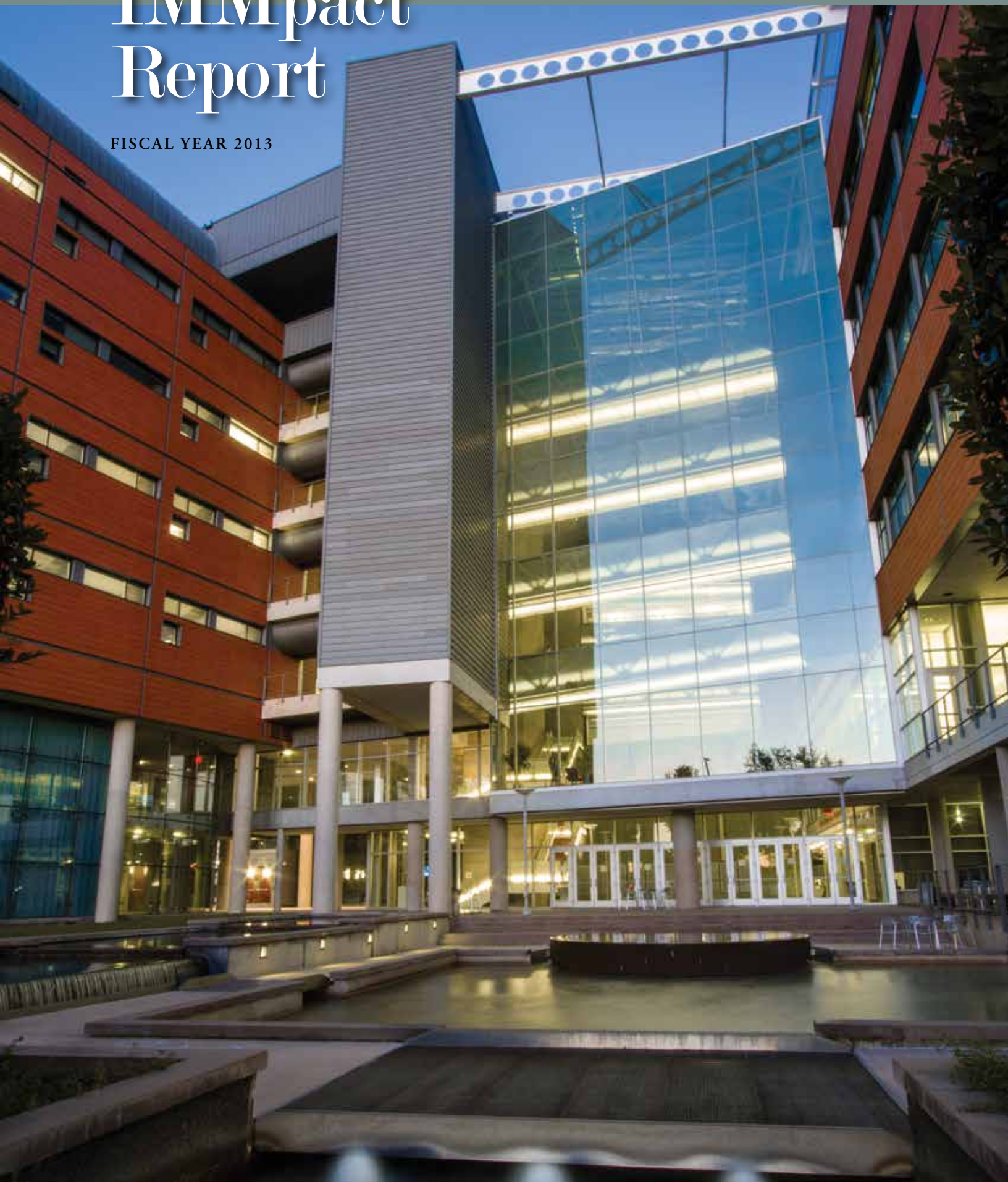
THE UNIVERSITY *of* TEXAS MEDICAL SCHOOL *at* HOUSTON'S
BROWN FOUNDATION INSTITUTE *of* MOLECULAR MEDICINE FOR THE PREVENTION *of* HUMAN DISEASES

IMMpact Report

FISCAL YEAR 2013



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 Fayez S. Sarofim Research Building is shown in this cover photo.

IMMpact Report is published by
The University of Texas Medical School at Houston.
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Director's Message

The IMM has two major objectives:

1. Discovery is the highest priority for the IMM faculty. This is a major challenge, since diabetes, 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.

The Brown Foundation Institute for Molecular Medicine for the Prevetion of Human Diseases (IMM) is pleased to provide our latest edition of the IMMpact Report for your review.

The IMM is a stand-alone research institute that is embedded within The University of Texas Medical School at Houston (a part of UTHealth). Our mission is to provide real translational outcomes that benefit patients. To this end, we have assembled teams comprised of basic scientists and clinicians, leveraging the best talents that the IMM and the UTHealth Medical School can offer. The Bentsen Stroke Center and our flagship programs in drug development, molecular imaging and regenerative medicine, which you will read about in this report, provide excellent examples of these collaborative teams. In the current issue, we have some in-depth feature articles on recent developments and specific information on every IMM faculty member and the innovative research in which they are engaged.

Just as we are breaking new ground in our discoveries, we aim to improve and develop our relationships with existing friends and donors and to forge new ones with people who value the aspirations of our science and appreciate our mission to translate molecular discoveries into new therapies for human disease. This year we have re-launched the IMM advisory council, under the leadership of Mr. Dudley Oldham to assist in the continued growth and development of the IMM.

At a time when basic and clinical science can potentially offer so much, especially in the areas of personalized and regenerative medicine, we face real challenges. As you are probably aware, the federal government continues to significantly reduce funding for scientific research. It is a remarkable testament to the quality and creativity of our scientists that the IMM faculty remains so successful in attracting research funds from this ever-diminishing pool. Never before, however, has the successful implementation of our mission been so dependent on seeking funding from alternative sources, including research charities, industry collaborations, and, most importantly, the continuing generosity of our friends and donors.

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 pleased to see you at the upcoming IMM symposium on March 20, 2014, when you can hear some exciting stories directly from our faculty; details are in the IMMpact report.

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.

The Institute of
Molecular Medicine for the
Prevention of Human Diseases

IMMpact Symposium

Personalized Medicine in Action

Thursday
March 20, 2014
6-8 p.m.

Fayez S. Sarofim Research Building
1825 Pressler Street

Bad Blood, Bad Lymph?

Eva Sevick, Ph.D.

Professor and Director, Center for Molecular Imaging
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research

Beyond Metal Joints

Brian Davis, Ph.D.

Professor and Director, Center for Stem Cell and Regenerative Medicine
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

Mining Texas Microbes for New Drugs

Gerald Bills, Ph.D.

Professor, Texas Therapeutics Institute

Save the Date

Research Looks at Connection Between Stress, Alzheimer's Disease

Anyone who has had a loved one with Alzheimer's knows that the disease causes stress. Caring for, or interacting with, someone with severe memory loss is challenging. But IMM researchers are looking at the other side of the coin, studying how stress can impact Alzheimer's.

"When it comes to Alzheimer's, most people focus on the loss of memory," says Nick Justice, Ph.D., assistant professor in the IMM's Center for Metabolic and Degenerative Diseases. "But there are also emotional components and neuropsychiatric changes that begin at the earliest stages of the disease."

Recent studies have linked depression and Alzheimer's disease. A University of California San Francisco study found that those with late-life depression were more than twice as likely to get Alzheimer's. An Alzheimer's Disease Neuroimaging Initiative study showed that those who report depression at the onset of Alzheimer's display faster loss of brain tissue density.

"Depression is the clinical manifestation of stress," Dr. Justice explains. "We are looking at the molecular and cellular responses to stress in order to control them and decrease their impact on this disease."

Scientists have discovered that animals with Alzheimer's make more corticotropin releasing factor (CRF), which is the initiator of the endocrine stress response.

These chemical changes in the body, brought about by stress, can change how the body functions and affect the brain's neurons. When one is under stress, CRF is released by the brain and causes the adrenal gland to release cortisol, which changes the way every tissue in the body functions, including the brain.

"Not living a chronically stressed life is beneficial to the health of your brain," Dr. Justice summarizes.

Half of 85-year-olds suffer from dementia, most often Alzheimer's disease, which is a debilitating disease marked by severe loss of memory and cognitive function. Clinical evaluation by a physician can lead to a diagnosis of mild cognitive impairment in the early stages of the disease, which will progress to dementia of the Alzheimer's type. Only 2 percent of Alzheimer's disease cases are caused by known genetic mutations, and a definitive diagnosis of Alzheimer's disease can only be made post-mortem.

"Alzheimer's disease can affect the way the brain functions in different ways. We want to understand how and when Alzheimer's disease causes emotional disturbances so that we can apply pharmacologic therapies to those patients, with the hope that this might slow progression of the disease," Dr. Justice says.

Dr. Justice joined the IMM in May 2013, following graduate work at UCSF and postdoctoral training at the Salk Institute. He came to Houston first as an instructor at the Baylor College of Medicine's Huffington Center on Aging.

Dr. Wylie Vale, with whom Dr. Justice completed his post-doctoral training at the Salk Institute, discovered CRF, and initiated development and testing of CRF receptor antagonist drugs for depression, which are currently in Phase 3 clinical trials.

"These drugs target hormonal disturbances and therefore have broader implications than just the treatment of depression. CRF receptor antagonists could prevent hormonal changes that occur during Alzheimer's disease and thereby limit or slow loss of cognitive ability," Dr. Justice says.

Does stress cause Alzheimer's?

"I would never say that stress causes Alzheimer's disease, but an overactive stress response accelerates the disease process that eventually becomes Alzheimer's disease," he says.

Nick Justice, PhD., is working to further clarify the links between stress and Alzheimer's disease.

Creating Cartilage

Scientists look to stem cells to fill in the gaps

The agony of knee pain. Or hip pain. Or elbow pain. Unless you have experienced it, you probably haven't given joint cartilage much thought.

Fortunately Naoki Nakayama, Ph.D., and his lab in the IMM's Center for Stem Cell and Regenerative Medicine are dedicated to cartilage regeneration and restoration.

"Once injured or degraded, joint cartilage in humans, especially in adults, does not heal by itself," says Dr. Nakayama, associate professor of molecular medicine. "In fact, once injured, there is continual deterioration of the cartilage."

Unlike other cells in the body that can be replenished, cartilage cells do not "turn over" frequently so the joint cartilage components will renew, on average, only once during our lifetime.

"When we are young, joint cartilage is white. As we age, so does the cartilage, turning yellow and looking old," Dr. Nakayama says.

There are two different types of joint injury that affect cartilage: post-traumatic osteoarthritis and general osteoarthritis. The post-traumatic type, as a result of sport or combat, takes a short amount of time and results in severe cartilage injury. The wear and tear associating with general osteoarthritis is a long-term process linked to aging or misalignment of the bones.

"Whatever the cause of injury, the joint cannot restore itself," Dr. Nakayama explains.

To repair these injuries, joints may be replaced with artificial instruments or smaller injuries may be treated by cracking the underlying bone, causing new tissue to grow in and fill the breaks.

"Unfortunately, a joint replacement does not last forever and can induce secondary pain, causing stress on other parts of the body. Creating tissue through breaking the bone is not a complete solution as this tissue is fibrous and not a typical joint cartilage," Dr. Nakayama says. "Cellular therapy that uses bone marrow stromal cells is in clinical trials, but scientists are finding that this material is not easy to use, since they can end up in a fibrotic cartilage or cartilage that is on the way to bone, both of which are not suitable for a stable joint cartilage."

Dr. Nakayama, who was recruited to the IMM in 2008 from the Australia Stem Cell Center, and his team are looking to a novel type of stem cells as a future solution for joint injury patients.

"Our question is how to recreate joint cartilage cells," he says.

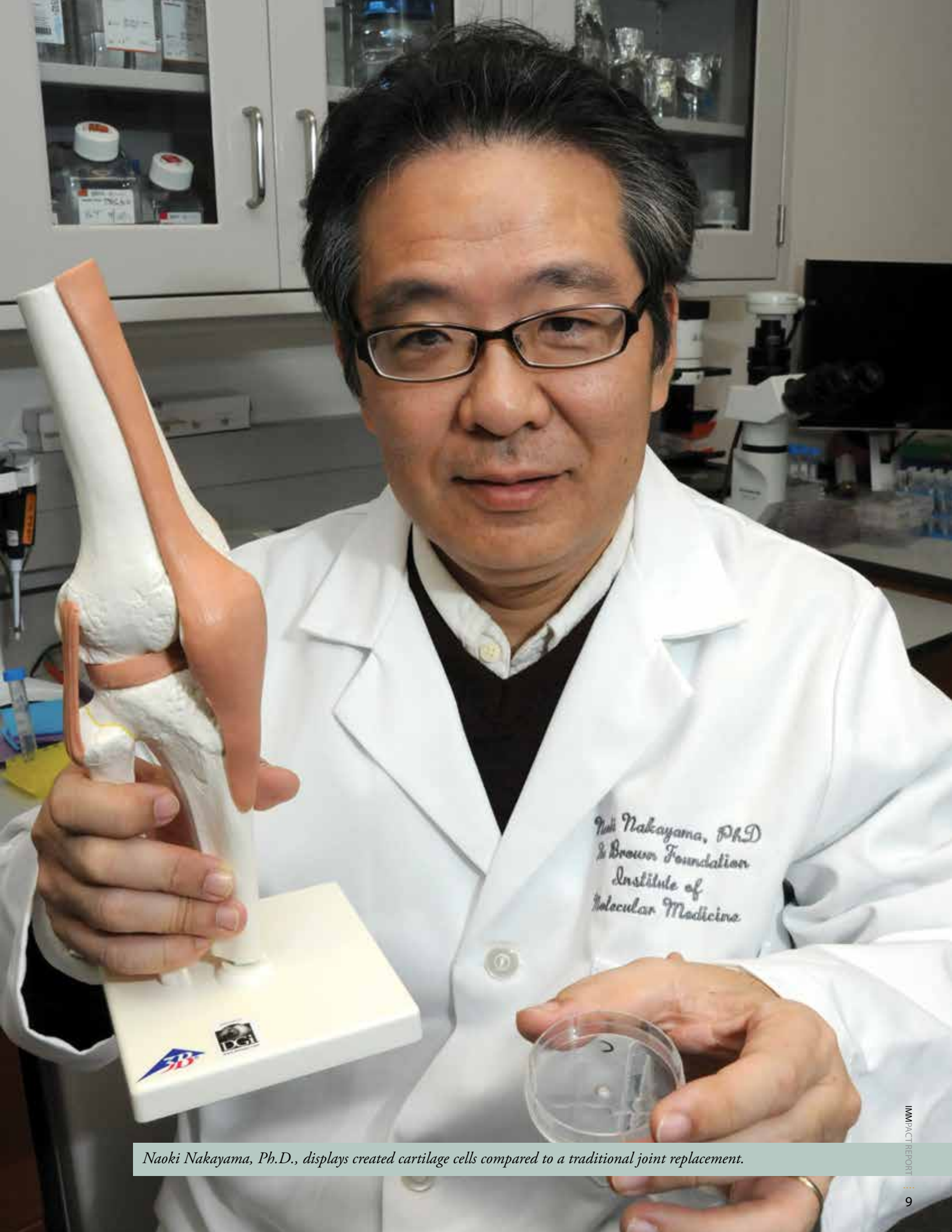
The scientists are using induced pluripotent stem cells (iPS) that behave like embryonic stem cells to create new cartilage cells. The iPS cells are not natural – they are taken from adult skin or blood cells and genetically manipulated to become stem cells that can turn into any type of cell – in this case joint cartilage cells.

"Joint cartilage is designed and formed during our fetal life. There is a good possibility that the proper joint cartilage-forming cells only exist prior to birth. The iPS cell system is thus by far the best tool for us to create such embryonic joint cartilage-forming cells," he explains.

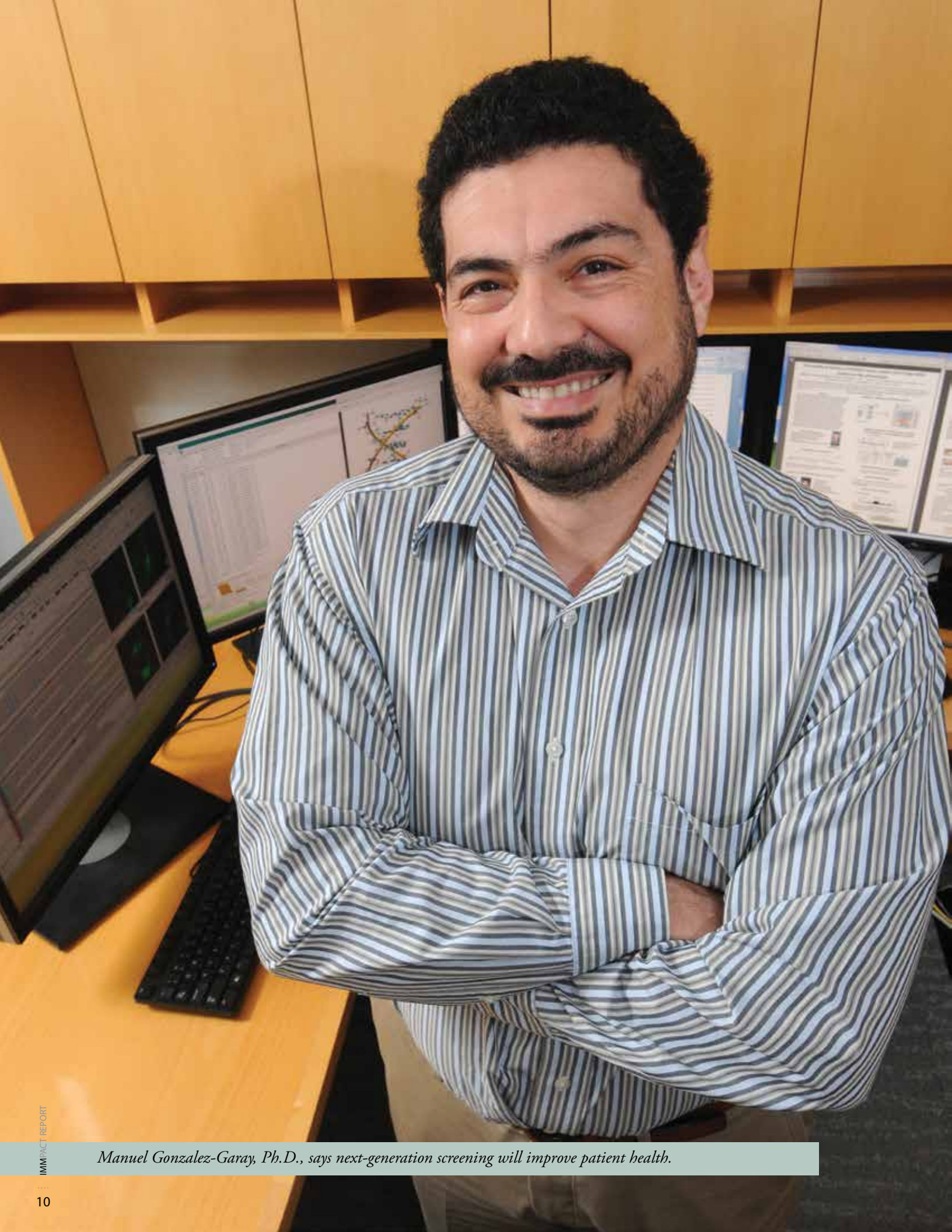
Researchers are placing the human iPS cell-derived joint cartilage cells into mice to evaluate the cells' activity and determine if they replace the adult cells and act more like them to form joint cartilage.

"Our long-term goal is looking at therapy. Since we are dealing with human stem cells, if we can verify the joint cartilage cells' activity in a preclinical model, it can move forward to clinical trials," Dr. Nakayama explains.

> "Once injured or degraded, joint cartilage in humans, especially in adults, does not heal by itself." — Dr. Naoki Nakayama



Naoki Nakayama, Ph.D., displays created cartilage cells compared to a traditional joint replacement.



Manuel Gonzalez-Garay, Ph.D., says next-generation screening will improve patient health.

Study Demonstrates Benefits of Genetic Screening

What if a simple blood draw or a drop of saliva could tell you what diseases you are at risk for in your lifetime and which medical treatments will best prevent them?

This is the future of clinical genomics and pharmacogenetics – identifying the unique chemical structure of your genes and their mutations.

Discovering how genes are expressed and mutated will help explain genetic links to disease and further tailor treatment options. Completed in 2004, the internationally collaborative Human Genome Project took 13 years, with the participation of 20 international centers at a cost of $3 billion. The first use of next-generation sequencing to detect a genetic defect in humans was in 2010 by Dr. Jay Shendure, of the University of Washington.

While next-generation sequencing has not yet become a routine part of the annual physical, 81 Houstonians recently were a part of a genetic research study undertaken by Manuel Gonzalez-Garay, Ph.D., and his colleagues.

"We completed a full genome analysis of each volunteer and compared the genetic findings to their medical history," explains Dr. Gonzalez-Garay, assistant professor in the IMM's Center for Molecular Imaging.

"This work represents a proof-of-concept to demonstrate that genomic sequencing has clinical value for a healthy individual."

Half of those tested showed known recessive gene mutations. All of those tested had previously undescribed mutations in known recessive genes. A single recessive mutation in a gene does not affect the health of the patient, it makes him/her a carrier. Since there are two copies of each gene, we have redundancy in our cells. However, if two carriers of a mutated gene have a baby, there is a one-in-four chance that the child will have an autosomal recessive disorder, which has devastating effects in newborns.

For dominant mutations, only one mutation is needed to develop a disorder.

From the 81 study participants, researchers were able to match the disorder of the participant to a previously known mutation in 24 cases. Scientists also identified the presence of genetic risk markers in multiple disease categories, such as cardiovascular, cancer, metabolic, and neurological. Genetic risk markers are mutations that have been associated with a disorder that develops at a late stage of life and can be used as an early predictor of an increased risk to develop the disorder.

"The main challenge in this area of research is our limited knowledge," says Dr. Gonzalez-Garay, who was recruited to the IMM in 2010. "Of the 25,000 genes, only 2,500 are known to be associated with a disorder. And from the millions of possible mutations, there are only approximately 100,000 gene mutations that are officially accepted as responsible for monogenic disorders."

One member of the study group asked for his nephew's genome to be sequenced as part of the project.

"The child was having issues and despite all of the medical tests, they couldn't pinpoint a disease," Dr. Gonzalez-Garay says, adding that the boy was suspected to have Prader-Willi syndrome.

Children with Prader-Willi syndrome are born small and have issues with feeding, thriving, and sexual development. As they age, they may become morbidly obese.

"This child had some of the qualifications but not all. Upon his genetic analysis, we found a new gene associated with the disorder 'MAGEL2.' This is the first time that a mutation in a single gene has been associated with Prader-Willi syndrome and autism," Dr. Gonzalez-Garay explains.

The publication of the finding in the prestigious journal *Nature Genetics* included three additional cases of Prader-Willi patients from Baylor College of Medicine.

"We expect that this finding will help in the near future to find a cure for this debilitating disorder," Dr. Gonzalez-Garay says.

"This technology has the potential to change someone's life."

Taking Microbes to the Next Level

In a lab unique in Houston and Texas, a group of researchers is isolating, growing, and studying fungi and the chemicals they make.

Whether they're from a dead leaf of a holly bush, found in silage or compost, or from a hot spring in the Yunnan Province of China – Texas Therapeutics Institute researchers are dissecting and evaluating molds for their therapeutic properties.

"Fifty to 80 percent of all marketed drugs are either a natural product, a derivative of a natural product, or of a synthetic structure based on a natural product," explains Gerald Bills, Ph.D., professor in the Texas Therapeutics Institute. "And 85 percent of our antibiotics are based on natural products."

Fungal chemistry has intrinsic biological activity in animal systems, Dr. Bills explains, as 30 percent of yeast genes have homologs – genes with a common ancestral DNA – in mammals.

"Looking to nature for healing properties is a prehistoric idea," Dr. Bills says. "Probably plants were used first because humans could see them and enough quantity is there. With microorganisms, like fungi, we have to grow enough new cells to make the product."

