THE UNIVERSITY *of* TEXAS MEDICAL SCHOOL *at* HOUSTON'S
BROWN FOUNDATION INSTITUTE *of* MOLECULAR MEDICINE FOR THE PREVENTION *of* HUMAN DISEASES

IMMpact Report

FISCAL YEAR 2014

About the cover

The University of Texas Medical School at Houston's Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases was established in 1995 to cure the diseases of our time in our time. The James T. Willerson, M.D., Discovery Hall is shown in this cover photo.

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Contents

Features

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, cancer, schizophrenia, Alzheimer's, and cardiovascular diseases are unsolved, common, and not caused by a single gene. 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 to achieve this goal of patient benefit from discovery.

I am pleased to provide the latest edition of the IMMpact report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). The current issue includes in-depth feature articles on recent developments and specific information on every IMM faculty member and the innovative research in which they are engaged. I trust that you will find the report interesting and informative.

The IMM is a stand-alone research institute that is embedded within The University of Texas Health Science Center at Houston, Medical School, which in turn is part of UTHealth. Our unique mission is to deliver translational outcomes from research in molecular medicine that benefits patients. We have teams of outstanding basic and translational scientists who collaborate closely with clinicians in UTHealth. The centers for metabolic and degenerative diseases, molecular imaging, and two of our flagship programs in regenerative medicine and drug development, plus others that are detailed in the report, provide excellent examples of these collaborative teams.

I am pleased to report that despite a persistent, very challenging environment for scientific research funding marked by significant reductions in the NIH budget by Congress over the past few years, IMM faculty have nevertheless been extremely successful. Over the financial year just ended, our new grants and contracts were up some 40% over the preceding year. It is truly a remarkable testament to the quality and creativity of our scientists that the IMM faculty remains so successful in attracting research funds from what is an ever-diminishing pool. That said, full implementation of our mission remains heavily dependent on attracting support from alternative sources, including research charities, industry collaborations, and, most importantly, the continuing generosity of our friends and donors.

In addition to advancing science and medicine, we therefore wish to further develop our relationships with all in our community who value the aspiration of our mission to translate molecular discoveries into new therapies for human disease. In this regard we are deeply appreciative of the strong work and dedication of the IMM advisory council, under the leadership of Mr. Dudley Oldham, which plays a key role in the continued growth and development of the IMM.

If you would like to investigate how you can be involved, we would be delighted to talk with you personally, so please feel free to contact us here at the IMM. Alternatively we would be delighted to see you at the upcoming IMM symposium, which will be held on April 1, 2015. Please mark this date in your calendar because you will hear exciting research stories directly from our faculty and have the opportunity to meet with them and discuss their science and its implications for the future of medicine and health care.

John Hancock, M.A., M.B., B.Chir., Ph.D., Sc.D.
Executive Director, Institute of Molecular Medicine
John S. Dunn Distinguished University Chair in Physiology and Medicine

Mission

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

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

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

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

As an institute of The University of Texas Medical School at Houston, 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.

Researchers target blood circulation issues

When the body has difficulty pumping blood to legs, arms, and other extremities, bad things can happen.

In the United States, millions have a circulatory problem of the legs called peripheral vascular disease. It can be painful and may even require surgery in serious cases. This disease can lead to severe skeletal muscle wasting and, in turn, limb amputation.

Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases scientists tested a non-surgical, preventative treatment in a mouse model of the disease and found it associated with increased blood circulation. Their proof-of-concept study appeared earlier this year in the journal *Cell Reports*.

Unlike previous studies in which other investigators used individual stimulatory factors to grow blood vessels, IMM researchers identified and turned off a genetic switch that stifles blood vessel development, says Vihang Narkar, Ph.D., senior author and assistant professor in the Center for Metabolic and Degenerative Diseases.

"We discovered an inhibitory switch that degrades blood vessels," Dr. Narkar says. "We were able to genetically turn it off to prevent peripheral vascular disease in a preclinical study."

Not limited to peripheral vascular disease, this discovery also could aid in the treatment of other conditions affecting blood vessels like diabetic retinopathy, diabetic nephropathy, and atherosclerosis.

The prevalence of peripheral arterial disease rises with age, affecting more than 5 percent of individuals between 50 and 60 years of age, and 10 to 20 percent of people more than 70 years of age.

More than 29 million people in the United States have diabetes and its related complications. Approximately eight million of them do not know they have diabetes.

Millions have hardening of the arteries – atherosclerosis – that is serious yet asymptomatic.

Dr. Narkar is now testing this genetic switch in mouse models of diabetic vascular complications.

"We found that this inhibitory switch is naturally turned on in a mouse of model of diabetes. We're doing tests to see what happens when it is turned off," he says.

The switch is called peroxisome proliferator-activated receptor gamma co-activator 1 beta (PGC1beta).

As for his vision for this research, Dr. Narkar says, "We are trying to identify cellular pathways that we can target with disease-selective drugs to treat vascular complications and build healthy blood vessels."

"Eighty percent of limb amputations are due to lack of adequate blood flow," says Kristofer Charlton-Ouw, M.D., assistant professor in the Department of Cardiothoracic and Vascular Surgery.

"The lack of blood flow is primarily due to blockages in the arteries from a build-up of atherosclerotic plaque. Sometimes, clinicians can crush open the plaques using balloons or stents. Other times, we can reroute the blood flow around the blocked arteries using bypass surgery. Unfortunately, many patients have failed treatment or they are not candidates for it and the result is limb loss."

Dr. Charlton-Ouw adds, "Dr. Narkar and colleagues provide an exciting new potential treatment for these patients by stimulating new blood vessel growth.

"The method is innovative in that they blocked the natural inhibitors and tipped the balance in favor of new growth. This has the potential to treat millions of people with painful and debilitating blood flow problems."

Dr. Vihang Narkar is working to help those with peripheral vascular disease.

Obesity - is it all in your brain?

Those concerned about their weight know that major lifestyle changes – improving diet and increasing exercise – are the keys to long-term weight-loss success. But one researcher at the Brown Foundation Institute for Molecular Medicine is taking a microscopic approach to controlling weight and preventing obesity.

Qingchun Tong, Ph.D., and his team are working to identify specific neurons and neural pathways in the brain that stimulate or inhibit feeding. The goal is to identify drug therapies to target these brain cells to ultimately regulate weight.

"About one-third of the population in the United States is obese, posing a great economic burden to our society," Dr. Tong says. "We are working on neuron-specific manipulation in mice to activate, or inactivate, specific groups of neurons, or key genes in the brain, to delineate neurocircuits important for feeding and energy expenditure."

Current drugs used to curb obesity are few in number, rife with side effects, and are not very effective. Instead of blindly throwing a dart on a board, hoping to hit a target, Dr. Tong is working on finding very specific targets to create the most effective drug therapies.

"Ultimately, we want to use the brain as the drug target, as the brain is what coordinates the other organs," he says.

With billions of neurons in the brain, Dr. Tong and his team's work seems like seeking the proverbial needle in a haystack.

"To achieve our goals, we are using the optogenetic approach, which allows us to activate or inhibit a specific group of neurons with millisecond resolution. This way of using light control of neuron activity provides a direct assessment of inter-neuronal communication," Dr. Tong says.

An associate professor in the Center for Metabolic and Degenerative Disease, Dr. Tong joined the IMM in 2009. He earned his Ph.D. from SUNY Downstate Medical Center and completed postdoctoral training at Beth Israel Deaconess Medical Center and Harvard Medical School.

His lab's research on how the brain controls feeding, energy expenditure, and glucose homeostasis recently was published in *Cell*, *Cell Metabolism*, and *Molecular Metabolism*.

"We are generating new mouse tools and combine mouse genetics with optogenetics to identify novel types of neuron in the brain for body weight regulation and feeding," Dr. Tong explains.

The group also is investigating the underlying actions of the hormone leptin in glucose homeostasis to identify potential drug targets for use in patients with Type 1 diabetes.

Leptin, known as the "fullness" hormone because it increases metabolism, curbs appetite, and maintains body weight homeostasis, is secreted from fat tissues in proportion to fat mass. Recently, it was found that leptin action in the brain fully restores glucose levels to normal in Type 1 diabetes.

In Type 1 diabetes, an autoimmune disease, patients' blood glucose levels are too high and the body does not make insulin, which is the hormone that regulates glucose use. As many as 3 million Americans have Type 1 diabetes.

"Something goes wrong in Type 1 diabetes – a very low leptin concentration is observed and thus, the leptin action in the brain to control glucose is inactive. We are researching how the brain senses, responds, and transmits the signal for leptin in glucose homoestasis," Dr. Tong says.

Dr. Tong and his team are studying this pathway in order to identify a new drug target, with a goal of using the target alone, or in concert with insulin, to control the uncontrolled glucose level in Type 1 diabetes.

"We are generating animal models to determine the neurons, transmitters, and pathways involved in Type 1 diabetes," Dr. Tong says. "The beneficial effect of leptin is that it doesn't cause hypoglycemia or fat accumulation, the typical side effects associated with insulin treatment."

Dr. Qingchun Tong and his team seek to identify neurons that inhibit and stimulate feeding.

Stem cell therapy holds promise for muscular dystrophy

For 45 years, viewers would be glued to the TV as Jerry Lewis would signal the timpani and watch the tote board rise higher and higher as he led the annual Muscular Dystrophy Association (MDA) telethon. Many people first learned of muscular dystrophy through this fundraising show, but what is the status of muscular dystrophy research today?

Thousands of researchers around the globe continue to seek the answers that will one day provide treatment for this debilitating genetic disease. There are still no cures available for patients afflicted with one of the nine types of muscular dystrophy – only palliative measures are offered to improve the quality of life.

Radbod Darabi, M.D., Ph.D., assistant professor at the IMM's Center for Stem Cell and Regenerative Medicine, has dedicated his research career to develop therapies for Duchenne Muscular Dystrophy (DMD). During the last decade, his research has pioneered use of stem cells for muscle regeneration in mice models of muscular dystrophies. As a recipient of a three-year MDA research award, his goal is to develop a treatment that uses healthy stem cells to replace the muscle cells destroyed by this aggressive disease.

DMD affects about one in 3,500 male births worldwide. Since the disease is caused by a defective gene on the X chromosome, and girls have two copies – one of which can be used as a backup, few girls are affected.

Onset of the disease, characterized by muscle weakness, usually starts in early childhood, and by age 12, most patients are confined to a wheelchair. Heart and respiratory muscles are affected by DMD, and few patients live past their 30s.

"In addition to developing stem cell therapies, we want to use patient's own reprogrammed stem cells for gene correction and therapy," Dr. Darabi explains.

One of the challenges DMD presents to researchers is that the DMD gene is the largest known human gene, which makes its correction even harder. This gene is responsible for encoding the muscle protein dystrophin. Patients with DMD cannot make the dystrophin protein in their muscles. This deficiency eventually leads to progressive muscle damage and its gradual replacement by fat and fibrotic tissues.

"Stem cells are the one of the best candidates for treatment of degenerative disorders like this, as they can be differentiated to the any needed cell type and be reproduced endlessly," Dr. Darabi says.

This research involves the use of induced pluripotent stem cells (iPS), which are derived from the patient and then re-programmed into stem cells. The iPS cells are taken from adult skin or blood cells and genetically manipulated to become stem cells that can turn into any type of cell – in this case muscle cells.

"Our aim is to replace the damaged cells with newly created healthy cells," Dr. Darabi says.

Darabi and his lab currently use a mouse model with DMD to test the human iPS cells – correcting the defective genes, generating muscle progenitor cells from the iPS cells, and then delivering the cells systemically to affected muscle groups.

"We are also looking to improve vascular circulation in the muscle where the stem cells are transplanted, to provide a healthier environment for them," he adds.

Therapy must first be validated in mice before it can move to a clinical trial in humans. In order to translate this research to the human patient, a clinical grade safe cell preparation is needed – a very pure myogenic population without any animal product.

Due to new advances in this field, especially new gene editing technologies, Dr. Darabi estimates it won't be very long until we see clinical trials in the United States begin.

Dr. Darabi has been with the IMM for just over a year. He completed his postdoctoral training at UT Southwestern at Dallas and was a research faculty at the University of Minnesota before joining the IMM faculty at the Center for Stem Cell and Regenerative Medicine (CSCRM).

Muscular dystrophy treatment via stem cells is a focus of Dr. Radbod Darabi's lab.

TTI adds medicinal chemist to arsenal

The importance of the health care team is ingrained into the students of each of our six health-related schools. It's not just the doctor, they learn, but a team of health experts who are dedicated to improving the patient's well-being.

What we do not hear a lot about is the importance of a well-rounded research team. Research doesn't happen in a silo, there are postdoctoral fellows, lab assistants, and technicians. But what about the researchers themselves – what kind of specialists must they be to move the chains on research?

The Texas Therapeutics Institute (TTI) of the IMM is comprised of investigators mostly from pharmaceutical and biotechnology companies whose goal is to identify and validate drug targets. These researchers have backgrounds rich in biology.

What TTI has lacked is a medicinal chemist who can support the existing research or develop new platforms.

Enter Kyoji Tsuchikama, Ph.D., who was recruited to TTI as an assistant professor in July to provide such chemistry expertise for the institute.

Dr. Tsuchikama hails from Japan, but most recently was at the Scripps Research Institute in La Jolla, Calif., where he completed a postdoctoral training in organic synthesis and chemical biology. He received his doctoral degree in organic chemistry from Waseda University in Japan.

"Chemistry is the basis of drug development, and until now TTI lacked chemists who can support such research," Dr. Tsuchikama explains.

TTI researchers have identified proteins of interest for drug development. "But without a chemical tool, they suffer from difficulty validating the biochemical process. Such chemical tools are occasionally not commercially available, and a chemist is needed to make compounds and tools," Dr. Tsuchikama says.

As the only organic chemist in the TTI, Dr. Tsuchikama says he will be expected to have a cooperative program, "filling the gaps" throughout the institute. In addition, he will do his own original research.

He is already in discussions with Gerald Bills, Ph.D., to pursue natural products and chemical agents to suppress microbial infection, and with Xiaodong Cheng, Ph.D., on a kinase inhibitor project that will focus on creating chronic pain and cancer-fighting drugs.

"My goal is to identify promising therapeutics using chemical methods," Dr. Tsuchikama says.

Dr. Tsuchikama's areas of research interest include treating and stopping the spread of infectious diseases, like staph and Ebola.

"There are so many proteins involved in cell physiology and diseases. We seek to understand the network of protein function – how the proteins work together to control cell function, or progress or suppress disease," he says.

Including medicinal chemists on the biomedical research team is catching on with other progressive institutes. The University of Texas MD Anderson Cancer Center has such programs, whereas most traditional universities do not.

"Medicinal chemists bring knowledge, expertise, and techniques to the translational field of research. Our presence will give the TTI more insights to development drug candidates," he says.

Dr. Tshukiama says he enjoys being able to bring a new perspective to TTI's research.

"I really like this environment of having biology experts as colleagues. It's more important than being with just chemists," he says. "With our complementary interests and expertise, this place has a lot of chances."

> "Medicinal chemists bring knowledge, expertise, and techniques to the translational field of research."
>
> — Dr. Kyoji Tsuchikama

As a medical chemist, Dr. Kyoji Tsuchikama brings a new perspective to the Texas Therapeutics Institute.

Loss brings IMM donor's generosity to life

Sixteen years ago, Steve Gordon and his wife, Janice, lived in a dream world—a world that ended in one day.

"I guess we all think that if you get sick, you go to the doctor, the doctor cures you, and you go home," he says. "And then one day you're told there's no cure."

At age 54, Janice Gordon's colonoscopy revealed stage four colon cancer. While the disease can usually be overcome in its early stages, it is almost always fatal if caught later.

"She knew from the minute they came out of the room," Steve Gordon says.

Doctors gave Janice Gordon six months to live, but she and Steve Gordon fought the disease for two years before she passed. As her main caregiver, he saw the toll the cancer took.

"The tragic things that happen in your life motivate you to do something," he says.

Since his wife's passing 14 years ago, Gordon has worked with UTHealth to fight the disease that took her life. He pledged $250,000 in 2004 to establish an endowed faculty position, called the Janice Davis Gordon Distinguished Professorship in Bowel Cancer Research, at the UTHealth Medical School's Brown Foundation Institute of Molecular Medicine (IMM). After fulfilling the pledge in 2009, he enhanced the position to an endowed chair with an additional $250,000 commitment.

"The goal in my mind is to find, if not a cure, at least a treatment for advanced colon cancer that would allow people to live sort of like when people are diagnosed with diabetes," Gordon says.

Dr. Qingyun "Jim" Liu is the current faculty holder of the Janice Davis Gordon Chair in Bowel Cancer Research, and he is exploring treatments that could give new hope to patients. One particular avenue is called "targeted therapy," which introduces medicine directly into the cancer to kill it without harming surrounding tissue.

"This approach could potentially treat patients much more effectively than we have been able to do so far," Dr. Liu says.

Gordon knows it's a tough disease to fight. He remembers his wife undergoing chemotherapies that would work for a few months before the cancer found a way around them. But he is encouraged by Dr. Liu's work indicating major treatment advances may finally be on the way.

"I don't want to give up," Gordon says. "I don't know any other route than to try to help the research go forward."

Recently remarried, Gordon is determined that he will not be stricken with colon cancer. He touts his bi-annual colonoscopy appointments like badges of honor and insists that everyone over 50 should have the test done frequently.

"They told me if my wife had gotten a colonoscopy six months or a year before she did, she would probably be alive today," he says. "It's that important."

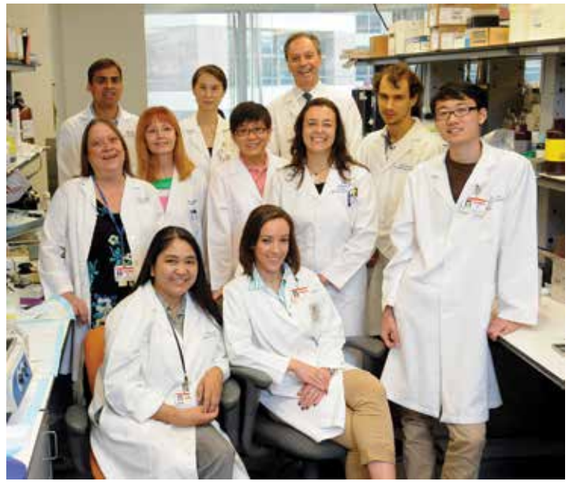
In addition to giving to support colon cancer research, Gordon dedicates his time to fighting disease by serving on the UTHealth Development Board and the IMM Advisory Council.

"You don't just stand by as a bystander and hope somebody else is doing something," he says. "You can feel like you've done a little bit in the way of helping mankind toward a cure for a deadly disease."

It's a mindset he shares with many others; most of the donors he meets at dinners and meetings have had similar experiences, shaken out of complacency and into action when they or someone they love were struck with a serious illness. They know, as he does, that even if they can't do it all, they can make a difference.

"In the big picture, what I'm giving is not even a water drop, but you try to do what you can, and maybe in the future at some point there will be a solution to this," he says. "That's what we hope for."

Steve Gordon and Susan Atkins are doing their part to support bowel cancer research.



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 Outpatient Clinic. The Center also has a Research Clinic, which is utilized for clinical research activities.

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

Faculty: Raffaella Lombardi, M.D., Ph.D. Assistant Professor; Priyatansh Gurha, Ph.D. Instructor; AJ Marian, M.D. Professor

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 and molecular genetic studies. The main objective of these activities is to identify the causal genes for hereditary cardiovascular diseases, primarily cardiomyopathies. The discoveries are then applied in cell culture systems and animal models in order to delineate the molecular links between the causal variants and the phenotype. Upon elucidation of the molecular links between the genetic mutations and the clinical phenotypes, genetic and pharmacological interventions are pursued to block the linking pathways in order to prevent and reverse the phenotype. The initial intervention studies are pursued in cell and animal models and then extended to humans through pilot randomized placebo-control trials.

Research Programs: The research programs are categorized into four categories:

I. Human molecular genetics/genomics: These studies are designed to delineate the molecular genetic and genomic basis of cardiovascular diseases in humans with a specific focus on three most common forms of hereditary cardiomyopathies; namely Hypertrophic Cardiomyopathy, Dilated Cardiomyopathy, and Arrhythmogenic Cardiomyopathy. The studies entail recruitment and clinical characterization of patients with hereditary cardiovascular diseases, genetic testing through nucleic acid sequencing, and studies to test for the causality of the variants.

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

III. Experimental Therapies: Upon delineation of the molecular mechanisms specific pathways that are responsible for the induction of the phenotype are pharmacologically and genetically targeted in myocytes through *in vitro* and *in vivo* studies.

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

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

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

Molecular genetics and pathogenesis of hereditary cardiomyopathies

Our long-standing research objectives have been to delineate the molecular genetics and pathogenesis of hereditary cardiomyopathies in humans and apply the discoveries to prevent the evolving and reverse the established phenotypes of heart failure and sudden cardiac death in patients with cardiomyopathies. 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 the 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 human molecular genetic studies through high throughput genomic DNA sequencing to identify the causal genes and mutations followed by molecular mechanistic studies, including in genetically modified animal models and cultured cells to identify the pathways that link the causal mutations to the disease phenotype. The mechanistic discoveries are complemented with genetic and pharmacological intervention targeting the pathways that link the causal mutations to the phenotype, in order to prevent and reverse the phenotype initially 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 changes including non-coding RNAs and histone modifications in hereditary cardiomyopathies.
- Identification and characterization of the molecular pathways that link the genetic mutations to the clinical phenotype in patients with cardiomyopathies including delineation of the mechanical signaling pathways regulated at the intercalated discs.
- HALT-HCM (Hypertrophic Regression with N-Acetylcysteine in Hypertrophic Cardiomyopathy) clinical trial (ClinicalTrial.Org NCT01537926).
- An industry -sponsored clinical trial to improve symptoms and exercise tolerance in patients with hypertrophic cardiomyopathy.

KEY PUBLICATIONS

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Faculty – Instructor: Priyatansh Gurha
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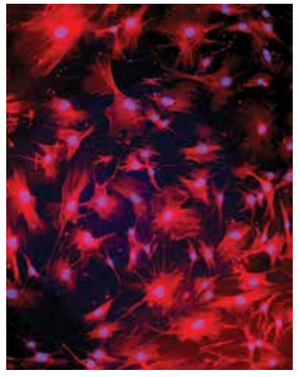


Expression of sarcomere protein α-tropomyosin in cardiac progenitor cells isolated from the heart of a mouse model of arrhythmogenic cardiomyopathy. Red: α tropomyosin; Blue:DNA.

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

Molecular genetics and pathogenesis of hereditary cardiomyopathies

The focus of my research is the delineation the pathogenesis of cardiomyopathies. Cardiomyopathies are genetically transmitted diseases of the heart muscle, associated with high risk of sudden cardiac death and heart failure.

Although significant progresses have been accomplished over the past two decades in revealing the causal genes, at the present time there is no effective pharmacological or non-pharmacological therapy for these disorders. In fact, even if the current pharmacologic approaches provide some benefits for symptom control, they have been unable to fundamentally change the natural history of these diseases.

We study the 3 most common forms of cardiomyopathies, namely Hypertrophic Cardiomyopathy (HCM), Arrhythmogenic Cardiomyopathy (AC), and Dilated Cardiomyopathy (DCM).

Our group has developed cell culture models as well as genetically modified animal models of human cardiomyopathies. The mechanistic findings in the *in vitro* and *in vivo* models have led to the identification of key molecular pathways implicated into the pathogenesis of these diseases.

More recently, my studies have addressed the pathogenesis of the unique phenotype of ARVC characterized by replacement of the cardiac myocytes with fat cells and fibrosis. The main focus of these studies is the identification of the cellular origin of excess adipocytes in AC. Through genetic fate-mapping experiments, I have identified a subset of cardiac progenitor cells from the second heart field (the embryonic source of the right ventricle) as a cell source of adipocytes in AC. Furthermore, I have identified molecular key pathways implicated in the differentiation of cardiac progenitor cells to adipocytes. These findings could lead to the development of new therapies aimed at preventing cardiac precursor cells from switching from a muscle cell fate to a fat cell fate and therefore, prevent this potentially deadly disease.

Whenever possible, I always confirm my findings in animal and cellular models also in human patients with cardiomyopathy. Furthermore, the efficacy of some of the treatments I tested in animal models has been and is currently being tested in human patients through pilot randomized placebo-control trials.

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Chen SN*, Gurha P*, Lombardi R*, Alessandra Ruggiero, Willerson J T, Marian AJ. The Hippo Pathway Is Activated And Is a Causal Mechanism For Adipogenesis in Arrhythmogenic Cardiomyopathy. *Circ Res* 2013 (in press).
* Authors contributed equally to this work

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

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

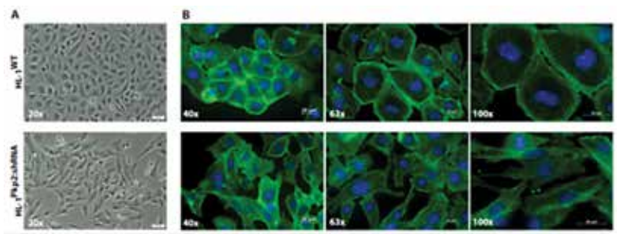
Lombardi R, Dong J, Rodriguez G, Bell A, Leung TK, Schwartz RJ, Willerson JT, Brugada R, Marian AJ. Genetic fate mapping identifies second heart field progenitor cells as a source of adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res.* 2009 May 8; 104(9):1076-84.

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

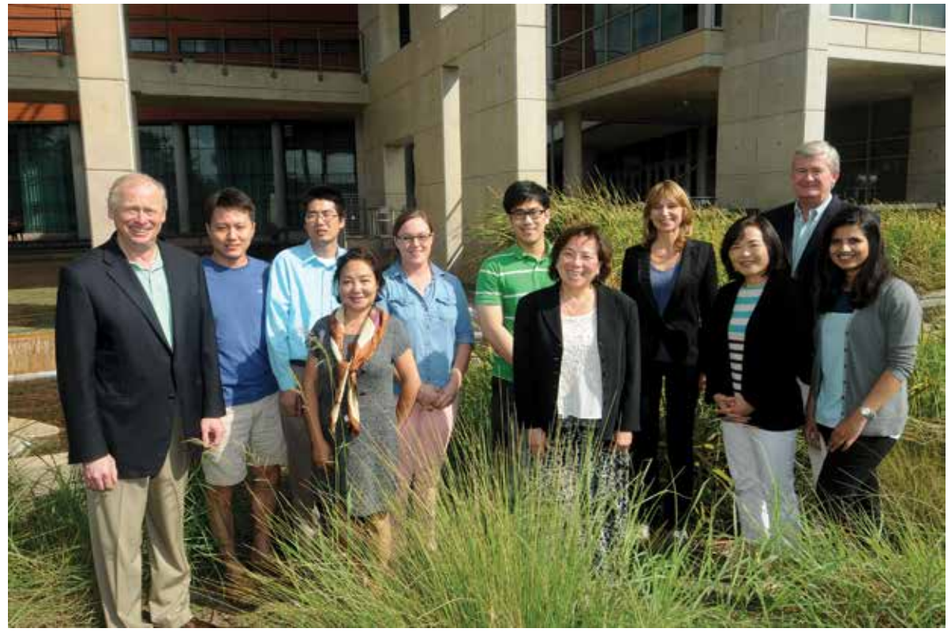
Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ. Resolution of Established Cardiac Hypertrophy and Fibrosis and Prevention of Heart Failure in the β-Myosin Heavy Chain-Q403 Transgenic Rabbits with N-Acetylcysteine. *Circulation* 2009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

LAB MEMBERS

Priyatansh Gurha, Ph.D., Instructor
Suet Nee Chen, Ph.D., Post-doctoral fellow
Chen, Xiaofan, M.D., Post-doctoral fellow
Karmouch Jennifer, Ph.D., Post-doctoral fellow
Rodionov Edward, Research Assistant
Grazyna Czernuszewicz, Research Associate



Morphological changes in the HL-1$^{PKP2:shRNA\ myocytes}$: Bright field (A) and Phalloidin staining of HL-1 myocytes at 3 sets of magnifications (B). The HL-1$^{PKP2:shRNA}$ exhibit altered cellular morphology and cytoskeletal organization.



The investigators of the Center for Human Genetics work to understand and reduce common cardiovascular diseases. Heart and kidney diseases, high blood pressure, and stroke are linked together and together have a larger impact on the health of our population than any other disease process. Heredity impacts our risk of these diseases. By discovering how variation in our genes produces disease risk, we identify pathways of disease and new avenues for prevention and treatment.

