICE CENTER

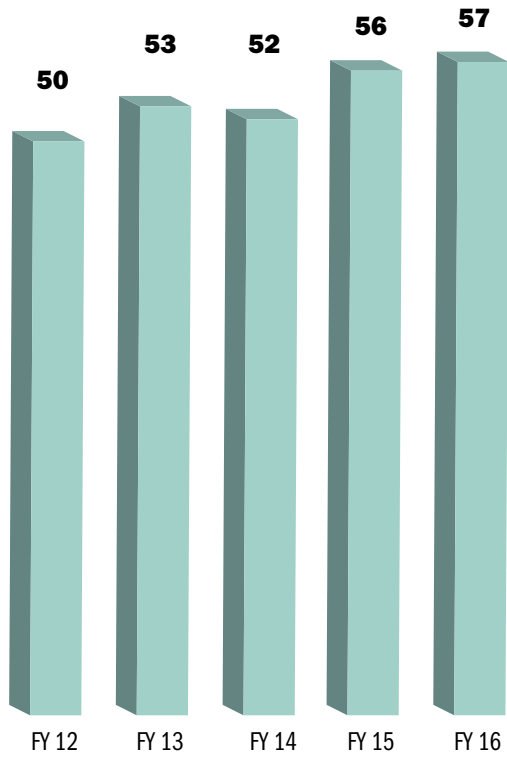
Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cells service center which was established in 1998. It has generated over 750 new transgenic and knock-out mouse animal models for all research investigators from UTHealth and external organizations on a fee-for-service basis.

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.

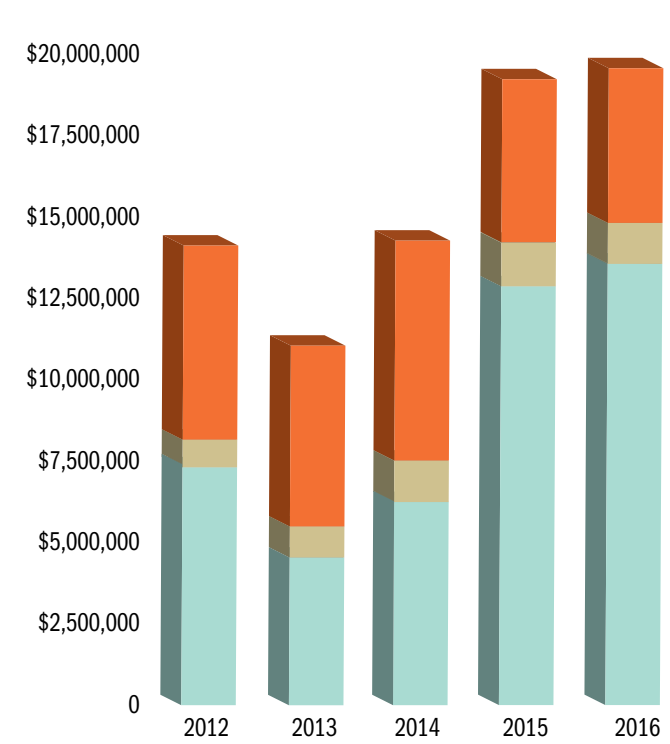
NANOCHEMISTRY SERVICE CENTER

The Nanochemistry Service Center was established in 2012 and is located on the third and fourth floors of the Faye S. Sarofim Research Building. It specializes in the discovery, synthesis, and purification of DNA aptamers, X-Aptamers, and RNA for specific targeting of proteins for the delivery of chemotherapeutic agents or the down-regulation of the protein. While most of our projects target cancers, such as ovarian, breast, and pancreatic cancers, we also develop aptamers for the modulation of other diseases. The center also offers nanoparticle production, and chemical conjugation services. Recently, we have added two large-scale, high-resolution (14 microns) 3D printers capable of producing both prototype models and final products for both industry and academics. The 3D printers can use standard STL files and medical imaging files such as MRI data.

NUMBER OF FACULTY



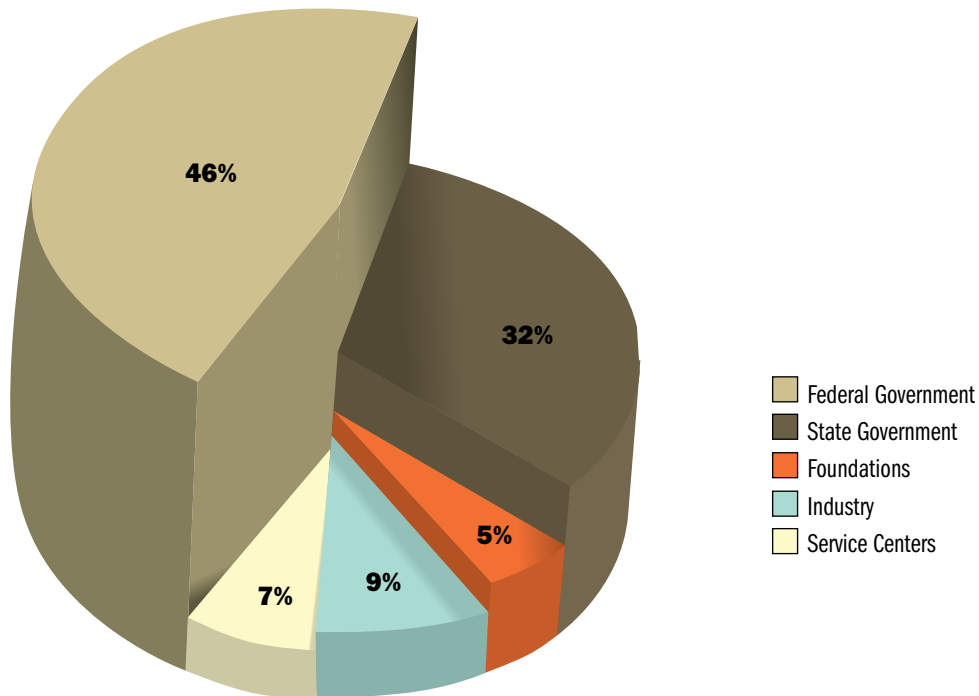
TOTAL FUNDS SUPPORTING RESEARCH



- Endowment/Gifts
- Service Center
- Sponsored Projects

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

TOTAL EXPENSES SUPPORTING RESEARCH



GIFT REPORT

New Gifts and Bequests Fiscal Year 2016

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Applied Economics Consulting Group, Inc	Marilyn Alice McDonald
Beverly and Dan Arnold	Patricia and John McDonald
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Office of Communications
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