The modern use of microbes for therapy started with penicillin and streptomycin.

"These discoveries and applications helped lay the foundation for the modern pharmaceutical industry," Dr. Bills says.

Bills, who received his Ph.D. in botany and mycology from Virginia Tech, completed a postdoctoral fellowship at the USDA Agriculture Research Service and at the University of Wyoming. He joined the IMM in 2012, recruited by Zhiqiang An, Ph.D., with whom he had worked at Merck.

"I worked in natural products drug discovery at Merck for 20 years. I was lucky to have learned in a department where they really pioneered natural products research and developed such drugs as lovastatin, the first cholesterol drug, and thienamycin, one of the most potent antibiotics known," he says. "That department no longer exists, so it is important that somebody carries on their work and teaches young people about the methods for drug discovery from microbes."

The IMM and the Texas Medical Center are providing the collaborative environment for Dr. Bills and his colleagues to take microbes to the next level.

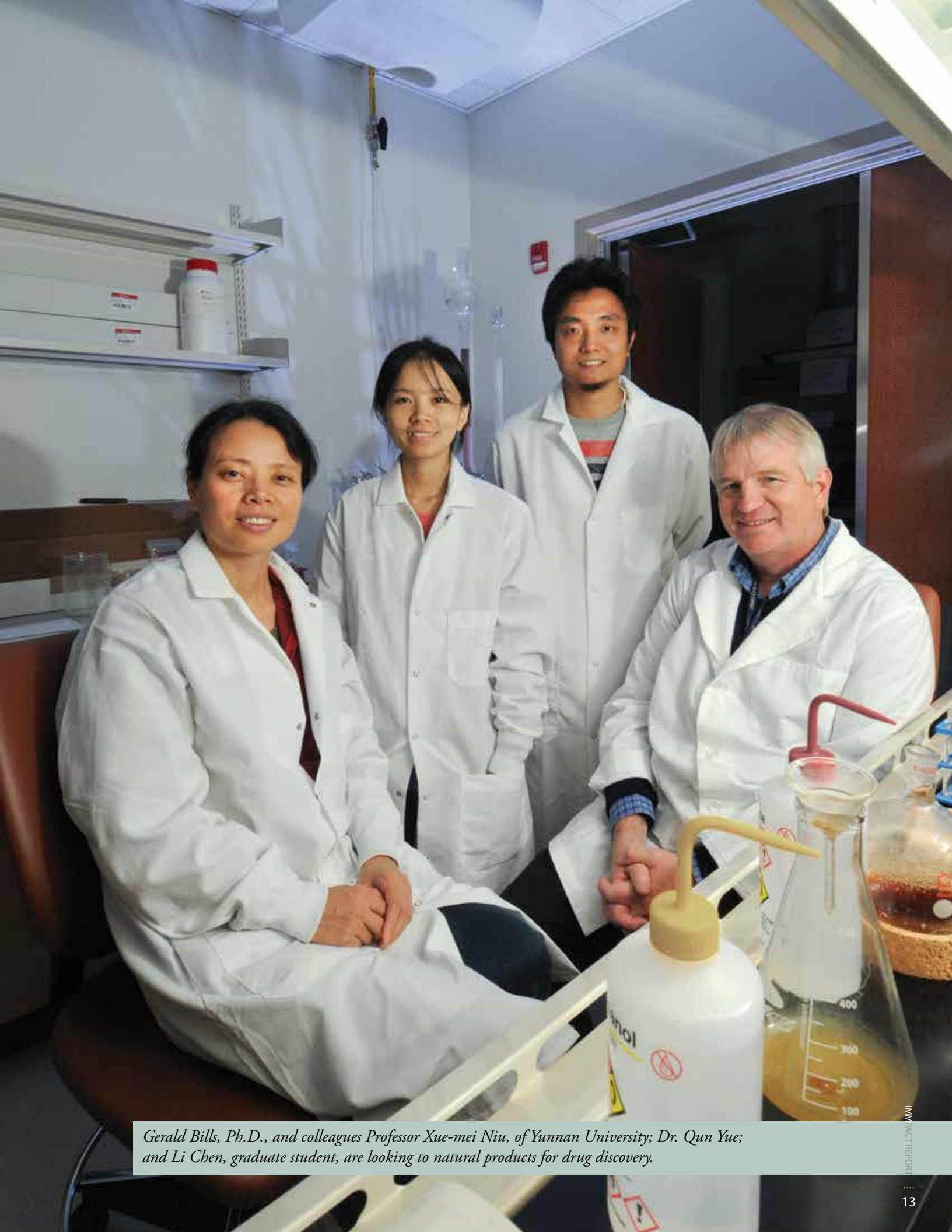
"One of our goals is to build a microbial chemical collection for drug discovery," Dr. Bills says, adding that the Texas Medical Center is home to two large-scale drug screening facilities – one at the Texas A&M Health Science Center and one at the MD Anderson Cancer Center.

The lab is dissecting the biosynthetic machinery of a fungus whose fermentation products kill roundworms. They also are looking to create new antifungals that would treat infections in the lungs, throat, or bloodstream. The research involves taking an important Merck product, caspofungin, one step further, by sequencing the fungus' genome and carrying out gene knockout experiments that alter biosynthetic expression, resulting in new variants of this successful antifungal drug.

"We are trying to understand how and why microorganisms make their secondary metabolites," Dr. Bills explains. "The most exciting part is when we find a new application for one of these molecules, which takes chemists and biologists working together."

Dr. Bills says he has collected microscopic fungi from across the United States and in many other countries. "But we have everything we need in Texas," he says, adding that there are more than enough natural products in the Lone Star State for all researchers.

> "Fifty to 80 percent of all marketed drugs are either a natural product, a derivative of a natural product, or of a synthetic structure based on a natural product."
>
> — Dr. Gerald Bills



Gerald Bills, Ph.D., and colleagues Professor Xue-mei Niu, of Yunnan University; Dr. Qun Yue; and Li Chen, graduate student, are looking to natural products for drug discovery.

Annie and Bob Graham: Leadership in Giving

The design of the successful New Frontiers Campaign, the largest fundraising initiative in the history of The University of Texas Health Science Center at Houston, included not only the resources for construction of a superior physical environment for research, but the long-term financial commitment to help UTHealth recruit top faculty.

By the time the Fayez S. Sarofim Research Building was dedicated as the new home of The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM), 27 philanthropists had stepped forward to provide the endowments to enable UTHealth to bring outstanding scientists to the Institute. Starting in 2001 and spanning five years, the New Frontiers Campaign raised a total of $240 million to benefit the IMM.

Annie and Bob Graham were among those who created one of the campaign's founding endowments, The Annie and Bob Graham Distinguished Chair in Stem Cell Biology.

Annie Graham observes, "We believe in the mission of the UT Health Science Center and knew that this was the right thing to do."

The couple's $1 million gift created a permanent endowment, held now by Brian R. Davis, Ph.D., associate professor and director of the IMM Center for Stem Cell & Regenerative Medicine. Funds from the endowment are used to further research involving stem cells, which can be used in transplantation medicine, to regenerate tissues, and to provide treatments for a number of chronic diseases, including heart and vascular disease.

Bob Graham notes, "We feel that the future of medicine depends upon opening new frontiers. Stem cell biology is a critical 'first step' in unlocking paths toward cures for many illnesses. Without the research necessary, that first step cannot be taken."

Both Annie and Bob Graham are strong supporters of UTHealth leadership and of UTHealth as a whole.

"We feel that it is important to contribute to the efforts of the Health Science Center because it is a driving force in this community," Annie Graham says. "The six schools have the opportunity to educate thousands of potential doctors, nurses, and health care providers. In our opinion, it is a shining star in our community."

"We are so grateful to Annie and Bob for their commitment not only to our research programs but also to our entire university and our role as educators. They are loyal friends of UTHealth, and their enthusiasm and commitment are priceless to me," says President Giuseppe N. Colasurdo, M.D.

The Grahams also support the School of Biomedical Informatics, creating the Robert H. Graham Professorship in Entrepreneurial Biomedical Informatics and Bioengineering in 2009. Bob also serves on the School of Biomedical Informatics Advisory Council.

Both Annie and Bob Graham serve on the UTHealth Development Board. Bob Graham, who received his BS and MS in electrical engineering and an MBA in finance from The University of Texas at Austin, will become chair in 2014.

> "We are so grateful to Annie and Bob for their commitment not only to our research programs but also to our entire university and our role as educators."
>
> — President Giuseppe N. Colasurdo, M.D.

Bob and Annie Graham are founding supporters of the IMM.

Center for Cardiovascular Genetics

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 Medical Center, 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.

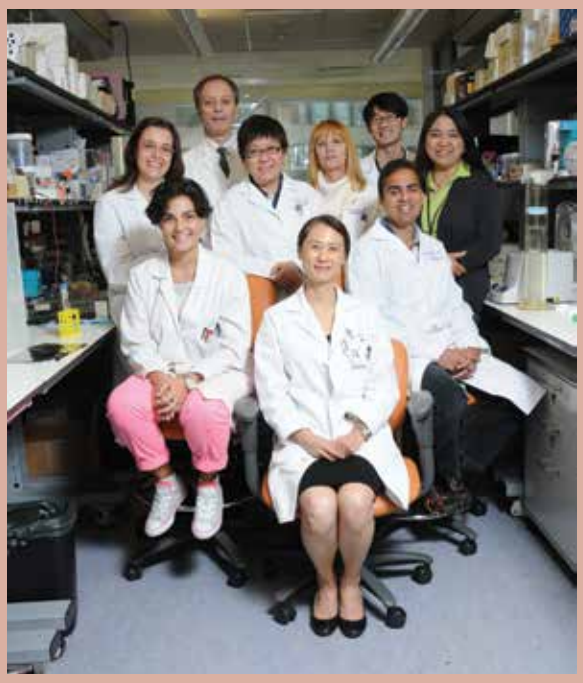
MISSION: To prevent cardiovascular diseases in humans through elucidating their molecular genetic causes. Genetic studies afford the opportunity to prevent the disease prior to the development of clinical manifestations of the disease. Delineation of the molecular pathways that link the mutations to 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 studies: These studies are designed to delineate the molecular genetic and genomic basis of cardiovascular diseases in humans with a specific focus on three most common forms of hereditary



cardiomyopathies; namely Hypertrophic Cardiomyopathy, Dilated Cardiomyopathy, and Arrhythmogenic Cardiomyopathy. 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 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 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 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

- Signaling mechanisms regulated by the intercalated discs in hereditary cardiomyopathies
- Molecular genetics and pathogenesis of cardiomyopathies caused by mutations in LMNA (Lamin A/C)
- Genetic fate-mapping and lineage tracing studies to identify and characterized the cell source of pathogenic myocytes, fibrosis and adipocytes and identify the responsible paracrine mechanisms in hereditary cardiomyopathies
- HALT-HCM (Hypertrophic Regression with N-Acetylcysteine in Hypertrophic Cardiomyopathy) clinical trial (ClinicalTrial.Org NCT01537926).

KEY PUBLICATIONS

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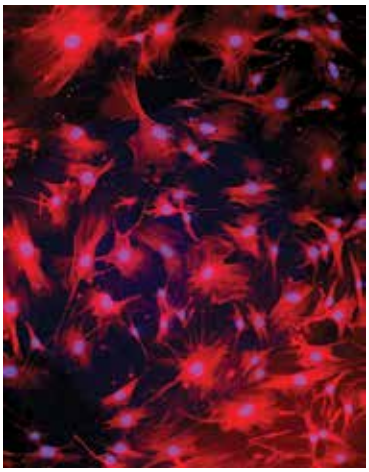
Student: Xiaofan Chen
Faculty – Instructor: Priyatansh Gurha
Faculty – Assistant Professor: Raffaella Lombardi



Expression of sarcomere protein α-tropomyosin in C Kit+ cardiac progenitor cells isolated from heterozygous plakoglobin- deficient (PG+/-) mouse heart, a model for arrhythmogenic right ventricular 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 genetic cardiomyopathies, which are important causes of heart failure and sudden cardiac death (SCD) in the young.

Although significant progress has been accomplished in revealing the causal genes, at the present time there is no effective pharmacological or non-pharmacological therapy for these genetic cardiac disorders.

We study the 3 most common forms of cardiomyopathies, namely Hypertrophic Cardiomyopathy (HCM), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), 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 these *in vitro* and *in vivo* models provide hints about the pathways implicated into the pathogenesis of these diseases and give the opportunity to identify targets for the development of therapeutic interventions in order to prevent or reverse the phenotype.

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 ARVC. 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 ARVC. 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.

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

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.

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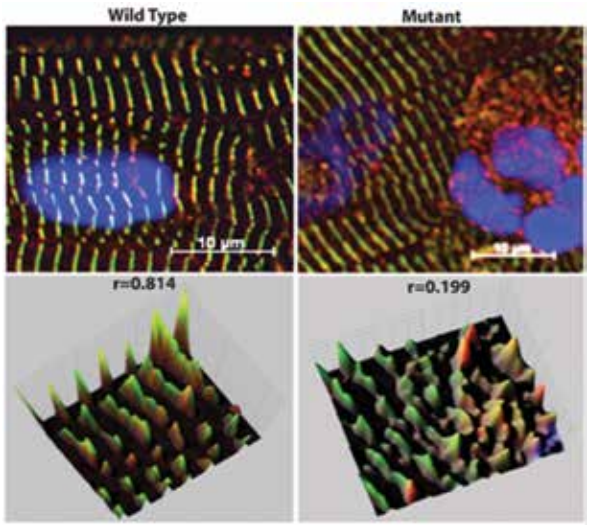
LAB MEMBERS

Priyatansh Gurha, Ph.D., instructor
Suet Nee Chen, Ph.D., post-doctoral fellow
Chen, Xiaofan, M.D., post-doctoral fellow
Grazyna Czernuszewicz, research associate



Reduced localization of mutant TRIM63 (novel causal gene for HCM) to Z-disks in cardiac myocytes. Upper panels. Immunofluorescence images of transduced adult cardiac myocytes expressing either a WT or a mutant TRIM63 protein after costaining with anti-α ACTININ (green) and anti-Flag (red) antibodies. Lower panels. Quantitative spectral display of colocalization of α-ACTININ and TRIM63 at the Z-disks. Correlation coefficient (r value) between the 2 colors in each image is shown (a value of 0 indicates no correlation and 1 a perfect colocalization).

Center for Human Genetics

The Investigators of the Research Center for Human Genetics focus their work on common cardiovascular diseases, such as heart and kidney diseases, high blood pressure, and stroke.

These diseases have a large impact on the health of our population.

Work in our center combines modern genetic and genomic methods with large-scale human population and animal model studies.

Progress in the laboratories of our investigators has provided important new understanding of susceptibility to atherosclerosis, coronary artery disease, stroke, and high blood pressure.

The ultimate goal of our center is to unravel the critical pathways that increase the likelihood an individual will experience common forms of cardiovascular disease and to allow the development and application of new and existing therapeutic and preventive approaches in a way best tailored to individual risk.

This work places us at the forefront of genomic medicine. Someday, these advances will allow doctors to target treatments to the underlying cause of disease, not just the symptoms.

Rapid progress is facilitated by the application of next-generation, whole-genome DNA sequencing and genome-wide genetic association studies that have been made possible by innovations in DNA analysis technology. This has sharpened the power of genetic studies to uncover precise regions of the genome containing genetic variation causing disease risk and to seek the specific DNA changes that generate risk.

The key to modern biomedical discovery is the ability to combine and interpret vast amounts of clinical and genomic data. Scientists in the Center for Human Genetics are among the world's leaders in the rapidly advancing areas of informatics and "Big Data."

We also develop and use a variety of laboratory models of cardiovascular disease, including stroke, atherosclerosis, and chronic kidney disease. These experimental studies allow us to address aspects of the disease process inaccessible to investigation in human populations.

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.

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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
Cullen Chair in Molecular Medicine

High blood pressure: causes and consequences

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 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 combines to play a key role in susceptibility to injury. We can prevent disease with immunosuppressant drugs in these animals. We also have obtained the first rat knockout model. This knockout eliminates immunoglobulin expression and we are transferring the null allele into our injury-prone animals to provide further assessment of the role of the immune system in this disease. These observations are leading toward the conclusion that, while high blood pressure may injure the kidney, it is the response of the immune system to this injury that determines whether normal renal function is sustained or lost. We have now obtained very high quality whole genome sequence from these two inbred rat lines. This has allowed us to identify one additional recent mutation in the disease-prone line that may be a key initiator of disease and that has important and predictable functional consequences both for immune system function and calcium signaling. 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. Furthermore, even mild loss of kidney function greatly amplifies the risk of death from other cardiovascular diseases.

RESEARCH PROJECTS

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

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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

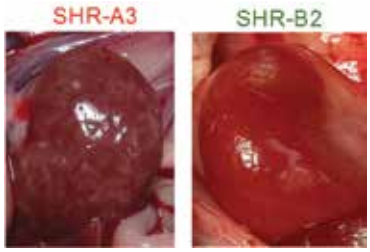
Braun, M.C. and P.A. Doris. Mendelian and transgenerational inheritance in hypertensive renal disease. *Annals Medicine,* 44 Suppl 1:S65-73, 2012.

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

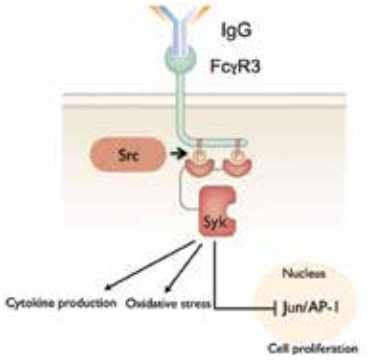
LAB MEMBERS

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



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.



Immunoglobulin signaling pathway. Within the 13% of the genome that is not identical lie genes (IgG, Src, Syk and JunD) that contribute to renal injury susceptibility. We have discovered important functional genetic differences in this pathway across our lines that are associated with renal injury susceptibility. Signaling in the IgG pathway leads to increased production of oxidative radicals that can injure tissues, increased production of cytokines that increase tissue damage from inflammation and increased proliferation of activated immune cells.

Myriam Fornage, Ph.D.
Associate 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. While patients with symptoms of acute stroke represent the easily-recognized "tip of the iceberg," it is well accepted that the deleterious effects of brain vascular disease begin well before clinical symptoms become apparent. Brain vascular 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 brain vascular disease both in its clinical and preclinical forms in well characterized populations from young adulthood to old age. Research strategies combine genetic epidemiology and functional genomic approaches using the latest genome resources and technologies. In recent years , I have used the power of genome-wide association 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 vascular health and disease.

RESEARCH PROJECTS

- Identifying 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 next-generation sequencing (work supported by R01-AG033193 and R01-HL093029)
- Identifying 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 (work supported by U01-NS069208)
- Identifying common and rare genetic loci influencing variation in blood pressure and integrate blood methylome profile to functionally characterize variants in these loci (work supported by R01-HL086694)
- Identify common and rare genetic loci influencing cardiovascular traits in diverse ethnic groups as part of the NHGRI Population Architecture and Genomic Epidemiology (PAGE) consortium (work supported by U01-HG004803)

KEY PUBLICATIONS

Ikram MA*, Fornage M*, Smith AV*, Seshadri S*, Schmidt R*, Debette S, Vrooman HA, Sigurdsson S, Ropele S, Taal HR, Mook-Kanamori DO, Coker LH, Longstreth WT, Niessen WJ, DeStefano AL, Beiser A, Zijdenbos A, Struchalin M, Jack CR, Rivadeneira F, Uitterlinden AG, Knopman DS, Hartikainen A-L, Pennell CE, Thiering E, Steegers EAP, Hakonarson H, Heirich J, Palmer LJ, Jarvelin M-R, McCarthy MI, Grant SFA, Sovio U, St Pourcain B, Timpson NJ, Davey Smith G, Nalls M, Au R, Hofman A, Gudnason H, van der Lught A, Harris TB, Meeks WM, Vernooij MW, van Buchem MA, Catellier DJ, Jaddoe VWV, Gudnason V, Windham BG, Wolf PA, van Duijn CM, Mosley TH, Schmidt H, Launer LJ, Breteler MMB, DeCarli C. Genome-wide association studies implicate loci on 6q22 and 7q2 in intracranial volume and early life brain growth. *Nature Genetics* 2012; 44:539-544

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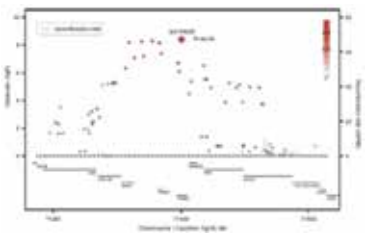
LAB MEMBERS

Li-An Lin, B.S.; Ph.D. student
Aron Joon, M.S.; statistician
Ping Wang, Ph.D.; research associate



Region of chromosome 17 associated with white matter lesion burden. Each square represents a polymorphism and its associated p-value.

Ba-Bie Teng, Ph.D., FAHA
Associate Professor

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 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

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.

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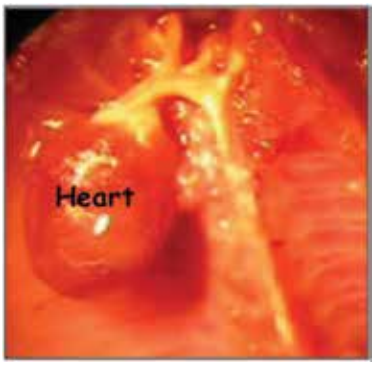
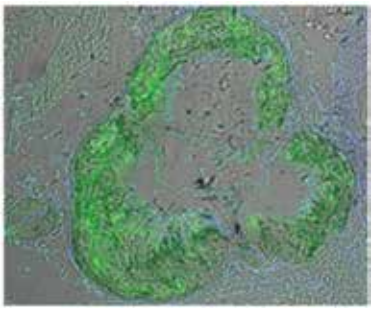
LAB MEMBERS

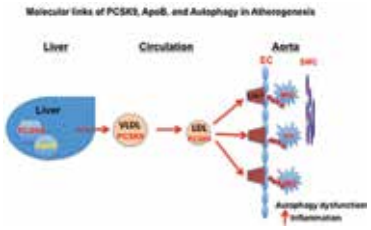
Post Docs: Hua Sun, Ph.D.
Research Assistants: Hersharan Nischal and Guohua Ji
Summer Intern: Sherri Hong from University of Houston



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



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.

Center for Immunology and Autoimmune Diseases

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
William S. Kilroy, Sr., Chair in Pulmonary Disease

Rick Wetsel, Ph.D.
Professor and Director of the Center for Immunology and Autoimmune Diseases
William S. Kilroy, Sr. Chair in Pulmonary Disease

Innate immunology and inflammation, lung disease, 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 anaphylatoxins in the pathogenesis of lung disease. For example, studies using mice in which the C3a receptor has been deleted have demonstrated that C3aR is an 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 recently were 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

Yan Q, Quan Y, Sun H, Peng X, Zou Z, Alcorn JL, Wetsel RA, Wang D. A site-specific genetic modification for induction of pluripotency and subsequent isolation of derived lung alveolar epithelial type II cells. *Stem Cells.* 2013, 10.1002/stem 1570, PMID: 24123810.

Hoyong L, Kim Y-UK, Drouin SM, Mueller-Ortiz S, Yun K, Wetsel RA, Chung Y. Negative regulation of pulmonary Th17 respsponses by C3a anaphylatoxin during allergic inflammation in mice. *PLoS ONE.* 2012, 10.1371/journal.pone.0052666, PMID: 23285141.

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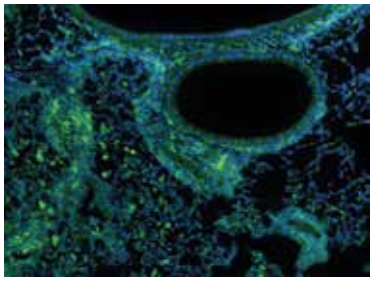
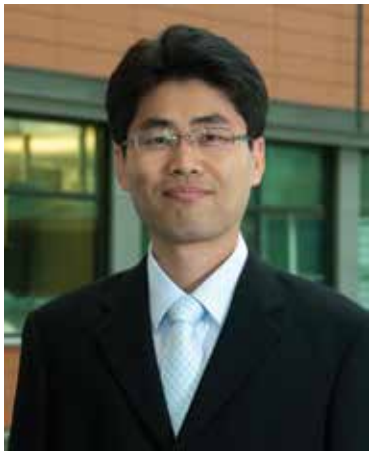
Wang D, Haviland DL, Burns AR, Zsigmond E, Wetsel RA. A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *Proc Natl Acad Sci.*, 2007, 104: 4449-4454, PMID: 17360544.