Work in our center targets DNA sequence, gene expression, and gene function using modern genetic and genomic methods. Our work involves large-scale studies of genetic variation and disease in human populations. We also use model organisms to understand how changes in the sequence of the genome affect protein function and how the resulting abnormalities advance to produce disease.

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

Why does disease risk run in families and what can the understanding of genetic risk do to allow improved health? This key question provides the focal point of our center's work to identify the natural genetic variation in our DNA that will allow us to know the pathways that increase the likelihood an individual will experience common forms of cardiovascular disease. It leads to insight into how disease arises and where to apply our efforts to impede the initiation and progression of disease. The advances we pursue will allow doctors to target treatments to the underlying cause of disease in each affected individual, not just the symptoms. It will allow us to see how different elements of lifestyle and environment shape the processing and expression of information contained within our genomes.

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

Eric Boerwinkle, Ph.D.
Professor and Director of the Center for Human Genetics
Kozmetsky Family Chair in Human Genetics

Genomic sciences to promote human health

I am the director of the Research Center for Human Genetics. My laboratory is identifying genes involved in the causes of human disease: both simply inherited Mendelian diseases and common complex diseases. Advances in laboratory technologies open the possibility that each and every one of us may have to read our own DNA sequence. At the same time, computers to store and analyze those data have grown in size and speed. The advent of "cloud computing" pushes this envelop even further. Concurrent with these scientific advances, the population of Texas and the United States continues to grow and age. Therefore, the burden of common chronic diseases, such as coronary heart disease, kidney disease, and stroke, is increasing. Our research is discovering the genes and mutations that increase the risk of developing common chronic disease and understanding how these genes interact with the environment to determine health and disease. This work is leading to novel approaches to both treat these conditions in the elderly and prevent their onset in our children. This research combines three powerful biomedical forces: large-scale DNA sequencing, computational analysis, and large samples of individuals with extensive clinical measurements.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Yang J., Loos R.J., Powell J.E., Medland S.E., Speliotes E.K., Chasman D.I., Rose L.M., Thorleifsson G., Steinthorsdottir V., Mägi R., Waite L., Smith A.V., Yerges-Armstrong L.M., Monda K.L., Hadley D., Mahajan A., Li G., Kapur K., Vitart V., Huffman J.E., Wang S.R., Palmer C., Esko T., Fischer K., Zhao J.H., Demirkan A., Isaacs A., Feitosa M.F., Luan J., Heard-Costa N.L., White C., Jackson A.U., Preuss M., Ziegler A., Eriksson J., Kutalik Z., Frau F., Nolte I.M., Van Vliet-Ostaptchouk J.V., Hottenga J.J., Jacobs K.B., Verweij N., Goel A., Medina-Gomez C., Estrada K., Bragg-Gresham J.L., Sanna S., Sidore C., Tyrer J., Teumer A., Prokopenko I., Mangino M., Lindgren C.M., Assimes T.L., Shuldiner A.R., Hui J., Beilby J.P., McArdle W.L., Hall P., Haritunians T., Zgaga L., Kolcic I., Polasek O., Zemunik T., Oostra B.A., Junttila M.J., Grönberg H., Schreiber S., Peters A., Hicks A.A., Stephens J., Foad N.S., Laitinen J., Pouta A., Kaakinen M., Willemsen G., Vink J.M., Wild S.H., Navis G., Asselbergs F.W., Homuth G., John U., Iribarren C., Harris T., Launer L., Gudnason V., O'Connell J.R., Boerwinkle E., Cadby G., Palmer L.J. James A.L., Musk A.W., Ingelsson E., Psaty B.M., Beckmann J.S., Waeber G., Vollenweider P., Hayward C., Wright A.F., Rudan I., Groop L.C., Metspalu A. Tee Khaw K., van Duijn C.M., Borecki I.B., Province M.A., Wareham N.J., Tardif J.C., Huikuri H.V., Cupples L.A., Atwood L.D., Fox C.S., Boehnke M., Collins F.S., Mohlke K.L., Erdmann J., Schunkert H., Hengstenberg C., Stark K., Lorentzon M., Ohlsson C., Cusi D., Staessen J.A., Van der Klauw M.M., Pramstaller P.P., Kathiresan S., Jolley J.D., Ripatti S., Jarvelin M.R., de Geus E.J., Boomsma D.I., Penninx B., Wilson J.F., Campbell H., Chanock S.J., van der Harst P., Hamsten A., Watkins H., Hofman A, Witteman J.C., Zillikens M.C., Uitterlinden A.G., Rivadeneira F., Zillikens M.C., Kiemeney L.A., Vermeulen S.H., Abecasis G.R., Schlessinger D., Schipf S., Stumvoll M., Tönjes A., Spector T.D., North K.E., Lettre G., McCarthy M.I., Berndt S.I., Heath A.C., Madden P.A., Nyholt D.R., Montgomery G.W., Martin N.G., McKnight B., Strachan D.P., Hill W.G., Snieder H., Ridker P.M., Thorsteinsdottir U., Stefansson K., Frayling T.M., Hirschhorn J.N., Goddard M.E., Visscher P.M. (2012) FTO genotype is associated with phenotypic variability of body mass index. *Nature* 490(7419):267-72.

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

Voight B.F., Peloso G.M., Orho-Melander M., Frikke-Schmidt R., Barbalic M., Jensen M.K., Hindy G., Hólm H., Ding E.L., Johnson T., Schunkert H., Samani N.J., Clarke R., Hopewell J.C., Thompson J.F., Li M., Thorleifsson G., Newton-Cheh C., Musunuru K., Pirruccello J.P., Saleheen D., Chen L., Stewart A.F., Schillert A., Thorsteinsdottir U., Thorgeirsson G., Anand S., Engert J.C., Morgan T., Spertus J., Stoll M., Berger K., Martinelli N., Girelli D., McKeown P.P., Patterson C.C., Epstein S.E., Devaney J, Burnett M.S., Mooser V., Ripatti S., Surakka I., Nieminen M.S., Sinisalo J., Lokki M.L., Perola M., Havulinna A., de Faire U., Gigante B., Ingelsson E., Zeller T., Wild P., de Bakker P.I., Klungel O.H., Maitland-van der Zee A.H., Peters B.J., de Boer A., Grobbee D.E., Kamphuisen P.W., Deneer V.H., Elbers C.C., Onland-Moret N.C., Hofker M.H., Wijmenga C., Verschuren W.M., Boer J.M., van der Schouw Y.T., Rasheed A., Frossard P., Demissie S., Willer C., Do R., Ordovas J.M., Abecasis G.R., Boehnke M., Mohlke K.L., Daly M.J., Guiducci C., Burtt N.P., Surti A., Gonzalez E., Purcell S., Gabriel S., Marrugat J., Peden J., Erdmann J., Diemert P., Willenborg C., König I.R., Fischer M., Hengstenberg C., Ziegler A., Buysschaert I., Lambrechts D., Van de Werf F., Fox K.A., El Mokhtari N.E., Rubin D., Schrezenmeir J., Schreiber S., Schäfer A., Danesh J., Blankenberg S., Roberts R., McPherson R., Watkins H., Hall A.S., Overvad K., Rimm E., Boerwinkle E., Tybjaerg-Hansen A., Cupples L.A., Reilly M.P, Melander O., Mannucci P.M., Ardissino D., Siscovick D., Elosua R., Stefansson K., O'Donnell C.J., Salomaa V., Rader D.J., Peltonen L., Schwartz S.M., Altshuler D., Kathiresan S. (2012) Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet* 380(9841):572-80. *Erraum in Lancet* 380(9841):564, 2012.

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

LAB MEMBERS

A large group of physicians, scientists and students collaborate to articulate and achieve a shared vision for a better understanding of the genetic basis of health and disease.

Peter Doris, Ph.D.
Professor & Deputy Director
Cullen Chair in Molecular Medicine

Genetic variation in immune function determines how high blood pressure injures the kidney

As we age, our kidney function declines. 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 end stage renal disease. This indicates that inherited factors influence risk of renal disease. At present, there are no therapies that provide kidney protection. This is because 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. We have developed and study a rat model of renal injury in the presence of high blood pressure. We have two very closely related rat lines that share similar genetic elevation of blood pressure, but one line gets renal disease and stroke, the two major end organ injuries produced by high blood pressure, while the other does not. The renal disease is similar in every way to that present in humans with high blood pressure. By combining functional studies with genetic studies, this model is yielding fascinating insight into the mechanism of disease.

We have found that functional genetic variation in immune system genes combine to play a key role in susceptibility to injury. We also have obtained the first rat gene knockout model. This knockout eliminates immunoglobulin expression, and we are transferring the null allele into our injury-prone animals to further assess the role of the immune system in this disease. Our recent whole genome sequence analysis has uncovered a truncating mutation in a key immune signaling gene, Stim1. Humans with truncating mutations in this gene have severe defects in immune function and autoimmunity. The mutation we have identified permits Stim1 to continue to function in its role linking activation of cell surface receptors in immune cells to calcium signaling that drives cytokine release and other elements of the immune response. However, the mutation strongly impedes the efficacy of Stim1 in this role. Lymphocyte calcium signaling is strongly affected with implications for functional alterations in many elements of the immune response and its regulation. We have pharmacologically confirmed the role of altered immune function in renal disease: Treatment of disease-susceptible animals with an immunosuppressant blocks the ability of hypertension to injury the kidney in these animals. These observations lead us to the conclusion that, while high blood pressure may initiate kidney injury, it is the response of the immune system to this injury that determines whether normal renal function is sustained or lost. This disease is important: more people die in the United States each year from loss of renal function than from breast and prostate cancer combined. Even partial loss of kidney function greatly amplifies the risk of death from other cardiovascular diseases. By identifying how genes shape disease, we can now predict targets for drug action to prevent disease.

RESEARCH PROJECTS

- Inherited susceptibility to renal and cardiovascular end-organ disease.
- Genetic mechanisms of elevated blood pressure.
- Non-genomic mechanisms of trans-generational trait sharing.

KEY PUBLICATIONS

R.I. Dmitrieva, C.A. Hinojos, M. Grove, R.J. Bell, E. Boerwinkle, M. Fornage and P.A. Doris. Genome-wide identification of allelic gene expression in hypertensive rats. *Circulation* (Cardiovascular Genetics) 2:106-115, 2009

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

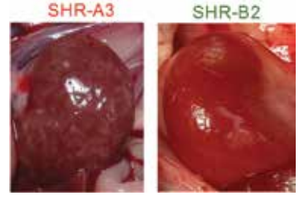
Herring, S.M., N. Gokul, M. Monita, R. Bell, E. Boerwinkle, S.E. Wenderfer, M.C. Braun and P.A. Doris. The rat immunoglobulin locus is associated with serum IgG levels and albuminuria. *J. Amer. Soc. Nephrol.* 22:881-9, 2011

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

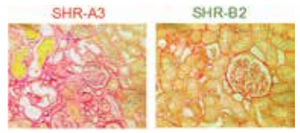
Gonzalez-Garay, M.L., S.M. Cranford, M.C. Braun, and P.A. Doris. Diversity in the pre-immune immunoglobulin repertoire of SHR lines susceptible and resistant to end organ injury. *Genes and Immunity*, In Press

LAB MEMBERS

Collaborating faculty: Eric Boerwinkle (IMM), Myriam Fornage (IMM), Manuel Gonzalez-Garay (IMM), Oleh Pochynyuk (UTH-MS), Michael Braun (Baylor College of Medicine), Scott Wenderfer (Baylor College of Medicine), M. John Hicks (Baylor College of Medicine), Roland Buelow (Open Monoclonal Technology Inc)
Technicians: Yaming Zhu, Stacy Herring
Post-doctoral fellow: Isha Dhande, Ph.D.



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



As kidneys become injured, the functional tissue is irretrievably replaced with scar tissue (fibrosis). The images below show the high level of scarring in the injury-prone SHR-A3 animal at 24 weeks of age, compared with the injury-resistant SHR-B2 animal.

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

Genetic basis of brain vascular disease and brain aging

My research interests focus on the genetic basis of common chronic diseases with an emphasis on vascular disease of the brain and brain aging. Patients with acute stroke and dementia represent the easily recognized "tip of the iceberg" but the deleterious effects of vascular and neurodegenerative disease on the brain begin well before clinical symptoms become apparent. Brain abnormalities, readily detectable by magnetic resonance imaging (MRI), are common in asymptomatic populations beginning in middle age. My research program investigates the genetics and genomics of vascular and neurodegenerative disease of the brain both in its clinical and pre-clinical forms in well characterized populations from young adulthood to old age. Research strategies combine genetic epidemiology and functional genomic approaches using the latest genome resources and technologies. In recent years, I have used the power of genome-wide association and sequencing studies in collaboration with researchers in the United States and Europe to identify genetic loci influencing the risk for stroke, dementia, and related phenotypes. Current work aims at identifying the specific genes and mutations that underlie these discoveries and to understand the function of these genes in brain health and disease.

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 (R01-AG033193, U01-AG049506, and R01-NS087541)
- Discovering novel epigenetic variants that influence risk for brain small vessel disease and its related neurocognitive outcomes (R01-NS087541)
- Discovering common and rare genetic variants influencing risk for ischemic stroke and its etiologic subtypes in well-characterized clinical samples from the NINDS Stroke Genetics Consortium (U01-NS069208)
- Discovering common and rare genetic loci influencing cardiovascular traits (lipids and blood pressure) in diverse ethnic groups as part of the NHGRI Population Architecture and Genomic Epidemiology (PAGE) consortium (U01- HG007416)
- Discover additional genetic loci for cardiovascular traits using gene-lifestyle interactions, pleiotropy analysis of correlated traits, and pathway analysis (R01-HL118305).

KEY PUBLICATIONS

Fornage M*, Debette S*, Bis JC*, Schmidt H*, Ikram MA*, et al. A locus on chromosome 17q25 influences burden of MRI-defined cerebral white matter hyperintensities in individuals of European descent. *Ann. Neurol.* 2011, 69: 928-939 (* denotes equal authors contribution)

Ikram MA*, Fornage M*, Smith AV*, Seshadri S*, Schmidt R*, et al. Genome-wide association studies implicate loci on 6q22 and 7q2 in intracranial volume and early life brain growth. *Nature Genetics* 2012; 44:539-544

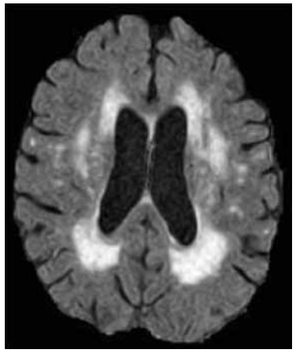
Bis JC*, DeCarli C*, Smith AV*, van der Lijn F*, Crivello F*, et al., for the CHARGE consortium. Genome-wide association studies implicate loci on Chromosome 2 in hippocampal volume. *Nature Genetics* 2012; 44:545-551

Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, et al., The Australian Stroke Genetics Collaborative, Wellcome Trust Case Control Consortium (WTCCC), Hofman A, Mosley TH, Mitchell BD, Furie K, Clarke R, Levi C, Seshadri S, Gschwendtner A, Boncoraglio GB, Sharma P, Bis JC, Psaty BM, Rothwell P, Meschia JF, Gretarsdottir S, Dichgans M, Markus HS. Genome-wide Associations with Ischaemic Stroke and its Subtypes - the Metastroke Collaboration. *Lancet Neurology* 2012; 11:951-962

Meschia JF, Arnett D, Ay H, Brown Jr. RD, de Bakker P, et al. Stroke Genetics Network (SiGN) Study: Design and rationale for a genome-wide association study of ischemic stroke subtypes. *Stroke* 2013; 44, 2694-702

LAB MEMBERS

Xueqiu Jian, PhD; Postdoctoral Fellow
Taebeom Kim; PhD Student
Li-An Lin, BS; PhD Student
Melissa Richard, PhD; Postdoctoral Fellow
Ping Wang, PhD; Research Associate



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

Ba-Bie Teng, Ph.D., FAHA
Professor
The Jerry and Maury Rubenstein Foundation
Distinguished Professorship in Heart Disease Research

Molecular genetics of atherogenesis and the development of genetic and cell therapies for the treatment of atherosclerotic vascular diseases

Cardiovascular disease is the leading cause of death globally. My laboratory is interested in the discovery of mechanisms contributing to the complex process of atherosclerosis in humans and in animal models.

Our laboratory investigates the molecular pathogenesis of atherosclerosis, and we study genes involved in the onset and progression of this disease. Recently, we discovered that PCSK9 (proprotein convertase subtilisin/kexin type 9) is the mediator for the development of atherosclerosis. PCSK9 activates the scavenger receptor LOX-1 (Lectin-like oxLDL receptor-1) in the vascular endothelial cells, promotes inflammatory responses from monocytes and macrophages, resulting in dysfunction of autophagy signaling pathway. Our study uncovers a novel link connecting PCSK9 to autophagy in atherosclerosis. The understanding of this regulatory mechanism of responses would provide new therapeutic target to manage the progression of atherosclerosis.

We engineer novel hammerhead ribozymes as therapeutic agents to inhibit gene expression to prevent/delay the disease process. Furthermore, we explore cell therapies to repair vascular injury. To better diagnosis of onset or progression of disease development, we use new technologies including metabolomics and miRNA profiling to identify new disease markers. These markers would provide valuable information to predict disease events in patients.

RESEARCH PROJECTS

- The role of PCSK9 (proprotein convertase subtilisin/kexin type 9) in lipid metabolism and atherosclerosis development.
- Investigating the action of novel Ribozyme molecules in regulating the production of apolipoprotine B and lipoprotein-associated phospholipase A2 (Lp-PLA2) mRNAs.
- The regulation of PCSK9 miRNAs in atherosclerosis development.
- Identify disease markers by metabolomics and miRNA profiling.
- Development of viral vectors for therapeutics.

KEY PUBLICATIONS

Hoyong Lim, Young Uk Kim, Hua Sun, Joyce H. Lee, Joseph M. Reynolds, Shino Hanabuchi, Huaizhu Wu, Ba-Bie Teng, and Yeonseok Chung. Proatherogenic conditions promote autoimmune T helper 17 cell responses in vivo. (2013) *Immunity* 40:1-13.

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

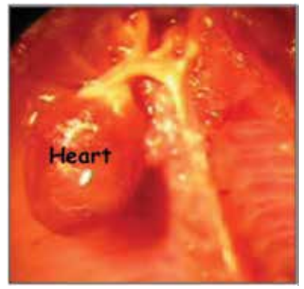
Hua Sun, Amin Samarghandi, Ningyan Zhang, Zemin Yao, Momiao Xiong, and Ba-Bie Teng. Proprotein Convertase Subtilisin/Kexin Type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. (2012) *Arterioscler Thromb Vasc Biol;* 32: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

Solida Mak, Hua Sun, Frances Acevado, Lawrence Shimmin, Lei Zhao, Ba-Bie Teng*, and James Hixson: Differential expression of genes in calcium signaling pathway underlies lesion development in the LDb mouse model of atherosclerosis. (2010) *Atherosclerosis* 213: 40-51. (*corresponding author)

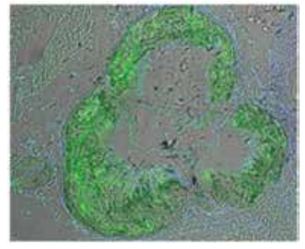
Shumei Zhong, Chichi Liu, David Haviland, Peter A. Doris, and Ba-Bie Teng: Simultaneous Expression of Apolipoprotein B mRNA Editing Enzyme and Scavenger Receptor BI Mediated by a Therapeutic Gene Expression System. (2006) *Atherosclerosis.* 184: 264-275.

LAB MEMBERS

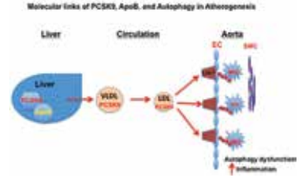
Mentor: Pei-Ying Chung, PhD (Assistant professor at School of Nursing)
Research Assistants: Michael Tan
Summer Intern: Jinwoo Peter Jung, Medical student from UTHealth.



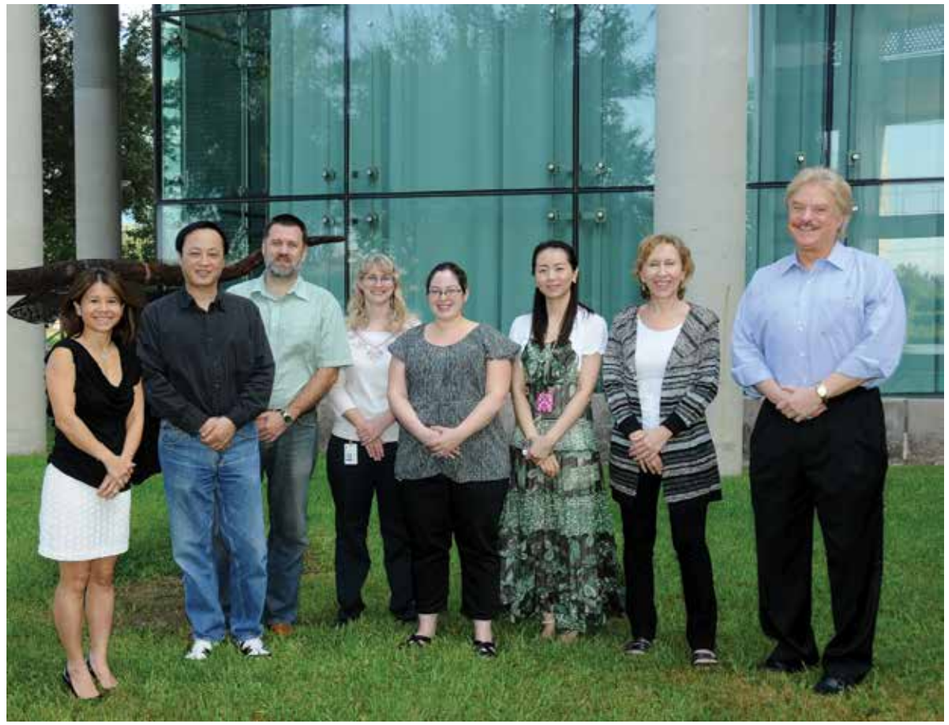
The severe atherosclerotic lesions are shown in the aorta of an LDb mouse. LDb mice are developed in our laboratory. They are excellent model for studying the pathogenesis of atherosclerosis.



A cross-section of aortic sinus of an LDb mouse with severe atherosclerotic lesions. The section is stained with macrophage marker CD68 (green color), which indicates the lesions contain large amount of macrophages.



A hypothesis: The new role of PCSK9 in the development 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.

As part of its interest in pulmonary immunity, the Center recently established a robust research program focused on the development of stem cell therapeutics for the treatment of acute and chronic lung diseases and for genetic deficiencies that affect normal lung function.

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

Research interests include:

- Asthma and Sinusitis
- T-Cells & Cytokine Biology
- Mucosal Immunology & Autoimmunity
- Microbial Infectious Disease
- Acute Lung Injury and COPD
- Surfactant Deficiencies
- Lung Stem/Progenitor Cells
- 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

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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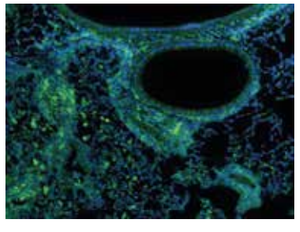
Wang D, Haviland DL, Burns AR, Zsigmond E, Wetsel RA. A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *Proc Natl Acad Sci.* 2007, 104:4449-4454.

LAB MEMBERS

Senior Research Scientist: Dr. Stacey Mueller-Ortiz
MD/PhD 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

Adenosine signaling and the regulation of chronic lung disease

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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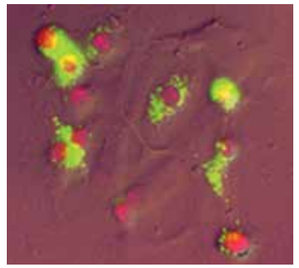
Weng, T., Poth, J. M., Karmouty-Quintana, H., Garcia-Morales, L. J., Melicoff, E., Chen, N. T., Evans, C. M., Bunge, R. R., Bruckner, B. A., Loebe, M., Volcik, K. A., Eltzschig, H. K. and Blackburn, M. R. (2014) Hypoxia-induced deoxycytidine kinase contributes to epithelial proliferation in pulmonary fibrosis. *Am. J. Crit. Care Med.* (Epub ahead of print) PMID: 25358054

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

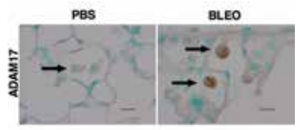
Karmouty-Quintana, H., Weng, T., Garcia-Morales, L. J., Chen, N. Y., Pedroza, M., Zhong, H., Molina, J. G., Bunge, R., Bruckner, B. A., Xia, Y., Johnston, R. A., Loebe, M., Zeng, D., Seethamaraju, H., Belardinelli, L. and Blackburn, M. R. (2013) ADORA2B and hyaluronan modulate pulmonary hypertension associated with chronic obstructive pulmonary disease. *Am. J. Respir. Cell. Mol. Biol.* 49, 1038-1047. PMID: 23855769

LAB MEMBERS

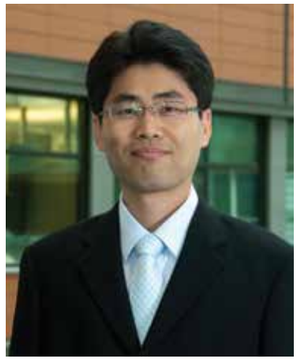
Harry Karmouty-Quintana, Ph.D., Assistant Professor
Tingting Weng, Ph.D., Assistant Professor
Kelly Volcik, Ph.D., Senior Research Scientist
Jonathan Davies, M.D., Visiting Scientist
Frank Lou, Ph.D., Postdoctoral Fellow
Kemly Philip, M.D./Ph.D. Student
Ning-Yuan Chen, Research Associate
Jose Molina, Sr. Research Scientist
Luis Garcia-Morales, Research Assistant



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).



Yeonseok Chung, Ph.D.
Assistant Professor

T cell regulation and function in immune disorders

Different types of helper T cell response mediate multiple arms of immune function to efficiently generate protective immunity against infectious disease and malignancy. However, most chronic inflammatory diseases also are associated with aberrant helper T cell responses. Understanding the regulation of helper T cell responses therefore is necessary not only for optimizing protective immunity but also for preventing aberrant inflammatory responses. In this aspect, we are particularly interested in the mutual regulation and contribution of each helper T cell subsets in disease settings, including allergic asthma, autoimmune disorders, and cancers. Among diverse helper T cell subsets, we are currently focusing on the regulation and function of follicular helper T cells (Tfh) and IL-17-producing helper T cells (Th17) as they are associated with many types of immune disorders.

Mucosal areas, including gut and lung, are always exposed to non-self environmental components such as commensals, food- or air-borne infectious agents, allergens, or food. The immune system in these mucosal tissues differs from that of non-mucosal lymphoid tissue. We are currently investigating the cross-talk between mucosal immune components and helper T cell responses by using diverse animal models.

Regulatory T cells are essential for preventing autoimmune disorders but also play a detrimental role in anti-tumor activity. Our recent study has identified a unique subset of regulatory T cells –termed 'follicular regulatory T cells'- that function to specifically suppress germinal center responses and subsequent antibody production from B cells. Considering many autoimmune diseases are mediated by autoreactive antibody responses, the use of follicular regulatory T cells might be beneficial for the treatment of autoimmune diseases by suppressing the production of the autoantibodies. We are actively investigating the developmental pathway of this regulatory T cell subset, and whether cellular therapy with follicular regulatory T cells can cure autoimmune diseases in animal models. Ultimately we hope to provide a fundamental basis for the use of this novel cell population in a clinical setting.

Another major focus in our group includes understanding the regulation of T cell responses by non-immune factors such as obesity, cholesterol, or hormones. The hypothesis here is that the immune system and metabolic pathway mutually regulate the other and contribute to complex disease phenotypes. We are primarily focusing on the changes of innate and T cell immunity in animal models of metabolic diseases. Outcomes of this study will allow us to better understand metabolic and immune-mediated disorders with multiple scientific angles.

RESEARCH PROJECTS

- Understanding helper T cell responses in mucosal area.
- Molecular regulation of follicular regulatory T cells and its application.
- Role of metabolic factors in shaping T cell responses and autoimmunity.
- Developing novel vaccine approaches for cancer and infectious agents.
- Regulation of type II innate lymphoid cells in the airway.