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



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 are also 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')

Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, Wang YH, Dong C. Bcl6 mediates the development of follicular helper T cells. *Science*. 2009: 325: 1001

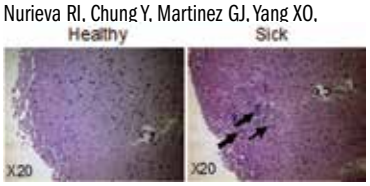
Chung Y*, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, Wang YH, Lim H, Reynolds JM, Zhou XH, Fan HM, Liu ZM, Neelapu SS, Dong C*. Follicular regulatory T cells expressing Foxp3 and Bcl6 suppress germinal center reactions. *Nature Medicine*. 2011: 17: 983 (* co-corresponding authors)

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

Chung Y, Yamazaki T, Kim BS, Zhang Y, Reynolds JM, Martinez GJ, Chang SH, Lim H, Birkenbach M, Dong C. Epstein Barr Virus-Induced 3 (EBI3) Together with IL-12 Negatively Regulates T Helper 17-Mediated Immunity to Listeria monocytogenes Infection. *PLoS Pathogens*. 2013;9:e1003628.

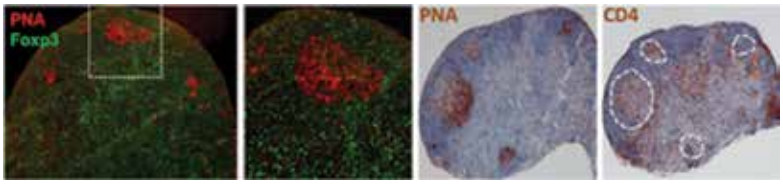
LAB MEMBERS

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



Inflammation in the spinal cords by autoimmune T cells

Subsets of helper and regulatory T cells



Germinal center reaction

Amber Luong, M.D., Ph.D.
Assistant Professor

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. 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. 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).

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. In addition, we are investigating translational implications of addressing IL-33 in CRS.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Clark D, Wenaas AE, Luong A, Citardi MJ, and Fakhri S. Staphylococcus aureus Prevalence in Allergic Fungal Rhinosinusitis Versus Other Subsets of Chronic Rhinosinusitis with Nasal Polyps. *International Forum of Allergy and Rhinology*, 2013 Feb;3(2):89-93. PMID:23038642

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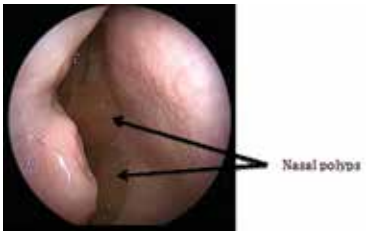
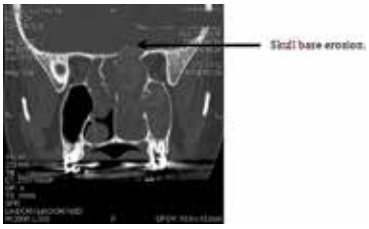
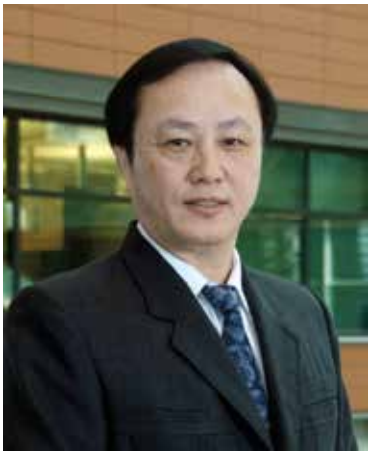
Shaw JL, Fakhri S, Citardi MJ, Porter PC, Corry DB, Kheradmand F, Liu YJ, Luong A. IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med*. 2013 Aug 15;188(4):432-9.



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 hemostasis 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 for developing 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) isolating and characterizing human and mouse ES cell derived distal lung stem/progenitor cell types both *in vitro* and *in vivo*; 2) generating "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) identifying and characterizing 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

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*. 2013. In press (* corresponding author)

Sun H., Quan Y., Yan Q., Peng X., Mao Z., Wetsel R.A., and Wang D*. Isolation and characterization of alveolar epithelial type II cells derived from mouse embryonic stem cells. *TISSUE ENGINEERING*. 2013. In press (* corresponding author)

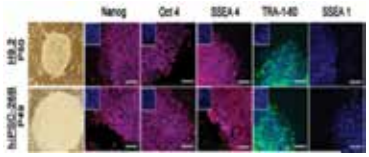
Wetsel RA. Wang D and Calame DG. Therapeutic Potential of Lung Epithelial Progenitor Cells Derived from Embryonic and Induced Pluripotent Stem Cells. *Annu. Rev. Med*. 2011. 62:30.1-30.11

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

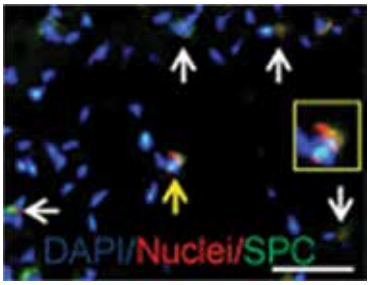
Wang D., Haviland D.L., Burns A.R., Zsigmond E., and Wetsel R.A. A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *PNAS,* Mar 13;104(11):4449-54 (2007).

LAB MEMBERS

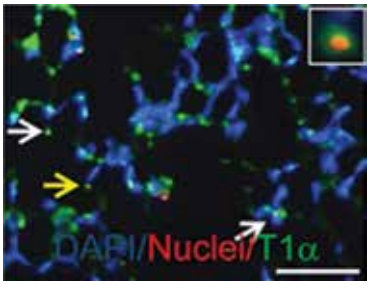
Research assistant: Dr. Yuan Quan



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



Engraftment of human iPS cell-derived alveolar type II cells in lungs.



Differentiation of the engrafted type II cells in lungs.

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 and knock-out mouse animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions. The laboratory has derived more than 20 new cell lines that have been highly effective for the generation of knock-out/ knock-in mice and for cellular differentiation studies. The Core Facility has had a 100% success rate of germline transmission in the production of knock-out mice when using mouse embryonic stem cells that have been derived in the laboratory. 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 PROJECTS

- Microinjection of DNA, BAC or YAC clones for the production of transgenic, knock-out and knock-in mice
- Microinjection of DNA for the production of transgenic rats
- Cryopreservation of fertilized mouse and rat eggs and sperm
- Re-derivation of mice and rats from fertilized eggs
- Gene targeting, selection, expansion, cryopreservation of mouse ES cells
- Derivation of novel mouse ES cells and other cell lines
- Availability of mouse ES cell lines and mouse fibroblast feeder layer cells

KEY PUBLICATIONS

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

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

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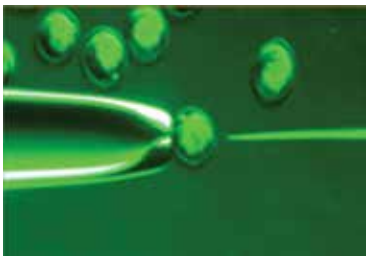
Wetsel, R.A., Kildsgaard, J., Zsigmond, E., Wei L. and Chan, L.: Genetic deficiency of Acylation Stimulating Protein (ASP/C3ades Arg) does not cause hyperapobetalipo- proteinemia in mice. *J. Biol. Chem*. 274: 19429-19433, 1999.

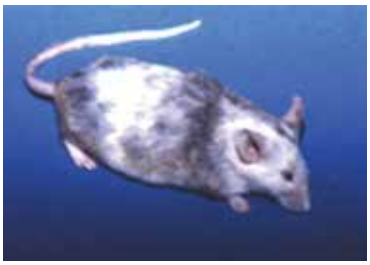
Kildsgaard, J., Zsigmond, E., Chan, L. and Wetsel, R. A.: A critical evaluation of the putative role of C3adesArg, ASP in lipid metabolism and hyperapobetalipoproteinemia. *Molec. Immunol.* 36: 869-876,1999.

LAB MEMBERS

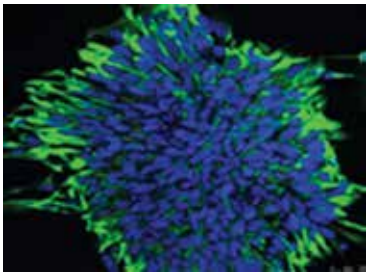
Manager: Aleksey Domozhirov



Microinjection of mouse zygotes for the production transgenic mice

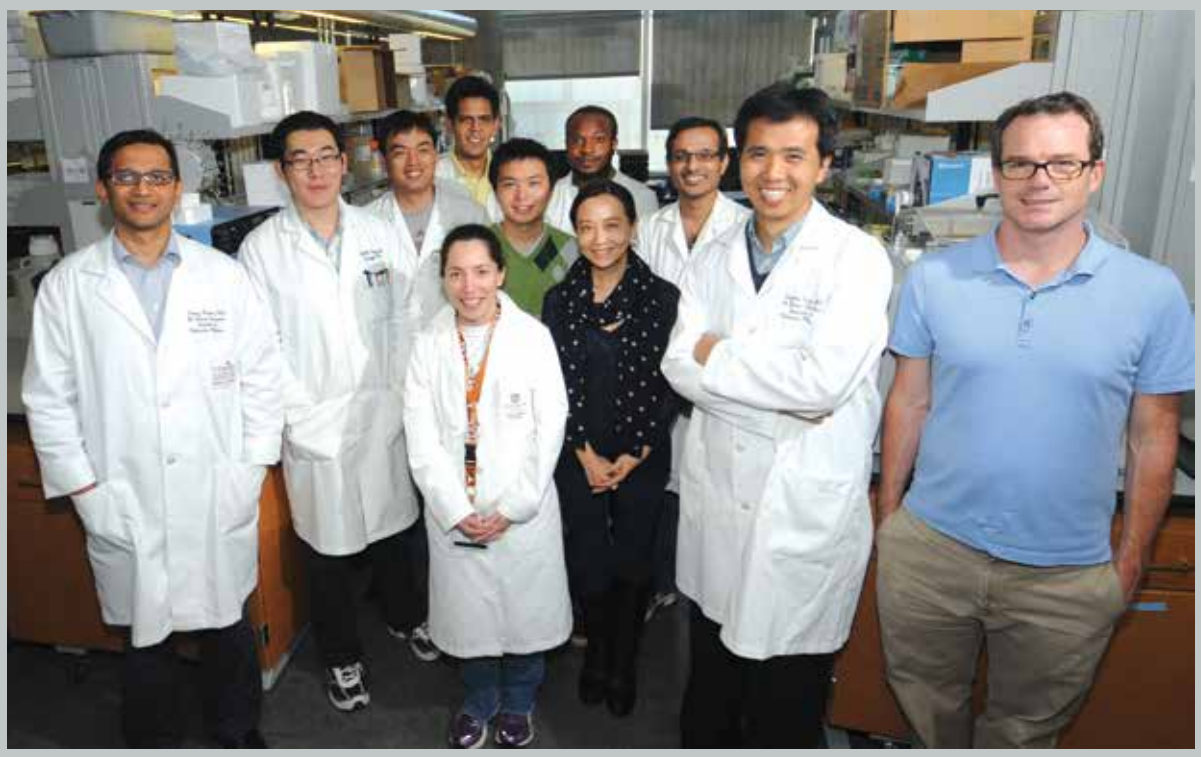


Chimeric mouse



Mouse ES cell colony undergoing neural differentiation

Center for Metabolic and Degenerative Diseases



The Center for Metabolic and Degenerative Diseases takes an integrative and collaborative approach to some of the most pressing health challenges of this century: diabetes, obesity, as well as aging and stress-related neurological diseases such as Alzheimer's and Parkinson's. The center continues to grow. In the past year, the Center has successfully recruited Dr. Nicholas Justice as an assistant professor from Baylor College of Medicine. Key questions being addressed by the center's faculty include the following:

• How does the brain control the body's energy balance and glucose metabolism?

• What are the gene regulatory pathways responsible for exercise endurance and benefits, and can these genes can be pharmaceutically activated to mimic exercise in treating obesity, diabetes, cardiovascular diseases as well as orphan muscle degenerative diseases?

• How do mutations in a small set of genes lead to specific brain degenerative disorders, such as Parkinson's and Huntington's, and what are the normal cellular functions of these genes and intra-cellular signaling pathways that these genes are involved in?

• How do stress and other stressful experiences affect neuronal function and cause degeneration that underlie resulting aberrant behaviors?

To tackle these important questions, the Center employs diverse model organisms including *Drosophila* (the fruit fly) and mice, and state-of-the-art methods and technology including sophisticated *Drosophila* and mouse genetics, optogenetics, electrophysiology, and behavior assays. The Center is fully equipped for comprehensive characterization of both mouse and invertebrate fly models, with instruments such as a new CLAMS system to assess mouse metabolic rate, an electrophysiological recording set-up to monitor brain neuron activity, a remote telemetry system to monitor mouse body temperature and locomotion, as well as regular mouse behavior testing modules and a full setup of *Drosophila* system.

The Center is well funded and each faculty is currently funded by multiple extra-mural grants from the National Institutes of Health, the Juvenile Diabetes Foundation, the American Heart Association, the American Diabetes Association, and the Muscular Dystrophy Association. The Center's faculty has extensive collaborative interactions with Drs. Mikhail Kolonin (Stem Cells) and Rebecca Berdeaux (Integrative Physiology and Pharmacology) who also work on metabolism. The center faculty have recently published in prestigious journals such as *Cell, Cell Metabolism, Molecular Metabolism, Endocrinology, Circulation Research* and *Faseb J*. The upcoming year will see a series of new discoveries from and more grants awarded to the Center.

John Hancock, M.A., M.B., B.Chir., Ph.D., Sc.D.
Vice-Dean for Research
Executive Director, The Brown Foundation Institute of Molecular Medicine
Professor and Chairman, Department of Integrative Biology and Pharmacology
John S. Dunn Distinguished University Chair in Physiology and Medicine

Plasma membrane nanostructure and signal transduction

Our group studies mammalian intracellular signaling. We are especially interested in the function of Ras proteins. These small GTP binding proteins operate as molecular switches in signal transduction pathways and are present in a mutant, activated state in many human tumors. Understanding the basic biology of Ras has major implications for the development of novel anticancer therapeutics.

Specifically, we are investigating how the Ras membrane anchors cooperate with the G-domain and peptide sequences flanking the anchor to drive lateral segregation. Our work suggests new models are needed to explain how lipidated proteins interact with, and use, the plasma membrane to generate signaling platforms. We are interested in how confinement of signaling complexes onto a 2D surface in general and in plasma membrane nanodomains in particular regulates the kinetics and sensitivity of Ras signal signal output. Similarly, as we develop our spatial and proteomic maps of the plasma membrane, we can address how the composition and organization of the membrane alters in response to specific growth factors.

We also have a major interest in characterizing the K-Ras endoplasmic reticulum to plasma membrane trafficking pathway. A recent focus of our work is to search for inhibitors of K-Ras plasma membrane association that may have utility as novel anticancer agents.

RESEARCH PROJECTS

- Molecular mapping of the proteins and lipids of plasma membrane nanodomains
- Electron microscopic visualization and quantitative characterization of surface nanodomains
- Investigation of the dynamic regulation of nanodomain localization of Ras and Ras-interacting proteins in response to physiological stimuli
- Characterization of the mechanism(s) whereby K-ras is transported to the plasma membrane
- Development of anti-K-ras drugs

KEY PUBLICATIONS

Fendiline inhibits K-Ras plasma membrane localization and blocks K-Ras signal transmission (2013) van der Hoeven D, Cho KJ, Ma X, Chigurupati S, Parton RG, Hancock JF. *Mol Cell Biol.* 33, 237-251

Andrographolide derivatives inhibit guanine nucleotide exchange and abrogate oncogenic Ras function (2013) Hocker H, Cho KJ, Maharaja N, Stanslas J, Hancock JF, Gorfe AA. *Proc Natl Acad Sci USA*.110, 10201-10206

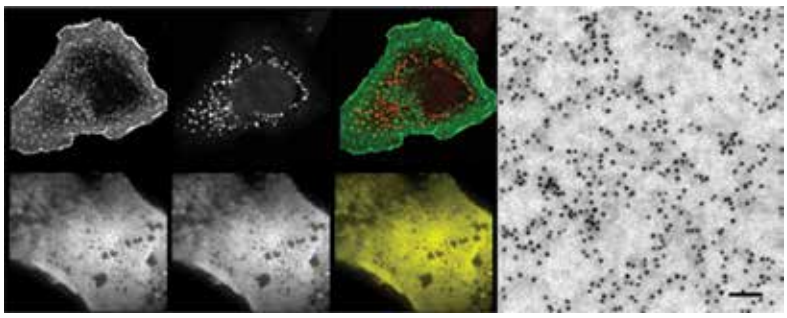
Temporal production of the signaling lipid phosphatidic acid by phospholipase D2 determines the output of ERK signaling in cancer cells (2013) Zhang F, Wang Z, Lu M, Yonekubo Y, Liang X, Zhang Y, Wu P, Zhou Y, Grinstein S, Hancock JF, Du G.Mol Cell Biol. [Epub ahead of print]

Cho K-j, Kasai RS, Park J-H, Chigurupati S, Heidorn SJ, van der Hoeven D, Plowman SJ, Kusumi A, Marais R, Hancock JF (2012) Raf inhibitors dysregulate the spatiotemporal dynamics of Ras proteins on the plasma membrane. *Curr Biol.* 22, 945-955

Cho KJ, Park JH, Piggott AM, Salim AA, Gorfe A, Parton RG, Capon RJ, Lacey E, Hancock JF (2012) Staurosporines disrupt phosphatidylserine trafficking and mislocalize Ras proteins. *J Biol Chem.* 287, 43573-43584

LAB MEMBERS

Instructor: Yong Zhou, Ph.D.
Post Docs: Kwang-jin Cho, Ph.D., Travis Rodkey, Ph.D.
Technicians: Xiaping Ma, Wei Chen, Hong Liang
Students: Kelsey Maxwell, Lingxiao Tan



Ras localization imaged by confocal, TIRF and electron microscopy

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 a 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 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

- Mechanisms of CRF system perturbation in Alzheimer's disease
- The coincidence of PTSD and Alzheimer's disease
- Defining neuronal circuits that respond to stress

KEY PUBLICATIONS

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

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

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

Justice NJ, Blount AL, Pelosi E, Vale W, Bilezikjian LM. Impaired FSHβ expression in Foxl2 Mutant Pituitaries, *Mol Endocrinol.* 2011 Aug; 25 (8):1404-15.

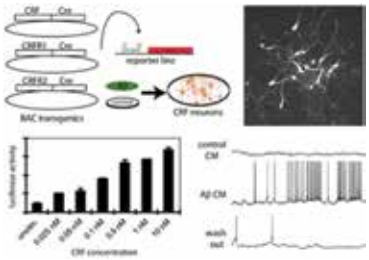
Sztainberg, Y, Kuperman, Justice NJ, Chen, A. An anxiolytic role for CRF receptor type 1 in the globus pallidus. *J Neurosci.* 2011 Nov 30;31 (48):17416-24.

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. Neurosc*i. 29(50):15660-8.

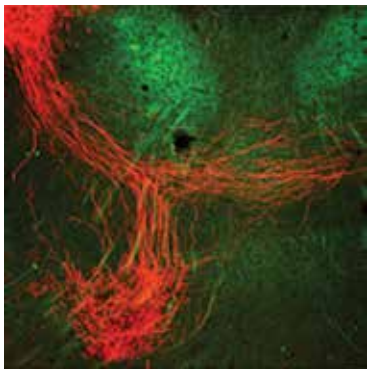
Justice NJ, Yuan ZF, Sawchenko PE, and Vale W. "Reconciling Ligand-Receptor Misalignment in the Central CRF System: Insights from a Transgenic Mouse Line Reporting Type 1 Corticotropin-Releasing Factor Receptor Expression." *J. Comp. Neurol* 2008 Dec 1;511(4):479-96.

LAB MEMBERS

Melissa Pruski, Research Technician



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



Injection of floxed-stop-tomato^{td} rAAV into the Central Nucleus of the Amygdala in CRFR1-cre animals allows selective tracing of neurons that express CRFR1. This projection leaves the CeA and projects caudally to brainstem targets.

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

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

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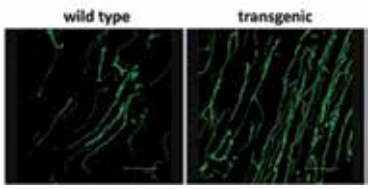
Matsakas A, Macharia R, Otto A, Elashry MI, Mouisel E, Romanello V, Sartori R, Amthor H, Sandri M, Narkar V, Patel K (2012) Exercise training attenuates the hypermuscular phenotype and restores skeletal muscle function in the myostatin null mouse. *Exp Physiol.* 97(1): 125-40.

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

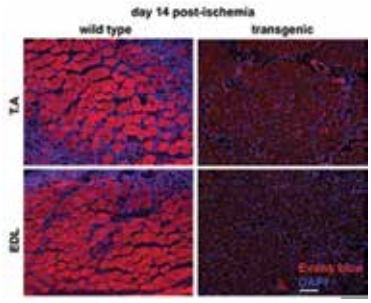
Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM. (2008) AMPK and PPARdelta agonists are exercise mimetics. *Cell.* 134(3): 405-15.

LAB MEMBERS

Post-doctoral fellows: Antonios Matsakas (*Recently was recruited to Hull University-UK as faculty), Vikas Yadav, Pierre-Marie Badin
Technicians: Sabina Lorca, Manik Kurvalekar
Summer Student: Christian Willrich



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.
Assistant 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. A better understanding of the mechanisms regulating body weight and glucose homeostasis is required to develop new therapeutic strategies. 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 (e.g. POMC neurons, AgRP neurons, etc.) and a few hypothalamic genes (POMC, AgRP and MC4R, etc.) in feeding, energy expenditure, and glucose homeostasis. However, the mechanisms and the neural pathways with which the brain and hypothalamus regulates energy balance are not well understood.

The long-term research goal of my group is to understand how neurocircuitry in the brain regulates energy balance and glucose homeostasis. My current research focus is to understand the role of glutamate, GABA and monoamines (dopamine) release from distinct groups of neurons in the regulation of energy balance. Glutamate and GABA are the main excitatory and inhibitory neurotransmitters, respectively, in the brain. However, research efforts that address the mechanisms' underlying energy balance have been largely focusing on the roles of neuropeptides, while the roles of glutamate and GABA have been overlooked. We generate and use mouse models with specific disruption of glutamate or GABA release, as well as other important genes, from discrete groups of neurons. These mice will be used to examine the contributions of glutamate, GABA and other neurotransmitters released from the targeted groups of neurons to the maintenance of energy balance. In addition, novel mouse genetic technology includes inducible and reversible inhibition, and activation of discrete groups of neurons will also be utilized to interrogate the role of these neurons in physiologic/pathological conditions.

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

- Role of GABA and glutamate release in mediating leptin action on body weight
- Brain mechanisms underlying leptin action in restoring blood glucose in type 1 diabetes
- Role of glutamate release in mediating melanocortin 4 receptor action
- Role of GABAergic action in body weight regulation using an inducible and reversible approach

KEY PUBLICATIONS

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*.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.

Wu Z, Xu Y, Zhu Y, Zhao R, Sutton A, Lowell BB, Olson DP, Tong Q. An obligate role of oxytocin neurons in energy expenditure regulation. *PLOS ONE,* 2012, 7(9) e45167. PMID: 23028821. Corresponding author.