KEY PUBLICATIONS

Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Li Ma L, Watowich SS, Jetten A, Tian Q, Dong C. Critical regulation of early Th17 cell differentiation by IL-1 signaling. *Immunity* 2009; 30: 576 (selected as a 'featured article of the month')

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Lim H, Kim YU, Sun H, Lee JH, Reynolds JM, Hanabuchi S, Wu H, Teng B, and Chung Y. Proatherogenic Conditions Promote Autoimmune T Helper 17 Cell Responses In Vivo, *Immunity* (2014), http://dx.doi.org/10.1016/j.immuni.2013.11.021

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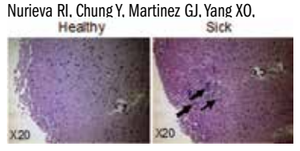
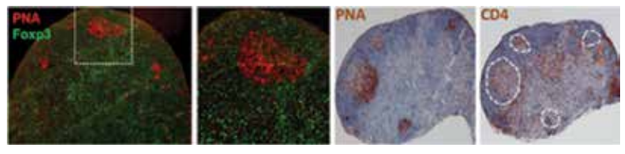
LAB MEMBERS

Post Doc: Hoyong Lim, Ph.D.
Ph.D. Student: Young Uk Kim

Subsets of helper and regulatory T cells



Inflammation in the spinal cords by autoimmune T cells



Germinal center reaction

Amber Luong, M.D., Ph.D.
Associate Professor, Center for Immunology and Autoimmune Diseases and Department of Otorhinolaryngology – Head and Neck Surgery

Environmental triggers regulating innate immune responses in chronic airway inflammation

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

CRS is clinically classified into 2 groups defined by the absence or presence of nasal polyps (see image 1). This clinical classification has been supported by immunologic profiles of the inflamed sinus tissue in which CRS without nasal polyps are characterized by predominance of neutrophils and elevated T helper cell type 1 (Th1) cytokines while CRS with nasal polyps (CRSwNP) have high presence of eosinophils, mast cells, and basophils and expression of T helper cell type 2 (Th2) 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 (see image 2).

Epithelial cells

Respiratory epithelial cells represent the first line of defense against the environment for sinonasal mucosal. Recent studies have shown that epithelial cells serve an active role through regulation of cytokines and release of anti-microbials. Three identified epithelial cell derived cytokines, thymic stromal lymphopoietin, interleukin (IL)-25 and IL-33, have been linked to the Th2 immune response.

Our lab has focused on the role of IL-33 in the Th2 immune response characteristic of CRS with nasal polyps. We recently confirmed that the receptor of IL-33 is upregulated in the diseased sinonasal mucosa of CRSwNP. In a recent publication, we demonstrated that innate lymphoid type 2 cells (ILC2) are preferentially found in CRSwNP patients relative to health controls and patients with CRS without nasal polyps. 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.

We are currently interested in expanding these initial observations. Ongoing studies focus on clarifying the molecular pathway responsible for the fungal signaling and characterizing innate lymphoid cells in CRS subtypes. In addition, we are investigating translational implications of addressing IL-33 in CRS and asthma.

RESEARCH PROJECTS

- Immunologic characterization of important cell types involved in the Th2 immune response.
- Molecular signaling through respiratory epithelial cells of fungi alone and with other environmental triggers responsible for initiating and/or maintaining the characteristic Th2 immune response.
- Clinical characterization and identification of biomarkers for CRS subtypes.

KEY PUBLICATIONS

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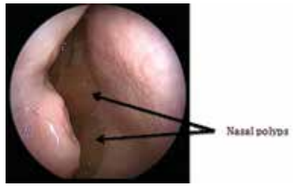
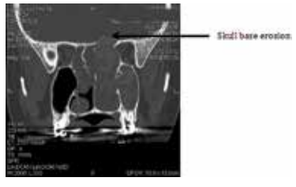


Nasal polyps seen by nasal endoscopy within nasal cavity of CRSwNP patient.



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

Dachun Wang, M.D.
Assistant Professor

Lung stem/progenitor cells and tissue regeneration

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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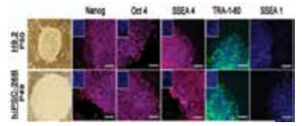
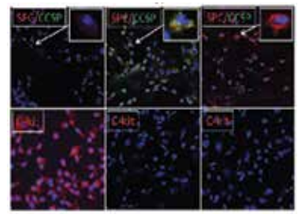
LAB MEMBERS

Senior research assistant: Dr. Yuan Quan



Genetic mutation-free and reprogramming factor-free human iPS cells.

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



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

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

Transgenic and stem cells core facility

The Transgenic and Stem Cells Core Facility was established in 1998 and since that time, it has generated over 700 new transgenic, knock-out and knock-in animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions.

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

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

RESEARCH SERVICES

- Microinjection of DNA, BAC or YAC clones for the production of transgenic animal models.
- Microinjection of ES cells for the production of knock-out and knock-in mice.
- Re-derivation of mice and rats from fertilized eggs.
- Cryopreservation of fertilized mouse and rat eggs.
- CRISPR/Cas9 mediated genome editing in mice.
- Gene targeting, selection, expansion, 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

- Generation of more than 750 transgenic, knock-out and knock-in animal models.
- Consistently high transgenic rates (average 23%).
- 100% success rate of germline transmission in the production of knock-out mice when using ES cells derived at the facility.
- 100% success rate in re-derivation of mice.
- Derivation of more than 20 mouse and human cell lines, including human ES cells approved for NIH-funded research.

KEY PUBLICATIONS

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

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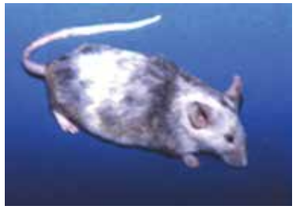
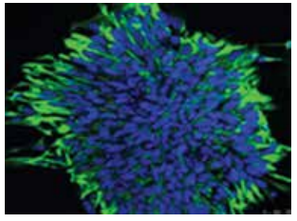
LAB MEMBERS

Manager: Aleksey Domozhirov



Chimeric mouse



Mouse ES cell colony undergoing neural differentiation.

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

- How are progenitors of fat-storing and fat-burning adipocytes regulated?
- How do cells from adipose tissue promote progression of certain cancers?
- Can pharmacological depletion of adipose tissue cells be used therapeutically?
- How does the brain control the body's energy balance?
- What are the transcriptional pathways that can be targeted to treat muscle diseases?
- How is angiogenesis molecularly regulated in health and disease?
- 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?
- How can we ameliorate the neuropsychiatric symptoms of Alzheimer's disease?

To address these questions, the Center employs state-of-the-art methods in model organisms, including the mouse and the fruit fly. Collaboration among the Center's laboratories promotes research synergy, thereby increasing productivity and innovation. The Center's faculty also collaborate with epidemiologists, biochemists, and clinicians to speed the translation of their discoveries for the benefit of patients with metabolic and degenerative diseases.

Mikhail Kolonin, Ph.D.
Associate Professor
Director, Center For Metabolic and Degenerative Diseases
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

Mikhail Kolonin, Ph.D.

Associate Professor
Director, Center For Metabolic and Degenerative Diseases
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

Adipocyte progenitor cells in pathology

The Kolonin Laboratory is investigating the association between obesity and such life-threatening diseases as type-2 diabetes and cancer. We have discovered that white adipocyte progenitor cells serve as the mechanistic link between fat tissue overgrowth and obesity pathogenesis. In clinical studies and in animal models, we have shown that white adipocyte progenitors are mobilized, traffic to tumors, and stimulate cancer progression. Studies elucidating the molecular mechanisms of intercellular interactions in fat tissue and of adipocyte progenitor migration are underway. Our group also has taken the lead in the exploration of pathogenic functions of adipose cells and in developing approaches to their suppression. Based on the expertise in cell population separation and high throughput combinatorial peptide library screening methods, we have identified tissue-specific cell surface receptors and peptide probes for their targeting. Based on them, we are developing a strategy to deplete white adipocyte progenitors for obesity prevention and cancer treatment. A distinct, recently discovered, population of adipocyte progenitors giving rise to metabolically advantageous beige adipocytes is also being explored as a prospective therapy target.

RESEARCH PROJECTS

- Adipose tissue markers and mechanisms of intercellular communication.
- Adipocyte progenitors and dedifferentiated adipocytes in tumor microenvironment.
- Development of therapies targeting white adipocyte progenitors.
- Induction of beige adipocyte progenitor recruitment for metabolic reprograming.

KEY PUBLICATIONS

Daquinag A., Zhang Y. Amaya F., Simmons P.J. and Kolonin M.G. An Isoform of Decorin is a Resistin Receptor on the Surface of Adipose Progenitor Cells, Cell *Stem Cell*. 9(1):74-86, 2011.

Zhang Y., Daquinag A., and Kolonin M.G. Stromal Progenitor Cells from Endogenous Adipose Tissue Contribute to Populations of Pericytes and Adipocytes in Tumor Microenvironment, *Cancer Research*, 15;72(20):5198-208, 2012.

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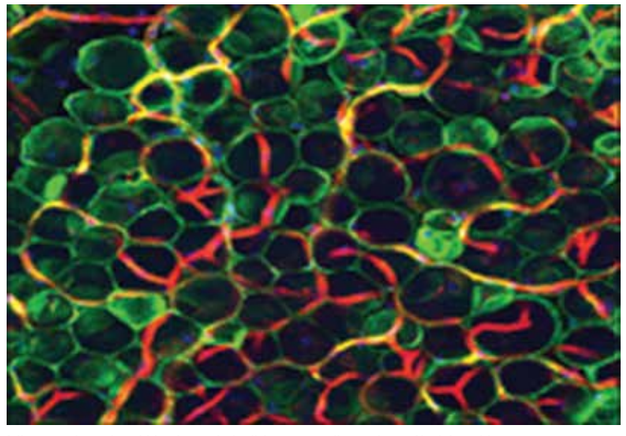
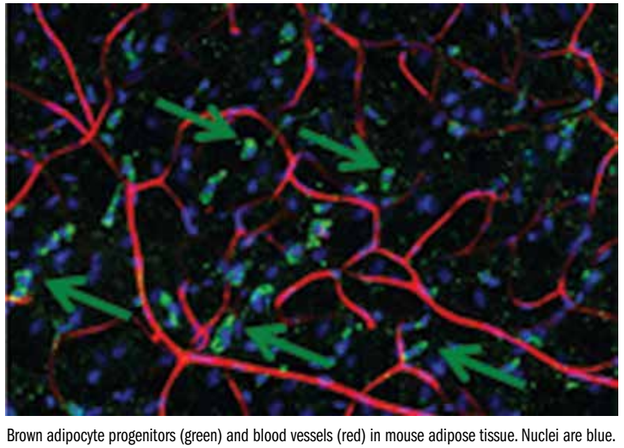
Daquinag A., Tseng C., Salameh A., Zhang Y., Tong Q. and Kolonin M.G. Depletion of white adipocyte progenitors induces beige adipocyte differentiation and suppresses obesity development, *Cell Death and Differentiation*, in press.

LAB MEMBERS

Alexis Daquinag: Sr. research scientist
Zhanguo Gao: Sr. research scientist
Zhang Tao: Postdoctoral fellow
Chieh Tseng: graduate student
Ali Dadbin: senior research assistant



Adipocytes (green) and blood vessels (red) in mouse adipose tissue. Nuclei are blue.



Brown adipocyte progenitors (green) and blood vessels (red) in mouse adipose tissue. Nuclei are blue.

Rebecca Berdeaux, Ph.D.

Assistant Professor

Signaling pathways regulating glycemic control and muscle performance

Many hormones act within the body to respond to internal and external challenges, such as food availability, danger or injury. Our lab is interested in how metabolic tissues respond to hormonal cues by activating or repressing genes. We study pathways and proteins regulated by cAMP, a small molecule that mediates cellular hormone responses, with the overall aim of identifying new targets to improve blood glucose control and skeletal muscle performance in states of diabetes, aging, and muscle injury.

Type 2 diabetes is an endocrine disease caused by over-nutrition, weight gain, and insulin resistance. One of the major problems in type 2 diabetic patients is hyperglycemia (elevated blood glucose), due in part to excessive activation of cAMP-regulated pathways in the liver. Salt inducible kinase 1 (SIK1) is an enzyme known to inhibit new glucose synthesis by liver cells, and we found that SIK1 protein abundance is very strictly regulated in this cell type. We deleted the Sik1 gene in mice to study its impact on glycemic control in normal and obese states. Through this work, we have identified surprising and complex functions of SIK1 on blood glucose control. Current work is focused on understanding how SIK1 and its partners regulate blood glucose in diabetic animals. Using tissue specific knockout mice, we will determine where SIK1 function is most important for regulation of blood glucose in diabetic states.

Our other major project focuses on skeletal muscle, which comprises 45% of body mass and is responsible for 25% of glucose disposal. Maintenance of skeletal muscle mass is thus critical for both mobility and glycemic control. We aim to unravel how cAMP-activated pathways could be harnessed to promote healthy aging and improve muscle strength and regeneration in individuals with muscle disease or atrophy.

cAMP-activating hormones have long been known to promote muscle growth (hypertrophy) and muscle regeneration, and we showed that the cAMP-activated transcription factor CREB promotes division of muscle stem cells. We are currently studying how CREB-stimulated genes, such as SIK1, contribute to muscle regeneration, hypertrophy and performance. We are carefully examining muscle phenotypes in SIK1 knockout mice. We are also undertaking an unbiased approach to identification of genes and proteins that mediate cAMP action in muscle by creating mice that express a "designer" cAMP-activating cell surface receptor that will only respond to a "designer" synthetic drug. We hope that our studies will identify new pathways that can be targeted to promote muscle growth and performance in humans.

RESEARCH PROJECTS

- Role of SIK1 in development and severity of type 2 diabetes.
- Regulation of exercise performance by SIK1.
- Chemical-genetic methods to stimulate muscle stem cell proliferation and muscle regeneration to uncover new pathways that promote muscle regeneration and hypertrophy.
- Role of *Sik1* in endocrine regulation of growth and body temperature.

KEY PUBLICATIONS

Akhmedov D and Berdeaux R. (2013) The effects of obesity on skeletal muscle regeneration. Front. *Physiol*., 4: 371 (PMC3865699).

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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. (PMC3404564)

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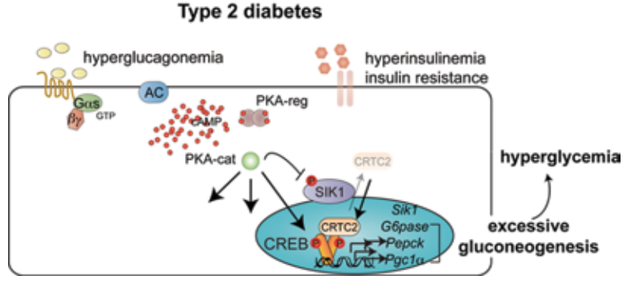
[can be omitted] Berdeaux R, Goebel N, Banaszynski L, Takemori H, Wandless T, Shelton GD, and Montminy M. (2007) SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. *Nature Medicine.* 13(5): 597-603. (Cover illustration)

LAB MEMBERS

Postdoc: Dmitry Akhmedov
Doctoral student: Randi Stewart
Research assistants: Kavitha Rajendran and Maria Mendoza
Medical student (summer): Micah Gibson



Model of CREB and SIK1 action in type 2 diabetes. SIK1 inhibits CREB activity through phosphorylation of the CREB co-activator CRTC2. In diabetes, enhancing SIK1 action is hypothesized to inhibit glucose output.

Nicholas Justice, Ph.D.
Assistant Professor

The role of stress in Alzheimer's disease pathogenesis

We study how stress, anxiety, and depression impact the progression of neurodegenerative disease. Our focus is the Hypothalamic-Pituitary-Adrenal (HPA) axis, the endocrine axis that responds to stress with the release of the hormone cortisol. The HPA axis is activated in early-stage Alzheimer's disease patients, and we are trying to understand both how this occurs and what impact it has on the emotional status of Alzheimer's disease patients. In addition, high levels of circulating cortisol can cause neurons to be sensitive to neurodegeneration caused by ongoing Alzheimer's disease related pathogenesis. We are focusing on strategies to limit HPA axis responses in Alzheimer's disease with the goal of controlling anxiety and depression associated with early stage Alzheimer's disease, along with the hope that blocking HPA axis activity and reducing the levels of circulating cortisol will have decelerating influence on disease progression.

The strategies we are pursuing to address neuropsychiatric symptoms of Alzheimer's disease hinge upon manipulating the neuropepetide Corticotropin Releasing Factor (CRF), the initiator of the HPA axis, as well as its two receptors. CRF acts in the pituitary to activate the HPA axis, but also acts centrally in the brain where it drives anxiety, fear, and addictive behavior. We have recently discovered that Aβ, the aggregating peptide fragment that makes up amyloid plaques in Alzheimer's Disease, can act directly to hyperexcite CRF neurons. This suggests a parsimonious model in which CRF neurons become overactive in Alzheimer's disease causing increased anxiety and depression and driving elevated cortisol levels. We are currently testing this hypothesis *in vivo* using newly designed genetic strategies in mice.

Problems with HPA axis responses and CRF signaling dynamics are thought to be key to the etiology of Post-Traumatic Stress Disorder (PTSD). When our colleagues at the VA here in Houston found that veterans with PTSD were almost twice as likely to suffer from dementia as they age, we used our Alzheimer's disease mouse models to investigate this finding. By modeling PTSD in mice, we have found that Alzheimer's disease mice are sensitive to PTSD-like induction, again suggesting that ongoing Alzheimer's Disease is perturbing stress responses. We are now using PTSD modeling to attempt pharmacologic intervention after trauma exposure to prevent later susceptibility to dementia and neurodegeneration in the context of PTSD.

RESEARCH PROJECTS

- How does stress impact the progression of Alzheimer's disease.
- How does ongoing Alzheimer's disease result in changes in the regulation of stress hormones and alter anxiety levels.
- How does Post Traumatic Stress Disorder accelerate the progression of Alzheimer's disease.
- Targeting amyloid pathology to hypothalamic circuits to understand hormonal misregulation in Alzheimer's disease.
- Local neural circuits that regulate Hypothalamic-Pituitary-Adrenal axis output.
- Functional characterization of neural circuits that respond to stress.
- Modeling Amyotrophic Lateral Sclerosis (ALS) in mouse using a newly discovered expansion in the c9orf72 locus.

KEY PUBLICATIONS

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

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

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

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

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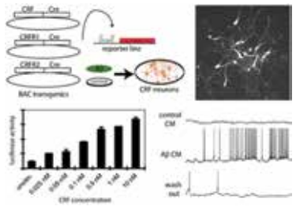
Sztainberg, Y, Kuperman, Justice NJ, Chen, A. An anxiolytic role for CRF receptor type 1 in the globus pallidus. *J Neurosci.* 2011 Nov 30;31(48):17416-24.

Yang L, Wang Z, Wang B, Justice NJ, Zheng H. 2009. Amyloid precursor protein regulates Cav1.2 L-type calcium channel levels and function to influence GABAergic short-term plasticity. *J. Neurosci.* 29(50):15660-8.

Justice NJ, Yuan ZF, Sawchenko PE, and Vale W. "Reconciling Ligand-Receptor Misalignment in the Central CRF System: Insights from a Transgenic Mouse Line Reporting Type 1 Corticotropin-Releasing Factor Receptor Expression." *J. Comp. Neurol* 2008 Dec 1;511(4):479-96.

LAB MEMBERS

Melissa Pruski, Research Technician
Albert Hunt, Graduate Student



Strategy for testing the direct activation of CRF neurons by toxic Aβ species.

Vihang Narkar, Ph.D.
Assistant Professor

Exercise mimicry in vascular, metabolic & degenerative diseases

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

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

RESEARCH PROJECTS

- ERRγ and diabetes
- ERRγ and skeletal muscle ischemic disease
- ERRγ and Duchenne Muscular Dystrophy
- Nuclear receptor atlas in muscle degenerative diseases

KEY PUBLICATIONS

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

Lee CS, Georgiou DK, Dagnino-Acosta A, Xu J, Ismailov II, Knoblauch M, Monroe TO, Ji R, Hanna AD, Joshi AD, Long C, Oakes J, Tran T, Corona BT, Lorca S, Ingalls CP, Narkar VA, Lanner JT, Bayle JH, Durham WJ, Hamilton SL. (2014) Ligands for FKBP12 increase Ca2+ influx and protein synthesis to improve skeletal muscle function. *J Biol Chem*. 289(37): 25556-70.

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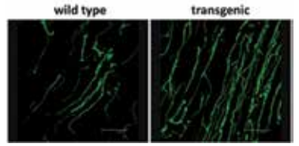
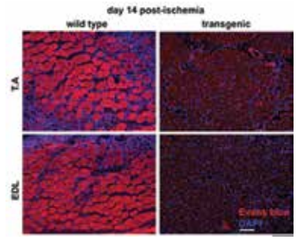
Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM. (2008) AMPK and PPARdelta agonists are exercise mimetics. *Cell.* 134(3): 405-15.

LAB MEMBERS

Post-doctoral fellows: Antonios Matsakas, Vikas Yadav, Pierre-Marie Badin
Technician: Sabina Lorca
Summer Students: Annum Sadana, Patrick Ruggles



Muscle vascularization by ERRγ. Microangiography shows that ERRγ over-expression in the skeletal muscle enhances vascular supply.



Reversal of post-ischemic muscle damage by ERRγ. Evans blue dye (red) exclusion test showing that ischemic muscles from ERRγ transgenic mice recover within 14 days compared to the ischemic muscles from the wild type mice, which remain extensively damaged.

Muscle cross-section showing myofibers surrounded by capillaries (yellow). Cover image of our publication on ERRγ and reparative angiogenesis in Circulation Research.

Qingchun Tong, Ph.D.
Associate Professor
Becker Family Professorship in Diabetes Research

Mechanisms underlying brain control of body weight and glucose homeostasis

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

Specific groups of neurons, especially those in the hypothalamus, receive and integrate nutritional status signals, and then adjust food intake and energy expenditure accordingly to maintain energy balance. Previous research has identified important functions of a few groups of hypothalamic neurons expressing key sets of in feeding, energy expenditure, and glucose homeostasis. Using novel techniques, including Cre-lox P-based mouse genetics, optogenetics, and pharmacogenetics, we aim to identify novel groups of neurons and neural pathways in the brain that are crucial to regulate feeding, diet-induced obesity and glucose homeostasis.

To link neuron function with behavior, we specifically activate or inhibit a distinct group of neurons with various channelrhodopsins (ChRs) by light 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 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.
- Neural pathways responsible for differential responses in diet-induced obesity between males and females.
- Role of GABAergic action in body weight regulation using an inducible and reversible approach.
- Identification of factors that control differential diet-induced obesity.

KEY PUBLICATIONS

Cao X, Xu P, Oyola MG, Yan X, Saito K, Zou F, Xia Y, Wang C, Yang Y, Hinton A. Jr., Yan C, Ding H, Zhu L, Yu L, Yang B, Feng Y, Clegg DJ, Khan S, DiMarchi R, Mani SK, Tong Q, Xu Y. Estrogens stimulate serotonin neurons to inhibit binge-like eating in mice. *J. Clinical Investigation*. 2014, 124 (10): 4351-4362.

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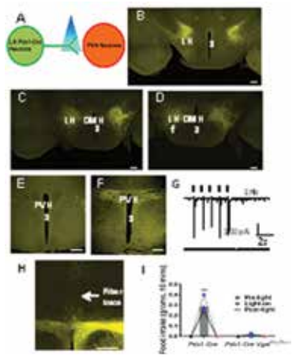
Xu Y, Wu Z, Sun H, Zhu Y, Kim ER, Arenkiel RA, Lowell BB, Xu Y and Tong Q. Glutamate Mediates the Function of MC4Rs on Sim1 Neurons in Body Weight Regulation. *Cell Metabolism*. 2013, 18 (6): 860-870. Corresponding author.

Xu Y, Kim ER, Zhao R, Myers MG, Munzberg H and Tong Q, Glutamate release mediates leptin action on energy expenditure, *Mol. Metabolism*, 2013, 2:109-115. Corresponding author.

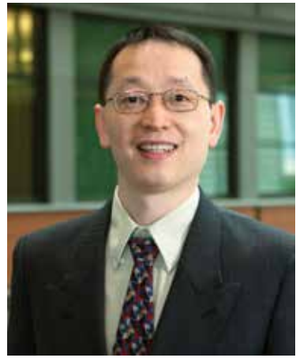
Kong D*, Tong Q*, Ye P, Koda S, Fuller PM, Krashes MJ, Vong L, Ray RS, Olson DP and Lowell BB. GABAergic Rip-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. *Cell,* 2012, 151 (3): 645-657. PMID: 23101631. *: Co-first author.

LAB MEMBERS

Instructor: Yuanzhong Xu
Post Docs: Eun Ran Kim, Hao Sun
Graduate Student: Leandra Mangieri
Visiting Scientists: Shengjie (Holy) Fan, Dakan Liu



Optogenetic stimulation of GABAergic fibers from LH Pdx1-Cre neurons in the PVH promotes feeding in well-fed animals. (A) A diagram showing ChR2-assisted circuitry mapped. (B-D) AAV-FLEX-ChR2-YFP expression pattern in bilateral LH of Pdx1-Cre mice at 2 weeks post viral delivery at the levels of bregma -1.34 mm (B), -1.46 mm (C) and -1.58 mm (D). (E-F) YFP-expressing fibers in the anterior (E) and the posterior PVH (F)F. (G) IPSCs elicited by blue laser (black ticks) at 1 Hz in *Pdx1-Cre* (top) and *Pdx1-Cre:Vgat*$^{flox/flox}$ mice (bottom). (H) A representative implantation of optic fiber cannula above the posterior PVH. (I) Feeding response to laser stimulation during 10-min period in well-fed *Pdx1-Cre* and *Pdx1-Cre:Vgat*$^{flox/flox}$ mice. Data presented as mean ± SEM, n=4-6, **p<0.01, 2-way ANOVA tests. LH: lateral hypothalamus; DMH: dorsomedial hypothalamus; PVH: paraventricular nucleus of hypothalamus; 3: the third ventrical. Scale bar=250 μM.



Sheng Zhang, Ph.D.
Assistant Professor

Molecular mechanisms of human brain degenerative diseases

With a longer life expectancy, a pressing challenge to our society is how to preserve a healthy life, not only physically but also mentally, for the increasing number of elderlies who are more susceptible to aging-related brain degenerative diseases, such as Alzheimer's and Parkinson's.

Our laboratory is studying the mechanisms responsible for these neurodegenerative diseases by combining both mammalian cell culture and the genetic model organism *Drosophila* (fruit flies). The fly, although small and simple, bears many remarkable similarities to humans and is easily manipulated experimentally (Figure 1), providing an excellent animal model for studying human diseases.

Currently our laboratory is focusing on the following directions:

Huntington's disease (HD)

HD is a genetically well-defined brain disease, caused by an abnormal expansion of a polyglutamine tract in the disease protein Huntingtin. However, how this unique mutation leads to relatively restricted destruction of striatal neurons in HD is still not known. The fly also has a Huntingtin-like gene, which we had removed from the fly genome and established the first-reported fly Huntingtin mutant line. We are using this unique tool to study how Huntingtin protein itself, which is known to harbor a neuronal protective activity, normally works in the cell (Figure 1) and how its dysregulation contributes to HD pathogenesis.

Protein misfolding, aggregation, and clearance in the cell

Most neurodegenerative diseases are marked by abnormal protein deposits (e.g., plaques and tangles) in the brain, a pathological feature that can be recapitulated and studied in the fly by targeted expression of human disease proteins in its brain (Figure 2). We are studying how protein aggregates develop and contribute to neuronal loss, and how to employ existing cellular clearance mechanisms such as chaperones and autophagy to prevent their accumulation in the cell (Figure 2).

Intracellular handling of neurotransmitters

In neurons, neurotransmitters, such as dopamine and serotonin, need to be packaged into specialized membrane-enclosed vesicles for their proper regulation and function, while disruption of this cellular process contributes to a spectrum of disorders such as Parkinson's, ADHD, and schizophrenia. The fruit fly has a highly conserved dopaminergic system with similar cellular machineries that control the formation and function of these vesicles (Figures 1 and 3). We are studying how this specialized cellular event is regulated and its potential implication in brain diseases.

RESEARCH PROJECTS

- Huntington's disease and the normal cellular function of Huntingtin.
- Formation of protein aggregates and their clearance in neurons.
- Intracellular handling of neurotransmitters and their dysfunction in brain diseases.

KEY PUBLICATIONS

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*: Huntingtin is a Scaffold Protein Promoting Macroautophagy. In revision. (* corresponding authors)

Rui YN, Xu Z, Chen ZH, and Zhang S*: The GST-BHMT Assay Reveals A Distinct Mechanism Underlying Proteasome Inhibition-Induced Macroautophagy in Mammalian Cells. In revision. (* corresponding authors)

Zhang S*, Binari R., Zhou R., Perrimon N*. (2010) A *Drosophila* genome-wide RNAi screen for modifiers of protein aggregate formation. *Genetics*, 184(4): 1165 - 1179. (* corresponding authors).