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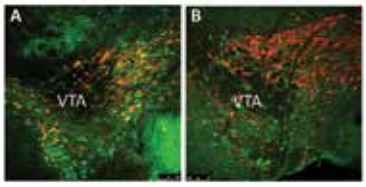
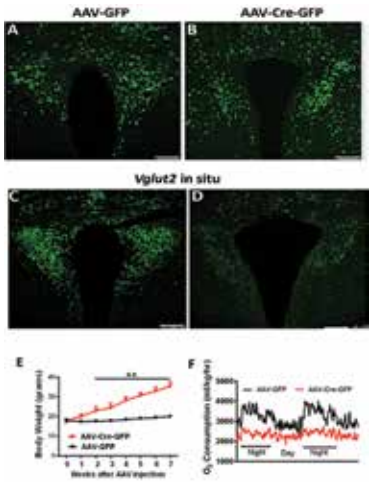
Song J, Xu Y, Hu X, Choi B, and Tong Q. Brain Expression of Cre Recombinase Driven by Pancreas-specific Promoters. *Genesis*, 2010, 48(11): 628-634, PMID: 20824628. Corresponding author.

LAB MEMBERS

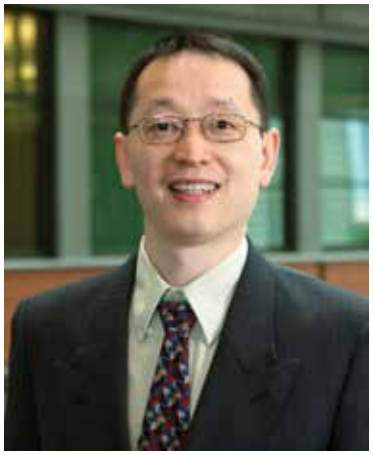
Post Docs: Yuanzhong Xu, Zhaofei Wu, Eun Ran Kim, Hao Sun
Graduate Students: Osita Benedict, Igwe, Leandra Mangieri (Rotation)
Visiting Student: Shengjie (Holy) Fan



Specific deletion of vesicular monoamine transporter 2 (VMAT2, required for dopamine release) in Leptin receptor-expression (LepR) neurons in ventral tegmental region (VTA). (A) A subset of deopaminergic neurons (green) express LepR (Red). Neurons labelled yellow are those neurons expressing both VMAT2 and LepR. (B) In *LepR-Cre:VMAT2*$^{flox/flox}$ mice, VMAT2 (green) is absent from LepR neurons (Red). These mice are being used to study the function of dopamine release from LepR neurons in mediating leptin action.



Disruption of glutamate release from Paraventricular nucleus of hypothalamus (PVH) leads to rapid obesity development and reduced energy expenditure. Bilateral injections of either AAV-Cre-GFP (A) or AAV-GFP (B) were made in PVH of Vglut2$^{flox/flox}$ mice . *Vglut2* in situ signal in the PVH of Vglut2$^{flox/flox}$ mice that received bilateral AAV-GFP (C) and AAV-Cre-GFP (D). (E) Weekly body weights following AAV vector injections. (F) Energy expenditure assessed by O2 consumption.



Sheng Zhang, Ph.D.
Assistant Professor

Molecular mechanisms of human brain degenerative diseases

While enjoying longer expectancy, our society is also facing a pressing challenge on how to help protect the increasing number of the elderlies from 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 simpler, 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 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 highly conserved 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, Chen ZH, Chen DS, Sun YM and Zhang S: Huntingtin is a Scaffold Protein Promoting Macroautophagy by a Dual Mechanism. In revision.

Rui YN, Xu Z and Zhang S: Characterization of Selective Macroautophagy Induced by Proteasome Inhibition by GST-BHMT Assay in Mammalian Cells. In revision.

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).

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

LAB MEMBERS

Post Docs: Dr. Zhen Xu, Dr. Yanning Rui, Dr. Dongsheng Chen
Ph.D. Students: Antonio Tito, Ryan Singer
Technicians: Zhihua Chen, Ph.D., Research Associate, Lili Ye, Research Assistant I

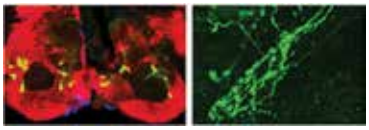


Figure 1. (A) Dopamine (green) and serotonin (blue) neurons in a fly brain (outlined in red by a neuronal marker). (B) Axonal terminal morphology (green) of a label neuron in a huntingtin knockout fly brain.

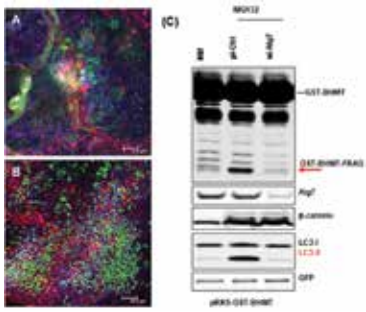


Figure 2. (A & B) High-magnification view of fly brains expressing human mutant Huntingtin protein (green) with a 47 (A) or 72 (B) polyglutamine tract. Note the prominent aggregates (green in B) that partially co-localize with autophagy markers ubiquitin (blue) and Ref(2) P (red). (B) BHMT-based biochemical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

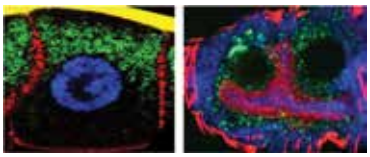


Figure 3. Cellular organelle formation. (A) An enrichment of membrane-bound vesicles (green) on the apical side (top) of a polarized secretory cell. Cell boundary is outlined in red and its nucleus labeled in blue. (B) Non-polarized distribution of organelles of lysosomal-origin (green) in three *Drosophila*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

Center for Molecular Imaging

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 NIR 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 instruments and agents to improve clinical utility of diagnostics.

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

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

Molecular imaging and diagnostics

The Center for Molecular Imaging (CMI) consists of an interdisciplinary team of scientists and engineers who focus upon multi-modality molecular imaging including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and our specialty, near-infrared (NIR) fluorescence to enable new understandings in several disease states. In addition to having its own basic science and clinical research projects, the team also operates a "collaboration" center where clinicians and basic scientists from across the Texas Medical Center partner with CMI members to effectively apply diagnostics in preclinical and clinical studies. 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*, to appear March 2014.

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.

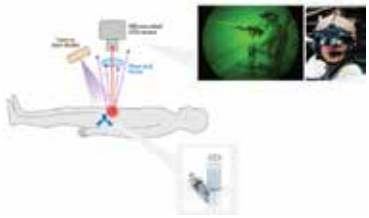
Burrows, P.E., Gonzalez-Garay, M.L., Rasmussen, J.C., Aldrich M.E., Guilliod R., Maus, E.A., Fife, C.E., Kwon, S., Lapinski, P.E., King, P.D., and E.M. Sevick-Muraca, "Lymphatic abnormalities are associated with RASA1 mutations in mouse and man," *Proc Natl Acad Sci*, epub ahead of print May 6, 2013.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

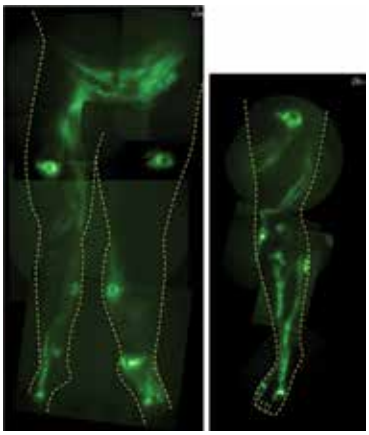
E.M. Sevick-Muraca, "Translation of near-infrared fluorescence imaging technologies: emerging clinical applications," *Ann Rev Med*, 63: 217-31, 2012, Epub 2011 Oct 27. PMID: 22034868

LAB MEMBERS

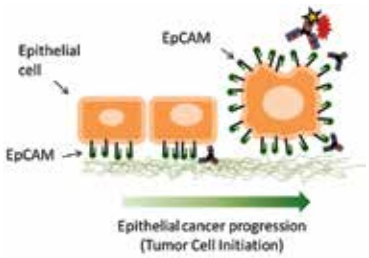
Co-Director of Flow Cytometry Service Unit: Amy Hazen
Chief Histology Technician of Tissue Histology Service Unit: Sarah Amra
Research Coordinators: Holly Robinson, Nathaniel Wilganowski, Karen Gore, Grace Wu
Postdoctoral Fellow: Dr. Chinmay Darne (co-advised)
Graduate Students: Germaine Agollah (co-advised)chance,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

Program for imaging in immunology

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

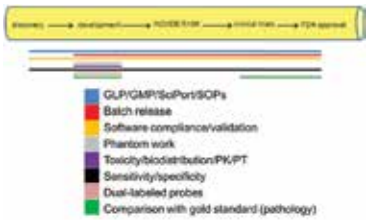
Aldrich MB, Guilliod RG, Fife CE, Maus EA, Smith L, Rasmussen JC, Sevick-Muraca EM. Lymphatic abnormalities in the normal contralateral arms of subjects with breast cancer-related lymphedema as assessed by near-infrared fluorescent imaging. 2012. *Biomedical Optics Express* 3:1256-65.

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.

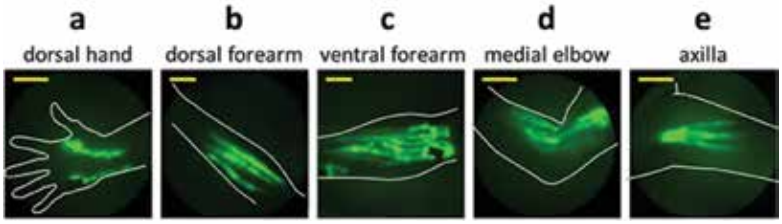
Aldrich MB, Sanders D, Lapteva N, Huang, XF, and Chen SY. SOCS1 downregulation in dendritic cells promotes memory T cell responses. 2008. *Vaccine* 26(8):1128-35.

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. Our lab uses radiometal-based positron emitters, such as Gallium-68 and Copper-64, for labeling of peptides, proteins, and antibody-based agents. His lab 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. We are 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.

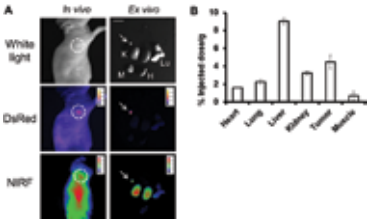
Sevick-Muraca, E.M., Akers, W.J., Joshi, B.P., Luker, G.D., Marnett, L.J., Contag, C.H., Wang, T.D. and Azhdarinia, A. Advancing the translation of optical imaging agents for clinical medical imaging. *Biomedical Opt Express.* 4(1): 160-70, 2013. PMID:23304655

Zhu, B., Wu, G., Robinson, H., Wilganowski, N., Hall, M.A., Ghosh, S.C., Pinkston, K.L., Azhdarinia, A., Harvey, B.R., Sevick-Muraca, E.M. Tumor Margin Detection Using Quantitative NIRF Molecular Imaging Targeting EpCAM Validated by Far Red Gene Reporter iRFP. *Mol Imaging Biol.* 15(5):560-8, 2013. PMID: 23619897

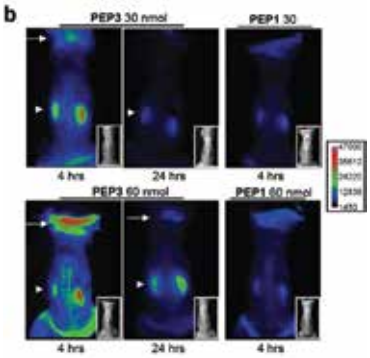
Azhdarinia, A., Daquinag, A.C., Tseng, C., Ghosh, S.C., Ghosh, P., Amaya-Manzanares F., Sevick-Muraca, E.M., Kolonin, M.G. Probes for targeted brown adipose tissue imaging. *Nat Commun.* 4:2472, 2013. PMID: 24045463

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. (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. (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. Hundred of scientists worldwide collaborated in this project using sequence technology that was developed in the early 1970s by Frederick Sanger. It took over 10 years and over $3 billion to sequence the human genome for the first time. 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 a 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 as we linked personal disease histories with causative disease genes in 18 volunteers. In addition, we identified risk alleles for breast, ovarian, colon, and 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 genetic disorders like schizophrenia, 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
- Identification of markers for schizophrenia in patients from Houston
- Identification of new alleles for Tuberous Sclerosis Complex (TSC) and Spina Bifida Cystica. Collaborator of Hope Northrup
- Dercum's disease, Adiposis dolorosa, Madelung's disease. Collaborator of Karen L Herbst, M.D. UC San Diego

KEY PUBLICATIONS

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.

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.

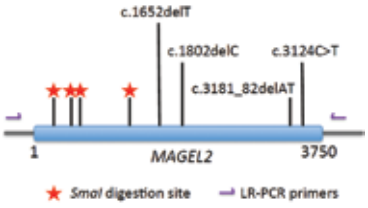
Gonzalez-Garay M.L.*#, Schaaf CP*#, Xia, F*., Potocki, L., Gripp, K. W., Zhang, B., Peters, B. A., McElwain, M. A., Drmanac, R., Beaudet, A. L., Caskey, C. T., Yang, Y. (2013) Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet*. 45:1405-1408.

Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. 2008. *Nature* 455:1061-1068. PMID: 18772890

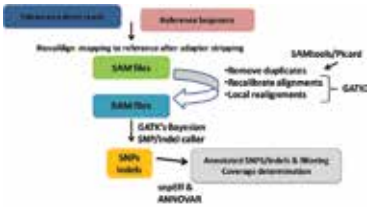
Ding L, et al. Somatic mutations affect key pathways in lung adenocarcinoma. 2008. *Nature* 455:1069-1075. PMID:18948947; PMCID: PMC2694412

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, and 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, 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*, August 2013. PMID: 23974022.

Hall, M.A., Pinkston, K.L., Wilganowski, N., Robinson, H., Ghosh, P., Azhdarinia, A., Vazquez-Arreguin, K., Kolonin, A.M., Harvey, B.R.* 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 Nuc Med*, 2012. Sep;53(9):1427-37 PMID: 22872743

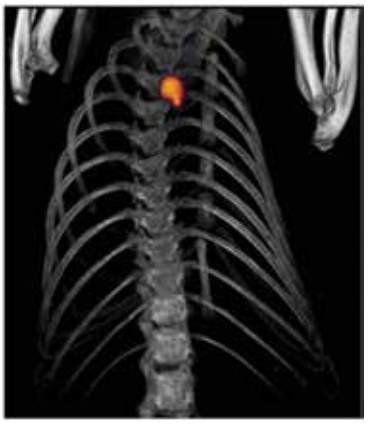
Pinkston KL, Gao P, Diaz-Garcia D, Sillanpää J, Nallapareddy SR, Murray BE, and BR Harvey. "Regulated *gelE* Expression Through the Fsr Quorum-Sensing System of *Enterococcus faecalis* Modulates the Surface Collagen-Binding MSCRAMM Ace, Affecting Collagen Adherence." *Journal of Bacteriology*, 2011. PMID: 21705589

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

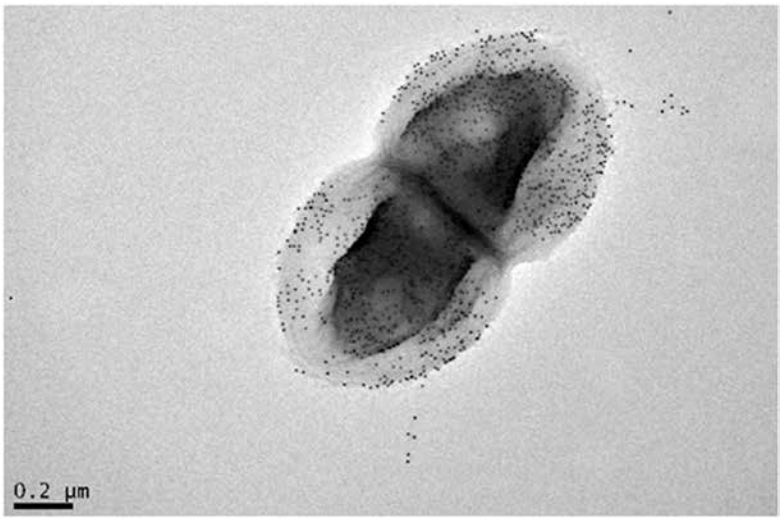
Harvey BR, Georgiou G, Hayhurst A, Iverson BL, and GK Rogers, "Anchored Periplasmic Expression (APEx), a Versatile Technology for the Isolation of High Affinity Antibodies from *E.coli* Expressed Libraries," *Proc Natl Acad Sci U S A,* 101(25): 9193-8, 2004. PMID: 15197275

LAB MEMBERS

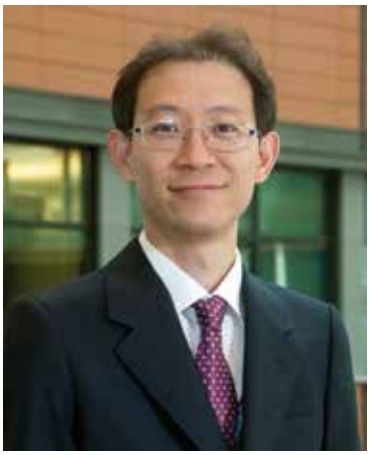
Kenneth L. Pinkston – Research Coordinator II
Dr. Peng Gao – Research Instructor
Emily Stinemetz – Graduate Student



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.



Sunkuk Kwon, Ph.D.
Assistant Professor

Functional lymphatic imaging in animal models of lymphovascular disorders

I lead the development and application of small animal imaging techniques to address biological questions in unique animal models of vascular disease, with an emerging emphasis of gastrointestinal disease. My main research interest focuses on investigating the microcirculatory movement of fluid and macromolecules, particularly in the lymphatic system using fluorescence optical imaging techniques. The lymphatic system plays an important role in edema prevention, immune surveillance, cancer metastasis, as well as fluid/protein homeostasis. 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.

Another area of interest is to non-invasively and quantitatively image gastrointestinal motility. Our team recently demonstrated for the first time intestinal motions using autofluorescence induced by standard murine diet, containing chlorophyll without an exogenous imaging agent. Based upon preliminary data, my research focuses upon imaging altered intestinal contractile function in genetically engineered models of GI motility disorders/dysfunction and in animal models of post-infectious and post-inflammatory irritable bowel syndrome.

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

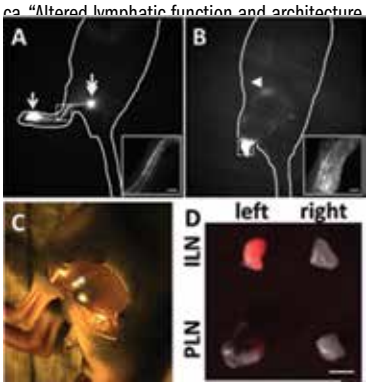
RESEARCH PROJECTS

- 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, C. Davies-Venn, and E. M. Sevick-Muraca, "In vivo dynamic imaging of intestinal motions using diet-related autofluorescence," *Neurogastroenterology & Motility*, 24; 494-497, 2012. (Feature on the editorial)

S. Kwon, S, G.D. Agollah, and E. M. Sevick-Muraca, "Altered lymphatic function and architecture



Images of the effect of lymph node (LN) metastasis from melanoma (B16F10) on the lymphatic structure. Fluorescent images of a mouse prior to (A) and 21 days post inoculation (B) showing additional lymphatic drainage to the inguinal LN (ILN, arrowhead), mainly due to tumor blockage of normal lymphatic drainage to the popliteal LN (PLN, double arrow). Arrow, ICG injection site. The insets show magnified fluorescent images of the rectangles of A and B. A color image (C) after a skin incision above the PLN shows the melanoma-filled PLN (asterisk). Fluorescent (D) images of dissected PLNs and ILNs on the tumor and contralateral sides of a mouse. Note that the melanoma-filled PLN has low ICG fluorescence as compared to the left ILN due to rerouting of normal lymphatic drainage to the left ILN. Scale, 1 mm.

in salt-induced hypertension assessed by near-infrared fluorescence imaging," *Journal of Biomedical Optics*, 17; 080504, 2012.

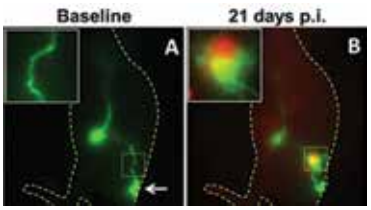
S. Kwon, G. D. Agollah, G. Wu, and E. M. Sevick-Muraca, "Direct visualization of changes of lymphatic function and drainage pathways in lymph node metastasis of B16F10 melanoma using near-infrared fluorescence imaging," *Biomedical Optics Express*, 30; 967-977, 2013.

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.

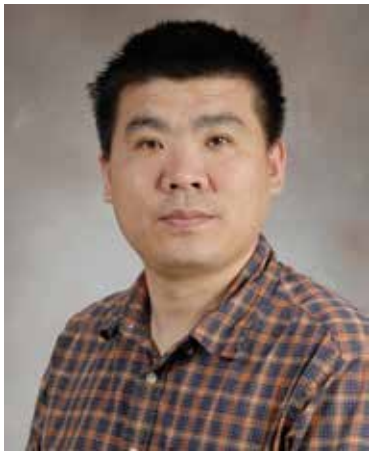
E. M. Sevick-Muraca, S. Kwon, and J. Rasmussen, "Emerging Lymphatic Imaging Technologies for Mouse and Man," *Journal of Clinical Investigation*. Accepted for publication, 2013.

LAB MEMBERS

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



Images of the effect of breast carcinoma on lymphatic structure. (A) A baseline NIRF image of the lymphatics prior to inoculation of inflammatory breast carcinoma (IBC) cells transfected with the iRFP gene reporter. The inset figure is a magnification of the lymphatic structure proximal the site of ICG administration (arrow). (B) An overlay of a NIRF image (green) of the lymphatics over a non-invasive iRFP image of the gene reporters in the primary tumor 21 days post-inoculation of the SUM149 cells. Note the reorganization of the lymphatics surrounding the tumor (inset).



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 ultimately 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

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).

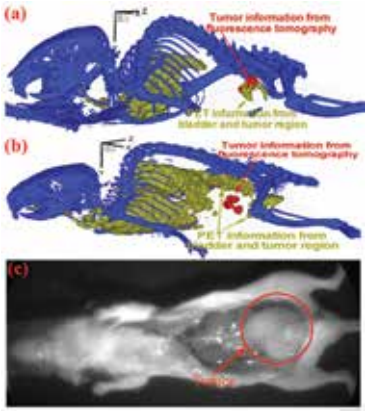
Darne, C.D., Lu, Y., and Sevick-Muraca, E.M. "Small animal fluorescence and bioluminescence tomography: a review of approaches, algorithms, and technology update," *Physics in Medicine and Biology* (In press) (Invited Review Paper).

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)

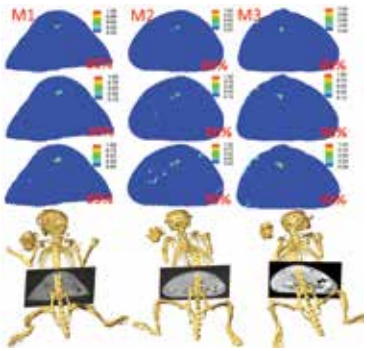
Lu, Y., Machado, H.B., Bao, Q., Stout, D., Herschman, H., and Chatziioannou, A.F., " In vivo Mouse Bioluminescence Tomography with Radionuclide-Based Imaging Validation," *Molecular Imaging and Biology*, 13:53-58, 2011.

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.

Specifically, my work focuses upon the development of NIRF imaging methodologies and their 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) identify 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, including 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

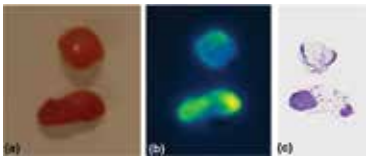
Sevick-Muraca, E.M., Kwon, S.K., and J.C. Rasmussen, "Emerging lymphatic imaging technologies for mouse and man," *Journal of Clinical Investigations*, accepted for publication, 2013.

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.

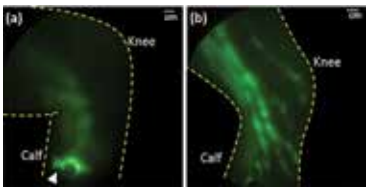
Zhang, J., Xiang, X., Zhou, S.K., Bautista, M., Nicom, B., Dickinson, G., Tan, I-C., Chan, W., Sevick-Muraca, E.M., and J.C. Rasmussen, "Validation of AFLIA for quantitative lymphatic imaging analysis," *Biomedical Optics Express*, 3(7):1713-1723, 2012.

Rasmussen, J.C., Kwon, S., Sevick-Muraca, E.M., and J.N. Cormier, The Role of Lymphatics in Cancer as Assessed by Near-Infrared Fluorescence Imaging, *Annals of Biomedical Engineering*, 40(2):408-421, 2012 (Invited, Cover).

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) Color image of resected lymph nodes, (b) near-infrared fluorescence image, and (c) image of the corresponding slide from pathology. The presence of two distinct nodes in the elongated tissue sample in (a) are clearly seen in (b) and (c). Note, the scale and orientation of the specimens in (c) are not necessarily the same as in (a) and (b) due to pathologic processing. (Accepted for publication in *Biomedical Optics Express*)



NIRF images of lymphatic vessels in the medial left knee of (a) a subject with Dercum's disease, a rare disorder of the subcutaneous fatty tissues, and (b) a normal control subject of with a similar body mass index (BMI). Note the dilated vessels in and the fluorescent lymphatic capillaries (arrowhead) radiating from the injection site in (a). (a) is pending publication and (b) is reproduced from Rasmussen, J.C., et al., PNAS 2013.

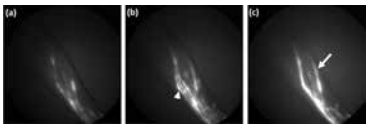


Image stabilization algorithm which reduces errors due to subject motion when quantifying lymphatic propulsion. (a) A single snapshot illustrating the actual lymphatics in the arm, (b) image showing the appearance of 'phantom' lymphatic vessels (arrowhead) in the aggregated image, and (c) image showing the enhanced lymphatics (arrow) following image stabilization. Reproduced from Zhang, J., et al., BOE, 3(7):1713-1723, 2012.

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 is limited due to the lack of a suitable imaging technique that has sufficient spatial and/or temporal resolutions. Recently, we 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 lymphatic function in a compassionate case of head and neck lymphedema and 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 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, 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.

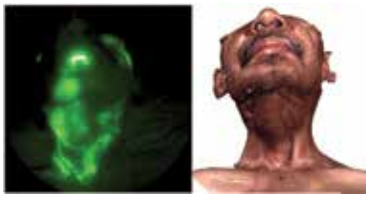
B. Zhu, I. C. Tan, J. C. Rasmussen, and E. M. Sevick-Muraca, "Validating the sensitivity and performance of near-infrared fluorescence imaging and tomography devices using a novel solid phantom and measurement approach," *Technol Cancer Res Treat,* vol. 11, pp. 95-104, 2012.

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.

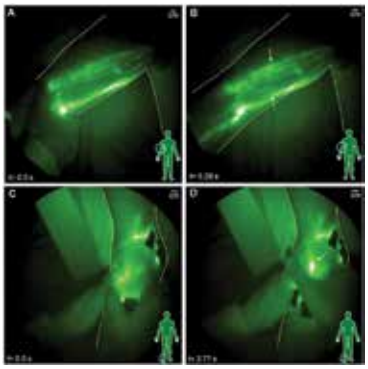
J. C. Rasmussen, I. C. Tan, M. V. Marshall, C. E. Fife, and E. M. Sevick-Muraca, "Lymphatic imaging in humans with near-infrared fluorescence," *Curr Opin Biotechnol*, vol. 20, pp. 74-82, 2009.

LAB MEMBERS

Co-advised: Chinmay Darne, Rodney John Morrow



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).



Sequential near-infrared fluorescence images during manual lymphatic drainage (MLD). [A, B] a wave of fluorescent packets (arrows) in multiple vessels moving toward axillary lymph nodes. [C, D] lymph in a vessel (arrow) being pushed toward the ankle during MLD. (Reproduced from Tan, *et al.* 2011)

Banghe Zhu, Ph.D.
Assistant Professor

Program for instrumentation for near-infrared fluorescence-guided tumor detection

Although much progress has been made to develop new effective cancer therapeutics, surgery remains the foundation of cancer treatment for either complete resection of primary lesions, or for debulking (or cytoreductive surgery) that enables more efficacious radiation and/or chemotherapy. Residual tumor burden after surgery is strongly correlated with reduced survivor rates. When possible, wide-field surgical excision can reduce the chance of positive tumor margins and residual disease, although it can also lead to disfigurement and enhanced risk for surgical morbidities. Accurate, intraoperative detection of margins is needed to minimize the amount of resected normal tissue required to achieve a negative margin. Near-infrared fluorescence (NIRF) imaging with molecularly targeted agents provide the "low hanging fruit" of molecular medicine by providing a method to add molecular diagnostics and to improve surgery by reducing the amount of residual disease left behind. Our program mainly focuses upon developing and applying the NIRF imaging devices for detecting prostate and breast cancers targeted with NIRF molecular imaging agents in tumor mouse models expressing either DsRed or iRFP gene reporter.

Since the combinational drug/device product first requires validation for detecting NIRF from agents targeting disease markers at pico- to femto-molar concentrations, I am developing and deploying a National Institute of Standards and Technology (NIST) traceable phantom that can be used to quantify the general platforms of NIRF molecular imaging devices. In addition, I have expanded the utility of fluorescence imaging into far-red gene reporter (IFP1.4) to longitudinally track *B. anthracis* infection with collaborators at BCM.

RESEARCH PROJECTS

- Developing NIRF imaging devices using both continues wave and frequency-domain measurement approaches
- Applying the developed devices for NIRF-guided cancer detection in tumor mouse models expressing either DsRed or iRFP gene reporter
- Validating the tumor-specific NIRF agents through receiver operating characteristic analysis
- Tracking *B. anthracis* infection using IFP1.4 gene reporter
- Working with NIST to develop a traceable fluorescent solid phantom

KEY PUBLICATIONS

Zhu, B., Wu, G., Robinson, H., Wilganowski, N., Hall, M. A., Ghosh, S. C., Pinkston, K. L., Azhdarinia, A., Harvey, B. R., and E.M. Sevick-Muraca, "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. PMID:23619897.

Zhu, B., and A. Godavarty, "Functional connectivity in the brain in joint attention skills using near infrared spectroscopy and imaging," *Behavioural Brain Research,* 250(1): 28-31, 2013. PMID:23624192.

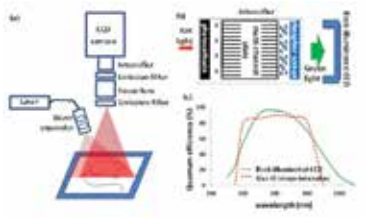
Sevick-Muraca, E. M., and Zhu, B, "The need for performance standards in clinical translation and adoption of fluorescence molecular imaging", *Medical Physics,* 40, 040402, 2013. PMID:23556867; PMCID:PMC3612123.

Zhu, B., Tan, I.C., Rasmussen, J.C., and E.M. Sevick-Muraca, "Validating the sensitity and performance of near-infrared fluorescence imaging and tomography devices using a novel solid phantom and measurement approach," *Technol Cancer Res Treat*, 11(1): 95-104, 2012. PMID: 22181335

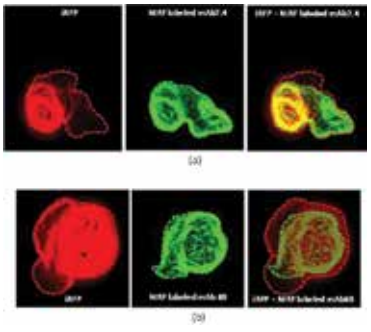
Darne, C.D., Lu Y, Tan IC, Zhu, B., Rasmussen, J.C., Smith, A.M., Yan, S., Sevick-Muraca, E.M., "A compact frequency-domain photon migration system for integration into commercial hybrid small animal imaging scanners for fluorescence tomography," *Phys Med Biol*. 57(24), 8135-52, 2012, PMID:23171509; PMCID:PMC3533362.

LAB MEMBERS

Coadvised: Grace Wu, Nathaniel Wilganowsk, Holly Robinson



(a) A schematic of the ICCD-based NIRF imaging system operating at two different wavelengths; (b) key components of the NIRF imaging system are an NIR intensifier coupled to a CCD chip, where the intensifier collects far red and NIR light and amplifies an electronic signal to produce green light that is optimally collected and integrated by the back illuminated CCD; and (c) the spectral responses of both the Gen III image intensifier and back-illuminated CCD, showing the advantage of CCD collection of green light over 720 nm (iRFP) and 830 nm (NIRF) light that is sensitively collected by the intensifier.



The comparison of margins of two primary lesions by NIRF labeled specific mAb 7.4 (a) and isotype control mAb 69 (b).

Center for Proteomics and Systems Biology

The Center for Proteomics and Systems Biology connects research efforts across the university in systems biology, clinical and translational sciences, protein chemistry, genomics, and proteomics, bringing together people to promote intellectual exchange and the transfer of expertise in these key fields and beyond.

While genomics has been highly successful at cataloging genetic variations, for the vast majority of genes, it is the protein products that are functional. Further, proteins are the targets for essentially all the drugs on the market today. Gene sequences give us a starting point, but most cellular proteins are extensively processed and modified. To understand cellular regulation, elucidate disease processes, and identify drug targets, we need the detailed characterization of proteins that now appear 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, and systems biology research.

The Mass Spectrometry Facility is located in the IMM and houses four state-of-the-art mass spectrometers that allow the identification and quantification of peptides and proteins for in-depth proteomic analysis of cells, tissues or biological fluids.

Hubs of Research Collaboration with the Center include:

- Protein Chemistry
- Proteomics
- Systems Biology and Bioinformatics
- Proteomics Core Laboratory of the Center
- CLIA Molecular Diagnostics Laboratory
- NCI Center for Cancer Nanomedicine Excellence

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 and E-Selectin.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Somasunderam, Anoma; Thiviyanathan, Varatharasa; Tanaka, Takemi; Li, Xin; Neerathilingam, Muniasamy; Lokesh, G; Mann, Aman; Peng, Yang; Ferrari, Mauro; Klostergaard, Jim; Gorenstein, David, "Combinatorial selection of DNA thioaptamers targeted towards the HA binding domain of human CD44", *Biochemistry*, 2010 Oct 26;49(42):9106-12. PMC2981344

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).

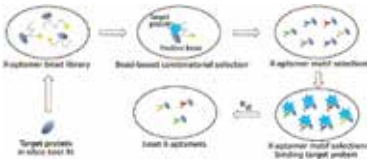
Xianbin Yang, Li Na, David G. Gorenstein, Strategies for the discovery of therapeutic aptamers, *Expert Opinion in Drug Discovery*, Volume 6, Number 1, January 2011, pp. 75-87(13). PMID: 21359096; PMCID: PMC3045091. doi: 10.1517/17460441.2011.537321

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).

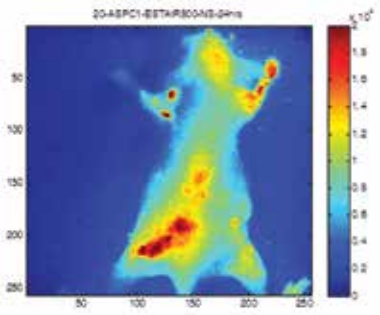
Weiguo He, Miguel-Angel Elizondo-Riojas, Xin Li, Ganesh Lakshmana Rao Lokesh, Anoma Somasunderam, Varatharasa Thiviyanathan, David E. Volk, Ross H. Durland, Johnnie Englehardt, Claudio N. Cavasotto, and David G. Gorenstein "X-Aptamers: A bead-based selection method for random incorporation of drug-like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

LAB MEMBERS

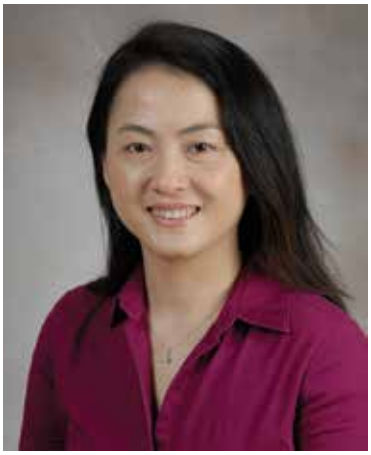
Research Scientists: Lokesh Rao, Ph.D., Hongyu Wang, Ph.D., Li Li, Ph.D.
Research Assoc.: Xin Li, M.S.
Post Docs: Miguel-Angel Elizondo-Riojas, Sai Gandham
Graduate Student: Kurtis Anderson



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

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, *In Press* (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).

A. Hanifi, X. Bi, X. Yang, B. Kavukcuoglu, PC Lin, E DiCarlo, RG Spencer, MP Bostrom, N Pleshko, Infrared fiber optic probe evaluation of degerative cartilage correlates to histological grading, 2012, *The American Journal of Sports Medicine,* 40(12): 2853-61 (2012).

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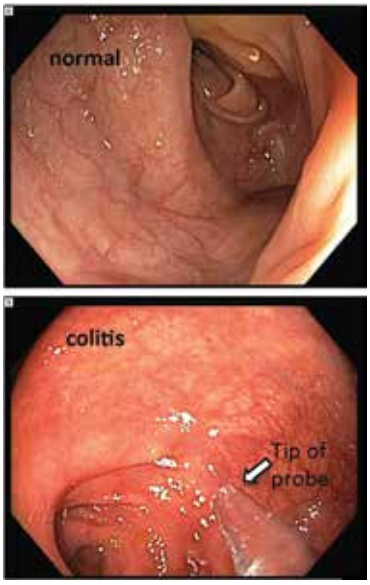
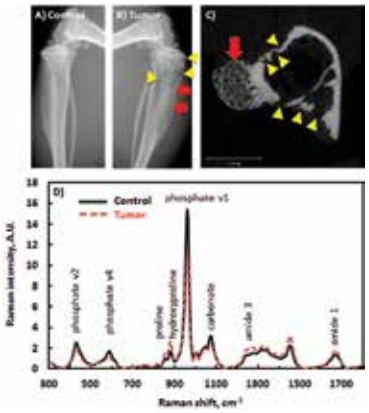
X. Bi, A. Walsh, A. Mahadevan-Jansen and A. Herline, Development of spectral markers for the discrimination of ulcerative colitis and Crohn's disease using Ramans spectroscopy, 2011, *Disease of the Colon and Rectum*, 54(1), 48-53 (2011).

LAB MEMBERS

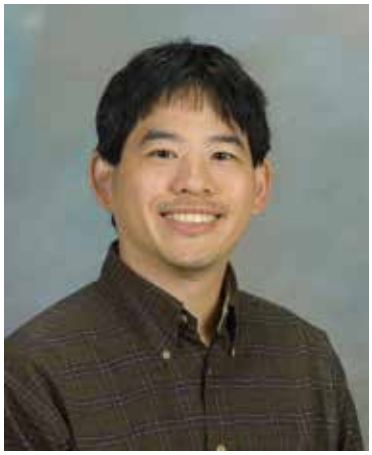
Postdoc: Hao Ding; Rameshwar Rao Tatavarty
Research Scientist: Zhiyong Wang
Technician: Guijin Lu



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
Assistant Professor

Cell signaling networks in cancer

Our lab deciphers cell-signaling programs. 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 that we are now investigating as potential treatments for breast cancer metastasis.

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.* 2013. Accepted.
* 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

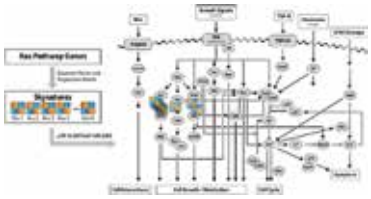
Chang JT. Deriving transcriptional programs and functional processes from gene expression databases. *Bioinformatics* 28(8), 2012.

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.

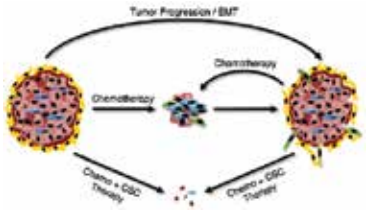
Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, Olson JA, Marks JR, Dressman HK, West M, and Nevins JR. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 439(7074): 353-357, 2005.

LAB MEMBERS

Postdocs: Weina Zhao, Ph.D., Sarah Prjic, Ph.D., Bettina Urban, Ph.D.
Bioinformaticians: Xiaoling Chen, Ph.D., Emily Lu
Research Assistants: Jessie Sjol, Mike Tisza



Gene expression signatures predict pathway activation.



The plasticity of cellular phenotypes complicates cancer treatments.

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 hrs EVA preparation down to two hrs. 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-hr 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

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

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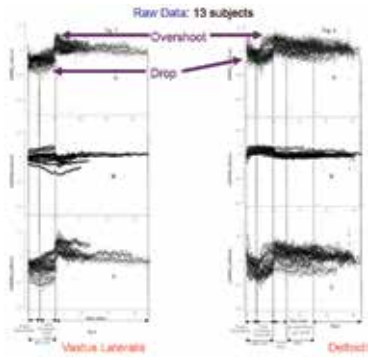
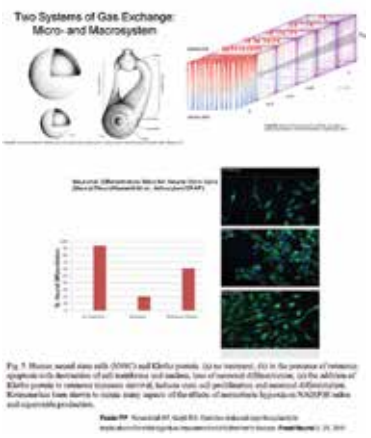
Foster PP, Pollock NW, Conkin J, Dervay JP, Caillot N, Chhikara RJ, Vann RD, Butler BD, and Gernhardt ML. Protective Mechanisms in Hypobaric Decompression. *Aviat. Space Environ. Med.* 84:3, 212-25, 2013.

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 Distinguished Professorship 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 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-βB activation pathway. *Journal of Biological Chemistry* 286: 37187-37195. PMID: 21900239.

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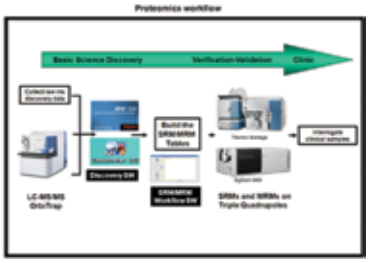
Voelkl, J., Alesutan, I., Leibrock, C.B., Quintanilla-Martinez, L., Kuhn, V., Feger, M., Mia, S., Ahmed, M.S., Rosenblatt, K.P., Kuro-O, M., and Lang, F. (2013) Spironolactone ameliorates PIT1-dependent vascular osteoinduction in klotho-hypomorphic mice. *Journal of Clinical Investigation,* Epub 2013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

Metabolomics, proteomics and nanomedicine

By combining powerful statistical analysis and bioinformatics methods with NMR- or MS-based measurements of metabolite or protein levels in living systems, mechanisms of disease pathways or onset of disease can be studied. Most recently we have used such techniques to investigate the effects of ethanol and fatty liver disease, and the ingestion of chemicals used to refine uranium and plutonium and the resulting metabolite profile. We also provide bioinformatics services through our newly created UTHealth Bioinformatics Service Center, which just recently developed Aptaligner©, a new DNA sequencing analysis program.

We also use nuclear magnetic resonance spectroscopy (NMR) to study the structures of large molecules, such as DNA or proteins, and their interactions with each other. Most recently we solved the structure of thymosin alpha-1, a peptide adjuvant used to treat viral infections. Previously, we have solved the solution structures of the envelope protein domain III, a key binding site for neutralizing antibodies, of West Nile, Omsk, Yellow Fever, and Dengue 4 viruses, and other proteins and carcinogenic DNA adduct structures. The structures formed by the co-mixing of non-steroidal anti-inflammatory drugs (NSAIDS) with phospholipids and bile salts also are being studied to determine the mechanism behind NSAID-induce ulcerations of the upper and lower GI-tracts and ways to reduce their rates of occurrence.

Another area of development includes DNA-based targeting/imaging agents (called aptamers) for attachment to nanoparticles to enhance delivery of chemotherapy directly to tumors. The aptamers target proteins that are over-expressed on the tumor surface, such as the CD44 and E-selectin proteins, and our most recent development, X-aptamers, contain drug-like appendages to increase specificity and binding affinity. By combining near-infrared dyes to such nanoparticles, these agents can simultaneously be used for chemotherapy using liposome nanoparticles or for image-guided laser destruction of cancerous tumors using gold nanoparticles.

RESEARCH PROJECTS

- Statistical analysis of proteomics data
- Develop targeting DNA molecules for drug delivery and imaging of tumors
- Structural studies of non-steroidal anti-inflammatory drug complexes
- Develop next-generation X-aptamers (DNA) and Aptaligner© program

KEY PUBLICATIONS

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, D.G. Gorenstein, *Biochemistry* 2012, 51(42):8321-8323.

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Insight into NSAID-induced membrane alterations, pathogenesis and therapeutics: characterization of interaction of NSAIDs with phosphatidylcholine, L.M. Lichtenberger, V. Jayaraman, J.R. Doyen, R.G. O'Neil, E.J. Dial, Y. Zhou, D.E. Volk, D.G. Gorenstein, U. Marathi, M.B. Boggara and R. Krishnamoorti, "", *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1821(7): 994-1002, 2012.

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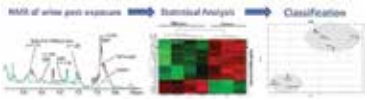
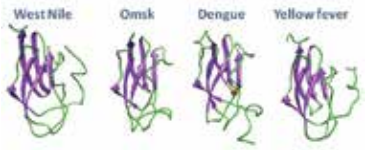
Thioaptamer Conjugated Liposomes for Tumor Vasculature Targeting, A.P. Mann, R.C. Bhavane, A. Somasunderam, B.L. Montalvo-Ortiz, K.B. Ghaghada, D. Volk, R. Nieves-Alicea, K.S. Suh, M. Ferrari, A. Annapragada, D.G. Gorenstein, T. Tanaka, *Oncotarget* 2(4), 298-304, 2011.

LAB MEMBERS

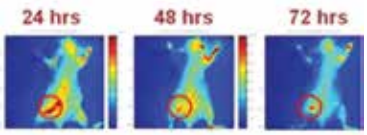
Bioinformatician: Emily Lu, PhD
Research Scientists: Chuantao Jiang, PhD, Lokesh Rao, PhD, Hongyu Wang, PhD
Research Associates: Xin Li, MS, Li Li, PhD
Post Docs: Miguel-Angel Elizondo-Riojas, PhD, Weiguo He, PhD, Ana Maria Zaske, PhD
Medical Students: Angela Sung, Max Polansky



Metabolite (or protein) profiles after chemical insult or disease progression, together with mathematical analysis and clustering methods, provide powerful biomarker classification to verify exposure or monitor disease.



NMR solution structures of Flavivirus envelope protein domain III, which is critical for binding to cells and recognition by neutralizing antibodies.



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

Center for Stem Cell and Regenerative Medicine

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 lung such as cystic fibrosis), 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.