Zhang S*, Feany M., Saraswati S, Littleton J.T., Perrimon N*. (2009) Inactivation of *Drosophila* Huntingtin affects long-term adult functioning and the pathogenesis of a Huntington's disease model. *Disease Models & Mechanisms*. 2 : 247-266 (* corresponding authors).

LAB MEMBERS

Instructor: Dr. Zhen Xu,
Post Doc: Dr. Yan-Ning Rui,
Technicians: Zhihua Chen, Ph.D., Research Associate,Lili Ye, Research Assistant I
Ph.D. Students: Antonio Tito, Jian Xiong (rotating)
Undergraduate Students: Mohammad Alsheikh-Kassim (Rice), Shebna Cheema (University of Houston), Daniel Colchado (Rice), Wenting Li (Rice), Zoe Tao (Rice), Sam Louis Vallagomesa (Rice), Kira Wegner-Clemens (Rice)

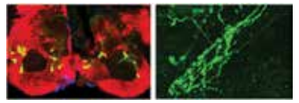


Figure 1. (A) Dopamine (green) and serotonin (blue) neurons in a fly brain (outlined in red by a neuronal marker). (B) Axonal terminal morphology (green) of a label neuron in a huntingtin knockout fly brain.

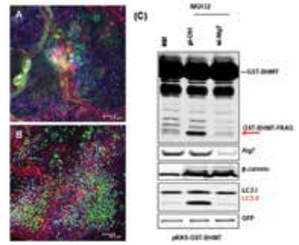


Figure 2. (A & B) High-magnification view of fly brains expressing human mutant Huntingtin protein (green) with a 47 (A) or 72 (B) polyglutamine tract. Note the prominent aggregates (green in B) that partially co-localize with autophagy markers ubiquitin (blue) and Ref(2)P (red). (B) BHMT-based biochemical assay for autophagy activity in mammalian cells, as revealed by significant accumulation of autophagy markers LC3 and GST-BHMT-FRAG in test condition.

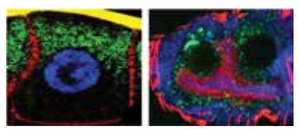


Figure 3. Cellular organelle formation. (A) An enrichment of membrane-bound vesicles (green) on the apical side (top) of a polarized secretory cell. Cell boundary is outlined in red and its nucleus labeled in blue. (B) Non-polarized distribution of organelles of lysosomal-origin (green) in three *Drosophila* cells (labeled in blue) surrounded by muscles (red).

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

The CMI houses a diverse, interdisciplinary team of scientists and engineers who develop and use multi-modality molecular diagnostics and imaging techniques, including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and near-infrared (NIR) fluorescence to enable new understandings in several disease states.

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

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

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

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

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

CENTER FOR **MOLECULAR IMAGING**

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

Molecular imaging and diagnostics

The Center for Molecular Imaging (CMI) consists of an interdisciplinary team of scientists and engineers who focus upon multi-modality molecular imaging including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and our specialty, near-infrared (NIR) fluorescence to enable new understandings in several disease states. In addition to having its own basic science and clinical research projects, the team also operates a "collaboration" center where clinicians and basic scientists from across the Texas Medical Center partner with CMI members to effectively apply diagnostics in preclinical and clinical studies. Our team effectively translates new NIR molecular imaging technologies literally from "bench-to-bedside" and back again in order to make discoveries in translational research. The CMI is one of four Centers in the United States comprising the National Cancer Institute's Network for Translational Research and has won the 2013 Most Promising Crossover Technology Award from the Rice Alliance.

Discoveries made from the translational near-infrared lymphatic imaging studies conducted by the CMI team, include identifying key signaling pathways and regulators associated with aberrant processes of lymphangiogenesis in human diseases and in animal models of human disease. In addition, the team has incorporated new far red gene reporters into novel animal models for small animal imaging and tomography with unique instrumentation that enables visualization of never-before-seen biological phenomenon.

RESEARCH PROJECTS

- Developing, building, and translating NIR fluorescence imaging instrumentation and algorithms for multi-modality molecular imaging and tomography in preclinical and clinical studies.
- Designing, producing, and validating unique NIR and nuclear imaging probes for assessing molecular pathways in preclinical studies and for enhanced diagnostics in Phase I and Phase I/II combination device/drug clinical studies.
- New molecular imaging agents for non-invasive diagnostic imaging for nodal staging in breast, prostate, melanoma, and other cancers.
- Using molecular imaging to understand the process of lymphangiogenesis involved in cancer metastasis, infection, injury and trauma, vascular diseases, and hereditary disease in unique animal models.
- Evaluating molecular signaling in the process of tissue re-organization in health and disease, including bone fracture, athlerosclerosis, and cancer.
- Combining molecular imaging and unique knockout animal models to understand the molecular genetics of disease.

KEY PUBLICATIONS

Sevick-Muraca, E.M., Kwon, S.K., and J.C. Rasmussen, "Emerging lymphatic imaging technologies for mouse and man," *Journal of Clinical Investigation*.

Darne, C., Lu, Y., and E.M. Sevick-Muraca, "Small animal fluorescence and bioluminescence tomography: new approaches, algorithms, and technology update," *Physics in Medicine and Biology* (accepted, invited review), 2013.

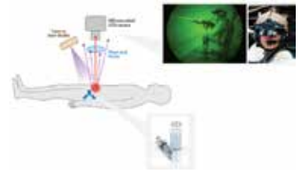
Burrows, P.E., Gonzalez-Garay, M.L., Rasmussen, J.C., Aldrich M.E., Guilliod R., Maus, E.A., Fife, C.E., Kwon, S., Lapinski, P.E., King, P.D., and E.M. Sevick-Muraca, "Lymphatic abnormalities are associated with RASA1 mutations in mouse and man," *Proc Natl Acad Sci*, epub ahead of print May 6, 2013. PMID: 23650393

Prabhakar, U., Maeda, H., Jain, R.K., Sevick-Muraca, E.M., Zamboni, W., Farokhzad, O.C., Barry, S.T., Gabizon, A., Grodzinski, P., and D.C. Blakey, "Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology," *Cancer Res*, 73(8): 2412-17, 2013. PMID: 23423979

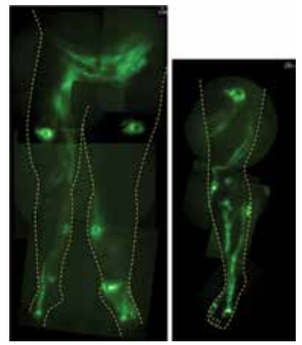
E.M. Sevick-Muraca, "Translation of near-infrared fluorescence imaging technologies: emerging clinical applications," *Ann Rev Med*, 63: 217-31, 2012, Epub 2011 Oct 27. PMID: 22034868

LAB MEMBERS

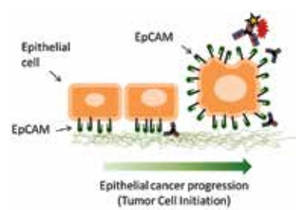
Co-Director of Flow Cytometry Service Unit: Amy Hazen
Chief Histology Technician of Tissue Histology Service Unit: Sarah Amra
Research Coordinators: Holly Robinson, Nathaniel Wilganowski, Karen Gore, Grace Wu
Postdoctoral Fellow: Dr. Chinmay Darne (co-advised)
Graduate Students: Germaine Agollah (co-advised)chance, Cynthia Davies-Venn



Near-infrared fluorescence imaging device and drug combination



Lymphatic imaging in case of Parkes Weber Disease (from Burrows, et al. 2013)



Target for combined NIR/PET imaging agent development (With Harvey and Azhdarinia)

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

Imaging in immunology

I bring a combination of expertise in translational science and immunology to lead the program of imaging of the lymphatics, the circulatory system, which is critical to immune surveillance and response. Near-infrared fluorescence (NIRF) imaging delivers high-resolution, low-cost images of lymphatic vessel architecture and pumping. In disease states such as lymphedema, manifested by severe limb swelling, NIRF imaging can provide information for diagnosis and evaluation of treatment efficacy. As part of a translation team, I have conducted clinical measurements that prove the usefulness of NIRF imaging to investigate lymphatic vessel architecture and function in health and disease. Our 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.

"Translation" is a much-used term in research that stresses the importance of research that is relevant to medical practice. Truly crossing the "bench to bedside" chasm, however, requires skills that most basic science researchers are not taught. I am formally and practically trained in translation requirements.

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Burrows PE, Gonzalez-Garay ML, Rasmussen JC, Aldrich MB, Guilliod R, Maus EA, Fife CE, Kwon S, Lapinski PE, King PD, Sevick-Muraca EM. Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. 2013. *Proc Natl Acad Sci USA* 110:8621-6.

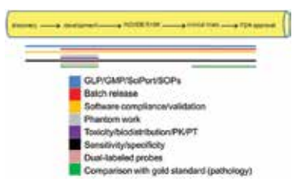
Aldrich MB, Guilliod RG, Fife CE, Maus EA, Smith L, Rasmussen JC, Sevick-Muraca EM. Lymphatic abnormalities in the normal contralateral arms of subjects with breast cancer-related lymphedema as assessed by near-infrared fluorescent imaging. 2012. *Biomedical Optics Express* 3:1256-65.

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

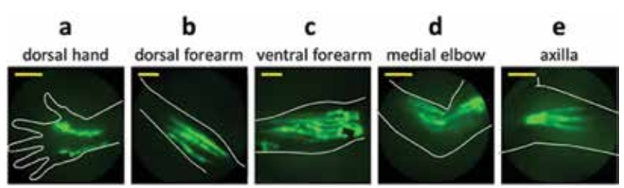
Aldrich MB, Sanders D, Lapteva N, Huang, XF, and Chen SY. SOCS1 downregulation in dendritic cells promotes memory T cell responses. 2008. *Vaccine* 26(8):1128-35.

LAB MEMBERS

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



Translation "pipeline" for optical imaging modalities



Normal arm lymphatic vessel architecture

Ali Azhdarinia, Ph.D.
Assistant Professor

Molecular imaging probe development

I am the faculty lead of the Radio- and Optical-Pharmaceutical development effort in the Center for Molecular Imaging (CMI). My research interests include the development of targeted agents for the visualization and treatment of cancer. I have served as the leader of the National Cancer Network's Network for Translational Research (NTR) Chemistry Core and am heavily involved in validation and qualification of preclinical studies prior to translation in both NTR-wide and CMI local studies. My work utilizes radioactive and near-infrared fluorescent (NIRF) contrast agents, which can be used for whole-body and intraoperative imaging, respectively, and may potentially improve surgical outcome while minimizing morbidities associated with current methods. The combination of both modalities into a single agent is a key area where I have focused my efforts through synthesis of a library of new multimodal chelation (MMC) platforms. My lab uses radiometal-based positron emitters, such as Gallium-68 and Copper-64, for labeling of peptides, proteins, and antibody-based agents and also conducts full pharmacological characterization of lead compounds to determine suitability for clinical translation. As part of the Center for Molecular Imaging, I have participated in establishing a dedicated clean room for production of probes under Current Good Manufacturing Practices (cGMP) to facilitate translational research. I am actively collaborating with clinical partners to establish creative approaches for translating "dual-labeled" agents.

RESEARCH PROJECTS

- Development of molecular imaging probes with radioactive and near-infrared labels
- Synthesis of novel chelation platforms for radiolabeling and drug design
- Optimization of NIRF labeling methods
- Pharmacological evaluation of imaging probes targeting tumors and other molecular processes

KEY PUBLICATIONS

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

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

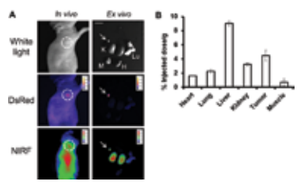
Sevick-Muraca, E.M., Akers, W.J., Joshi, B.P., Luker, G.D., Marnett, L.J., Contag, C.H., Wang, T.D. and Azhdarinia, A. Advancing the translation of optical imaging agents for clinical medical imaging. *Biomedical Opt Express*. 4(1): 160-70, 2013. PMID:23304655

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

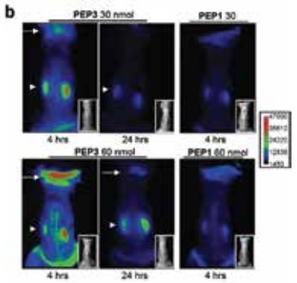
Cisneros, B.T., Matson, M.L., Law, J.L., Azhdarinia, A., Sevick-Muraca, E.M., Wilson, L.J. Stable confinement of PET & MR agents within carbon nanotube capsules for in vivo bimodal imaging. *Nanomedicine* (Lond). 2014 Mar 17. [Epub ahead of print]. PMID:24628687.

LAB MEMBERS

Research Scientist: Sukhen Ghosh



Representative multimodality images in a tumor-bearing mouse at 40 h post-injection of ^{64}Cu-labeled mAb7 (A). Focal tumor signal was visualized by DsRed and NIRF imaging in vivo (circle). Ex vivo imaging on selected tissues showed comparable fluorescence levels in the kidneys and tumor with low signal elsewhere. Quantification of ^{64}Cu-mAb7 uptake is represented in (B) and indicates highest signal in liver, tumor, and kidneys. Arrow indicates excised tumor. K = kidney, Lu = lung, H = heart, M = muscle. Scale bar = 1.6 cm. (from Ghosh, S.C. et al., *J Med Chem*, 56(2):406-16, 2013).



Biodistribution of PEP3 conjugated with a NIR fluorophore. (a) HPLC chromatograms of peptide probes. Left: UV detection of PEP3 and WAT-homing peptide CKGGRAKDC (containing two Dde protecting groups on internal Lys residues) at 280 nm. AU denotes relative absorption units. Right: fluorescence detection of PEP3-IRDye800, and CKGGRAKDC-IRDye800. (b) Whole-body NIR fluorescence imaging of cold acclimated mice 4 and 24 hrs after iv administration of indicated doses of IRDye800-conjugated PEP3 or control PEP1. Arrows: interscapular signal; arrowheads: perirenal signal. Insets show black/white photographs of mice that had skin removed from the back for imaging. Right: Plotted data analysis from n=3 mice per group (mean + s.e.m., *P<0.05, Student's t-Test). (c) NIR fluorescence imaging of ip WAT and interscapular BAT isolated from cold-acclimated mice iv-injected with increasing doses of IRDye800-conjugated PEP3 or a control peptide (PEP1) after 1 hr of circulation. Scale shows fluorescence intensity. Black/white photographs of tissues are shown below. Scale bar: 5 mm. Graph: plotted quantitative data corresponding to NIR images. (adapted from Azhdarinia, A. et al., *Nat Commun*. 4:2472, 2013).

Manuel L. Gonzalez-Garay, Ph.D.
Assistant Professor

Personalized medicine using bioinformatics and whole genome sequencing for early discovery and diagnosis of human disorders

My program is motivated by the unprecedented achievement in which the entire human genome was sequenced to near completion in 2000 by hundred of scientists worldwide using sequence technology that was develop in the early 1970s by Frederick Sanger. It took over 10 years and over $3 billion to sequence for first time the human genome. The development of massively parallel DNA sequencing technologies (Next Generation Sequencing, NGS) in 2005 brought a paradigm shift to biomedical research. NGS made it possible to sequence a human genome for few thousand dollars in few weeks, transferring the challenge of sequencing a genome to the bioinformatics analysis and interpretation of the information.

I foresee a day in the near future when getting your genome sequenced and interpreted will be standard practice. To get to this point, we need to develop tools to analyze the whole genome sequence, interpret the information and detect markers that will allow physicians to develop personalized treatment for every patient. My group recently published a proof of concept study of the usability of next generation sequencing (NGS) for genetic screening of healthy adults. For our study we specifically selected a group of middle age individuals with abundant medical records and strongly motivated to improve their health. There are many conditions that are detected at middle age for example cardiovascular disorders, eye disorders like cataracts, hearing loss, metabolic disorders, and many types of cancers. Many of our volunteers already suffered and survived many of these maladies but they lack of a molecular explanation for the disorder. Our findings were substantial, we linked personal disease histories with causative disease genes in 18 volunteers, in addition we identified risk alleles for breast, ovarian, colon, prostate cancer in many volunteers some of them with previous cancer diagnosis or/and strong family history.

Another main focus of my laboratory is to detect and associate genetic markers (variations) with rare genetic disorders. We recently published an important discovery of a new association between the gene MAGEL2, autism and Prader-Willi Syndrome. We also have been able to demonstrate that mutations in RASA1 gene are associated with lymphatic abnormalities in mouse and humans. My group currently has multiple collaborations with renowned scientists at UTHealth, who work in multiple areas like Drs. Eva Sevick (Lymphedema), Peter Doris (High blood pressure and kidney function), Brian Davis (Stem Cell), Hope Northrup (Pediatric disorders), Michael Lorenz (*Candida albicans*), etc. In addition, we are working with several other scientists from other institutions to identify genetic markers associated with familial panic disorders, Dercum's disease, Adiposis dolorosa and Madelung's disease.

RESEARCH PROJECTS
- Genome and Bioinformatics Analysis of patients with Lymphedema.
- Personalized medicine using next generation sequencing: The CEO Genome Project.
- Detection of markers for sudden death syndrome in a population from Venezuela. Collaborator of Dr. Rosalva Rodríguez, Instituto Venezolano de Investigaciones Cientificas.
- Identification of genetic markers for Panic disorders in Monterrey, N. L. Mexico. Collaborator of Dr. Augusto Rojas-Martinez, Universidad Autonoma de Nuevo Leon.
- Virulence factor identification by comparative transcriptomics in Candida species. Collaborator of Michael Lorenz, Ph.D. UTHealth.
- Hypertensive Renal Injury. Collaborator of Peter Doris, Ph.D. UTHealth.
- Dercum's disease, Adiposis dolorosa, Madelung's disease. Collaborator of Karen L Herbst, M.D. UC San Diego.

KEY PUBLICATIONS

Gonzalez-Garay M.L., Cranford SM, Braun MC, Doris PA. 2014. Diversity in the preimmune immunoglobulin repertoire of SHR lines susceptible and resistant to end-organ injury. *Genes Immun.* PMID: 25056448.

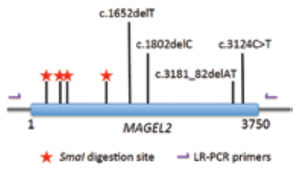
Brownstein CA, Beggs AH, Gonzalez-Garay M.L., et al. 2014. An international effort towards developing standards for best practices in analysis, interpretation and reporting of clinical genome sequencing results in the CLARITY Challenge. *Genome Biol.*15(3):R53. PMID: 24667040.

Gonzalez-Garay M.L., McGuire AL, Pereira S, & Caskey CT (2013) Personalized genomic disease risk of volunteers. *Proc Natl Acad Sci U S A.* 110(42):16957-16962.

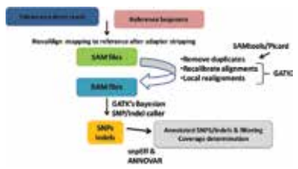
Gonzalez-Garay M.L.*#, Schaaf CP*#, Xia, F*., Potocki, L., Gripp, K. W., Zhang, B., Peters, B. A., McElwain, M. A., Drmanac, R., Beaudet, A. L., Caskey, C. T., Yang, Y. (2013) Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet.* 45:1405-1408.

Gonzalez-Garay M.L.*, Burrows P.E.*, Rasmussen J. C.*, Aldrich M. B., Guilliod R., Maus E. A., Fife C. E., Kwon S., Lapinski P. E., King P. D. & Sevick-Muraca E. M. (2013) Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man. *Proc Natl Acad Sci U S A* 110(21):8621-8626.

LAB MEMBERS
Research Coordinator: Karen Gore
Co-advised: Germaine Agollah



Mutations on MAGEL2 associated with Prader-Willi



Basic steps in our variant analysis pipeline.

Barrett Rowland Harvey, Ph.D.
Assistant Professor

Therapeutic and diagnostic antibody development

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

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

KEY PUBLICATIONS

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

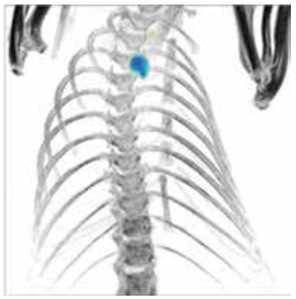
Pinkston KL*, Gao P*, Diaz-Garcia D, Sillanpää J, Nallapareddy SR, Murray BE, and Harvey BR. "The Fsr quorum-sensing system of Enterococcus faecalis modulates surface display of the collagen-binding MSCRAMM Ace through regulation of gelE." *Journal of Bacteriology,* Sep;193(17):4317-25 2011. PMID: 21705589

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

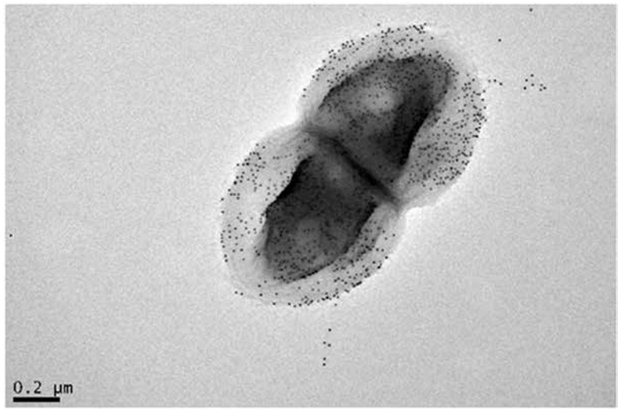
Pinkston, KL, Singh KV, Gao P, Wilganowski N, Robinson H, Ghosh SC, Azhdarinia A, Sevick-Muraca EM, Murray BE, Harvey, BR. "Targeting Pili in Enterococcal Pathogenesis" *Infection and Immunity*, 2014 Apr;82(4):1540-7. PMID: 24452680. (Featured article on April 2014 Cover).

Gao P, Pinkston KL, 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.* 2014 Aug 19. [Epub ahead of print] PMID: 25135058

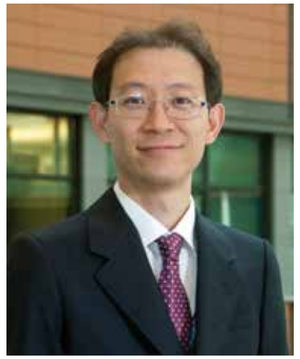
LAB MEMBERS
Kenneth L. Pinkston – Research Coordinator II
Dr. Peng Gao – Research Instructor
Emily Stinemetz – Graduate Student



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 pili structure on the bacterial target.



Labeling and electron microscopy evaluation of major pilin subunit, EbpC, on surface of *Enterococcus faecalis* using in-house generated high affinity monoclonal antibody.



Sun Kuk Kwon, Ph.D.
Assistant Professor
The Carolyn Frost Keenan Professorship in Cardiovascular Disease Research

Functional lymphatic imaging in animal models of lymphovascular disorders

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

Other directions of our 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, and inflammation and tracking response to therapeutic agents.
- Non-invasive imaging of gastrointestinal motility using a fluorescence optical imaging technique.
- Multi-modal molecular imaging.

KEY PUBLICATIONS

S. Kwon, G. D. Agollah, G. Wu, and E. M. Sevick-Muraca, "Direct visualization of changes of lymphatic function and drainage pathways in lymph node metastasis of B16F10 melanoma using near-infrared fluorescence imaging," *Biomedical Optics Express*, 30; 967-977, 2013.

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

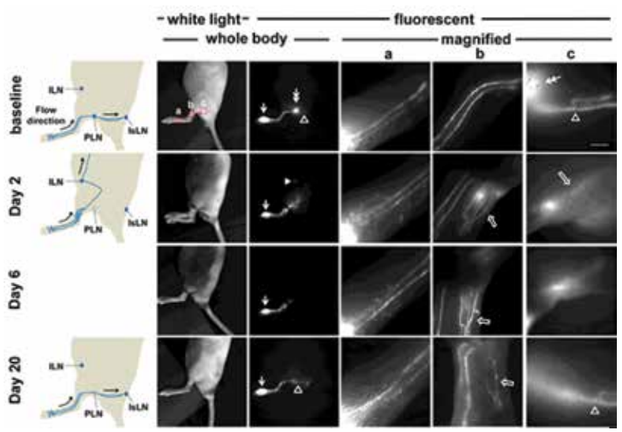
E. M. Sevick-Muraca, S. Kwon, and J. Rasmussen, "Emerging Lymphatic Imaging Technologies for Mouse and Man," *Journal of Clinical Investigation*. 124; 905-914, 2013.

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

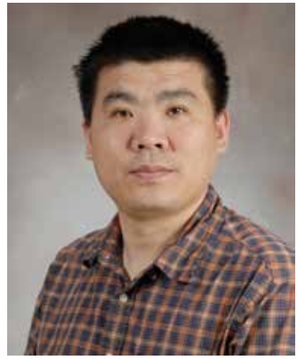
D. A. Germaine, G. Wu, E. M. Sevick-Muraca, and S. Kwon, "In vivo lymphatic imaging of a human inflammatory breast cancer model," *Journal of Cancer*. Accepted for publication, 2014.

LAB MEMBERS

Student co-advised: Germaine Agollah
Research Coordinators: Grace Wu, Holly Robinson



White light and fluorescent images in mice prior to and 2, 6, and 20 days after popliteal lymph node (PLN) removal. Images were acquired 10 mins after intradermal injection of ICG. At 2 days post-surgery, functional fluorescent lymphatic vessels branching from previously observed pre-existing vessels before surgery were detected. These vessels draining from the injection site to the inguinal LN (ILN) were not detected at day 20, whereas collecting lymphatic vessels from the injection site to the ischial LN (IsLN) through the site of PLN removal were visualized. Thus, alternate drainage pathways were detected due to redirection of lymph flow 2 days after lymphadenectomy and ICG-laden lymph moved within a continuous network of lymphatic vessels due to decreased flow resistance at 20 days post-lymphadenectomy. Magnified fluorescent images of the red rectangles (a, b, and c) were also acquired. Arrow, ICG injection site. Double arrow, PLN. Open arrowhead, popliteal efferent collecting lymphatic vessel. Arrowhead, ILN. Open arrow, newly detected fluorescent lymphatic vessels after PLN removal. Scale bar, 1 mm.



Yujie Lu, Ph.D.
Assistant Professor

Program for multimodal optical tomography and relevant preclinical applications and clinical translation

I lead the development of optical tomography in the Center for Molecular Imaging. Optical molecular imaging offers a new tool to monitor the occurrence and development of biological processes and has potential to provide early imaging diagnostic information in the clinic by making use of the specific probes to target specific biological targets and diseases at the molecular and cellular levels. Although the advanced imaging sensors such as high-sensitivity scientific charge-coupled device (CCD) cameras afford high-quality images detected from the surface of the small animal or patient, the acquired planar images cannot provide 3-D quantitative tomographical imaging information, which has leashed the development of optical molecular imaging.

My work is: (i) to exploit the state-of-art imaging theory and methods to develop the fast, robust, and accurate reconstruction algorithm for 3-D optical tomography; (ii) to develop simulated and experimental strategies and platforms to assess and optimize the optical imaging systems; (iii) to make use of the developed multimodal tomography imaging system to perform preclinical imaging research; and (iv) to ultimate translate tomography to pertinent clinical problems.

RESEARCH PROJECTS

- Develop the photon immigration simulation platform using Monte Carlo methods and radiative transfer-based models;
- Develop the fast, robust, and accurate reconstruction algorithms for the multimodal time-dependent fluorescence imaging system;
- Develop fluorescence gene reporter tomography to monitor the development of prostate cancer and relevant metastasis using the nanoparticle techniques;
- Perform multimodal fluorescence tomography for BMP2-based ossification for spinal fusion;
- Perform cancer nodal staging research using the developed fluorescence tomography in the clinical trials.

KEY PUBLICATIONS

Darne, C.D., Lu, Y., and Sevick-Muraca, E.M. "Small animal fluorescence and bioluminescence tomography: a review of approaches, algorithms and technology update." *Physics in Medicine and Biology* 59: R1-R64, 2014

Lu, Y., Darne, C.D., Tan, I., Zhu, B., Hall, M.A., Lazard, Z.W., Davis, A.R, Simpson, L., Sevick-Muraca, E.M., and Olmsted-Davis, E.A. "Far-red fluorescence gene reporter tomography for determination of placement and viability of cell-based gene therapies," *Optics Express* 21:24129-24138, 2013.

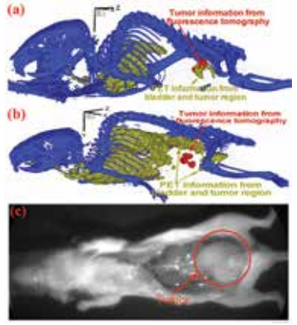
Lu, Y., Darne, C.D., Tan, I., Wu, G., Wilganowski, N., Robinson, H., Azhdarinia, A., Zhu, B., Rasmussen, J.C. and Sevick-Muraca, E.M. "*In vivo* imaging of orthotopic prostate cancer with far-red gene reporter fluorescence tomography and *in vivo* and *ex vivo* validation," *Journal of Biomedical Optics* 18, 101305-101305 (2013).

Darne, C.D.*, Lu, Y. *, Tan, I. *, Zhu, B., Rasmussen, J.C., Smith, A.M., Yan, S. and Sevick-Muraca, E.M. "A Compact Frequency-domain Photon Migration System for Integration Into Commercial Hybrid Small Animal Imaging Scanners for Fluorescence Tomography", *Physics in Medicine and Biology*, 57:8135-8152, 2012 ("*": equal contribution)

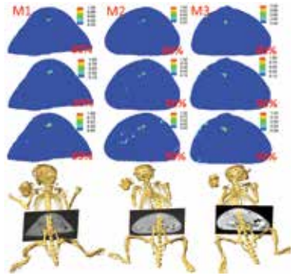
Lu, Y., Machado, H.B., Bao, Q., Stout, D., Herschman, H., and Chatziioannou, A.F., " In vivo Mouse Bioluminescence Tomography with Radionuclide-Based Imaging Validation," *Molecular Imaging and Biology*, 13:53-58, 2011.

LAB MEMBERS

Co-advisement: Chinmay Darne (post-doc), Holly Robinson, Nathaniel Wilganowski



iRFP gene reporter fluorescence tomography overlaid on CT and PET at different tumor stages. (a) and (b) are reconstructed results 4 and 10 weeks after cell implantation, respectively. Blue represents the skeletal information from CT images; yellow represents PET imaging information; and red represents the reconstructed results of fluorescence tomography. The artifacts on the mouse surface are removed for better demonstration. (c) *In situ* white light image for euthanized mouse depicted in (b) (the liver and intestine were removed).



The reconstructed IFP1.4 gene reporter distribution in the cross-sections with the maximal reconstructed values (the first, second and third rows). Top 80%, 90%, and 99% reconstructed values are shown, respectively. The fourth row shows the position of the cross-sections. "M1", "M2", and "M3" are Mouse 1, 2, and 3, respectively.

John Rasmussen, Ph.D.
Assistant Professor

Device translation for lymphatic imaging

I am the faculty lead of the instrumentation for translational fluorescence imaging. Traditional clinical imaging modalities, such as scintigraphy, X-ray, MRI, and ultrasound lack the spatial and/or temporal resolutions needed to resolve fine lymphatic architecture and contractile function and/or require quantities of contrast agent not easily introduced into the lymphatics. Over the past few years, my research interest has focused upon the development and translation of near-infrared fluorescence (NIRF) optical imaging as a way to noninvasively image and characterize human lymphatics and quantify their contractile function in health and disease using microdose amounts of fluorescent contrast agent.

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

RESEARCH PROJECTS

- Nodal staging of cancer using noninvasive NIRF imaging.
- Etiology of cancer-related lymphedema.
- Identification of genetic causes for lymphovascular diseases.
- Development of automated NIRF image analytical algorithms.
- Application driven enhancement of NIRF imaging systems.

KEY PUBLICATIONS

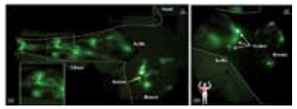
Rasmussen, J.C., Herbst, K.L., Aldrich, M.B., Darne, C.D., Tan, I-C., Zhu, B., Guilliod, R., Fife, C.E., Maus, E.A., Sevick-Muraca, E.M., "An abnormal lymphatic phenotype is associated with subcutaneous adipose tissue deposits in Dercum's disease," *Obesity,* 22(10): 2186-2192, 2014.

Sevick-Muraca, E.M., Kwon, S.K., and J.C. Rasmussen, "Emerging lymphatic imaging technologies for mouse and man," *Journal of Clinical Investigations*, 124(3): 905-914, 2014.

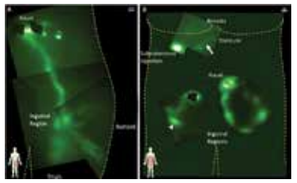
Rasmussen, J.C., Burrows, P.E., Gonzalez-Garay, M.L., Aldrich, M.B., Guilliod, R., Maus, E.A., Fife, C.E., Kwon, S., Lapinski, P.E., King, P.D., and E.M. Sevick-Muraca, "Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man," *Proceedings of the National Academy of Sciences*,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

Zhang, J., Xiang, X., Zhou, S.K., Bautista, M., Nicom, B., Dickinson, G., Tan, I-C., Chan, W., Sevick-Muraca, E.M., and J.C. Rasmussen, "Validation of AFLIA for quantitative lymphatic imaging analysis," *Biomedical Optics Express*, 3(7):1713-1723, 2012.

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.



(a) Image montage of lymphatic vasculature in the arm, axilla, and breast of subject. While most of the lymphatics are linear and well-defined, atypical tortuous lymphatics are noted in the arm (inset box) and the drainage areas from the areola towards both the sternum and the axilla. (b) Image of axillary lymph nodes. Reproduced from Meric-Bernstam, et al., Biomedical Optics Express, 5(1): 183-196, 2014.



(A) Montage of the lymphatic drainage to the inguinal region from intradermal injections near the navel, above and below the buttock, and from the leg in a normal subject. (B) Montage of the lymphatic drainage of the abdomen of a subject with Dercum's Disease. The arrow identifies the location of a dim lymphatic vessel draining the subcutaneous injection below the right breast. The arrowhead identifies the location of a fluorescent, painful fibrotic mass palpated in the right inguinal region. The dotted circles show locations of identified lipomas. Injection sites are covered by round bandages and/or black vinyl tape. Reproduced from Rasmussen, et al., Obesity, 22(10): 2186-2192, 2014.

I-Chih Tan, Ph.D.
Assistant Professor

Instrumentation and medical applications of NIRF imaging

My research program focuses upon the application-specific development of near-infrared fluorescence (NIRF) imaging technologies for unmet clinical needs, as well as broad-based development of technologies for basic science investigation.

In the first research arena, I work with clinicians to apply measurements of lymphatic function to understand the etiology of disease. So far our understanding of the lymphatic architecture and function and its role in many diseases in limited due to the lack of a suitable imaging technique that has sufficient spatial and/or temporal resolutions. Recently, our team developed and translated lymphatic imaging technology using NIRF optical imaging with microdose amounts of fluorescent contrast agent. It allowed visualization of the lymphatics and quantification of their contractile function in humans and animals.

My work currently focuses on developing and optimizing NIRF lymphatic imaging instrumentations and image analysis algorithm, as well as utilizing this technology in biomedical research and applications. For example, using this technology I studied the abnormal lymphatic drainage pattern in a pediatric postoperative chylothorax case. I also studied the lymphatic function in a compassionate case of head and neck lymphedema and have secured funding to expand the study to understand the role of surgery and radiation in the development of lymphatic dysfunction.

Another focus of my work is developing and optimizing the instrumentation for time-dependent optical tomography system and integrating the system into a commercial scanner to perform multi-modality (PET/CT/optical) molecular tomography in small animals. This hybrid imaging system allows us to validate the performance of the optical tomography system against the "gold standard" nuclear imaging using dual-labeled imaging agents developed by other faculty in the team. It also provides many opportunities to longitudinally study the molecular mechanisms of cells and diagnostic/therapeutic biological agents *in vivo*.

RESEARCH PROJECTS

- Developing, building, and translating NIRF lymphatic imaging instrumentation and image analysis algorithm in preclinical and Phase I/II clinical studies.
- Studying lymphatic drainage patterns in children with pleural effusion after heart surgery using NIRF imaging.
- Studying lymphatic architecture and functions before and after cancer treatment in head and neck cancer patients longitudinally using NIRF imaging.
- Evaluating the effects of conventional LE treatments and novel treatment devices using NIRF imaging.
- Developing and building time-dependent optical tomography system for hybrid molecular imaging in preclinical studies.

KEY PUBLICATIONS

I. C. Tan, D. Balaguru, J. Rasmussen, R. Guilliod, J. Bricker, W. Douglas, and E. Sevick-Muraca, "Investigational Lymphatic Imaging at the Bedside in a Pediatric Postoperative Chylothorax Patient," *Pediatric Cardiology,* vol. 35, pp. 1295-1300, 2014.

I. C. Tan, C. D. Darne, Y. Lu, B. Zhu, J. C. Rasmussen, A. M. Smith, S. Yan, and E. M. Sevick-Muraca, "A compact frequency-domain photon migration system for integration into commercial hybrid small animal imaging scanners for fluorescence tomography," *Phys Med Biol*, vol. 57, pp. 8135-52, 2012.

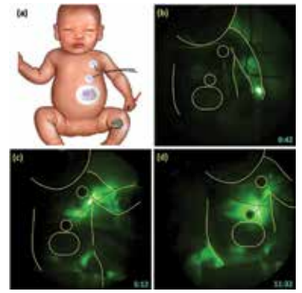
I. C. Tan, E. A. Maus, J. C. Rasmussen, M. V. Marshall, C. E. Fife, L. A. Smith, R. Guilliod, and E. M. Sevick-Muraca, "Near-infrared fluorescence imaging of lymphatics in head and neck lymphedema," *Head & Neck*, vol. 34, pp. 448-453, 2012.

I. C. Tan, E. A. Maus, J. C. Rasmussen, M. V. Marshall, K. E. Adams, C. E. Fife, L. A. Smith, W. Chan, and E. M. Sevick-Muraca, "Assessment of lymphatic contractile function after manual lymphatic drainage using near-infrared fluorescence imaging," *Arch Phys Med Rehabil*, vol. 92, pp. 756-764 e1, 2011.

J. C. Rasmussen, I. C. Tan, M. V. Marshall, C. E. Fife, and E. M. Sevick-Muraca, "Lymphatic imaging in humans with near-infrared fluorescence," *Curr Opin Biotechnol*, vol. 20, pp. 74-82, 2009.

LAB MEMBERS

Co-advised: Chinmay Darne, Rodney John Morrow



Time laps of near-infrared fluorescence lymphatic images (b-d) showing abnormal lymph drainage from left hand to axilla and retrograde into left chest in a pediatric postoperative chylothorax patient. Time-stamps indicate time after the injection in mm:ss. (Reproduced from Tan, *et al.* 2014)

Near-infrared fluorescence lymphatic imaging (left) and 3D photogrammetry (right) of a human subject with head and neck lymphedema (Reproduced from Maus, *et al.* 2012).

Banghe Zhu, Ph.D.
Assistant Professor

Program for instrumentation for near-infrared fluorescence (NIRF)-guided tumor detection in ambient light and for performance standard for clinical translation of NIRF imaging

Successful image-guided surgery based upon fluorescently labeled imaging agents requires exogenous fluorescent signals from diseased tissues to be above the noise floor and to have adequate contrast relative to background, or normal tissues. Since surgical resection is most facile with fluorescence imaging concurrent with illumination that preserves the surgeon's visual acuity, it is critical to eliminate ambient light to enable high imaging contrast. We are developing a modulated, intensified CCD (ICCD) fluorescence imaging system for rapid planar imaging that enables electronic filtering of non-modulated ambient light that can contribute to the noise floor. The technique is rapid because it requires acquisition at only three-phase delays between the radio frequency (RF) signal driving the image intensifier and laser diode in order to extract images of AC amplitude.

Although fluorescence molecular imaging is rapidly evolving as a new combinational drug/device technology platform for molecularly guided surgery and noninvasive imaging, there remains no performance standards for efficient translation of "first-in-humans" fluorescent imaging agents using these devices. We are developing a stable, solid phantom designed to exaggerate the confounding effects of tissue light scattering and to mimic low concentrations (nM–pM) of near-infrared fluorescent dyes expected clinically for molecular imaging in order to evaluate and compare the commonly used CCD camera systems employed in preclinical studies and in human investigational studies.

RESEARCH PROJECTS

- Developing NIRF imaging device having the capability of operating in ambient light with frequency-domain measurement approach.
- Collaborating with NIST to develop performance standard for accelerating the clinical translation of NIRF imaging.
- Validating NIRF imaging device using various area detectors and excitation light sources using the developed fluorescent phantom.
- Validating 3D fluorescence tomographic imaging system using the develop fluorescent phantom.

KEY PUBLICATIONS

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

Meric-Bernstam, F., Rasmussen, J.C., Krishnamurthy, S., Tan, I-C., Zhu, B., Wagner, J.L., Babiera, G.V., Mittendorf, E.A., and Sevick-Muraca, E.M., "Toward nodal staging of axillary lymph node basins through intradermal administration of fluorescent imaging agents," *Biomedical Optics Express,* 5(1): 183-196, (2014)

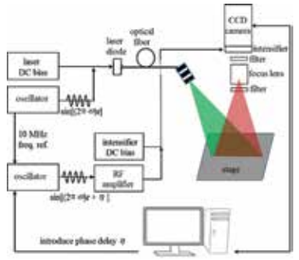
Zhu, B., Rasmussen, J.C., and Sevick-Muraca, E. M., "Non-invasive fluorescence imaging under ambient light conditions using a modulated ICCD and laser diode," *Biomedical Optics Express.* 5(2):562-572 (2014)

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

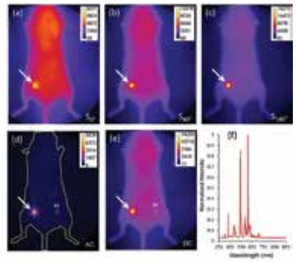
Darne, C., Lu, Y., Tan, I., Zhu, B., Rasmussen, J. C., Yan, S., Smith, A., Sevick-Muraca, E. M., "A compact frequency-domain photon migration system for integration into commercial hybrid small animal imaging scanners for fluorescence tomography" *Physics in Medicine and Biology,* 57(16): 8135-8152 (2012).

LAB MEMBERS

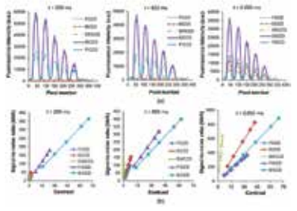
Coadvised: Grace Wu, Holly Robinson



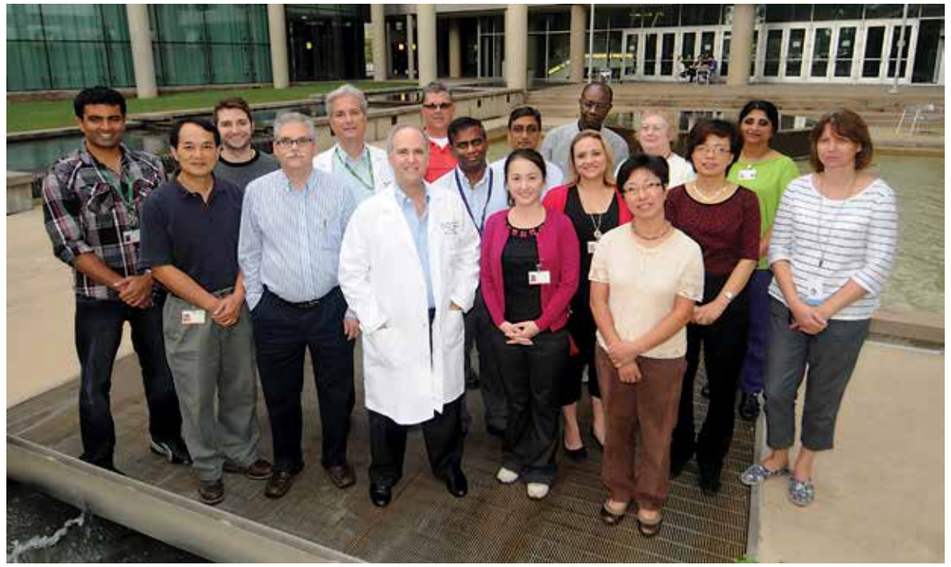
Schematic of fluorescence imaging device using a modulated ICCD camera and laser diode.



iRFP fluorescence images acquired at the phase delay (a) 0°, (b) 90° and (c) 180° from a representative mouse. (d) The extracted image of AC amplitude. (e) CW Image of DC. (f) Spectra of the fluorescent room lights acquired in the surgical suite showing far red spectral. The arrows point to the breast cancer location and the dashed circles represent the background (BK) ROIs.



Quantitative analysis of various CCD based NIRF imaging devices. (a) illustrates the measurement fluorescence intensity profiles using various CCD cameras under different integration times. (b) illustrates the plots of SNR vs contrast using various CCD cameras under different integration times.



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

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

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

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

Hubs of Research Collaboration with the Center include:

- Protein Chemistry
- Proteomics (including a UTMB/UTHealth NHLBI Proteomics Center)
- Systems Biology and UTHealth Bioinformatics Core Laboratory
- Clinical and Translational Proteomics Core Laboratory
- CLIA ProteoPath Molecular Diagnostics Laboratory
- NCI Center for Cancer Nanomedicine Excellence
- UT System-wide Proteomics Core Facility Network
- UTHealth / MDACC Clinical and Translational Center for Translational Technologies

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

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

NanoMedicine and proteomics in cancer and cardiovasculature disease

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Aman Mann, Rohan Bhavane, Anoma Somasunderam, Brenda Liz Montalvo-Ortiz, Ketan B. Ghaghada, David Volk, René Nieves-Alicea, K. Stephen Suh, Mauro Ferrari, Ananth Annapragada, David Gorenstein, Takemi Tanaka, "Thioaptamer Conjugated Liposomes for Tumor Vasculature Targeting", *Oncotarget*, April, Vol.2,pp. 298-304 (2011).

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

Aman P. Mann, Takemi Tanaka, Anoma Somasunderam Xuewu Liu, David G. Gorenstein, Mauro Ferrari, "Bone marrow targeted delivery of multistage vector via E-selectin", *Advanced Healthcare Materials*, 23, H278-H282 (2011) (Front page cover).

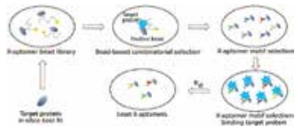
Weiguo He, Miguel-Angel Elizondo-Riojas, Xin Li, Ganesh Lakshmana Rao Lokesh, Anoma Somasunderam, Varatharasa Thiviyanathan, David E. Volk, Ross H. Durland, Johnnie Englehardt, Claudio N. Cavasotto, and David G. Gorenstein "X-Aptamers: A bead-based selection method for random incorporation of drug-like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

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

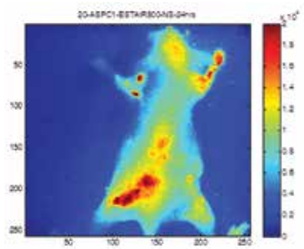
PubMed PMID: 24818768; PubMed Central PMCID: PMC4109393.

LAB MEMBERS

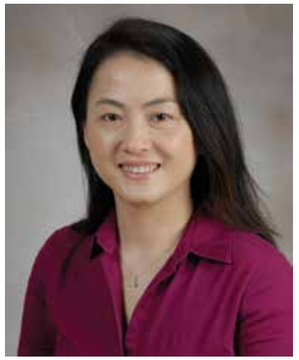
Research Scientists: Lokesh Rao, Ph.D., Hongyu Wang, Ph.D., Li Li, Ph.D.
Research Assoc.: Xin Li, MS
Post Doc: Sai Gandham
Graduate Student: Kurtis Anderson



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



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



Xiaohong Bi, Ph.D.
Assistant Professor

Optical spectroscopy and imaging for medicine

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

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

We have extensive experience in quantifying bone mineralization and composition, which are important determinants of bone strength. The effect of genetic variations and disease on bone compositional properties and mechanical function is constantly studied in the lab. In addition, we have developed RS spectral markers that are related to breast and prostate cancers induced bone alterations. These markers can be used to assess bone quality and to evaluate the response of metastatic bone to treatment. A noninvasive method is in development to test on animal model and patients based on the above findings.

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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)

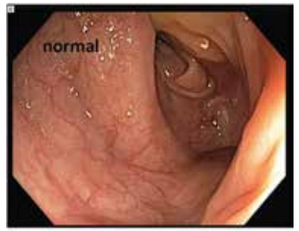
X. Bi, B. Rexer, C.L. Arteaga, M. Guo, A. Mahadevan-Jansen, Evaluating HER2 Amplification Status and Acquired Drug Resistance in Breast Cancer Cells Using Raman Spectroscopy. *Journal of Biomedical Optics*, 19(2):25001 (2014)

R. Tatavarty, H Ding, G Lu, RJ Taylor, X Bi, Synergistic Synergistic acceleration in the osteogenesis of human mesenchymal stem cells by graphene oxide-calcium phosphate nanocomposites, *Chem Commun* (Camb), 50(62): 8484-7 (2014)

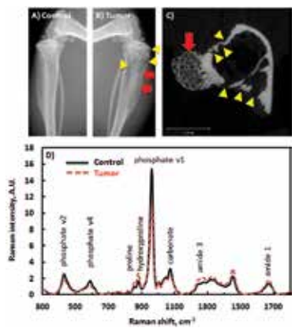
X. Bi, J.A. Sterling, A.R. Merkel, D.S. Perrien, J.S. Nyman, A. Mahadevan-Jansen, Prostate cancer metastases alter bone mineral and matrix composition independent of effects on bone architecture in mice – A quantitative study using microCT and Raman spectroscopy. *Bone*, 56(2):454-60 (2013)

LAB MEMBERS

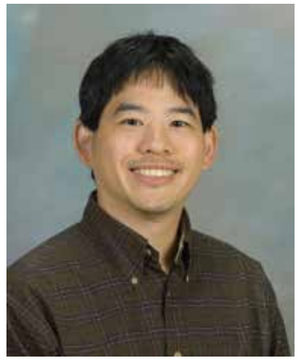
Postdoc: Hao Ding
Research Scientist: Zhiyong Wang
Technician: Guijin Lu



The endoscope pictures of normal (A) and colitis colon (B). Raman fiber optic probe was inserted through the accessory channel of the colonoscope. Tip of the probe is shown in the picture.



Osteoblastic (yellow arrow) and osteolytic (red arrows) lesions observed in the radiographs from a PBS-injected, non-tumor-bearing contralateral control (A) and a prostate tumor-bearing tibia (B), and a representative cross sectional microCT image (C) from the tibial metaphysis. D) Mean Raman spectra from the tumor-bearing tibiae (dashed line) and the contralateral controls (solid line). Selective Raman bands are marked with biochemical assignments.



Jeffrey Chang, Ph.D.
Assistant Professor

Genomic approaches for cancer therapies

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

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

1. Breast cancer metastasis. It is estimated that up to 90% of cancer deaths are due to metastasis, in part because metastatic cells do not respond to traditional therapies. To address this problem, we have used computational approaches to reposition drugs to target cells that exhibit phenotypes that promote metastasis. We have identified a selection of natural compounds and FDA-approved drugs targeting novel pathways that have shown the ability to inhibit metastasis in preclinical models.

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

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

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

RESEARCH PROJECTS

- Cancer metastasis, cancer stem cells, and the epithelial-to-mesenchymal transition.
- Alterations of drug sensitivity profiles in cancer stem cells.
- Genetic perturbations of Ras signaling.
- Transcriptional regulatory programs of E2F1-driven apoptosis.
- Automated planning of genomic data analyses pipelines with expert systems.

KEY PUBLICATIONS

Bild AH*, Chang JT*, Johnson WE*, and Piccolo SR. Emergent Scientist Phenotypes in Omic Research. *PLoS Biology.* 2014.
* Co-Corresponding Authors

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

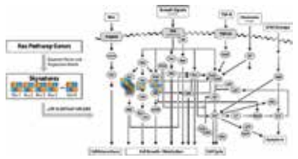
Chang JT. Deriving transcriptional programs and functional processes from gene expression databases. *Bioinformatics* 28(8), 2012.

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

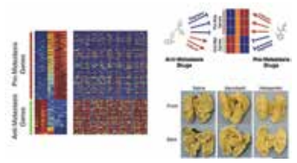
Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, Olson JA, Marks JR, Dressman HK, West M, and Nevins JR. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 439(7074): 353-357, 2005.

LAB MEMBERS

Postdocs: Weina Zhao, Ph.D., Sarah Prjic, Ph.D., Bettina Urban, Ph.D.
Bioinformatician: Xiaoling Chen, Ph.D.
Research Assistant: Jessie Sjol



Gene expression signatures predict pathway activation.



Genomic screening for novel anti-metastasis therapies.

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

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

One of our extraordinary scientific achievements from basic research to innovative human application was the success in hand-made assembly of the International Space Station in the most hostile environment that man ever had to face (spatial void, absence of gravity, extreme temperatures). During the preparation (O_2 prebreathe) for extra-vehicular activities (EVAs), the decompression from sea-level pressure to its third may lead to the presence of bona fide nano-, micronuclei of gases or microbubbles in blood, brain, or other tissues forming and growing *in situ* by cavitation or tribonucleation. This extensive collaborative effort between NASA and several North American institutions led to products and procedures that were delivered to NASA such as the decrease from 24-72 hours EVA preparation down to two hours. Members of the team received several awards from NASA for those achievements. Special skeletal muscle exercise prevents potential adverse events (neurological, pain,...) to occur. Non-invasive near infrared spectroscopy (NIRS) allowed observation of instantaneous variations of total, oxygenated and deoxygenated hemoglobin/myoglobin concentrations in microcirculatory networks of active limbs during the dynamic exercise that was used to for the successful two-hour O_2-prebreathe. In NanoMedicine, encapsulated gas microbubbles, e.g. drug-loaded liposomes targetting tissues (tumors, ...); cavitation-induced of encapsulated microbubbles are used to regulate the drug release. New challenges will be to study the role of gases (O_2, CO_2) potentially generating stress on neuronal oxygen consumption (effects on cerebral circulation and vigilance), by fMRI, evaluate the individual susceptibility gene variants to anxiety and characterize some molecular mechanisms that regulate O_2-induced neurogenesis and their applications in neurodegenerative diseases (e.g. Alzheimer's). Another challenge is to study the effects of O_2 and other physical variables on force fields for nucleic acids, aptamers or X-aptamers, and proteins with a specially custom-designed device. An extension of the previous goal is also to study the O_2-dependent molecular mechanisms and other physical variables that regulate cancer cells such as the effects of hypoxia on tumorigenic cells. Our focus is on patients with lung adenocarcinoma with metastatasis to the brain and Glioblastoma.

RESEARCH PROJECTS

Biology of Oxygen Applied to Three Fields of Research: 1). Spatial Environment (Basic & Operational Research); 2). Cancer; and 3). Cognitive Plasticity.

- Understand the role of gases (O_2, CO_2) generating stress on neuronal oxygen consumption (effects on cerebral circulation and vigilance), using functional MRI, and development of "stress-aptamers" (1). Characterize some molecular mechanisms that regulate O_2-induced neurogenesis (3): applications in neurodegenerative diseases (e.g. Alzheimer's).
- Study the effects of O_2 and other physical variables on force fields for nucleic acids, aptamers or X-aptamers, and proteins with a specially custom-designed device (2).
- Study the O_2-dependent molecular mechanisms and other physical variables that regulate cancer cells (2). Effects of hypoxia on tumorigenic cells.

KEY PUBLICATIONS

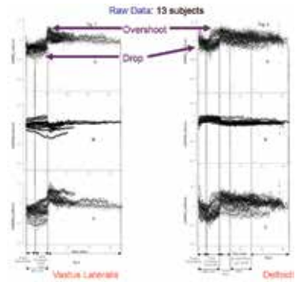
Jørgensen A, Foster PP, Brubakk A.O., Eftedal I. Effects of hyperbaric oxygen preconditioning on cardiac stress-markers in rats. *Physiological Reports*, 2013.

Foster PP. "How does dancing promote brain reconditioning in the elderly? *Front. Ag. Neurosci.*, doi: 10.3389/fnagi.2013.00004. Epub 2013 Feb 26.

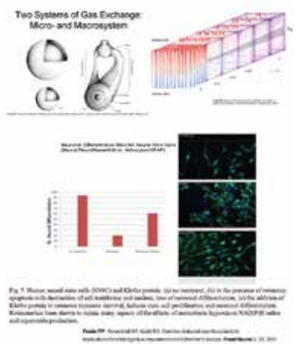
Foster PP. The "brain-skin connection" in protein misfolding and amyloid deposits: embryological, pathophysiological, and therapeutic common grounds? *Front Neurol* 3: 56, 2012.

Foster PP, Pollock NW, Conkin J, Dervay JP, Caillot N, Chhikara RJ, Vann RD, Butler BD, and Gernhardt ML. Protective Mechanisms in Hypobaric Decompression. *Aviat. Space Environ. Med.* 84:3, 212-25, 2013.