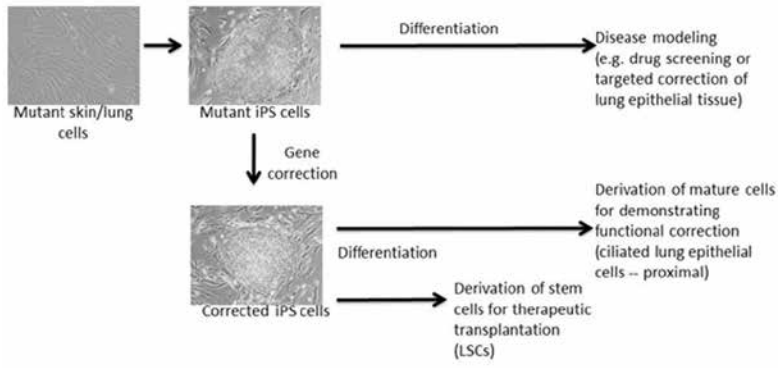
Our focus within the Center for Stem Cell and Regenerative Medicine is to study the fundamental properties of stem cells and to translate their unique biological properties into novel cellular therapies for tissue regeneration for currently intractable disorders. It is essential that such an endeavor 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 a multidisciplinary faculty with the appropriate breadth of expertise and scientific rigor in the discipline of stem cell biology 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 Center faculty in order to significantly increase the breadth and depth of our basic and translational 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 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
Annie and Bob Graham Distinguished Chair
in Stem Cell Biology

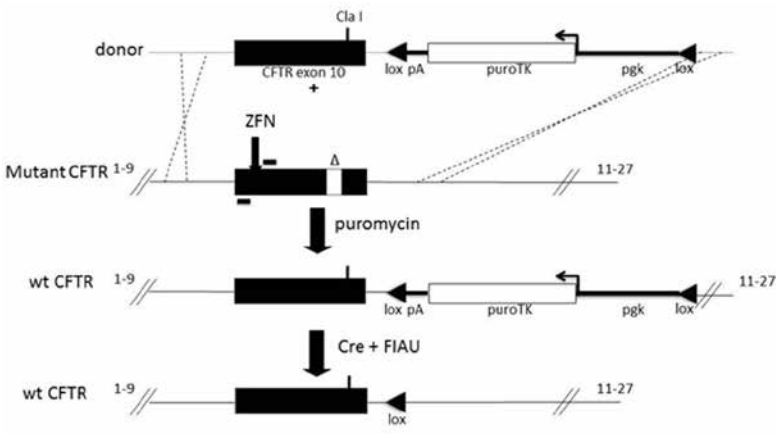
Brian Davis, Ph.D.
Associate Professor
Director of the Center for Stem Cell and Regenerative Medicine
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

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

Our 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," which 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



ZFN-mediated Correction of *CFTR* Mutation in CF iPS 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: Revertant somatic mosaicism in the Wiskott-Aldrich Syndrome. *Immunologic Research* 44:127-131, 2009.

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

LAB MEMBERS

Research Staff: Dr. Ana M. Crane, Wei Liao, Pooja Gandhi, Dr. Leila Rouhigharabaei, Dr. Daniela Mora Ortiz
Postdoctoral Fellows: Dr. Philipp Kramer, Dr. Xuan Shirley Li
Ph.D. Student: Tamara Laskowski

Laura A. Smith Callahan
Assistant Professor

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

The research in my laboratory focuses on the 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.

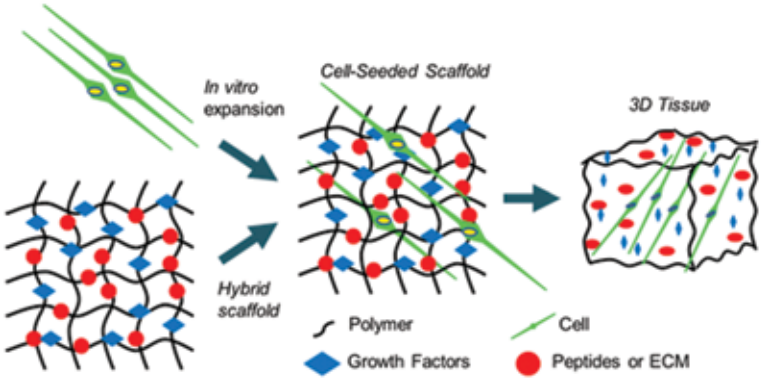
Smith Callahan LA, Policastro GM, Benard SL, Childers EP, Boettcher RM, Becker ML. Influence of Discrete and Continuous Culture Conditions on Human Mesenchymal Stem Cell Lineage Choice in RGD Concentration Gradient Hydrogels. *Biomacromolecules*. 14(9): 3047-3054, 2013.

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 Fellow: Yueh Hsun (Kevin) Yang



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) has 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 cells isolated from fetal cadaver brain tissue and neural progenitor cells derived from human embryonic stem cells (hESCs)-derived have been approved for clinical trials for SCI patients. However, these cells are associated with ethical controversy and graft rejection. Cells derived from hESCs have additional risk of teratoma formation. Human induced pluripotent stem cells (hiPSCs) are recently developed remarkable pluripotent, ESC-like cells reprogrammed from adult somatic cells by over-expression of four developmental/ pluripotency transcription factors. Compared with ESCs, hiPSCs offer significant additional advantages in terms of availability of source material without ethical concerns of embryo use, and especially the ability to generate isografts without the need of immunosuppression. But hiPSC-derived NSCs still share the risk of tumor formation with its counterpart from hESCs. More recently, somatic cells, such as fibroblasts, can be directly reprogrammed into functional neurons or NSCs without passing through pluripotent stem cell stage. The directly induced NSCs (iNSC) have all advantages of hiPSCs but without the risk of teratoma formation. However, the current protocols for producing iNSCs relied on using retroviral or lentiviral vectors to induce the transcription factors into the genome of host somatic cells, a process associated with risks including mutation, dysregulation of gene expression, and likely blocking its clinical translation. To overcome these limitations of retrovirus derived iNSC (rv-iNSC), we have successfully developed a novel approach to directly convert SCI patient fibroblasts into NSCs using recombinant proteins. The protein induced NSCs (p-iNSC) can proliferate over long time *in vitro* and be induced to differentiate into functional neurons, astrocytes, and oligodendrocytes. Importantly, p-iNSCs can survive and differentiate into both neurons and glias after transplantation into the contused spinal cord. Currently, we are studying the molecular mechanisms to regulate the proliferation and differentiation of p-iNSCs and developing standard methods to differentiate and purify ideal neural cells for different neurological diseases. We are testing the therapeutic potential and long-term safety of p-iNSCs in preclinical animal models of spinal cord injury, traumatic brain injury, and stroke. These studies will help us to develop novel stem cell-based therapies for these neurological disorders, which can be translated to clinical application in the near future.

RESEARCH PROJECTS

- Identification of molecular mechanisms for the direct reprogramming of human fibroblasts into neural stem cells
- The long-term therapeutic efficacy and safety of induced neural stem 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.
- 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.

Wang YP, Cheng XX, He Q, Kim DH, Whittemore SR and Cao QL (2011) Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult OPCs by increasing the expression of bone morphogenetic proteins. *J Neurosci* 31(16):6053– 6058.

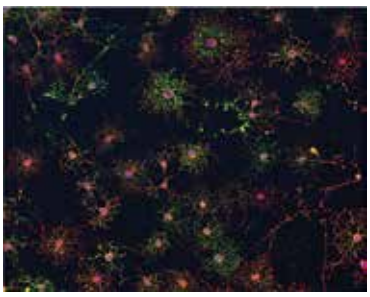
Cao Q and Whittrmore SR (2012). Cell transplantatin: stem cells and precursor cells. *Handb Clin Neurol.* 109: 551-61.

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.

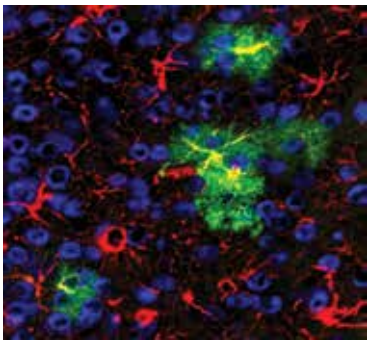
Chen KN, Deng SY, Lu HZ, Zheng YY, Yang GD, Kim DH, Cao QL* and Wu JQ* (2013). RNA-Seq characterization of spinal cord injury transcriptome in acute/subacute phases: a resource for understanding the pathology at the systems level. *Plus One* (in Press). * co-corresponding authors.

LAB MEMBERS

Postdoc Research Associate: Hezou Lu, Shaohui Wang, Yiyan Zheng
Research Assistant: Io Long Chan, Jun Li



Oligodendrocyte precursor cells in culture



Astrocyte differentiation of grafted induced neural stem cells in normal brain

Charles Cox, Jr., M.D.
Professor
Children's Fund Inc. Distinguished Professorship in Pediatric Surgery

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.

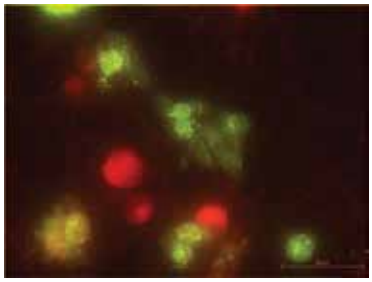
Bedi SS, Hetz R, Thomas C, Olsen A, Williams S, Smith P, Xue H, Aroom K, Uray K, Hamilton T, Mays RW, Cox CS. Intravenous MAPC therapy improves spatial learning after TBI. *Stem Cells/Translational Medicine*. 2:953-960, 2013.

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

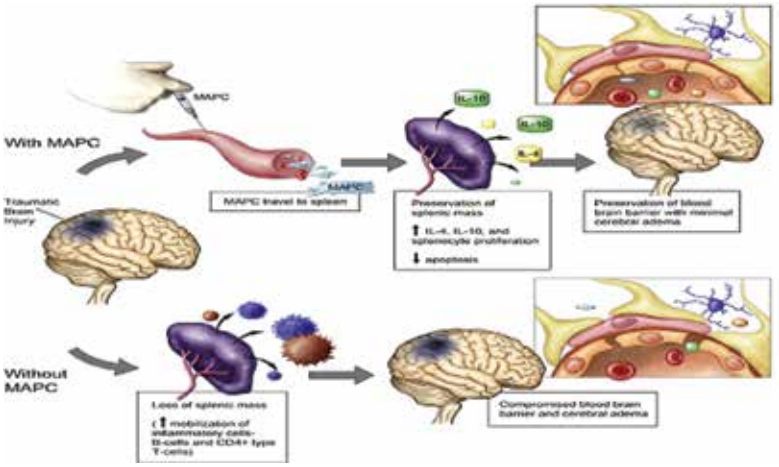
Walker PA, Bedi SS, Shah SK, Jimenez F, Xue H, Hamilton JA, Smith P, Thomas CP, Mays RW, Pati S, Cox CS. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: Modulation of microglia/macrophages. *J Neuroinflammation* 9: 228-240, 2012. PMID: 23020860

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

Our lab's main interest is using pluripotent stem cells for skeletal muscle regeneration. During the last few years, I have 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, our lab focuses on the approaches to improve stem cell therapies for skeletal muscle regeneration. My research will include optimizing cell delivery, survival, and engraftment, studying the 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 other goal of our lab.

Our 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 for skeletal muscle regeneration in a mouse model of Duchenne Muscular Dystrophy (DMD).

RESEARCH PROJECTS

- 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.

Darabi R, Baik J, Clee M, Kyba M, Tupler R, Perlingeiro RC. Engraftment of embryonic stem cell- derived myogenic progenitors in a dominant model of muscular dystrophy. *Experimental Neurology,* 2009 Nov; 220(1):212-6.

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

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

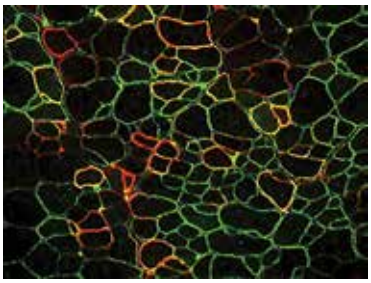
Darabi R, Arpke RW, Irion S, Dimos JT, Grskovic M, Kyba M, Perlingeiro RC. Human ES- and iPS-Derived Myogenic Progenitors Restore Dystrophin and Improve Contractility upon Transplantation in Dystrophic Mice. Cell *Stem Cell,* 2012 May; 10 (5), 610-619.

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

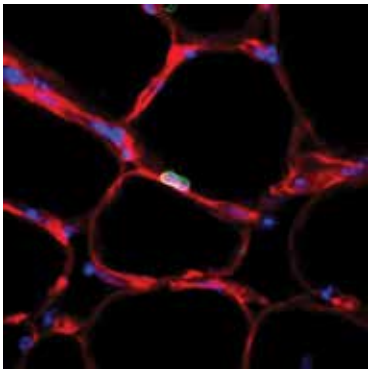
Arpke RW, Darabi R, Mader TL, Zhang Y, Toyama A, Lontree CL, Nash N, Lowe DA, Perlingeiro RC, Kyba M. A New Immuno- Dystrophin- Deficient Model, The NSG-mdx4cv Mouse, Provides Evidence for Functional Improvement Following Allogenic Satellite Cell Transplantation. *Stem Cells,* 2013 Apr 19. doi: 10.1002/stem.1402.

LAB MEMBERS

Postdoctoral Fellow: Jianbo Wu
Research Technician: Samuel D. Hunt



Expression of human (Red) vs. mouse (Green) dystrophin following engraftment of Human ES derived myogenic progenitors in the muscle.



Human ES derived satellite cell (human lamin A/C: Green, Pax7/D API merge: Pink) located under the basal lamina (laminin: Red) of a myofiber one month post- transplantation.

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

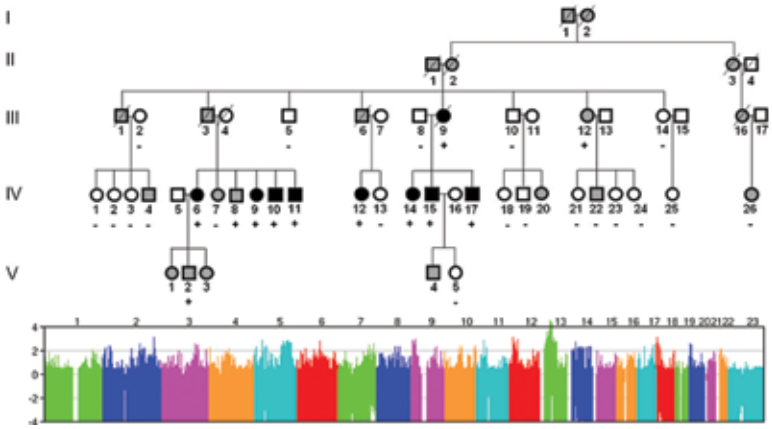
KEY PUBLICATIONS

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.

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.



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.

Mikhail Kolonin, Ph.D.
Associate Professor
Jerold B. Katz Distinguished Professor in Stem Cell Research
John S. Dunn Research Scholar

Adipocyte progenitor cells in pathology

My laboratory investigates the role of progenitor cells in obesity progression and reversion, as well as in the obesity link to cancer and other pathologies. Based on clinical specimens and mouse models, we discovered the phenomenon of adipose stromal cell mobilization and trafficking to tumors and characterized their stimulatory effects on cancer progression. Based on the expertise in cell population separation and high throughput combinatorial peptide library screening methods, we have identified a number of tissue-specific cell surface markers. Investigation of molecular mechanisms, leading to adipocyte 'browning', regulated by these molecules in white adipose tissue, is underway. Therapeutic peptides targeting markers of endothelial cells are in clinical trials. A strategy to target white adipocyte progenitors for obesity and cancer intervention is a new promising line of translational research in the laboratory. Other projects include the development of a targeted compound useful for non-invasive imaging of brown adipose tissue and approaches to three-dimensional adipose tissue engineering.

RESEARCH PROJECTS

- Role of adipose tissue cells in tumor microenvironment
- Development of experimental drugs targeting white adipocyte progenitors
- Adipose tissue markers and mechanisms of intercellular communication
- Development of approaches to target cells of brown adipose tissue

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, C*ancer Research*, 15;72(20):5198-208, 2012.

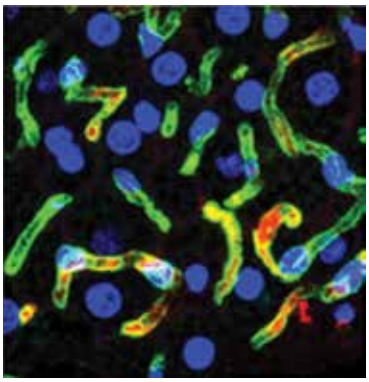
Daquinag A., Souza G. and Kolonin M.G. Adipose tissue engineering in three-dimensional levitation tissue culture system based on magnetic nanoparticles, *Tissue Engineering,* 19(5):336-344, 2013.

Zhang Y., et al. Lazar A.J., Pollock R.E., Simmons P.J., Lev D. and Kolonin M.G. Heterogeneity and immunophenotypic plasticity of malignant cells in human liposarcomas, *Stem Cell Research.* 11(2):772-781, 2013.

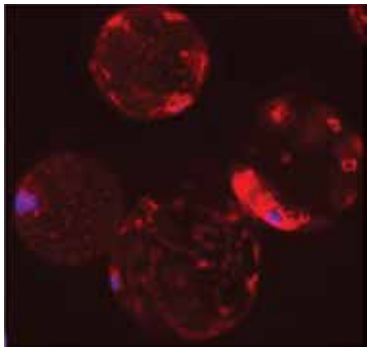
Azhdarinia A., Daquinag A.C., Tseng C., Ghosh S.C., Ghosh P., Amay-Manzanares F., Sevick-Muraca E., and Kolonin M.G. A peptide probe for targeted brown adipose tissue imaging, *Nature Communications.* 4:2472-2482, 2013.

LAB MEMBERS

Alexis Daquinag: research scientist
Zhang Tao: postdoctoral fellow
Chieh Tseng: graduate student
Ahmad (Nasser) Salameh: postdoctoral fellow
Ali Dadbin: senior research assistant



Blood vessels (green) labeled with a peptide (red) targeting brown adipose tissue. Nuclei (blue) reveal brown adipocytes.



Cultured white adipocytes displaying cell membrane (red) remodeling. Nuclei are blue.

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 behind aging processes in the musculoskeletal system and detecting candidate genes for aging prevention; and 3) use of 3D printer or updated bioengineering techniques to build 3D soft 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. 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 with 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 disease 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

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

Nozaki M, Ota S, Li Y, Uehare K, Gharaiben B, Fu FF, Huard J. Timing of the administration of suramin treatment after muscle injury. *Muscle & Nerve* 2012;46(1):70-79.

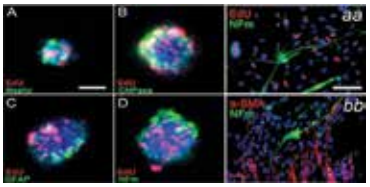
Lin B, Kim J, Li YX, Pan HY, Carvajal-Vergara X, Salama G, Cheng T, Li Y, Lo CW, Yang L. High purity enrichment of functional cardiac lineage cells from human iPS cells. *Circulation Research* 2012;95(3):327-335.

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.

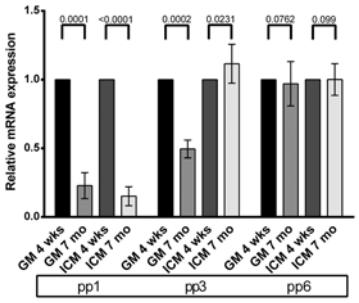
Vojnits K, Yang L, Zhan M, Cox CS, Li Y. Very small embryonic-like cells in the mirror of regenerative medicine. *Journal of Stem Cell* (Accepted 2013)

LAB MEMBERS

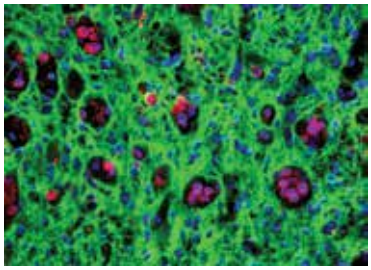
Administrator: Stephanie Baca
Lab senior technician/manager: Haiying Pan
Postdoc research fellow: Dr. Yohan Choi; Dr. Kinga Vojnits
Medical residency fellow: Dr. George P Liao
Medical student: Chen Fu, and Yunfeng Xue



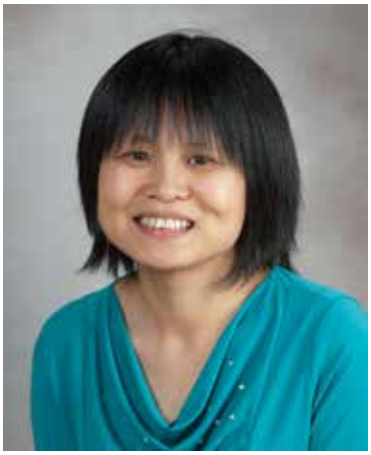
Cell dedifferentiation study.



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



Mechanisms behind fibrosis scar tissue formation.



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 a 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 set up systems to optimize 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. We also have developed efficient procedures to genetically label and purify hESC- and hiPSC-derived lineage specific cells for in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

- Generation of patient-specific, integration-free iPSCs
- Creation of neural lineage hESC and hiPSC reporters by gene targeting and genome editing tools with high efficiency for purification and transplantation tracking
- Identification of optimal neural lineage progenitors for cell-based therapy in spinal cord injury and stroke
- Analysis of ALS patient-specific iPSCs and their neural derivatives
- Characterization of the role of OLIG genes using patient iPSCs

KEY PUBLICATIONS

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)

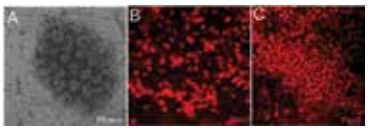
Liu, Y., Rao, M. (2011) Gene targeting in human pluripotent stem cells. *Methods Mol Biol.* 767:355-367.

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

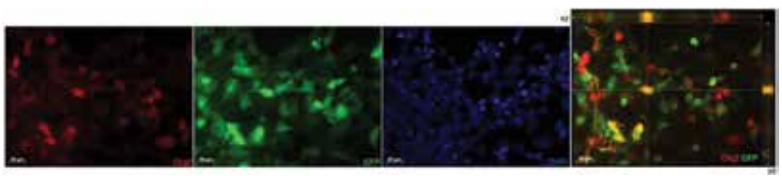
Liu, Y., Thyagarajan, B., Lakshmipathy, U., Xue, H., Lieu, P., Fontes, A., MacArthur, C.C., Scheyhing, K., Rao, M.S., and Chesnut, J.D. (2009). Generation of a platform human embryonic stem cell line that allows efficient targeting at a predetermined genomic location. *Stem Cells Dev* 18, 1459-1472

LAB MEMBERS

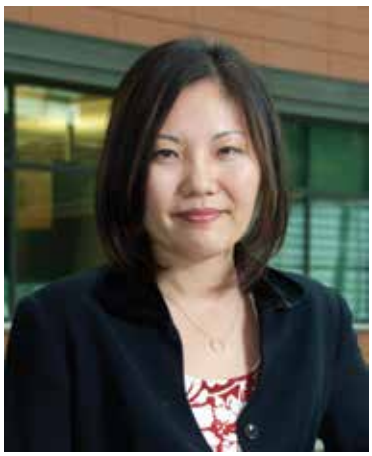
Postdoctoral Fellow: Shenglan Li
Research Associate: Haipeng Xue
Research Assistant: Jianhu Zhang
Visiting Scientist: Bo Long



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

Cellular and molecular heterogeneity in blood cancers

Various reports have identified stem-like cells as important mediators for tumor initiation and progression in hematological cancers and solid tumors. Malignant stem-like cells have the unique ability to proliferate and self-renew extensively. However, the mechanisms of the tumor initiation and rapid growth by these cells have been largely unknown. The current focus of my lab is to characterize molecular and cellular mechanisms that confer survival and drug resistance stem-like cells in various hematopoietic malignancies and how components of these pathways are functionally linked. We are currently using mantle cell lymphoma and multiple myeloma as model systems to investigate these issues.

Another project we are focusing on is how cancer cells evade the host immune functions to promote uncontrolled growth. These immune evasion phenomenons are also important in occurrence of stem cells, and understanding such mechanisms became a critical issue for stem cell-related therapies. Characterizing the immune surveillance mechanisms by cancer cells and stem cells will have important translational and preclinical implications.

RESEARCH PROJECTS

- Investigating the roles of stem-like cells in blood cancers
- Developing targeted therapies against signaling pathways in multiple myeloma and Non-Hodgkin's Lymphomas
- Characterizing the molecular and cellular mechanisms of malignant cell development and progression in blood cancers
- Analyzing immune escape mechanisms of malignant cells in blood cancers

KEY PUBLICATIONS

Chen, Z., Pittman, E.F., Romaguera, J., Fayad, L., Wang, M., Neelapu, S.S., Mclaughlin, P., Kwak, L.W., McCarty, N. (2013) Nuclear Translocation of B-cell-specific transcription factor, BACH2, modulates ROS mediated cytotoxic responses in Mantle Cell Lymphoma. *PLOS one.* 2;8(8):e69126. doi: 10.1371/journal.pone.0069126.

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

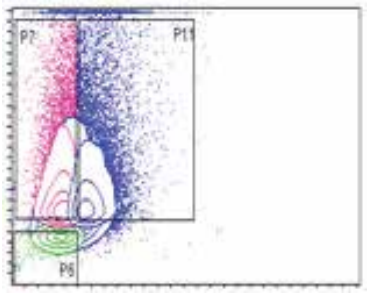
Jung, H-J., Chen, Z., McCarty, N. (2012) Synergistic antiproliferative effects of arsenic trioxide (ATO) with bortezomib in mantle cell lymphoma (MCL). *American Journal of Hematology.* 87:1057-1064.

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

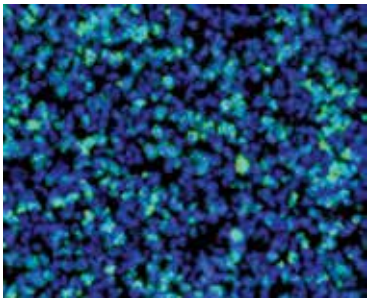
Jung, H-J., Zheng, C., Fayad, L., Wang, M., Romaguera, J., Kwak, L.W., McCarty, N. (2012) Bortezomib-resistant nuclear factor kappa B expression in stem like cells in mantle cell lymphoma (MCL). *Experimental Hematology.* 40:107-118.

LAB MEMBERS

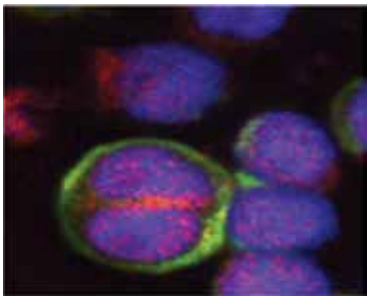
Senior Research Assistant: Judy Chen
Graduate student: Albert Teo



Transglutaminase 2 and NF-kB components are colocalized in MCL cells.



FACS analysis of cell cycle using Pyronin and Hoechst staining.



Ki-67 staining reveals proliferative capacity of MCL initiating cells.

Naoki Nakayama, Ph.D.
Associate Professor

Stem cell differentiation and lineage specification

Pluripotent stem (PS) cells, whether derived from an embryo or induced from adult cells, grow almost indefinitely without losing their developmental potential. PS cells are also "pluripotent" *in vivo*, and are thus expected to differentiate into any somatic cell-type *in vitro*, making human (h)PS cells a promising source of cells for regenerative medicine. The major challenges have been to direct their differentiation toward the cell type of interest and to isolate them in large quantity without introducing transgenes and mutations. The principle of our strategy is to apply to human cells what we have learnt from developmental biology of the mouse.

Development of human joint chondroprogenitor cells: 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 adult stem cell therapies face the problems of low yield of cells and their tendency to yield unsuitable and/or unstable cartilage. Joint is formed during embryogenesis. Therefore, embryonic chondroprogenitors responsible for limb and vertebral joint formation are likely to be the best source of cells for the regeneration of joint cartilage in the adult. We have previously developed and purified from hPS cells paraxial mesoderm and neural crest progeny with the capacity to expand and differentiate into chondroprogenitor cells. We also have established a condition where these progeny generate hyaline-like cartilage particles. We have recently established a way to selectively generate and expand, to a limited extent, from mesodermal progeny of hPS cells joint progenitor-like cells, which are supposed to be the common embryonic precursor of synovial joint components, including articular and meniscal chondrocytes and ligaments. We are currently focusing on the characterization of the joint progenitor-like cells, aiming to demonstrate their capacity to generate joint-type stable cartilage.

Development of hematopoietic stem cells (HSCs): Attempts to derive and isolate hematopoietic cells from PS cells began nearly 20 years ago using mouse embryonic stem cells, later moving to hPS cells. However, all early studies, including our own, failed to reproducibly generate hematopoietic cells that fulfill the stringent definition of stem cells: significant levels of multilineage marrow repopulation in serial transplants. One of the major sites where marrow-repopulating HSCs are born during embryogenesis is the endothelium of dorsal aorta. We have established defined culture conditions and methods that allow to generate and purify hemogenic, as well as non-hemogenic, endothelial progeny from hPS cells, the former of which display very weak marrow-repopulating activity in immunocompromised mice after co-culture with a mouse embryonic stromal cell line. We are currently interested in defining the key molecular mechanism by which hematopoietic cells are born from the hemogenic endothelial cells.

RESEARCH PROJECTS

- Specification, prospective isolation and expansion of three embryonic chondroprogenitors (sclerotome, limb mesenchyme and ectomesenchyme) from hPS cells
- Elucidation of molecular basis of long-term expansion without loss of chondrogenic activity of the hPS cell-derived chondroprogenitor cells
- Generation, detection, isolation, and expansion of joint progenitors from hPS cells using specific reporter PS cell lines
- Defining the process of chondrogenesis from the hPS cell-derived "general" chondroprogenitors cells and joint progenitor cells to elucidate molecular basis of articular chondrogenesis
- Establishment of orthotropic xenotransplantation model for cell-based cartilage repair
- Specification, prospective isolation, and expansion of hemogenic, as well as non-hemogenic, endothelial cells from hPS cells
- Elucidation of molecular basis of endothelial hemogenesis

KEY PUBLICATIONS

Zhao, J., Li, S., Tanaka, M., et al. (2013) "Directed specification of sclerotomal chondroprogenitor and induction of somitic chondrogenesis program from embryonic stem cells." Development in revision.

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.

Wang Y, Umeda K, and Nakayama N. (2010) "Collaboration between WNT and BMP signaling promotes hemoangiogenic cell development from human fibroblast-derived iPS cells". *Stem Cell Res*. 4:223-231.

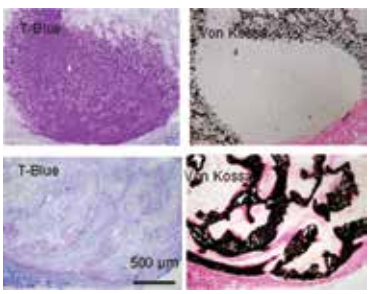
Tanaka, M., Jokubaitis, V., Wood, C., et al. (2009) "BMP inhibition stimulates WNT-dependent generation of chondrogenic mesoderm from embryonic stem cells". *Stem Cell Res*., 3:126-141.

LAB MEMBERS

Research Associate: Qing Yan, Ph.D.
Research Technician: Suprita Trilok
Animal Specialist: Nadine Matthias, D.V.M.

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)

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

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.

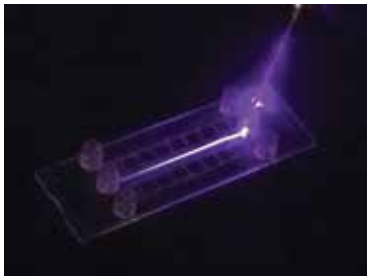
Wenzel, P.L.*, Chong, J.-L.*, Saénz-Robles, M.T., Ferrey, A., Hagan, J.P., Gomez, Y.M., Sharma, N., Chen, H.-Z., Robinson, M.L., and Leone, G. (2011). Cell Proliferation in the Absence of E2F1-3. *Developmental Biology* 351: 35-45. *Equal contribution.

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.

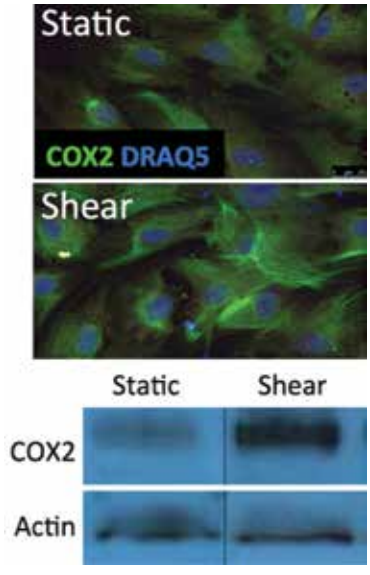
Adamo, L., Naveiras, O., Wenzel, P.L., McKinney-Freeman, S., Mack, P.J., Gracia-Sancho, J., Suchy-Dicey, A., Yoshimoto, M., Lensch, M.W., Yoder, M.C., Garcia-Cardeña, G., and Daley, G.Q. (2009). Biomechanical forces promote embryonic haematopoiesis. *Nature* 459: 1131-1135.

LAB MEMBERS

Research Associate: Miguel Diaz
Postdoctoral Fellow: Hyun Jung Lee, Ph.D.
Postdoctoral Fellow: Nan Li, Ph.D.
Research Assistant: Siobahn Evans
Administrative Assistant: Stephanie Baca (Pediatric Surgery)



Microfluidics are prepared for stem cell culture by corona treatment (emission of high energy to charge the culture surface).



Shear stress activates production of Cox2, an enzyme known to play a key role in mediating immunomodulatory function in mesenchymal stem cells.

Jiaqian Wu, Ph.D.
Assistant Professor

Gene transcription and regulation of stem cell differentiation

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Wu, J. Q., Du, J., Rozowsky, J., Zhang, Z., Weissman, S., Gerstein, M., Snyder, M. (2008). Systematic analysis of transcribed loci in selected ENCODE regions using RACE sequencing. *Genome Biology*. 9(1):R3

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.

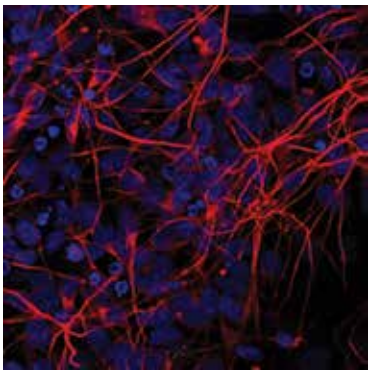
Wu, J. Q. (2011). Characterize mammalian transcriptome complexity. Deutschland, Germany: LAP LAMBERT Academic Publishing.

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.

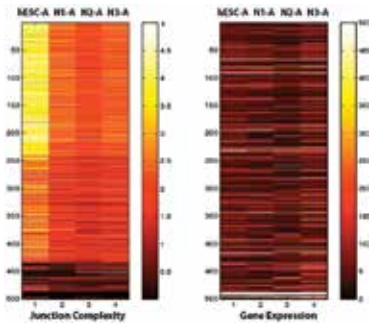
Chen, K., Deng, S., Lu, H., Zheng, Y., Yang, G., Kim, D., Cao, Q., and Wu, J.Q. (2013). RNA-Seq characterization of spinal cord injury transcriptome in acute/subacute phases: a resource for understanding the pathology at the systems level. *Plos One*. 8(8):e72567. PMC3739761

LAB MEMBERS

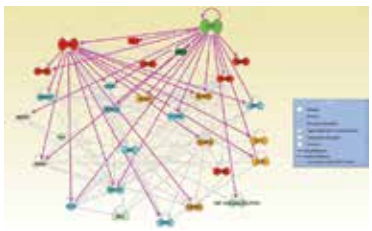
Postdoctoral Fellow: Kenian Chen
Postdoctoral Fellow: Xiaomin Dong
Research Assistant: Shuyun Deng
Postdoctoral Fellow: Shan Zong
Undergraduate student (University of Houston): Abdur Jamal



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

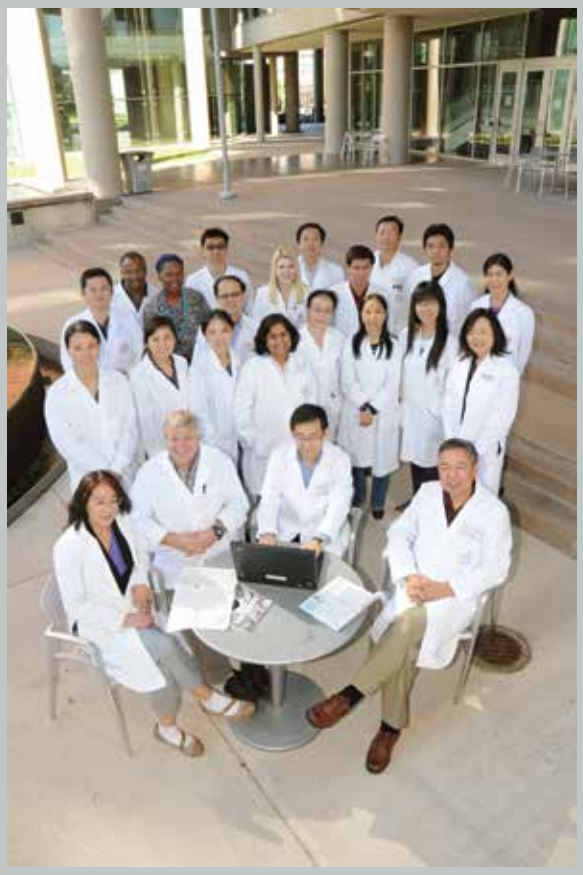
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston for the discovery, development, and commercialization of therapeutic agents and diagnostic tools.

To meet this goal, most of the TTI faculty were recruited from pharmaceutical and biotechnology companies. Research conducted at the center focuses on the identification and validation of drug targets and the establishment of proof-of-principle for therapeutics.

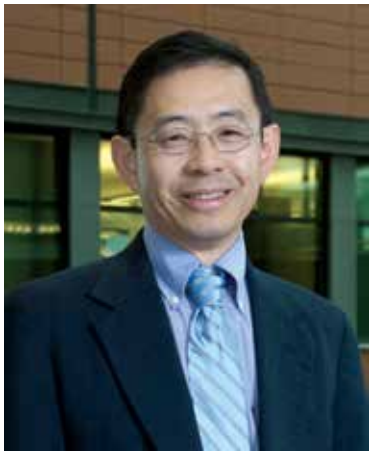
Current research activities at TTI-IMM include: 1) signaling mechanisms of receptors and enzymes that have critical roles in tumor initiation, progression, or metastasis; 2) discovery of biologics and natural products that modulate the activity of these targets as potential lead molecules for drug discovery; 3) characterization of antibodies from animals and humans in response to experimental vaccines; and 4) microbial natural products drug discovery.

TTI-IMM investigators have quickly brought in significant funding from the pharmaceutical industry, including Johnson & Johnson and Merck, the National Institutes of Health (NIH), and the Cancer Prevention and Research Institute of Texas (CPRIT), and have made significant scientific discoveries in the areas of cancer biology and biologics drug development. Some of the highlights are listed below:

- Discovery of a receptor in stem cell function resulted in funding at both the federal and state level
- New drug-resistant mechanism for cancer therapeutic antibodies discovered by TTI researchers resulted in collaboration and funding from Johnson and Johnson
- Therapeutic antibodies targeting viral infections resulted in collaboration and funding from Merck
- TTI research is funded by grants from federal and state agencies and private foundations such as NIH, CPRIT, ETF, UT System Star awards, and the Welch Foundation. More importantly, TTI have established significant collaborations with industry partners including Johnson & Johnson, and Merck
- As a result of the research activities, TTI scientists have filed nine disclosures and five patent applications on drug targets, biomarkers, and lead molecules.
- TTI has been building a comprehensive antibody drug discovery platform and is in the process of building a natural products drug discovery platform.
- Delineated the pneumocandin biosynthesis pathway using a combination of genomic, genetic, and chemical approaches.
- Drug discoveries targeting the metabolites of cancer are being led by TTI labs and involving researchers from UT Southwestern Medical Center at Dallas, which resulted a UT-spinoff biotech company.



Zhiqiang An, Ph.D.
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.

1. HER3 mediated cell signaling and HER3 targeting antibodies for cancer therapy. Ablated regulation in the HER/ErbB family receptor signaling has been implicated in various cancer types. Agents targeting EGFR and HER2 exhibited clinical benefits for the treatment of some cancer types, but drug resistance is widespread. Current understanding of the drug resistance mechanisms is limited, and HER3 has been implicated in the resistance to current EGFR and HER2 therapies. Our group is working on: 1) HER3 mediated cell signaling; 2) the role HER3 plays in resistance to current anti-HER2 and EGFR antibody therapies; and 3) generation of HER3 targeting antibodies and their mode of actions.

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

3. 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.

4. 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

Daniel C. Freed, Qi Tang, Aimin Tang, Fengsheng Li, Xi He, Zhao Huang, Weixu Meng, Lin Xia, Adam C. Finnefrock, Amy S. Espeseth, Danilo R. Casimiro, Ningyan Zhang, John W. Shiver, Dai Wang, Zhiqiang An, Tong-Ming Fu. 2013. A glycoprotein H complex is the primary target for potent neutralization by an experimental human cytomegalovirus vaccine. *Proc. Natl. Acad. Sci. USA*. 110(51):E4997–E5005

L. Chen, Q. Yue, X. Zhang, M. Xiang, C. Wang, S. Li, Y. Che, F. J. Ortiz-López, G. F Bills, X. Liu and Z. An. 2013. Genomics-driven discovery of the pneumocandin biosynthetic gene cluster in the fungus Glarea lozoyensis. *BMC Genomics* 14:339 doi:10.1186/1471-2164-14-339

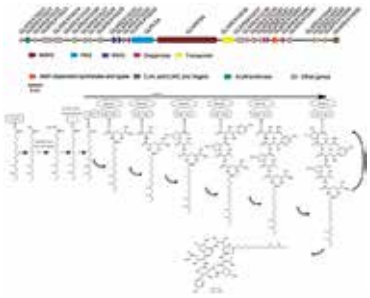
N. T. Redpath, Y. Xu, N. J. Wilson, A. E. Andrews, L. J. Fabri, M. Baca, H. Braley, P. Lu, C. Ireland, R. E. Ernst, A. Woods, G. Forrest, Z. An, et al. 2013. Production of a human neutralizing monoclonal antibody and its crystal structure in complex with the ectodomain 3 of the interleukin-13 receptor 1. *Biochemical Journal.* 451:165-175.

N. Zhang, M. E. Klegerman, H. Deng, Y. Shi, E. Golunski, Z. An. 2013. Trastuzumab-doxorubicin conjugate provides enhanced anti-cancer potency and reduced cardiotoxicity. *Journal of Cancer Therapy.* 4:308-322.

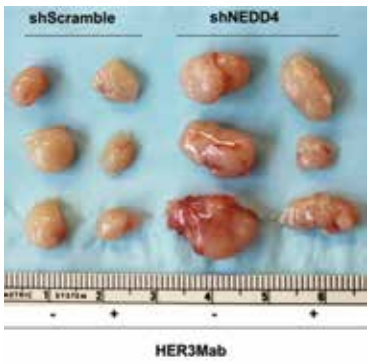
L. Schardl, C. A. Young, U. Hesse, S. G. Amyotte, K. Andreeva, P. J. Calie, D. J. Fleetwood, D. C. Haws, Neil Moore, B. Oeser, D. G. Panaccione, K. K. Schweri, C. R. Voisey, M. L. Farman, J. W. Jaromczyk, B. A. Roe, D. M. O'Sullivan, B. Scott, P. Tudzynski, Z. An, et al. 2013. Plant-symbiotic fungi as chemical engineers: multi-genome snalysis of the Clavicipitaceae reveals dynamics of alkaloid loci. *Plos Genetics* 9(2) e1003323.

LAB MEMBERS

Post Docs: Zhao (George) Huang, Yun Shi, Weixu (Ella) Meng, Qun Yue (jointly with Dr. Bills), Xiumei Niu (jointly with Dr. Bills)
Students: Lin Xia, Li Chen (jointly with Dr. Bills)
Scientists/Research Associates: Byung-Kwon Choi, Hui Deng, Xuejun Fan, Wei Xiong



The pneumocandin biosynthesis pathway. BMC Genomics 14:339, 2013.



Anti-HER3 antibody displayed higher efficacy in inhibiting the signaling and growth of shNEDD4 knockdown cancer cells. Oncogene (Under revision).

Gerald Bills
Professor

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 have recently characterized 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 will develop new genetic and physiological methods for expressing and un-regulating 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., 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*: (in press) (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.

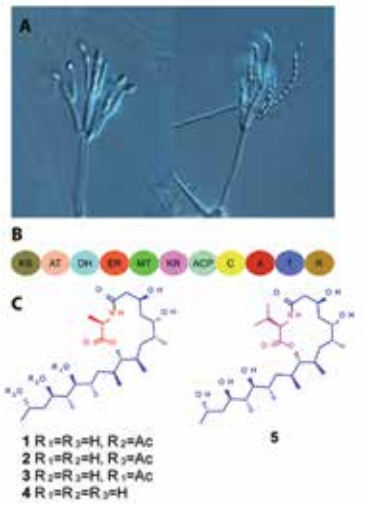
Singh, S.; J. Ondeyka, G. Harris, K. Herath, D. Zink, F. Vicente, Francisca, G. Bills, J. Collado, G. Platas, A. González de Val, J. Martín, F. Reyes, H. Wang, J. Kahn, S. Galuska, R. Giacobbe, G. Abruzzo, T. Roemer & D. Xu. 2013. Isolation, structure and biological activity of a cyclic lipodepsipeptide phaeofungin from a Phaeosphaeria sp. using the genome-wide Candida albicans Fitness Test. *Journal of Natural Products* 76: 334–345.

Bills, G.F., González-Menéndez, J. Martín, G. Platas, J. Fournier, D. Peršoh & M. Stadler. 2012. Hypoxylon pulicicidum sp. nov. (Ascomycota, Xylariales), a pantropical insecticide-producing endophyte. *PLoS One* 7: e46687. doi:10.1371/journal.pone.0046687.

Bills, G.F., A.W. Dombrowski & M.A. Goetz. 2012. The "FERMEX" method for metabolite-enriched fungal extracts. Methods in Molecular Biology. Fungal Secondary Metabolism. *N.P. Keller & G. Turner.* Eds. 944:79-96.

LAB MEMBERS

Students: Li Chen (visiting from Institute of Microbiology, Chinese Academy of Sciences). Scientists/Research Associates: Prof. Xue-Mei Niun (visiting from Yunnan University), Dr. Qun Yue, Dr. Yan Li (visiting from Institute of Microbiology, Chinese Academy of Sciences).



The thermolides, potent nematocidal compounds from the thermophilic fungus Talaromyces thermophilus. A. Photomicrograph of Talaromyces thermophilus isolated from hot spring in Yunnan Province, China. B. Putative domain structure of hybrid polyketide-nonribosomal peptide synthase gene cluster for biosynthesis of the thermolides; ketosynthase (KS), acyl-transferase (AT), dehydrogenase (DH), enoyl reductase (ER), methyl transferase (MT), keto-reductase (KT), acyl carrier protein, adenylation domain (A), reducing thio-esterase (T), reducing domain (R). C. Chemical structures (1-5) of the thermolides. C. Chemical structures of the thermolides (1-5).

Nathan S. Bryan, Ph.D.
Assistant Professor

The role of nitric oxide in health and disease

Nitric oxide (NO) is one of the most important signaling molecules produced in the human body. As we age, we lose our ability to generate NO. Loss of NO production and functionality is associated with a number of chronic diseases, including cardiovascular disease, type 2 diabetes, Alzheimer's disease, and many others diseases that occur later in life. Understanding mechanisms of NO production and metabolism is critical to developing new therapeutics and diagnostics. Furthermore, recognizing patient populations that may be NO insufficient and implementing strategies to restore NO production will hopefully allow for the prevention of human disease.

My lab is focused on the regulation of endogenous NO production from L-arginine and how this molecular complex becomes disrupted in disease. Understanding the molecular biology and biochemistry at each step in the pathway will allow for better strategies to restore normal NO production. More importantly, we have identified a redundant system for NO production and homeostasis that can overcome endothelial NO dysfunction. This human nitrogen cycle allows for nitrate in the diet to be reduced to nitrite and NO by commensal bacteria and mammalian enzyme systems, respectively. Understanding this system will allow for nutritional and probiotic strategies to restore NO homeostasis and rescue patients that may be NO insufficient from endothelial dysfunction.

We have the tools and methods to interrogate NO activity at every level and use cell culture, tissue organ baths, as well as animal models, to understand the regulation as the level of complexity increases. Through sensitive analytical methods involving HPLC, chemiluminscence and functional tissue assays, we can trace NO production and metabolism in different disease models and begin to develop rationale therapeutics.