Jørgensen A, Foster PP, Wisløff U, Paulsen G, Havnes MB, Eftedal I, and Brubakk AO. Eccentric exercise-induced myofibrillar disruption with sarcolemmal integrity prior to delayed diving has no effect on vascular bubble formation in rats, *Exp. Physiol.* 2012.



Extravehicular Activities (EVAs). Near infrared spectroscopy (deltoid & vastus lateralis muscles). Model of tructural & functional barriers for the transport-diffusion-delivery of O_2.



Functional MRI, Human neural stem cells, cognitive plasticity & biomarkers.

Kevin Rosenblatt, M.D., Ph.D.
Associate Professor
Levit Family Chair in the Neurosciences

Vimentin is a novel AKT1 target mediating motility and invasion

One of my areas of interest is in the discovery and validation of biomarkers and novel drug targets for molecular pathways of disease. This work is performed both as basic research in animal and cell models and as translational research in human biological fluids and tissues. Our group has focused on protein-based biomarkers and molecular targets because proteins are the "workhorses" of cells and tissues–i.e. proteins carry out the majority of the cell signaling and metabolic reactions necessary for normal physiology, and deranged protein networks are responsible for altered metabolism that results in disease. Thus, while genomics and transcriptomics studies are incredibly useful for understanding the molecular basis of many diseases, a knowledge of how protein expression is altered–which proteins, their relative levels, and their altered regulation at the posttranslational level–is necessary for a more complete understanding of a disease process. The team has developed several high-throughput screening methodologies, including discovery and validation approaches, such as mass spectrometry work flows and phosphoproteomic lysate microarrays, for uncovering the molecular protein networks that drive diseased cells. Their approaches have suggested new druggable protein candidates and signaling profiles that distinguish one disease subclassification from another. These insights are useful tools in this new era of personalized molecular medicine.

Because animal and cell line models are still a useful way to gain insight to human diseases and cellular physiology, our lab works in collaboration with basic researchers to apply their expertise to model systems to discern candidates that may be relevant to human disease. They then attempt to translate these findings into human diseased tissues and biological fluids to determine relevance for the human disease correlates. Along these lines, our lab has been using a variety of advanced techniques to elucidate the protein networks driving Klotho-dependent protein signaling cascades: Klotho is a novel protein family member that has been implicated in aging/longevity and oxidative stress pathways in mammals. Klotho is a single pass transmembrane protein, released into the blood and CSF, that has far reaching effects on cellular signaling and metabolism. Recent efforts and have concerned the identification of the Klotho "receptor" and some of the cytoplasmic and nuclear signals of Klotho activity and their biological consequences; we are now engaged in several translational projects to determine the role of this protein, if any, in human aging and in human age-related diseases, such as cancer and Parkinson's disease.

RESEARCH PROJECTS

- Development of BirthStat, a peripheral blood test for predicting and diagnosing pre-term birth in high-risk pregnancies.
- Neuroprotective effects of Klotho in Parkinsonian disease models.
- Role of Klotho in neural stem cell survival and differentiation.
- National Children's Study Proteomics Center.
- ProteoPath High-Complexity CLIA Laboratory for Clinical Proteomics and Metabolomics.

KEY PUBLICATIONS

Choudhary, S., Rosenblatt, K.P., Fang, L., Tian, B., Wu, Z., and Brasier, A.R. (2011) High-throughput siRNA screening of the human kinome identifies novel kinases controlling the canonical NF-κB activation pathway. *Journal of Biological Chemistry* 286: 37187-37195. PMID: 21900239.

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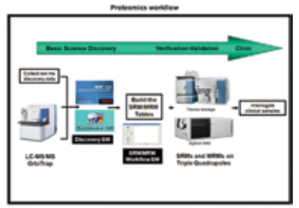
Rosenblatt, K.P., Huebschman, M.L., and Garner, H.R. (2012) Construction and Hyperspectral Imaging of Quantum Dot Lysate Arrays. *In Methods of Molecular Biology: Individualized Molecular Medicine.* Espina, V. and Liotta, L.A., eds. (New York: Humana Press, Inc.), pp 311-324.

Voelkl, J., Alesutan, I., Leibrock, C.B., Quintanilla-Martinez, L., Kuhn, V., Feger, M., Mia, S., Ahmed, M.S., Rosenblatt, K.P., Kuro-O, M., and Lang, F. (2013) Spironolactone ameliorates PIT1-dependent vascular osteoinduction in klotho-hypomorphic mice. *Journal of Clinical Investigation,* Epub 2013 Jan 9. PMID: 23298834

LAB MEMBERS

Post-Doctoral Fellows: Reynolds Brobery, Ph.D., Nataliya Bulayeva, Ph.D., Mehdi Dehghani, Ph.D.
Staff Scientist: Hongyu Wang, M.D., Ph.D.
Technical Staff: Li Li, M.S.



Protein Biomarker Discovery Workflow. Our approach rapidly moves newly discovered candidates into verification and clinical validation trials.

David Volk, Ph.D.
Assistant Professor

Targeting cancer with X-aptamers and nanoparticle conjugates

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

Thioaptamers targeting dengue virus type-2 envelope protein domain III, S.H.A. Gandham, D.E. Volk, G.L.R. Lokesh, M. Neerathilingam, D.G. Gorenstein, Biochem. & Biophys. *Res. Commun*. 2014. DOI: 10.1016/j.bbrc.2014.09.053

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

Thioaptamer Conjugated Liposomes for Tumor Vasculature Targeting. A.P. Mann, R.C. Bhavane, A. Somasunderam, B.L. Montalvo-Ortiz, K.B. Ghaghada, D. Volk, R. Nieves-Alicea, K.S. Suh, M. Ferrari, A. Annapragada, D.G. Gorenstein, T. Tanaka, *Oncotarget* 2(4), 298-304, 2011.

LAB MEMBERS

Research Scientists: Lokesh Rao, Ph.D., Hongyu Wang, Ph.D.
Research Associates: Xin Li, M.S., Li Li, Ph.D., Kathy Hoch, Sai Gandham, Ph.D.
Post Doc: Ana Maria Zaske, Ph.D.
Medical Student: Max Polansky

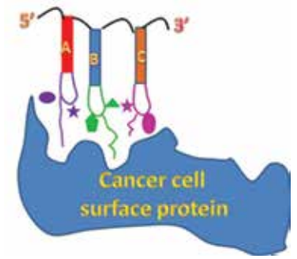
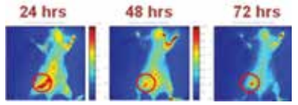


Diagram illustrating the interaction between an X-Aptamer and a cancer cell surface protein. Drug-like compounds (polygons) and protein-like side chains (squiggly lines) enhance binding to cancer cells for directed delivery of chemotherapeutic agents.



Real-time near infrared imaging of nanoparticles targeting the E-selectin protein on the surface of a human pancreatic tumor (circled) in a mouse.

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

Interventional oncology research

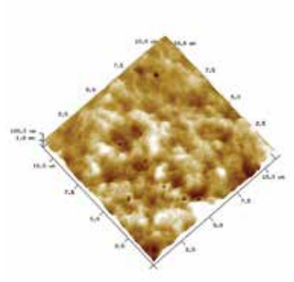
My research is focused on innovation and improvements in minimally invasive, image guided, oncologic treatments.

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

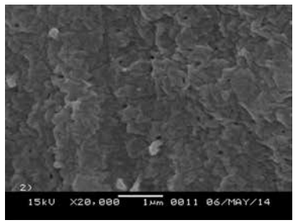
Another treatment that my research is focused is on radiogenomics in hepatocellular carcinoma. Radiogenomics is the correlation of imaging phenotypes to genomic genotypes. Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third most frequent cause of cancer-related death. The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

RESEARCH PROJECTS

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



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



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

KEY PUBLICATIONS

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

Yue Zhang, Sarah B. White, Jodi R. Nicolai, Zhuoli Zhang, Derek L. West, Dong-hyun Kim, A. Lee Goodwin, Frank H. Miller, Reed A. Omary, Andrew C. Larson. Multimodality Imaging to Assess Immediate Response to Irreversible Electroporation in a Rat Liver Tumor Model. *Radiology,* 2014, 271: 721-729.

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

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 deficiences), trauma (e.g. spinal cord injury), or aging (e.g. degeneration of the joints). Regenerative medicine has as its goal the replacement or regeneration of human tissues and/or organs to restore or establish normal function. Implicit in the successful design, implementation, and application of regenerative medicine approaches to the repair of a damaged tissue and/or organ is the reliance on the unique biological properties of specialized cells: stem cells.

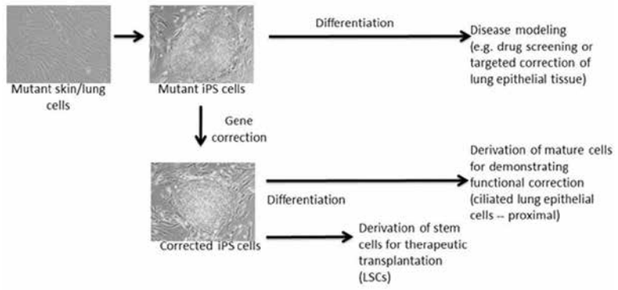
Our focus within the Center for Stem Cell and Regenerative Medicine is to study the fundamental properties of stem cells and to translate their unique biological properties into novel cellular therapies for tissue regeneration for currently intractable disorders. It is essential that such an endeavor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

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

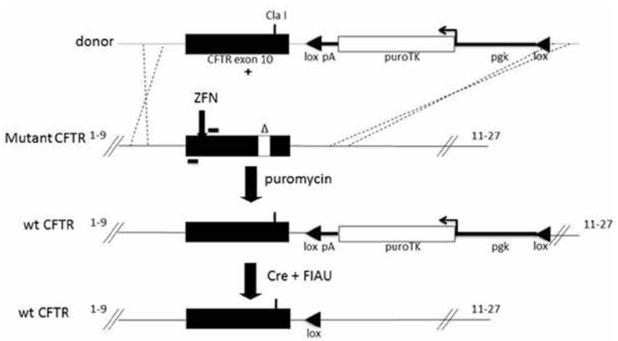
Brian Davis, Ph.D.
Associate Professor
Director of the Center for Stem Cell and Regenerative Medicine
C. Harold and Lorine G. Wallace Distinguished University Chair

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

My laboratory has as its primary objective the sequence-specific genetic correction of mutations in the chromosomal DNA of induced pluripotent stem (iPS) cells derived from patients with inherited disorders affecting the lung or blood system, with the ultimate goal of developing stem/progenitor cell-based therapeutic approaches. We have utilized Zinc Finger Nuclease-mediated Homology Directed Repair to correct the most common genetic mutations in iPS cell lines derived from patients with Cystic Fibrosis or Surfactant Protein B Deficiency – with the objective of demonstrating genotypic/phenotypic correction in lung epithelial cells derived from these corrected iPS cells. The second project in the laboratory focuses on the site-specific correction of gene mutations responsible for inherited blood disorders (e.g. Wiskott-Aldrich Syndrome) in patient-specific iPS cells – with subsequent differentiation to blood stem cells for transplantation. The third laboratory project focuses on "natural gene correction," that is when spontaneous mutations arising in blood cells bearing inherited genetic mutations result in functional restoration of the defective gene, followed by *in vivo* selection for the revertant corrected cells. This gives rise to the phenomenon of revertant somatic mosaicism. We are presently examining this natural gene correction particularly as it occurs *in vivo* in patients with the Wiskott-Aldrich Syndrome.



Applications of Cystic Fibrosis (CF) iPS Cells



Restored expression of mature, fully glycosylated *CFTR* protein in corrected, differentiated cells

RESEARCH PROJECTS

- Correction and lung differentiation of iPS cells from inherited lung diseases (Cystic Fibrosis, Surfactant Protein-B Deficiency, Alpha 1 Anti-Trypsin Deficiency).
- Correction and blood differentiation of iPS cells and blood stem cells from inherited blood disorders (Wiskott-Aldrich Syndrome, Pyruvate Kinase Deficiency).
- Characterization of spontaneous gene mutation resulting in correction of inherited Wiskott-Aldrich Syndrome defects.

KEY PUBLICATIONS

Davis BR, DiCola MJ, Prokopishyn NL, Rosenberg JB, Moratto D, Muul LM, Candotti F and Blaese RM. Unprecedented diversity of genotypic revertants in lymphocytes of a patient with Wiskott-Aldrich syndrome. *Blood* 111:5064-5067, 2008.

B.R. Davis and F. Candotti: Mosaicism – Switch or Spectrum. *Science* 330:46-47, 2010.

Davis BR, Yan Q, Bui JH, Felix K, Moratto D, Muul LM, Prokopishyn NL, Blaese RM and Candotti F. Somatic Mosaicism in the Wiskott-Aldrich Syndrome: Molecular and Functional Characterization of Genotypic Revertants. *Clinical Immunology* 135:72-83, 2010.

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

Amarijo E, Soto C, Davis BR. HIV/AIDS: modified stem cells in the spotlight. *Cellular and Molecular Life Sciences*. 14: 2641-2649, 2014

LAB MEMBERS

Research Staff: Dr. Ana M. Crane, Dr. Daniela Mora Ortiz
Postdoctoral Fellows: Dr. Philipp Kramer, Dr. Nadine Matthias, Dr. Leila Rouhigharabaei

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

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

The research in my laboratory focuses on developing tissue engineering approaches toward clinical treatments for spinal cord injury, traumatic brain injury, and cartilage defects using 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.

By examining cell-material interactions, we seek to understand which aspects of the native extracellular matrix facilitate tissue repair and integration with the surrounding host tissue. Once optimal composition, architecture (porosity, feature size, fiber alignment, etc.), mechanical properties, and bioactive signaling peptide concentrations have been identified using combinatorial methods, they will be integrated into advanced hybrid scaffolding systems. These scaffolding systems maximize the advantages of both synthetic (consistency in fabrication and cellular response) and natural (natural bioactive signaling) polymers, while mitigating their disadvantages, namely lack of bioactive signaling and batch to batch inconsistency in scaffold properties and cellular response, respectively. When combined with additional bioactive signaling and controlled architecture, these hybrid scaffolds can begin to emulate the native tissue microenvironment and support tissue development far better than traditional scaffolds. Preliminary studies have focused on optimizing the concentration of bioactive laminin fragments for the differentiation of stem cells to neurons and the development of novel synthetic polymers capable of displaying multiple bioactive signaling peptides at independent concentrations.

In order to advance tissue engineering to 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

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.

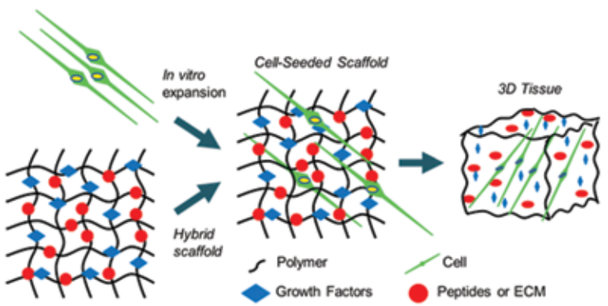
Smith Callahan LA, Policastro GM, Benard SL, Childers EP, Boettcher RM, Becker ML. Influence of Discrete and Continuous Culture Conditions on Human Mesenchymal Stem Cell Lineage Choice in RGD Concentration Gradient Hydrogels. *Biomacromolecules.* 14(9): 3047-3054, 2013.

Smith Callahan LA, Ganios AM, Childers EP, Weiner, SD, Becker ML. Primary Human Chondrocyte Extracellular Matrix Formation and Phenotype Maintenance using RGD derivatized PEGDM Hydrogels Possessing a Continuous Gradient in Modulus. *Acta Biomaterialia*. 9 (4): 6095–6104, 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

Postdoctoral Fellows: Yueh Hsun (Kevin)Yang and Hyun Ju Lim
Undergraduate Students (Rice University): Matthew Mosley and Zara Khan



Schematic of hybrid scaffold tissue engineering approach.

Qi Lin Cao, M.D.
Associate Professor

Stem cells for neurological diseases

Transplantation of neural stem cells (NSCs) is proved to be 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 a 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 glias after transplantation into the contused spinal cord and promote functional recovery. These studies suggest that transplantation of hiPSC-derived NSC is an effective therapy to preserve and restore neurological functions. Currently, we are testing the therapeutic efficacy and long-term safety of NSCs, neuronal or glial precursor cells to identify the optimal cell graft for SCI and stroke. Recently, we are testing whether we can directly reprogram the astroglial cells in the injured spinal cord or stroke brain into neurons. Astroglial scar are the major inhibitor for axonal regeneration. *In situ* reprogramming active astrocytes into neuronal precursor cells will decrease astrocyte inhibition to promote axonal regeneration. The newly reprogrammed neuronal precursor cells could replace the lost neurons after SCI or stroke. These two mechanisms may work synergistically to promote great functional recovery after SCI or stroke. Our long-term goal is to develop novel stem cell-based therapies to treat human SCI or stroke.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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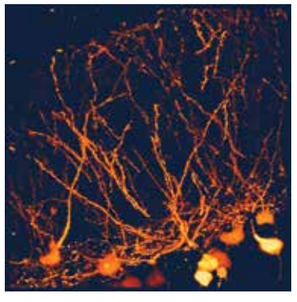
Fan CL, Zheng YY, Cheng XX, Qi XB, Bu P, Luo XG, Kim DH and Cao QL (2013) Transplantation of D15A-expressing glial-restricted-precursor-derived astrocytes improves anatomical and locomotor recovery after spinal cord injury. *Int J Biol Sci*. 2013;9(1):78-93.

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

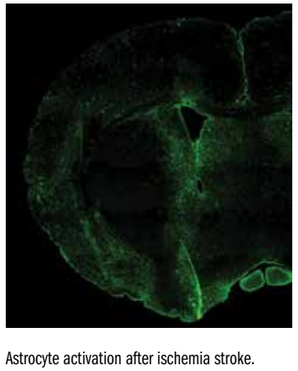
Fan CL, Wang H, Chen D, Cheng XX, Xiong K, Luo XG and Cao QL (2014) Effect of type-2 astrocytes on the viability of dorsal root ganglion neurons and length of neuronal processes. *Neural Regen. Res.* 9: 119-128.

LAB MEMBERS

Postdoc Research Associates: Michelle Wang, Yiyan Zheng
Senior Research Assistant: Jun Li



Transgenic labeling of dentate neurons in mice hippocampus.



Astrocyte activation after ischemia stroke.

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 MAPCs 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.

KEY PUBLICATIONS

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

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

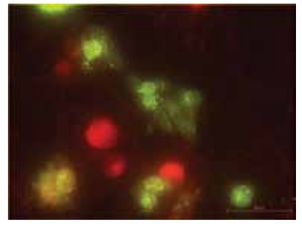
Bedi SS, Hetz R, Thomas C, Olsen A, Williams S, Smith P, Xue H, Aroom K, Uray K, Hamilton T, Mays RW, Cox CS. Intravenous MAPC therapy improves spatial learning after TBI. *Stem Cells/Translational Medicine*. 2:953-960, 2013.

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

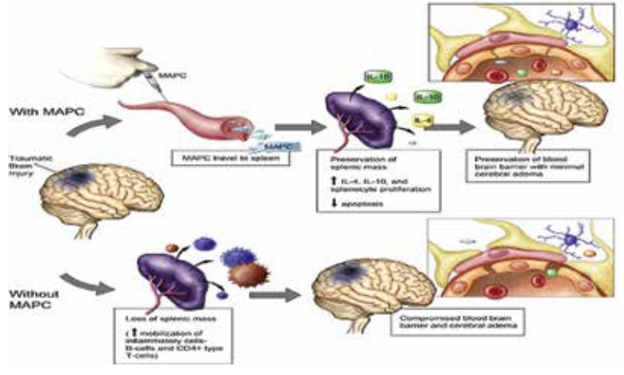
Walker PA, Bedi SS, Shah SK, Jimenez F, Xue H, Hamilton JA, Smith P, Thomas CP, Mays RW, Pati S, Cox CS. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: Modulation of microglia/macrophages. *J Neuroinflammation* 9: 228-240, 2012. PMID: 23020860

LAB MEMBERS

Supinder Bedi, Ph.D.-Instructor
Karen Uray, Ph.D.-Assistant Professor
Robert Hetz, M.D.-Brown Foundation Post-Doctoral Fellow
George Liao, M.D.-NIH T32 Post-Doctoral Fellow
Suchit Sahal, Ph.D.-Post-Doctoral Fellow
Phillipa Smith, M.S.-Flow Cytometry Technician
Chelsea Thomas, B.S.-Medical Student.
Henry Caplan, BS-Medical Studnet
Hasan Xue, M.D.-Research Scientist
Fabio Triolo, Ph.D.-GMP center director
Sufira Kiran, GMP-QA director



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



The cartoon above highlights our currrent paradigm of how cell-based therapies alter the innate immune response to injury and improve structural and functional outcomes.

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

Skeletal muscle regeneration using pluripotent stem cells/ new insights using knock-in reporter ES/iPS cells

My lab main interest is using pluripotent stem cells for skeletal muscle regeneration. During the last few years, I've developed novel methods for using mouse/human embryonic stem cells (ES cells) and induced pluripotent cells (iPS cells) for cell therapy in mouse models of muscular dystrophies.

Here at IMM, my lab focuses on the approaches to improve stem cell therapies for skeletal muscle regeneration. Currently the lab uses cutting edge gene editing technologies for generation of knock-in reporter lines in human ES/iPS cells for early myogenic genes (PAX7, MYF5). This will allow studying emergence of early myogenic progenitors from any human ES/ iPS cells; a crucial step to identify and isolate these cells for future iPS cell based therapies.

My research also will include optimizing cell delivery, survival and engraftment, study mechanisms involved in cell homing into the muscle after systemic cell delivery, as well as exploring the effect of local tissue perfusion in cell survival and engraftment. Generation of safe and integration free myogenic progenitors from ES and iPS cells would be our other goal.

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

RESEARCH PROJECTS

- Generation of knock-in human ES/iPS cell lines for early myogenic genes (PAX7, MYF5).
- Role of local tissue perfusion on survival and engraftment of human ES/ iPS derived myogenic progenitors in skeletal muscle.
- Generation of integration free and safe myogenic progenitors from human ES/ iPS cells.
- Systemic cell delivery approaches for cell therapy in muscular dystrophies.
- Using bio-scaffolds for cell delivery.

KEY PUBLICATIONS

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

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

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

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

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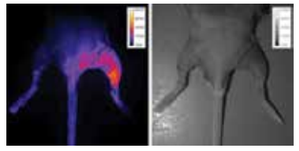
Arpke RW, Darabi R, Mader TL, Zhang Y, Toyama A, Lontree CL, Nash N, Lowe DA, Perlingeiro RC, Kyba M. A New Immuno- Dystrophin- Deficient Model, The NSG-mdx4cv Mouse, Provides Evidence for Functional Improvement Following Allogenic Satellite Cell Transplantation. *Stem Cells*, 2013 Aug; 31(8):1611-20.

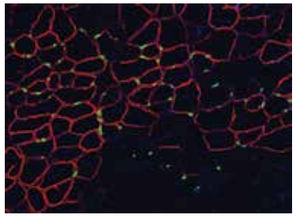
Skoglund G, Lainé J, Darabi R, Fournier E, Perlingeiro R, Tabti N. Physiological and ultrastructural features of human induced pluripotent and embryonic stem cell-derived skeletal myocytes in vitro. *Proc Natl Acad Sci USA.* 2014 Jun 3; 111(22):8275-80.

LAB MEMBERS

Postdoctoral Fellows: Jianbo Wu, Nadine Matthias
Research Technician: Samuel D. Hunt



NIR imaging demonstrates high efficiency hindlimb perfusion following intra-arterial (intra-femoral artery) canulation and perfusion in mouse.



(Human lamin A/C: Green, Dystrophin: Red) Human iPS derived myogenic progenitors engraft and restore the missing protein (dystrophin) in mouse model of Duchenne Muscular Dystrophy/DMD.

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

Mechanisms of memory formation and memory dysfunction

The research objective of my laboratory is to explore the molecular mechanisms contributing to working memory (lasting seconds), short-term memory (lasting minutes-to-hours) and long-term memory (lasting days to a lifetime), and the relationships among these types of memories. To accomplish this, we disrupt or augment specific biochemical events in discrete brain regions, such as the prefrontal cortex and the hippocampus, to determine the aspect of memory altered as a result of the manipulation. Human and animal studies have shown that the prefrontal cortex is required for holding information "online" for a period of seconds (referred to as working memory), which is used to guide goal-directed behavior. Working memory is critical for decision-making and coherent thought processes, and is often impaired as a result of normal aging, and diseases such as Parkinson's, Alzheimer's, and schizophrenia. Short-and long-term explicit memories are dependent on the function the hippocampus, a structure within the medial temporal lobe. We utilize a multi-disciplinary approach involving molecular, biochemical, genetic and behavioral techniques to manipulate molecular processes within the prefrontal cortex and hippocampus to determine their contribution to various memory processes.

Both the prefrontal cortex and hippocampus are highly vulnerable to insults, such as traumatic brain injury. Injury to these structures often results in memory loss and a lack of coherent thought processes. Biochemical and molecular cascades initiated as a result of trauma are thought to alter inter- and intracellular signaling, causing changes in the brain ranging from survival and growth to neuronal dysfunction and death. We use an experimental brain injury model in rodents to explore some of the molecular mechanisms contributing to injury-related memory deficits. The long-term goal of this research is to identify potential targets for therapeutic interventions to alleviate the memory disorders associated with brain injuries and degenerative diseases.

KEY PUBLICATIONS

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Dash PK, Hebert AE, Runyan JM. A unified theory for cellular and systems memory consolidation. *Brain Res. Review* 45:30-37.

Runyan JD, Moore AN, Dash PK. A role for prefrontal calcium-sensitive protein phosphatase and kinase activities in working memory. *Learn Mem.* 2005 Mar-Apr

Jeter CB, Hergenroeder GW, Ward Iii NH, Moore AN, Dash PK. Human traumatic brain injury alters circulating L-arginine and its metabolite levels: possible link to cerebral blood flow, extracellular matrix remodeling, and energy status. 2011 *J Neurotrauma*. Sep 26

Dong Kim, M.D.

Professor and Chairman
Department of Neurosurgery
Director, Mischer Neuroscience Institute
Memorial Hermann Hospital – TMC

Advancing the field of neuroscience

As director of the Mischer Neuroscience Institute (MNI) since October 2007, I lead the clinical neuroscience efforts for the Memorial Hermann Healthcare System as well as for The University of Texas Health Science Center at Houston.

Combining the strengths of an 11-campus hospital group with 3,600 patient care beds and the academic resources of the UT System, MNI provides the most specialized treatment available for diseases of the brain and is a national leader in research for new treatments.

My research focuses on the origin, development, and treatment of brain aneurysms. I lead basic science efforts, such as identifying the genes that lead to an inherited risk for aneurysms and genetic changes in brain tumors, and translational projects that directly affect clinical practice.

I have been honored with numerous awards and was named to America's Top Surgeons, Marquis Who's Who and Who's Who in America. I am the recipient of grants from the National Institutes of Health and the American Stroke Association and have authored studies published in journals such as *Nature Genetics, Brain Research, International Journal of Cancer, Neurology, Neurosurgery, Journal of Neurosurgery and Genes, Chrom, Cancer.*

I am a graduate of Stanford University and the University of California, San Francisco (UCSF) School of Medicine. After general surgery training at Harvard, I completed my neurosurgery training under Dr. Charles Wilson at UCSF. I went on to complete a fellowship in cerebrovascular surgery and skull-based tumors with Dr. Arthur Day.

I have held faculty and hospital appointments 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
- Arteriovenous malformations
- Skull base tumors and meningiomas
- Carotid disease
- Trigeminal neuralgia
- Chiari malformations

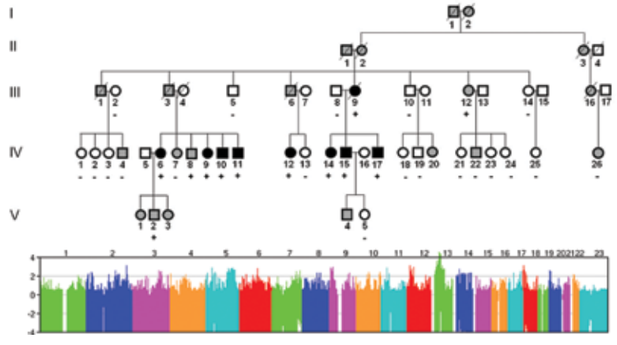
RESEARCH PROJECTS

- Stem cell therapy for spinal cord injury
- Genetic aneurysm research
- Neuro trauma research

KEY PUBLICATIONS

Tran-Fadulu V, Pannu H, Kim DH, Vick GW 3rd, Lonsford CM, Lafont AL, Boccaladro C, Smart S, Peterson KL, Hain JZ, Willing MC, Coselli JS, LeMaire SA, Ahn C, Byers PH, Milewicz DM: Analysis of multigenerational families with thoracic aortic aneurysms and dissections due to TGFBR1 or TGFBR2 mutations. *J Med Genet.* 46(9):607-613, 2009. Epub 2009 Jun 18.

Xiaoxin Cheng, Yaping Wang, Qian He, Yiyan Zheng, Dong Kim, Scott Whittemore, and Qilin Cao: Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult OPCs by increasing the expression of bone morphogenetic proteins. *J Neuroscience* 31(16)6053-6058, April 20, 2011.



Mapping for Intracranial Aneurysm Genes in Affected Families.
Figure A shows the pedigree of research family CVM presenting with autosomal dominant inheritance of intracranial aneurysms. Circles represent females, and squares represent males. Blackened symbols denote individuals with aneurysms while unblackened and grayed symbols denote unaffected and unscreened individuals, respectively. Genomewide linkage analysis and gene sequencing identified a potential mutation in a gene in Chromosome 13 that was detected (+) in all affected individuals, but not detected (-) in most other family members and thousands of controls. Results of linkage analysis demonstrating significant linkage to Chromosome 13 are shown in Figure B. We are currently investigating, through mouse models, the role of the mutated gene in aneurysm formation.

Dong Kim, Qian He, Yaping Wang, Yiyan Zheng, Xiaoxin Cheng, Scott Whittemore, Qilin Cao: Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult OPCs by increasing the expression of bone morphogenetic proteins. *J Neuroscience* 31(16)6053-6058, April 20, 2011.

Dong Kim, Qian He, Yaping Wang, Yiyan Zheng, Xiaoxin Cheng, Scott Whittemore, Qilin Cao: Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult OPCs by increasing the expression of bone morphogenetic proteins. *J Neuroscience* 31(16)6053-6058, April 20, 2011.

Guo, D., Regalado, E., Casteel, D. E., Santos-Cortez, R. L., Gong, L., Kim, J. J., Dyack, S., Horne, S. G., Chang, G., Jondeau, G., Boileau, C., Coucke, P. J., Choi, J., Bamshad, M., Khan, N., Grange, D. K., Mendoza-Londono, R., Bradley, T. J., Olney, A. H., Adès, L., Maher, J. F., Guo, D., Buja, L. M., Kim, D., Hyland, J. C. and Regalado, E. S. (2010), De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction. *American Journal of Medical Genetics Part A*, 152A: 2437–2443. doi: 10.1002/ajmg.a.33657

Cao, Qilin, He, Qian, Wang, Yaping, Cheng, Xiaoxin, Howard, Russell M., Zhang, Yiping, DeVries, William H., Shields, Christopher B., Magnuson, David S.K., Xu, Xiao-Ming, Kim, Dong H., Whittemore, Scott R. Transplantation of Ciliary Neurotrophic Factor-Expressing Adult Oligodendrocyte Precursor Cells Promotes Remyelination and Functional Recovery after Spinal Cord Injury. *J Neuroscience* 30(8) 2989-3001, 2010.

Yong Li, M.D., Ph.D.

Associate Professor

Pluripotent stem cell and regenerative medicine

This research team has developed several novel techniques for molecular, cellular, and animal-based studies to focus on few major areas of study: 1) exploring the properties of the dedifferentiation/transformation of terminally differentiated cells into pluripotent stem cell for regenerative medicine and tissue engineering applications; 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 translational study and clinical application of stem cells and engineered tissue for treating congenital diseases and traumatic injuries. 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 newts 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 a 3D printer by using natural proteins and cells to create a functional tissue compound for wound tissue repair.
- Dedifferentiation and Stem Cell Populations: The project aims to enlarge the pluripotent stem cells' pool without genetic modification as a cell source for regenerative medicine.
- 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.
- Newt model: Combination of mammalian cells with amphibian cells to investigate the potential of tissue/organ regeneration process in the Newt model and the mechanisms.
- 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 transcriptom expression and protein profile within the model system.

KEY PUBLICATIONS

Pan HY, Vojnits K, Meng FW, Liu T, Yang L, Wang YG, Huard J, Cox C, Li Y. MMP1 gene expression enhances myoblast migration and engraftment after implanting into mdx mice. *Cell Adhesion & Migration* (2014, Accepted).

Bellayr I, Holden K, Mu XD, Li Y. Matrix metalloproteinase inhibition negative affects muscle stem cell behavior. *J Clin Exp Pathol* 2013;6(2):124-141.

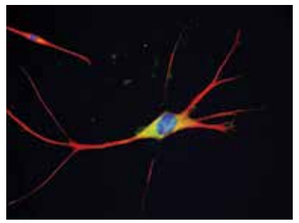
Mu XD, Bellayr I, Choi YH, Pan HY, Li Y. Regeneration of soft tissue is promoted by MMP1 after digit amputation in mice. *PLoS One*. 2013;8(3):e59105.

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

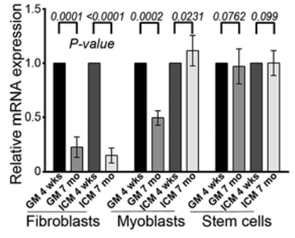
Vojnits K, Zhan M, Cox CS, Li Y. Small embryonic stem cell, a new type of stem cell. *Journal of Stem Cell* 2014; 9(1):1-16.

LAB MEMBERS

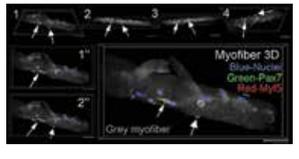
Administrator: Stephanie Baca
Lab senior technician/manager: Haiying Pan
Postdoc research fellows: Dr. Kinga Vojnits; Dr. Fanwei Meng.
Medical students: Chen Fu, Parsha Forouzan, Justin Pham



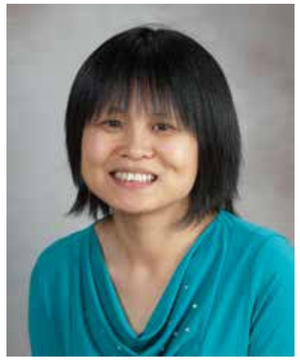
Cell dedifferentiation study



Aging prevention in the intercostal muscles (with telomere prevention) compare to the limb muscles.



3D visualization for studying muscle stem cells within *ex vivo* myofibers.



Ying Liu, Ph.D.
Assistant Professor

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

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

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are promising therapeutic tools for regenerative medicine. They can proliferate indefinitely in culture and have the capacity to differentiate into any cell types of the body. Protocols for directed differentiation of hESCs and hiPSCs into neural stem cells (NSCs) have been established. These NSCs can be maintained in chemically defined medium and proliferate in culture for at least 20 passages without going into senescence or changing their multipotential properties. When induced, they become functional neurons and glia as directed. The number of NSCs can be amplified to satisfy clinical demands. However, ethical issues, the possibility of immune rejection, and tumorigenicity have precluded hESCs and their derivatives to be applied to the clinical settings. hiPSCs, which are reprogrammed from somatic cells, have the potential to circumvent some of these problems. 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 pluripotent state and share many hESC characteristics. Most critically, hiPSCs 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. We perform directed differentiation of patient-specific iPSCs into NSCs, 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 CRISPR/Cas9 mediated lineage reporters for differentiation and purification of transcription factor-defined neural progenitors from hiPSCs. These neural lineage specific cells will be applied to in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

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

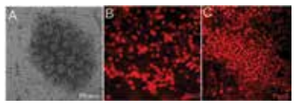
MacArthur, C.C., Xue, H., Van Hoof, D., Lieu, P., Dudas, M., Fontes, A., Swistowski, A., Touboul, T., Seerke, R., Laurent, L.C., Loring, J.F., German, M.S., Zeng, X., Rao, M.S., Lakshmipathy, U., Chesnut, J.D., and Liu, Y. (2012). Chromatin insulator elements block transgene silencing in engineered human embryonic stem cell lines at a defined chromosome 13 locus. *Stem Cells Dev.* 21: 191-205

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

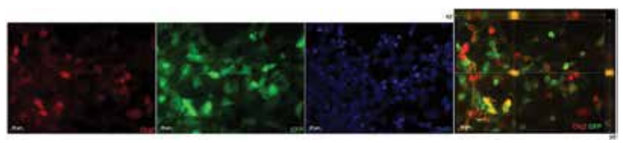
Xue, H., Wu, S., Papadeas, S., Spusta, S., Swistowska, A.M., MacArthur, C.C., Mattson, M.P., Maragakis, N.J., Capecchi, M., Rao, M.S., Zeng, X., and Liu, Y. (2009). A targeted neuroglial reporter line generated by homologous recombination in human embryonic stem cells. *Stem Cells,* 27, 1836-1846

LAB MEMBERS

Postdoctoral Fellow: Shenglan Li
Research Associate: Haipeng Xue
Visiting Scientist: Bo Long, Li Sun, Lihua Luo
Students: Tai Truong, Karen Y. He, Cheng Ma



Directed neural differentiation of human induced pluripotent stem cells (hiPSC).



Human induced pluripotent stem cells (hiPSCs) knockin GFP reporter recapitulates endogenous expression of targeted neural lineage specific transcription factor.

Nami McCarty, Ph.D.
Assistant Professor

Deciphering molecular and cellular mechanisms of pathogenesis and drug resistance in human lymphoma and multiple myeloma

The major goals of my research program are to decipher molecular pathways that confer selective growth and survival advantages to malignant B cells. We are also interested in understanding how stem-like cells contribute to drug resistance in these malignancies. Therefore, I began new lines of studies involving the identification and characterization of stem like cells in MCL (mantle cell lymphoma), termed MCL initiating cells (MCL-ICs). In 2009, I initiated collaborations with clinicians at neighboring MD Anderson Cancer Center (MDACC) to obtain multiple MCL patient samples, which we used to prospectively isolate stem-like cells in MCL. cDNA microarray analyses led to discovery of a signaling axis, comprised of NF-kB/ transglutaminase 2 (TG2) signaling, which contribute select survival of MCL-ICs. These stem-like populations are also highly resistant to drugs that are currently used in the clinics, such as R-CHOP, R-CVAD, R-DHAP and FluBR. These results emphasize that our findings are clinically relevant and further characterization of MCL-ICs may improve patient survival. Another line of research involves understanding how transcription factors that determine normal B cell lineage differentiation are involved in malignant B cell initiation and progression. One of those factors is paired box 5 (PAX5), a determinant of normal B cell lineage development. We discovered that PAX5 silencing in MCL leads to increased tumor formation in vitro and in xenograft mice, indicating that PAX5 is a potential tumor suppressor. We are currently conducting collaborative translational research efforts with clinicians at MDACC to correlate PAX5 levels with relapsed MCL cases and patient survival. We also conducted high throughput drug screening using libraries comprised of 3991 compounds of NCI oncology, custom clinical, and prestwick libraries. We discovered that select compounds target the survival pathways of PAX5 silenced cells. Given that PAX5 silenced cells are highly drug resistant, discovery of compounds that target drug resistance populations in MCL have direct translational applications. Downstream of PAX5 signaling is BACH2 (BTB and CNC homology), which is another transcription factor that is involved in drug responses in MCL. We discovered that BACH2 nucleo/cytoplasmic shuttling influences resistance to drugs that generate reactive oxygen species (ROS).

We also have developed a new line of research studying stem-like cells in multiple myeloma (MM) and their interaction with microenvironment. MM is heterogeneous disease due to their manifestation in the bone marrow compartment. We developed a new technology that can trace stem-like cells *in vivo*. Staining cells with lipophilic fluorescent dye PKH allows cells to cycle *in vivo*. Only cells that retain the dye are quiescent stem-like cells. Interestingly, stem-like MM cells preferentially reside within osteoblastic niche rather than vascular and spleen niches. These cells also were drug resistance and contributed to increased tumor formation in the secondary xenograft mice. We conducted gene profiling analyses for the quiescent PKH+ populations and the characterization of PKH+ cells and their interaction with microenvironment is underway. Stem-like cells in MCL or MM are relatively minor populations. Isolation and investigating characteristics of these cells is technically challenging, and we have developed systems to make seminal discoveries in the field of B cell cancers.

RESEARCH PROJECTS

- Transcription factor networks and pathogenesis of human lymphoma: We will continue to address roles for PAX5 signaling in MCL pathogenesis. PAX5 silencing increased bone marrow targeting *in vivo* and increased drug resistance in MCL. We will analyze PAX5-myc-p53 signaling using mice models of lymphomas and continue to investigate the roles of these signaling in lymphoma spread. Downstream of PAX5 is BACH2 transcription factor, which plays an important role in lymphoma drug resistance. We plan to use genetic silencing to test whether BACH2 has tumor suppressive roles in MCL. 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.
- Stem-like MM cells and microenvironment niche: We conducted microarray analyses to identify genes expressed in quiescent PKH+ cells from osteoblastic, vascular and spleen niches. We will continue to characterize functions of these genes in the MM interaction with bone marrow microenvironment. We will also collaborate with clinicians and basic scientists at MD Anderson to investigate niche competition between stem-like MM cells with hematopoietic stem cells.
- Targeted drug discoveries to increase survival of relapsed MCL patients: 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.

KEY PUBLICATIONS

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

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

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

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

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

Naoki Nakayama, Ph.D.
Associate Professor
Jerold B. Katz Distinguished Professorship in Stem Cell Research

Stem cell differentiation and lineage specification

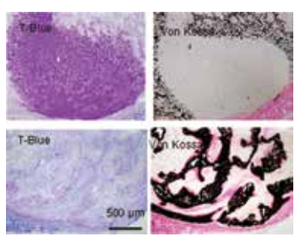
The cartilage of joints is not spontaneously repaired after injury in humans. There has been considerable interest in the clinical application of stem cells to the repair of damaged cartilage; however, current cell therapies using chondrocytes and mesenchymal stromal cells (MSCs) face the problems of low yield of cells and their tendency to yield unsuitable and/or unstable cartilage after expansion. Joint is formed during embryogenesis. Therefore, we hypothesize that the embryonic cell-type responsible for limb and vertebral joint formation: i.e. joint progenitor, the common precursor of synovial joint components including articular and meniscal chondrocytes and ligaments, would be the best for the regeneration of adult joint cartilage. Pluripotent stem cells (PSCs), whether derived from an embryo, or induced from adult cells, are expected to differentiate into any somatic cell-type in culture through a processes that mimicks embryogenesis *in vivo*, making human (h)PSCs a promising source of embryonic cells for regenerative medicine. The major challenges have been to direct their differentiation toward the cell type of interest (i.e. to obtain progeny of the right quality), and to isolate them in large quantities without introducing transgenes and mutations.

Quality cells – human joint progenitors: We have previously developed and purified from hPSCs paraxial mesoderm and neural crest progeny, two of the three embryonic origins of chondrocytes, with the capacity to expand and differentiate into sclerotomal and ectomesenchymal chondroprogenitors, respectively. We have recently established a condition to generate chondrogenic lateral plate mesoderm, the third embryonic origin of chondrocytes, from hPSCs, too. All these progeny generate hyaline-like cartilage particles in culture. However, except for cartilage particles developed from uncultured paraxial mesoderm progeny, most of them are unstable *in vivo* and are mineralized and turned into bone when ectopically transplanted into immunocompromised mice. We have recently discovered a way to selectively generate and expand, to a limited extent, joint progenitor-like cells that express syndetomal (ligament precursor) markers from the paraxial mesoderm progeny. We are currently focusing on the characterization of such joint progenitor-like cells, aiming to demonstrate their capacity to generate joint-type stable cartilage as predicted from animal studies.

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

RESEARCH PROJECTS

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



Human paraxial mesoderm-derived cartilage

Human neural crest-derived cartilage

Transplantation of cartilage generated with hPS cell-derived paraxial mesoderm (upper panels) and neural crest (lower panels) for 12 weeks in an immunocompromised mouse: The cartilage area is in purple (left panels) and bony part is in black (right panels).

KEY PUBLICATIONS

Umeda, K., Oda, H., Matthias, N., et al. (2014) "Long-term expandable SOX9+ chondrogenic ectomesenchymal cells from human pluripotent stem cells" *Stem Cell Rep*, in revision.

Yokoyama, K., Ikeya, M., Umeda, K., et al. (2014) "Enhanced chondrogenesis of iPS cells from neonatal-onset multisystem inflammatory disease occurs via the caspase-1-independent cAMP/PKA/CREB pathway" *Arthritis Rheum*, in press.

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

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

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

LAB MEMBERS

Senior Research Associate: Qing Yan, PhD
Animal Specialist: Nadine Matthias, DVM

Pamela Wenzel, Ph.D.
Assistant Professor

Regulation of stem cell potential by biomechanical force

Stem cell potential is tightly linked to biomechanical forces present in the microenvironment. Members of our lab study how extracellular cues, such as mechanical force, impact function, development, specification, and expansion of stem cells.

One arm of our research is designed to address how biomechanical force activates the hematopoietic program during embryogenesis and how we might use this information in the laboratory to expand improved sources of hematopoietic 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.

Shear stress, or frictional force, also modulates the behavior of mesenchymal stem cells, and impacts proliferation, cell survival, and fate decisions. Mesenchymal stem cells are emerging as powerful tools for regenerative medicine, and current research suggests that these types of cells positively impact inflammatory signaling and innate immune response. Consequently, our second area of interest is to determine how mechanical force alters the biology of mesenchymal stem cells, including their ability to modulate vascular permeability and inflammation. We utilize culture-based assays and therapy models of stroke and traumatic brain injury as readouts of stem cell response to mechanical stimuli.

Finally, fluid flow and hydrostatic pressure have been implicated in tumor biology, but it remains unclear what role lymphatic or vascular shear stresses may play in modulating the gene expression programs or metastatic potential of cancer cells. Using custom microfluidics, we modulate the shear stress present in the cancer cell microenvironment and evaluate its impact on invasive potential and activation of oncogenic pathways. Together, we hope that these approaches will translate to improved treatment options for pediatric and adult patients affected by immune disease, inflammation, or cancer.

RESEARCH PROJECTS

- Mechanobiology of blood development.
- Biomechanical modulation of anti-inflammatory genetic programs in mesenchymal stem cells.
- Role of force in initiation of metastatic programs.

KEY PUBLICATIONS

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

Li, N., Diaz, M.F., Wenzel, P.L. (2014) Application of Fluid Mechanical Force to Embryonic Sources of Hemogenic Endothelium and Hematopoietic Stem Cells. *Methods Mol Biol*, in press. DOI 10.1007/7651_2014_95.

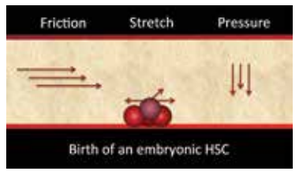
Lee, H.J., Li, N., Evans, S.E., Diaz, M.F., Wenzel, P.L. (2013) Biomechanical force in blood development: extrinsic physical cues drive pro-hematopoietic signaling. *Differentiation* 89: 92-103.

Gustafsson, K., Heffner, G., Wenzel, P.L., Curran, M., Grawe, J., McKinney-Freeman, S.L., Daley, G.Q., Welsh, M. (2013) The Src homology 2 protein Shb promotes cell cycle progression in murine hematopoietic stem cells by regulation of focal adhesion kinase activity. *Exp Cell Res*: 319: 1852-1864.

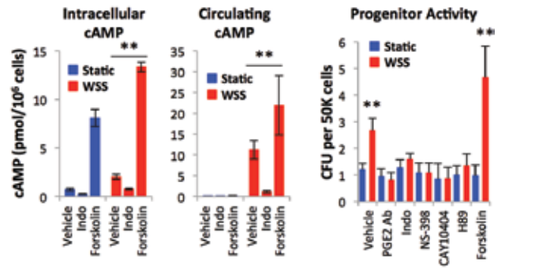
Chong, J.-L.*, Wenzel, P.L.*, Saénz-Robles, M.T.*, Nair, V., Ferrey, A., Hagan, J.P., Gomez, Y.M., Sharma, N., Chen, H.-Z., Ouseph, M., Wang, S.-H., Trikha, P., Culp, B., Mezache, L., Winton, D.J., Sansom, O.J., Chen, D., Bremner, R., Cantalupo, P.G., Robinson, M.L., Pipas, J.M. and Leone, G. (2009). E2F1-3 switch from activators in progenitor cells to repressors in differentiating cells. *Nature* 462: 930-934. *Equal contribution.

LAB MEMBERS

Research Associate: Miguel Diaz
Postdoctoral Fellows: Hyun Jung Lee, Ph.D., Nan Li, Ph.D.
Research Assistant: Abishek Vaidya, M.S.
Students: Katherine Price, Alexander Alexander, Joyce Ozuna, Hannah Willey
Administrative Assistant: Stephanie Baca (Pediatric Surgery)



Hematopoietic stem cells (HSCs) emerge from the lumen of blood vessels and are exposed to various biomechanical forces associated with cardiac output during embryogenesis.



Cyclic AMP (cAMP) production is activated by wall shear stress (WSS) in a PGE2-dependent manner to drive developmental signaling required for hematopoiesis (asterisks ** denote statistical differences by two-way ANOVA; p=0.001). Protein kinase A inhibition by H89 reduces progenitor activity, whereas stimulation of cAMP levels by an adenylyl cyclase agonist (forskolin) elevates hematopoietic activity above static vehicle controls.

Jiaqian Wu, Ph.D.
Assistant Professor

Gene transcription and regulation of stem cell differentiation

My laboratory combines stem cell biology and systems-based approaches involving genomics, proteomics, bioinformatics, and functional assays to unravel gene transcription and regulatory mechanisms governing stem cell differentiation. One major focus of our group is investigating stem cell neural differentiation and developing effective and safe treatment for spinal cord injury and neurological diseases. We are studying gene expression and the regulation of transcription factors and regulatory RNAs using next-generation sequencing technologies including RNA-Seq and ChIP-Seq. These studies are crucial in understanding the molecular mechanism of stem cell neural differentiation and its clinical implications. Our goal is to identify and modulate key regulators as therapeutic targets to direct the differentiation of stem cell into neural cells more efficiently, and to increase transplantation safety.

The other area of our research interest lies in the studies of the regulatory networks of hematopoietic precursor cell self-renewal and differentiation using multipotent EML (erythroid, myeloid, and lymphocytic) cell as a model system. We are using integrated genomic and proteomic approaches to identify key components that control the switch. We have identified TCF7, together with RUNX1 are important regulators in this process. Future study will generate a global interaction network and a novel and comprehensive view of the regulation of earl stages of hematopoietic precursor self-renewal and differentiation. This study can serve as a model for the analysis of cell self-renewal and differentiation in general and provide insight for efficient expanding and manipulating hematopoietic precursor and stem cells, including reprogramming partially differentiated cells to return them to a self-renewing state.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Wu, J. Q. (2011). Characterize mammalian transcriptome complexity. Deutschland, Germany: LAP LAMBERT Academic Publishing.

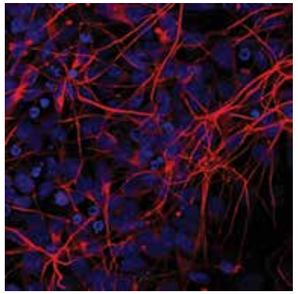
Wu, J. Q., Seay, M., Schulz, V., Hariharan, M., Tuck, D., Lian, J., Du, J., Shi, M., Ye, Z. J., Gerstein, M., Snyder, M., and Weissman, S. (2012). Tcf7 is a key regulator of the self-renewal and differentiation switch in a multipotential hematopoietic cell line. *PLoS Genet* 8(3): e1002565.

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

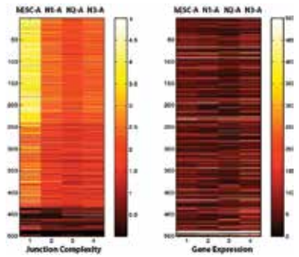
Zhang, Y., Chen, K., Sloan, S., Bennett, M., Scholze, A., O'Keeffe, S., Phatnani, H., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S., Zhang, C., Daneman, R., Maniatis, T., Barres, B., Wu, J.Q. An RNA-Seq transcriptome and splicing database of neurons, glia and vascular cells of the cerebral cortex. *Journal of Neuroscience*. In Press.

LAB MEMBERS

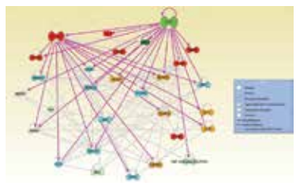
Postdoctoral Fellows: Kenian Chen, Xiaomin Dong, Xiaojing Dai, Zhihua Qi
Undergraduate student (University of Houston): Abdur Jamal



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



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



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

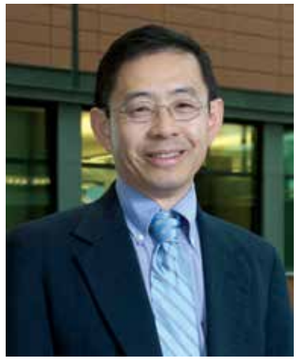
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston for the discovery, development, and 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 quickly brought in significant funding from the pharmaceutical and the biotechnology industry, including Johnson & Johnson, Merck and PanaMab, the National Institutes of Health, and the Cancer Prevention and Research Institute of Texas, and have made significant scientific discoveries in the areas of cancer biology 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.

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 are also support collaborative projects with scientists from the IMM, the Texas Medical Center, and other Texas-based institutions.

Zhiqiang An, PhD
Professor and 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) HER3mediated 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, denge, 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).

Pnemocandin 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 engineering analogues with improved oral availability or broader spectrum of antifungal activities.

Therapeutic monoclonal antibody drug discovery platform. Supported by a grant from the Texas Emerging Technology Fund and as part of the Texas Therapeutics Institute, our group has been building a comprehensive antibody drug discovery platform with a focus on antibody lead optimization technologies such as antibody phage display, deep sequencing of antibody encoding genes from individual antibody expressing B cells, affinity maturation and humanization.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Kai Sun, Jiyoung Park, William L. Holland, Olga Gupta, Pernille Landrock Auerbach, Ningyan Zhang, Roberta Goncalves Marangoni, John Varga, Thorkil Plough, Zhiqiang An and Philipp E. Scherer. 2014. Endotrophin Triggers Adipose Tissue Fibrosis, Inflammation and Metabolic Dysfunction. *Nature Communications* (2014 Mar 19;5:3485. doi: 10.1038/ncomms4485).

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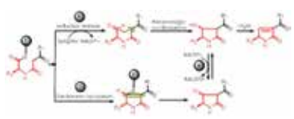
Yun Shi, Xuejun Fan, Weixu Meng, Hui Deng, and Ningyan Zhang, and Zhiqiang An. 2014. Engagement of immune effector cells by trastuzumab induces HER2 downregulation in cancer cells through STAT1 activation. *Breast Cancer Research* 16(2):R33.

Lina Wang Dunne, Zhao Huang, Weixu Meng, Xuejun Fan, Ningyan Zhang, Qixu Zhang, Zhiqiang An. 2014. Human decellularized adipose tissue scaffold as a model for breast cancer cell growth and drug treatments. *Biomaterials* 35(18):4940-9.

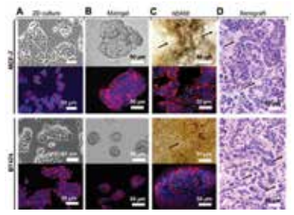
Xuemei Niu, Li Chen, Qun Yue, Baile Wang, Junxian Zhang, Chunyan Zhu, Keqin Zhang, Gerald F. Bills, and Zhiqiang An. 2014 Characterization of thermolide biosynthetic genes and a new thermolide from sister thermophilic fungi. *Org Lett* 16 (14), pp 3744–3747.

LAB MEMBERS

Post Docs: Ahmad S. Salameh, Weixu (Ella) Meng, Leike (Simon) Li, Shu (Selena) Zhang Qun Yue (jointly with Dr. Bills), Yan Li (jointly with Dr. Bills), Li Chen (jointly with Dr. Bills)
Student: Ziyi (Wendy) Huang



Release mechanisms of fungal PKS–NRPS hybrids to yield tetramic acids (Org Lett 16 (14), pp 3744–3747).



Cellular organization of breast cancer cells in 2D cultures, Matrigel, hDAM, and xenografts (Biomaterials 35(18):4940-9).

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

Fungi produce many bioactive secondary metabolites useful in medicine, including antibacterials (penicillin, cephalosporins), antifungals (pneumocandins, griseofulvin, and strobilurins), immunosuppressants (cyclosporin A, mycophenolic acid), antihypercholesterolemia agents (lovastatin), and migraine and obstetrics pharmacologics (ergot alkaloids).

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 are currently characterizing the polyketide synthase-nonribosomsal synthase pathway responsible for pneumocandin B0, the starting molecule for the antifungal drug CANCIDAS. Our goal is to develop methods to reprogram pneumocandin biosynthesis and produce new chemical derivatives that overcome resistance, or that have improved potency, spectrum and pharmacokinetics, while reducing fermentation production costs. Characterization of related lipopeptide pathways will enable us to recombine genes from these pathways to produce hybrid natural products with improved therapeutic properties.

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

RESEARCH PROJECTS

- Biosynthesis and pathway engineering of the pneumocandin lipopeptides for improved antifungals. Biosynthesis and production of the thermolides, potent nematocidal polyketide-amino acid macrolides from the thermophilic fungus, *Talaromyces thermophilus* (with Prof. Xue-Mei Niu).
- Development of methods for reprogramming transcription of biosynthetic genes of fungi to discover new natural products useful to treat human diseases.
- Development of a natural products 'chemical resource platform' for drug discovery for other investigators within the UT System, Texas and elsewhere.

KEY PUBLICATIONS

Bills, G.F., Y. Li, L. Chen, Q. Yue, X. Niu & Z. AN. 2014. New insights into the echinocandins and other fungal non-ribosomal peptides and peptaibiotics. *Natural Product Reports* 31:1348-1375.

Niu, X., L. Chen, Q. Yue, B. Wang, J. Zhang, C. Zhu, K. Zhang, G.F. Bills & Z. An. 2014. Characterization of thermolide biosynthetic genes and a new thermolide from sister thermophilic fungi. *Organic Letters* 16: 3744–3747.

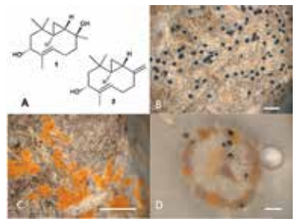
Igarashi, Y., T. Hanafusa, F. Gohda, S. Peterson & G. Bills. 2014. Species-level assessment of secondary metabolite diversity among *Hamigera* species and a taxonomic note on the genus. Mycology: An International Journal of Fungal *Biology* 5:102-109

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

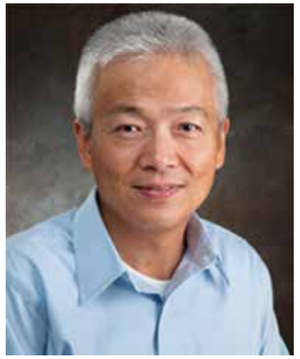
Chen, L., Q. Yue, X. Zhang, M. Xiang, C. Wang, S. Li, Y. Che, F.J. Ortiz-López, G.F. Bills, X. Liu & Z. An. 2013. Genomics-driven discovery of the pneumocandin biosynthetic gene cluster in the fungus *Glarea lozoyensis*. *BMC Genomics* 14:339.

LAB MEMBERS

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



Hypocoprins A-B, new sesquiterpene antibacterials from *Hypocopra rostrata* (Ascomycota, Xylariales) collected in Hitchcock, Galveston Co. Texas. A. Structures of hypocroprins A (1) and B (2). B. Ascomata sporulating on horse dung (Bar = 1 mm). C. Conidia sporulating on horse dungi (Bar = 5 mm). D. The fungus producing both ascomata and conidia in artificial culture (Bar = 1 cm). *Hypocopra rostrata* was first discovered on horse dung in Arizona in 1903. Its chemical properties have never been investigated. This specimen not only yielded new antimicrobial chemistry, but the collection represents the first report of this microorganism for the state.



Xiaodong Cheng, Ph.D.
Professor

cAMP - mediated cell signaling and drug discovery

Our laboratory studies intracellular signaling associated with second messenger cAMP. We apply multidisciplinary approaches, coupling biochemistry, biophysics and cell biology with pharmacology and chemical biology, to understand the structure and function of exchange proteins directly activated by cAMP (EPAC). Our goals are to unravel the signaling intricacies of EPAC proteins and to design pathway specific probes for these important signaling molecules so that their functions can be pharmaceutically exploited and modulated for the treatment of human diseases.

Our laboratory has developed first-in-class EPAC selective inhibitors and EPAC knockout mouse models to study the physiological functions and diseases relevance of this family of important signaling molecules. Recently, we have identified a potential use of EPAC inhibitors in the prevention and treatment of fatal rickettsioses. Currently, we are actively engaged in developing second generation isoform specific EPAC inhibitors and agonists and in exploring their potential uses in various human diseases including cancer, diabetes, chronic pain and infections.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

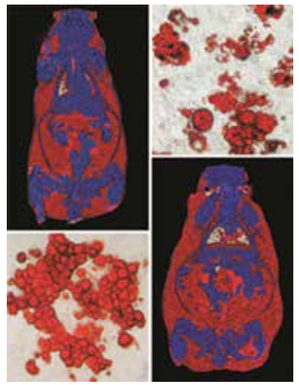
Almahariq, M., Tsalkova, T., Mei, F. C., Chen, H., Zhou, J., Sastry, S. K., Schwede, F., and Cheng, X. A novel EPAC-specific inhibitor suppresses pancreatic cancer cell migration and invasion. *Molecular Pharmacology*. 83:122-128, 2013.

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

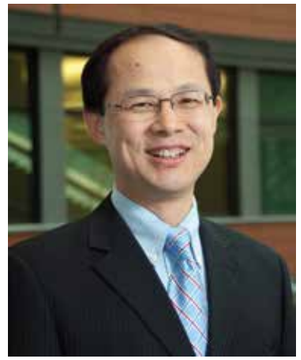
Tsalkova, T., Mei, F. C., Li, Sheng, Chepurny, O. G., Liu, T., Woods, Jr., V. L., Holz, G.G., and Cheng, X. Isoform-specific antagonists of exchange protein directly activated by cAMP. *Proc. Acad. Natl. Sci. USA.* 109:18613-18618, 2012.

LAB MEMBERS

Research Scientist: Yingmin Zhu
Post Docs: Hui Wang, Upasana Banerjee
Student: Yaohua Hu



Loss of EPAC1 increases leptin sensitivity and protects mice from high-fat diet induced obesity (*Molecular Cellular Biology*. 33:918-926).



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. My 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

- New regulators for cancer metastasis and their mechanisms of action.
- New regulators for EMT and their involvement in cancer progression.
- Development of precision medicine based on drug sensitivity of cultured circulating tumor cells from blood samples of cancer patients.
- Acquired resistance to cancer therapeutics.

KEY PUBLICATIONS

Li L, Liu C, Chang JT, Du G, Li W*. CDKL2 promotes epithelial-mesenchymal transition and breast cancer progression. *Oncotarget* (in press). *corresponding author

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

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

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

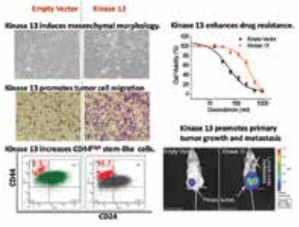
Grueneberg DA*, Degot S*, Pearlberg J*, Li W*, Davies JE*, Baldwin A*, Endege W, Doench J, Sawyer J, Hu Y, Boyce F, Xian J, Munger K, Harlow E. I. Comparing Kinase requirements across Various Cell types. *Proceedings of the National Academy of Sciences USA (PNAS)*. 2008 Oct 28;105(43):16472-7. *these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

Note: These three PNAS papers in 2008 were selected as Signaling Breakthroughs of 2008 selected by Science Signaling.

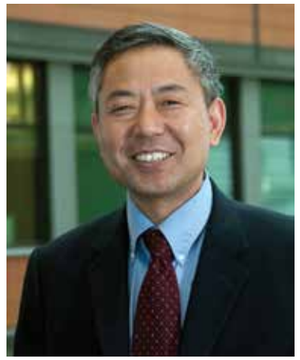
LAB MEMBERS

Postdoc Fellows: Meixiang Sang, Xueli Li, Zhi Li, Yan Zhang
PhD student: Mohit Hulsurkar
Visiting student: Xiaochong Zhang

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



An example of novel EMT regulators we identified that promote migration, stem cell-like phenotypes, drug resistance, primary tumor growth and metastasis.



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 also are believed to be the cells-of-origin for many types of cancer as they are programmed to divide indefinitely. Furthermore, tumor tissues are also heterogeneous in which only a subpopulation of cells can self-renew and provide daughter cells that make up the bulk of the tumor. These self-renewing cancer cells, designated cancer stem cells or tumor-initiating cells, often bear great similarity to normal stem cells in molecular profile and regulatory systems. Understanding of the mechanisms that govern the control of the self-renewal and differentiation of normal and cancer stem cells will provide crucial knowledge to the discovery and development of novel therapeutics for regenerative medicine and cancer treatment.

Our research is focused on delineating the function and mechanisms of a group of cell surface receptors called LGR4, LGR5, and LGR6 (LGR4-6) that play critical roles in the survival of normal stem cells and tumor cells. Previously, we discovered that LGR4-6 function as receptors of a group of stem cell factors called R-spondins (RSPOs) that are essential for the survival and growth of stem cells. We have now elucidated how RSPOs and LGRs work together to regulate cell growth and migration. Most recently, we uncovered that RSPO3-LGR4 has a major role in the aggressiveness of lung adenocarcinomas. Our current efforts are focused on identifying and characterizing drug leads targeting the RSPO-LGR system as potential treatment for colon and lung cancers.

RESEARCH PROJECTS

- Delineation of signaling mechanisms of stem cell receptors.
- Determination of the function and mechanism of the receptors in the control of normal and cancer cell growth.
- Investigation of the roles of aberrant expression of the RSPOs in the control of tumor metastasis of lung and colon cancer.
- Identification of lead molecules targeting the RSPO-LGR system as novel anticancer therapeutics.

KEY PUBLICATIONS

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

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

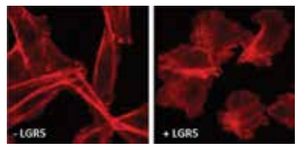
Carmon, K.S., Gong, X, Lin, Q., Thomas, A., and Liu, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A*, 108:11452-11457 (2011).

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

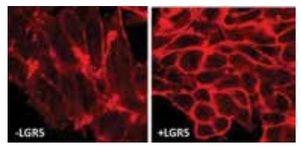
Carmon, K. S., Gong, X., Yi, J., Thomas, A., Liu, Q. RSPO-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proc Natl Acad Sci U S A.* 111: E1221-E1229 (2014).

LAB MEMBERS

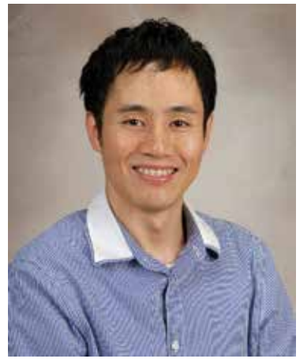
Instructors: Kendra Carmon and Xing Gong
Postdoctoral fellow: Jing Yi
Technician: Anthony Thomas



LGR5 alters cytoskeletal structure. CHO cells without (left panel) and with (right panel) expression of LGR5 stained for F-actin.



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 infectious diseases

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

With this background in mind, my lab is focused on two research projects by taking advantage of the power of organic chemistry and chemical biology. Firstly, we will conduct proteomic profiling using synthetic chemical probes based on antimicrobial metal complexes. In the past decades, metal complexes have attracted increasing attention as potential drugs for the treatment of cancer, autoimmune diseases, and, more recently, infectious diseases caused by drug-resistant pathogens. However, in general, their molecular targets are still unclear. Chemical probes based on such antimicrobial metal complexes will enable us to identify their protein targets and thus provide novel insights into pharmacological mechanisms and drug design for developing innovative antimicrobial therapeutics. Secondly, we will design, synthesize, and evaluate untraditional antimicrobial agents that could potentially circumvent drug resistance development. Our molecular design stems from the concept of "delivering catastrophic agents only to target pathogens." We will develop and evaluate various types of molecules consisting of "targeting" and "killing" motifs. Throughout these projects, we hope to drive our efforts toward innovative medical cures to save people suffering from serious diseases.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Tsuchikama, K.; Zhu, J.; Lowery, C. A.; Kaufmann, G. F.; Janda, K. D. C4-Alkoxy-HPD: A Potent Class of Synthetic Modulators Surpassing Nature in AI-2 Quorum Sensing. *J. Am. Chem. Soc.* 2012, 134, 13562-13564.

Tsuchikama, K.; Lowery, C. A.; Janda, K. D. Probing Autoinducer-2 Based Quorum Sensing: The Biological Consequences of Molecules Unable To Traverse Equilibrium States. *J. Org. Chem.* 2011, 76, 6981-7294. Selected as a Featured Article and featured on the front cover of Volume 76, Issue 17.

Tsuchikama, K.; Hashimoto, Y.; Endo, K.; Shibata, T. Iridium-Catalyzed Selective Synthesis of 4-Substituted Benzofurans and Indoles via Directed Cyclodehydration. *Adv. Synth. Catal.* 2010, 351, 2850-2854.

Tsuchikama, K.; Kasagawa, M.; Endo, K.; Shibata, T. "Cationic Ir(I)-Catalyzed sp3 C-H Bond Alkenylation of Amides with Alkynes", *Org. Lett.* 2009, 11, 1821-1823.

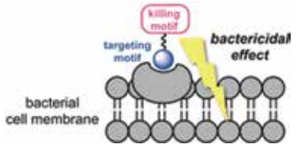
Shibata, T.; Tsuchikama, K. Recent advances in enantioselective [2+2+2] cycloaddition. *Org. Biomol. Chem.* 2008, 6, 1317-1323.

Tsuchikama, K.; Kuwata, Y.; Shibata, T. Highly Enantioselective Construction of a chiral Spirocyclic Structure by the [2 + 2 + 2] Cycloaddition of Diynes and exo-Methylene Cyclic Compounds. *J. Am. Chem. Soc.* 2006, 128, 13686-13687.

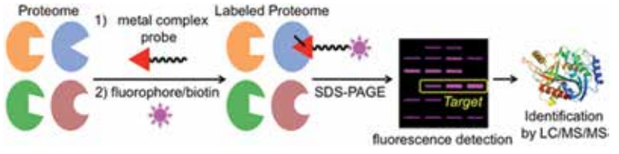
Shibata, T.; Tsuchikama, K. Ir-catalyzed almost perfect enantioselective synthesis of helical polyaryls based on an axially-chiral sequence. *Chem. Commun.* 2005, 6017-6019.

LAB MEMBERS

Dr. Tsuchikama joined TTI in July 2014 and is currently seeking postdoctoral fellows with experience in organic chemistry/medicinal chemistry.



Scheme of selective killing of pathogenic bacteria.



Scheme of target identification using antibacterial metal probes.

Ningyan Zhang, Ph.D.
Associate Professor

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

Monoclonal antibodies are becoming a major drug modality for cancer treatment and have shown clinical success for treatment of various types of cancer. Human epidermal growth factor receptor (EGFR) family consists of four closely related type 1 transmembrane tyrosine kinase receptors (EGFR/HER1, HER2, HER3 and HER4) and plays important roles in cell growth and signaling. Abnormal gene amplification and overexpression of EGFR and HER2 are well documented in many types of cancer and multiple therapeutic monoclonal antibodies such as, cetuximab, panitumumab targeting EGFR and trastuzumab and pertuzumab against HER2, are currently used in the clinic for treatment of different types of cancer. Similar to many molecular targeted cancer therapies, both innate and acquired resistance to those therapeutic monoclonal antibodies have been widely reported and present significant challenges in the clinic.

My research interest is to understand resistance mechanisms to cancer therapeutic antibodies targeting EGFR family members including the HER2 targeting antibody trastuzumab. Multiple mechanisms of action of trastuzumab have been proposed, including inhibition of HER2 signaling, prevention of HER2 extracellular domain shedding, and triggering immune effector function such as antibody dependent cellular cytotoxicity (ADCC) through the antibody Fc interaction with activating Fc gamma receptors expressed on immune effector cells. Our current research programs are focused on roles of immune modulation and evasion in cancer resistance to therapeutic antibodies such as trastuzumab. We have established cancer cells/immune cells co-culture system and *in vivo* mouse tumor models to investigate immune modulation in response to cancer therapeutic antibody treatment. We employ a wide array of experimental approaches including *in vitro* 2D and 3D cell culture, mouse tumor models, and studies with clinical samples from cancer patients. State-of-the-art technologies are used in our studies such as high-content fluorescence imaging, mass spectrometry, multi-color flow analysis, and fluorescence activated cell sorting (FACS). We also are studying the role of matrix metalloproteinases (MMPs) in cancer resistance to therapeutic antibodies. The long-term goal of our research is to identify key molecular markers that govern the dynamic interaction between cancer cells and immune cells in tumor microenvironment and to help 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 trastuzumab resistance.
- Modulation of anticancer immunity by antibody therapeutic treatment.

KEY PUBLICATIONS

Shi Y, Fan X, Meng W, Deng H, Zhang* NY, and An Z (2014) Engagement of immune effector cells by trastuzumab induces HER2 downregulation in cancer cells through STAT1 activation. *Breast Cancer Research* 16(2):R33.

Huang Z, Choi BK, Mujoo K, Fan X, Fa M, Mukherjee S, Owiti N, Zhang* NY, and An Z (2014) The E3 ubiquitin Ligase NEDD4 Negatively Regulates HER3/ErbB3 Level and Signaling. *Oncogene doi*: 10.1038/onc.2014.56.

Wang L, Huang Z, Meng W, Fan X, Zhang NY, Zhang Q, An Z (2014) Human decellularized adipose tissue scaffold as a model for breast cancer cell growth and drug treatments. *Biomaterials* 35(18):4940-9.

Kai S, Park J, Holland WL, Gupta O, Auerbach PL, Zhang NY, Marangoni RG, Varga J, Plough T, An Z and Scherer P (2014). Endotrophin Triggers Adipose Tissue Fibrosis, Inflammation and Metabolic Dysfunction. *Nature Communications doi:* 10.1038/ncomms4485).

Zhang* NY, Klegerman ME, Deng H, Shi Y, Golunski E, An Z (2013) Trastuzumab-doxorubicin conjugate provides enhanced anti-cancer potency and reduced cardiotoxicity. *J. Cancer Therapy* 4:308-322.

Fa M, Hoch K, Fan X, Dubinsky WP, An Z, Zhang* NY (2013) Novel approach for quantitative measurement of matrix metalloprotease-1 (MMP1) in human breast cancer cells using mass spectrometry. *J. Anal. Sci., Method and Instru.* 3: 54-61.

Freed DC, Tang Q, Tang A, Li F, He X, Huang Z, Meng W, Xia L, Finnefrock AC, Espeseth A, Casimiro DR, Zhang NY, Shiver JW, Wang D, An Z, Fu T (2013) A glycoprotein H complex is the primary target for potent neutralization by an experimental human cytomegalovirus vaccine. *PNAS* 110(51):E4997-E5005.

Fan X, Brezski RJ, Fa M, Deng H, Oberholtzer A, Gonzalez A, Dubinsky WP, Strohl WR, Jordan RE, Zhang* NY, An Z. (2012) A single proteolytic cleavage within the lower hinge of trastuzumab reduces immune effector function and *in vivo* efficacy. *Breast Cancer Res.* 14:R116.

Choi B-K, Fan X, Deng H, Zhang* NY, An Z. (2012) ERBB3 (HER3) is a key sensor in the regulation of ERBB3-mediated signaling in both low and high ERBB2 (HER2) expressing cancer cells. *Cancer Medicine. DOI:* 10.1002/cam4.10.

Choi B-K, Cai X, Yuan Y, Huang Z, Fan X, Deng H, Zhang* NY, An Z. (2012) HER-3 intracellular domains play a crucial role in HER3/HER2 dimerization and activation of downstream signaling pathways. *Protein & Cell* 3(10): 781–789.

Ha S, Ou Y, Vlasak J, Li Y, Wang S, Vo K, Du Y, Mach A, Fang Y and Zhang* NY (2011) Isolation and Characterization of IgG1 with Asymmetrical Fc Glycosylation. *Glycobiology* 21:1087-1096.

Zhang NY, Liu L, Dumitru CD, Cummings NR, Cukan M, Jiang Y, Li Y, Li F, Mitchell T, Mallem MR, Ou Y, Patel RN, Vo K, Wang H, Burnina I, Choi B, Huber H, Stadheim TA, Zha D (2011) Glycoengineered Pichia produced anti-HER2 is comparable to trastuzumab in preclinical study, *mAbs* 3:1-10.

LAB MEMBERS

Joined with Dr. Zhiqiang An's laboratory, see complete list of members on Dr. An's page.

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 UTH Health-IMM is a critical component of this commitment.

Antibody Engineering and Expression Service Center

Antibody therapeutics represents a major breakthrough in combating human diseases including cancer. Even though the pharmaceutical and biotechnology industries are in the center stage of drug discovery and development, academic researchers are increasingly engaged in discovering new antibody drug candidates. However, advancement of some of the promising antibodies in the early stage of discovery from academic research laboratories is often hindered by the lack of access to the expertise and infrastructure required for antibody lead optimization. Our antibody engineering and expression service center will fill the gap of the much needed expertise for the advancement of monoclonal antibodies from early discovery to lead optimization and preclinical development for the research and drug discovery communities. Objective of the service center is to provide technical support and services to advance proof-of-concept antibodies to the stage of preclinical development. Results generated from the core facility 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.

Director: ZHIQIANG AN, PHD,
Professor, Texas Therapeutics Institute
713-500-3011
Co-Director: NINGYAN ZHANG, PHD
Associate Professor, Texas Therapeutics Institute
713-500-3332

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 services and analysis 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.

Director: KEVIN ROSENBLATT, MD, PHD
Associate Professor, Center for Proteomics and System Biology
713-500-3611

Contact: LI LI,
Mass Spectrometry Specialist | Service Center Manager
713-500-2232

Collaboration Imaging Service Center

The IMM Center for Molecular Imaging is a facility that all researchers at UTHealth who are involved in small animal studies should be acquainted with. The Center is directed by Dr. Eva Sevick and led by eight engineering and basic science faculty members whose research focus 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 academic and industry researchers across the nation on small animal imaging projects in areas including 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, a Digi-Rad gamma camera, and an array of custom bioluminescence and fluorescence instrumentation that is paired with unique molecules for diagnosis and therapy. Generalized protocols are available to investigators to maximize benefit from the latest developments in molecular imaging.

Director: EVA M. SEVICK, PHD
Professor & Director
Center for Molecular Imaging
713-500-3560
Contact: Holly Robinson
Research Coordinator I
713-500-3606

Flow Cytometry Service Center

The Flow Cytometry Service Center is located on the sixth floor of the Fayez S. Sarofim Research Building and maintains four instruments: BD FACS Calibur, BD FACS Aria II, BCI FC500, and a Luminex 200.

These instruments are available on a fee-per-services charge to all research investigators from UTHealth or external organizations. These instruments allow scientist to evaluate a large number of samples in a short time frame and gather information on very rare populations of cells. The service center provides training, instrumentation, and technical expertise for both analysis and cell sorting.

Director: EVA M. SEVICK, PHD
Professor & Director
Center for Molecular Imaging
713-500-3560
Contact: AMY HAZEN, PHD
Assistant Director
713-500-3612

Tissue Histopathology Service Center

Our Center for Molecular Imaging is now providing in-house routine histology, special stain, and immunohistochemistry services in support of research projects to all research investigators from UTHealth or external organizations. With the growth of research activities that require histopathology services, the laboratory houses equipment for the preparation of thin sections; both paraffin and fresh frozen-tissue.

A full range of histopathology services is provided:

- Routine histology (process, embed, cut and stain)
- Section cut rolled and placed in microcentrifuge tub for DNA, RNA studies
- Multi-tissue embedding & sectioning
- Frozen tissue embedding & sectioning
- Blood smear stain
- Immunohistochemistry and special stain

Director: EVA M. SEVICK, PHD
Professor & Director
Center for Molecular Imaging
713-500-3560
Contact: SARAH AMRA, BS, HT (ASCP)
Chief Histology Technician
713-500-3386

Microscopy Service Center

The IMM Microscopy Service Center provides assistance in wide-field fluorescence microscopy, confocal microscopy, and image analysis. The facility is equipped with a Nikon Eclipse TE2000E inverted wide-field microscope, a Leica TSC SP5 upright confocal microscope with conventional and resonant scanner, and a dedicated computer workstation running Amira software for post-acquisition analysis of imaging data.

The Microscopy Service Center will support the research needs of all research investigators from UTHealth or external organizations on a fee-for-service basis by providing microscopy technical support, training, and consultation.

Director: EVA M. ZSIGMOND, PHD
Assistant Professor, Center for Immunology and Autoimmune Diseases
Director, Microscopy Service Center
713-500-2453
Contact: ZHENGMEI MAO, PHD
Manager
713-500-3389

Molecular Diagnostics Service Center

Our Molecular Diagnostic Laboratory, ProteoPath, provides diagnostic testing in a CLIA certified laboratory to all research investigators from UTHealth or external organizations on a fee-for-service basis. Major testing includes mass spectrometry (based on metabolites and Vitamin D) along with research testing. We serve as a diagnostic technology development site for The Brown Foundation Institute of Molecular Medicine, Clinical Laboratories, physicians, and other external organizations.

Director: KEVIN ROSENBLATT, MD, PHD
Associate Professor, Center for Proteomics and System Biology
713-500-3611
Contact: NATALIYA BULAYEVA, PHD (ASCP)
Lab Manager
713-500-3428

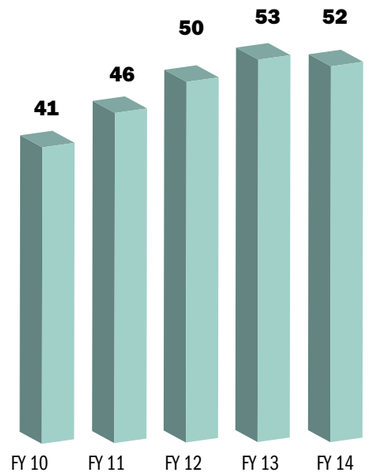
Transgenic and Stem Cell Services

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

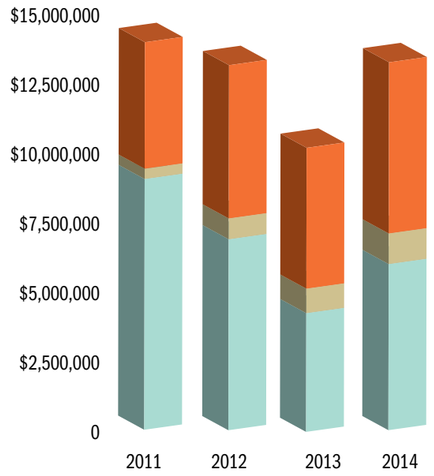
The stem cell lines that have been derived in the laboratory are highly effective for the generation of knock-out/ knock-in mice and for cell differentiation studies. In addition to the production, cryopreservation and re-derivation of genetically-engineered mice and rats, the services of the facility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

Director: EVA M. ZSIGMOND, PHD
Assistant Professor, Center for Immunology and Autoimmune Diseases
Director, Transgenic and Stem Cells Service Unit
713-500-2453
Contact: ALEKSEY DOMOZHIROV
Research Associate | Manager
713-500-2452

Number of Faculty

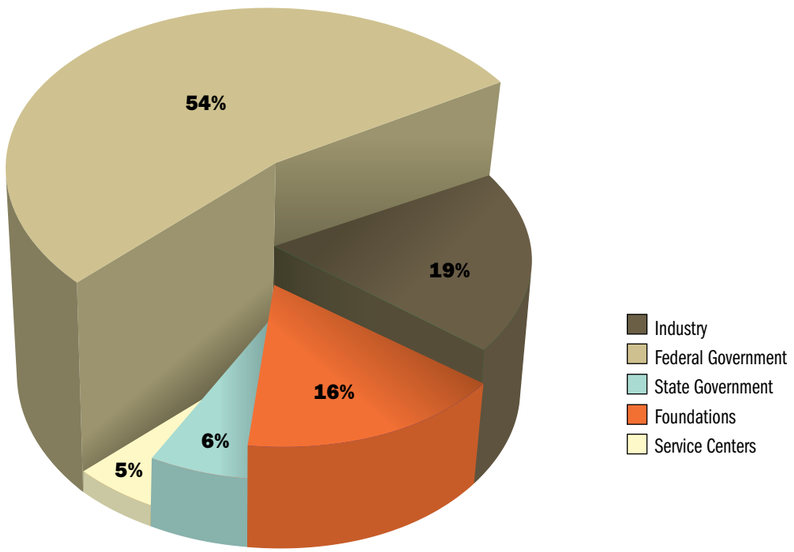


Total Funds Supporting Research



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

Total Expenses Supporting Research



Gift Report

New Gifts and Bequests Fiscal Year 2014

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