Discoveries in my lab have led to 3 issued US patents (8,298,589 8,303,995 & 8,435,570), and we have 7 more pending worldwide. We also have also been successful at commercializing these discoveries. Through the formation of Neogenis Labs, Inc, we have exclusively licensed the intellectual property from The University of Texas and brought to market a salivary nitric oxide test strip as an accurate non-invasive measure of NO bioavailability. This technology provides the first and only assessment of NO status in humans. Through the discovery of plant-based products that have profound NO activity, we also have developed and commercialized an over-the-counter dietary supplement that generates NO when activated by the saliva and restores NO homeostasis in humans. We have strong relationships and collaborations with clinicians and other researchers within the Texas Medical Center. This multi-institutional, multi-discipline approach is what drives innovation in my lab.

RESEARCH PROJECTS

- Identification and characterization of nitrate reducing bacteria in humans
- Determining NO status in select patient populations.
- Effects of novel inhibitors of S-nitrosoglutathione reductase (GSNOR) as a means to affect NO signaling

KEY PUBLICATIONS

Erez A, Nagamani SC, Shchelochkov OA, Premkumar MH, Campeau PM, Chen Y, Garg HK, Li L, Mian A, Bertin TK, Black JO, Zeng H, Tang Y, Reddy AK, Summar M, O'Brien WE, Harrison DG, Mitch WE, Marini JC, Aschner JL, Bryan NS, Lee B. Requirement of argininosuccinate lyase for systemic nitric oxide production. *Nat Med.* 2011 Nov 13;17(12):1619-26

Bryan NS. Application of nitric oxide in drug discovery and development. *Expert Opin. Drug Discov.* 2011

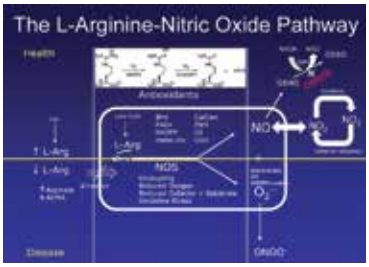
Hord NG, Ghannam J, Garg HK, Berens PD, Bryan NS: Nitrate and nitrite content of human, formula, bovine and soy milks: implications for dietary nitrite and nitrate recommendations *Breastfeeding Medicine* 2010 Oct 19.

Edited Books
Bryan NS (Editor): Food, Nutrition and the Nitric Oxide Pathway. DesTech Publishing – Pennsylvania ISBN: 978-1-932078-84-8, September 2009

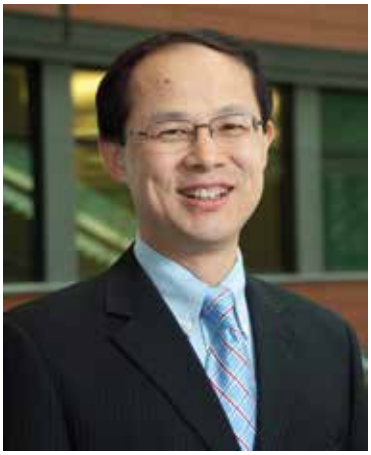
Bryan NS and Loscalzo J (Editors) Nitrite and Nitrate in Human Health and Disease – Springer Humana Press New York ISBN: 978-1-60761-615-3, May 2011

LAB MEMBERS

Hong Jiang, Ph.D. – Senior Research Scientist
Amy Potts, B.S. – MPH student



Nitric oxide production and biochemistry. There are a number of critical steps for the NOS production of NO from L-arginine. Under healthy conditions (top), enzymatic function proceeds normally. Under disease conditions (bottom), there can be a number of problems with L-arginine availability, transport and conversion to NO due to enzyme uncoupling or insufficient co-factor availability. Once produced, NO can form nitrosothiols or become oxidized to nitrite and nitrate which now recognized can be recycled to regenerate NO.



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. Interestingly, some of these genes may contribute to cancer progression promoted by chronic behavior stress. 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 our lab is involved in identifying and studying human kinases as novel regulators for EMT. Kinases play central roles in many aspects of signaling transduction, cell physiology, and diseases. They are also one of the most important gene families for cancer drug development. Our literature search indicated that the majority of >700 kinases in human genome are still poorly studied. Our lab is employing unbiased functional screens against hundreds of human kinases to identify novel regulators for EMT and linking them to stem cell phenotypes and cancer metastasis. Investigation of the molecular mechanisms of these kinases will have a significant impact in expanding our knowledge in the crossroad of exciting and critical areas, such as development, stem cell, drug resistance, and metastasis. These kinases also may 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 mechanism of actions
- New regulators for EMT and their involvement in stem cell, organ fibrosis, and cancer progression
- New players in cancer progression promoted by chronic behavior stress
- Acquired resistance to cancer therapeutics

KEY PUBLICATIONS

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) (in press). *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)

Bommi-Reddy A, Almeciga I, Sawyer J, Geisen C, Li W, Harlow E, Kaelin WG Jr, Grueneberg DA. III. Altered Kinase Requirements in VHL-/- Renal Carcinoma Cells Detected in a Pilot Synthetic Lethal Screen. *Proceedings of the National Academy of Sciences USA (PNAS)*. 2008 Oct 28;105(43):16484-9.

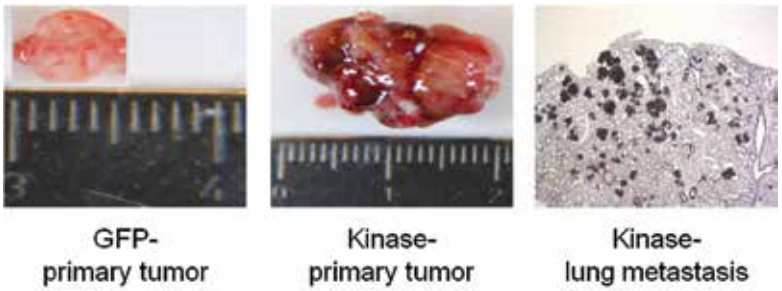
Baldwin A, Li W, Grace M, Harlow E, Münger K and Grueneberg DA. II. Genetic Interaction Screens Identify Alterations in Kinase Requirements Following HPV16 E7 Expression in Cancer Cells. *Proceedings of the National Academy of Sciences USA (PNAS)*. 2008 Oct 28;105(43):16478-83.

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)

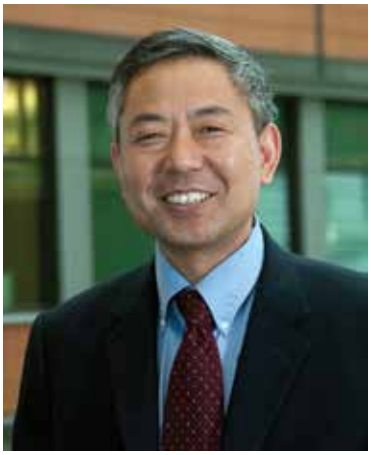
Note: These 4 *PNAS* papers I-IV were selected as Signaling Breakthroughs of 2008 selected by *Science Signaling*.

LAB MEMBERS

Postdoc Fellows: Haiping Song, Chunping Liu
Ph.D. student: Mohit Hulsurkar



A novel kinase we identified promotes prostate primary tumor growth and lung metastasis in mouse xenografts.



Qingyun (Jim) Liu, Ph.D.
Professor and Co-Director of the Texas Therapeutics Institute
Janice Davis Gordon Distinguished Professorship 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 mechanisms of a group of cell surface receptors called LGR4, LGR5, and LGR6 that potentially play critical roles in the survival of normal stem cells and determining their roles in the maintenance and proliferation of cancer cells. We also are engaged in the validation of these receptors and their ligands as potential drug targets and identification of lead molecular as potential anticancer therapeutics. Previously, we successfully identified a group of proteins called R-spondins (RSPOs) that are essential for the activation of the receptors, representing an important step toward the understanding of the mechanisms of these receptors. We have now discovered how these receptors control the proliferation and migration in normal and cancer stem cells, particularly in a subset of 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 cancer
- Identification of lead molecules for the discovery and development of 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.

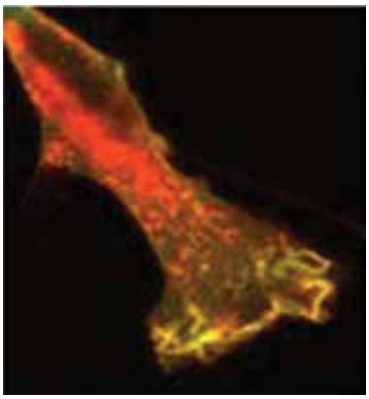
Gong X, Carmon KC, Lin Q, Thomas A, Yi J, and Liu Q (2012). LGR6 Is a High Affinity Receptor of R-Spondins and Potentially Functions as a Tumor Suppressor. *PLoS One* 7:e37137-e37146.

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).

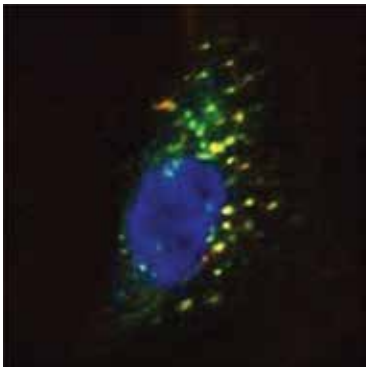
Yi J., Xiong W., Gong X., Bellister S., Ellis LM., and Li Q. (2013) Analysis of LGR4 Receptor Distribution in human and mouse tissues. *PloS One,* In press.

LAB MEMBERS

Instructors: Kendra Carmon and Xing Gong
Postdoctoral fellows: Jing Yi
Technicians: Anthony Thomas



Co-localization (yellow) of stem cell receptor LGR4 (red) with its signal transducer IQGAP1 (green) in MDCK cells.



Co-localization (yellow) of LGR4 (green) with its ligand RSPO1 (red) in lung cancer cells.

Kalpana Mujoo, Ph.D.
Assistant Professor

HER3 signaling in prostate cancer and NO-cGMP signaling in induced pluripotent stem cells

In the United States, approximately 239,000 new cases of prostate cancer will be reported and out of that ~ 30,000 will die of their disease (ACS, 2013). The treatment regimens for prostate cancer are prostatectomy, radiation therapy, and hormone ablation. However significant patient population becomes hormone refractory and metastatic leading to the death of patients within 2 years. Although mechanism(s) of the disease progression have been extensively studied, currently no molecularly targeted therapy has been approved for prostate cancer. Previous studies suggest that the EGFR family regulates proliferation and survival of prostate cancer and increase of HER3 expression and function has been reported in prostate cancer. However, the molecular mechanism of HER3-driven prostate cancer is poorly understood. Moreover, lack of responding biomarkers poses a challenge for the clinical development of HER3 targeting antibodies. Therefore, we are interested in understanding HER3 regulation and activation for the development of HER3 targeting cancer therapies. Recently, our laboratory identified NEDD4 as one of the novel HER3 interacting partner. NEDD4 (an E3 ubiquitin ligase) negatively regulates HER3 levels, signaling, migration and proliferation *in vitro* and *in vivo*. Further, our studies revealed an inverse correlation between HER3 and NEDD4 expression in several tested prostate cancer cell lines. Examination of NEDD4 and HER3 staining using IHC in prostate cancer tissue array slides (human anatomical samples) revealed that NEDD4 was mostly expressed in the epithelial cells surrounding the ducts, whereas, HER3 levels were low in the epithelial cells surrounding the prostate ducts. Positive staining of HER3 was mainly detected in membranous and cytoplasmic areas of cancerous tissues where NEDD4 often exhibited low or negligible staining, collectively suggesting that the association of NEDD4 and HER3 play an important role in regulation of HER3 in cancer cells. We currently are evaluating the anti-tumor efficacy of HER3 monoclonal antibodies in orthotopic mouse tumor model using bioluminescence imaging. Another novel HER3 interacting partner is DJ-1 which is a chaperone protein with some oncogenic functions. Our preliminary studies have shown that DJ-1 silencing leads to inhibition of HER3 signaling and phenotypic changes in the cells, such as inhibition of migration and proliferation. These studies are being conducted in collaboration with Drs. Zhiqiang An and Ningyan Zhang of TTI at IMM. We also are collaborating with Drs. Amato and Said of the Division of Oncology to validate our novel HER-3 interacting partners such as NEDD4 (an E3 Ubiquitin ligase) and DJ-1(known oncogene with multiple functions) as responding biomarkers for HER3-driven prostate cancers.

RESEARCH PROJECTS

- HER-3 signaling in prostate cancer
- Nitric oxide-cyclic GMP signaling in embryonic and induced pluripotent stem cells

KEY PUBLICATIONS

Huang, Z., Choi, B-K., Mujoo, K., Fan, X., Fa, M., Mukherjee, S., Owiti, N., Zhang, N., An, Z. The E3 ubiquitin ligase NEDD4 negatively regulates HER3/ErbB3 level and signaling. 2013 (submitted to *Oncogene*)

Mujoo, K.*, Nikonoff, L., Sharin, V., Bryan, N.S., Kots, A.Y., Murad, F. (2012). Curcumin induces differentiation of embryonic stem cells through possible modulation of nitric oxide-cyclic GMP pathway. *Protein Cell* 3:535-544.

Mujoo, K.*, Krumenacker, J.S., and Murad, F. (2011). Nitric oxide-cyclic GMP signaling in stem cell differentiation. *Free Radical Biology & Medicine* 51:2150-2157.

Sharin, V., Mujoo, K., Kots, A., Martin, E., Murad, F., and Sharina, I. (2011). Nitric oxide receptor soluble guanylyl cyclase undergoes splicing regulation in differentiating human embryonic stem cells. *Stem Cells & Development* 20:1287-1293.

Mujoo, K.*, Sharin, V.G., Marin, E., Choi. B-K., Sloan. C., Nikonoff. L.E., Kots. A., and Murad, F* (2010). Role of soluble guanylyl cyclase- cyclic GMP signaling in tumor cell proliferation. *Nitric Oxide: Biology and Chemistry* 22: 43-50.

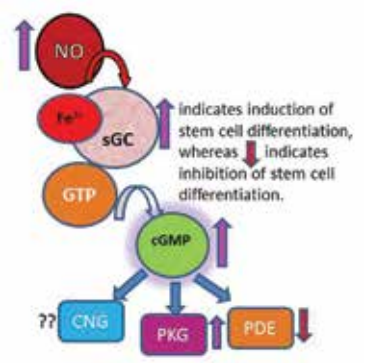
Mujoo, K.*, Sharin, V.G., Bryan, N., Krumenacker, J.S., Sloan, C., Parveen, S., Kots, A., Murad, F*. (2008). Role of nitric oxide signaling components in differentiation of embryonic stem cells into myocardial cells. *Proc Natl Acad Sci. USA,* 105: 18924-18929.

Mujoo, K., Krumenacker, J.S., Wada. Y., and Murad, F. (2006). Differential expression of nitric oxide signaling components in undifferentiated and differentiated human embryonic stem cells. *Stem Cells & Development* 15: 779-787.

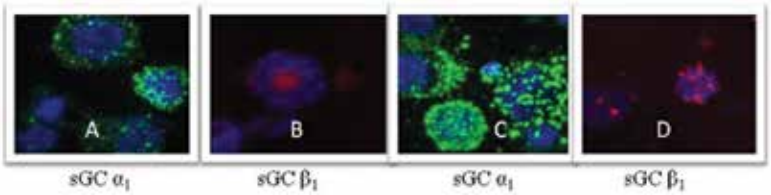
*Corresponding author

LAB MEMBERS

Graduate students (with Dr. An)
Seema Mukherjee
Pooja Dhupkar



Nitric oxide-cyclic GMP signaling in stem cell differentiation



Nuclear and cytoplasmic staining of NO receptor (sGC) in ES-derived myocardial cells in response to NOC-18 (NO donor; Panel A&B) and allosteric sGC activator (BAY41-2272; Panel C&D).

Ningyan Zhang, Ph.D.
Associate Professor

Immuno-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 development. Abnormal gene amplification and overexpression of EGFR and HER2 are well documented in many types of cancer and four therapeutic monoclonal antibodies, cetuximab, panitumumab targeting EGFR and trastuzumab and pertuzumab against HER2, are currently used in the clinic for treatment of different types of cancer. Trastuzumab has been approved by FDA for more than a decade for treatment of HER2 overexpressing breast cancer and has shown clinical success at both adjuvant and neoadjuvant settings. However, similar to many molecular targeted cancer therapies, both innate and acquired resistance to trastuzumab 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 are also studying the role of matrix matalloproteinases (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 therapeutic strategies for overcoming resistance and stimulation 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

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.

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.

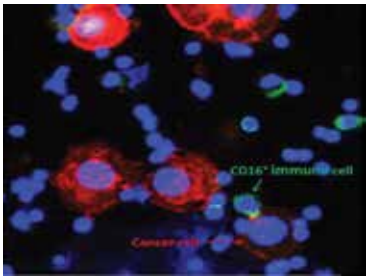
Choi B-K, Cai X, Yuan Y, Huang Z, Fan X, Deng H, Zhang* NY, An Z. (2012) HER-3 intracellular domains play a crucial role in HER3/HER2 dimerization and activation of downstream signaling pathways. *Protein & Cell* 3(10): 781–789.

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.

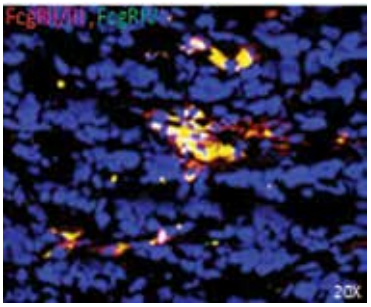
Zhang NY, Liu L, Dumitru CD, Cummings NR, Cukan M, Jiang Y, Li Y, Li F, Mitchell T, Mallem MR, Ou Y, Patel RN, Vo K, Wang H, Burnina I, Choi B, Huber H, Stadheim TA, Zha D (2011) Glycoengineered Pichia produced anti-HER2 is comparable to trastuzumab in preclinical study, mAbs 3:1-10.

LAB MEMBERS

Joined team with Dr. Zhiqiang An's laboratory, see the listed members in Dr. An's page.



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



Detection of tumor infiltrated immune cells by immunofluorescence microscopy in mouse xenograft tumor

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 the 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.

Clinical and Translational Proteomics

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 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, M.D., PH.D.
Associate Professor, Center for Proteomics and System Biology
713-500-3611
Contact: LI LI,
Mass Spectrometry Specialist
Service Center Manager
713-500-2232

Flow Cytometry

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, PH.D.
Professor & Director
Center for Molecular Imaging
713-500-3560
Contact: AMY HAZEN, PH.D.
Program Manager Research
713-500-3612

Tissue Histopathology

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, PH.D.
Professor & Director
Center for Molecular Imaging
713-500-3560
Contact: SARAH AMRA, B.S., H.T. (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, PH.D.
Assistant Professor, Center for Immunology and Autoimmune Diseases
Director, Microscopy Service Center
713-500-2453
Contact: ZHENGMEI MAO, PH.D.
Manager
713-500-3389

Molecular Diagnostics

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, M.D., PH.D.
Associate Professor, Center for Proteomics and System Biology
713-500-3611
Contact: ANTONIO FLORES
Medical Technologist III
713-500-3428

Transgenic and Stem Cell Services

Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cell service center, which was established in 1998. It has generated over 650 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, PH.D.
Assistant Professor, Center for Immunology and Autoimmune Diseases
Director, Transgenic and Stem Cells Service Unit
713-500-2453
Contact: ALEKSEY DOMOZHIROV
Research Associate
Manager
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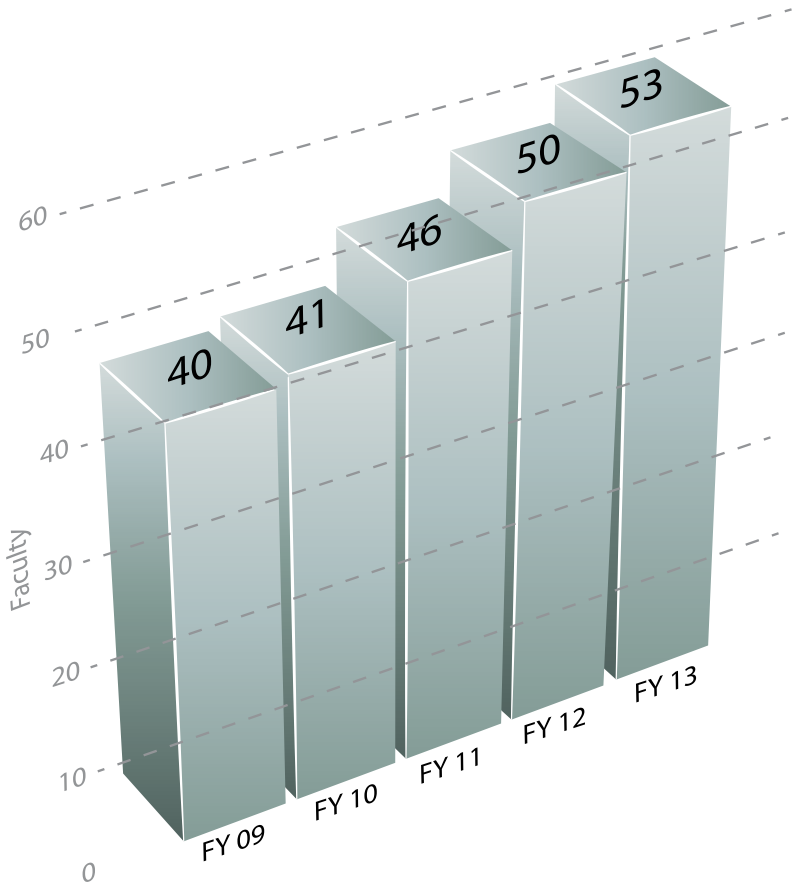
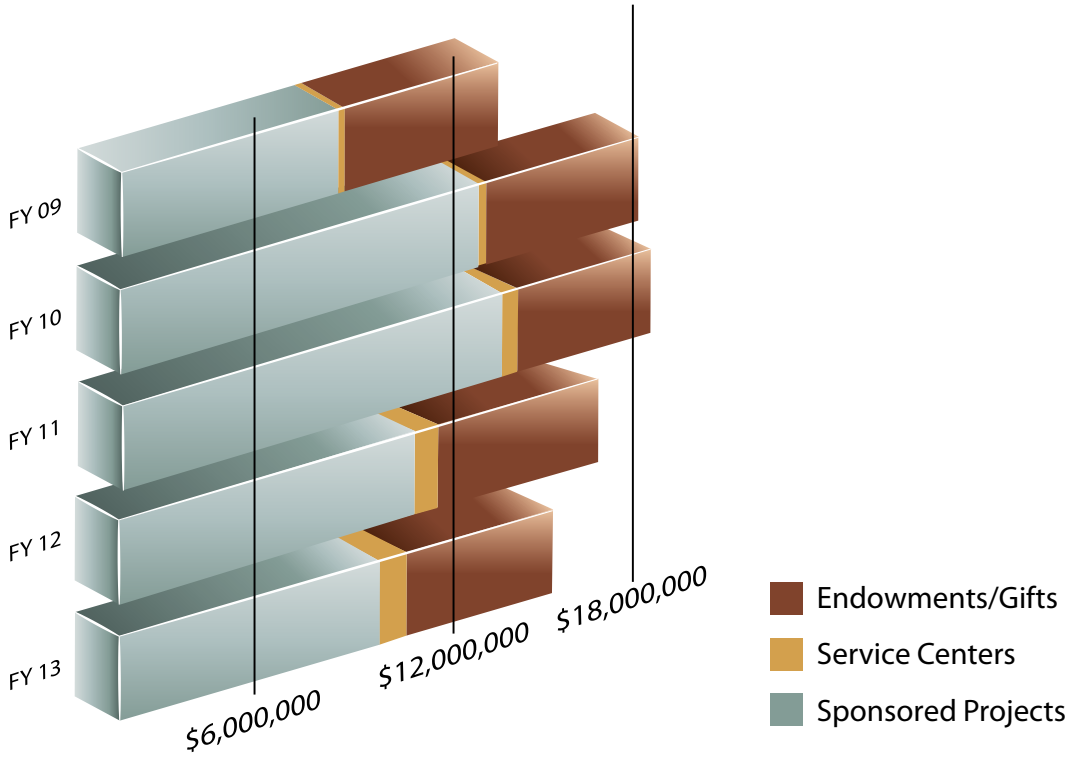
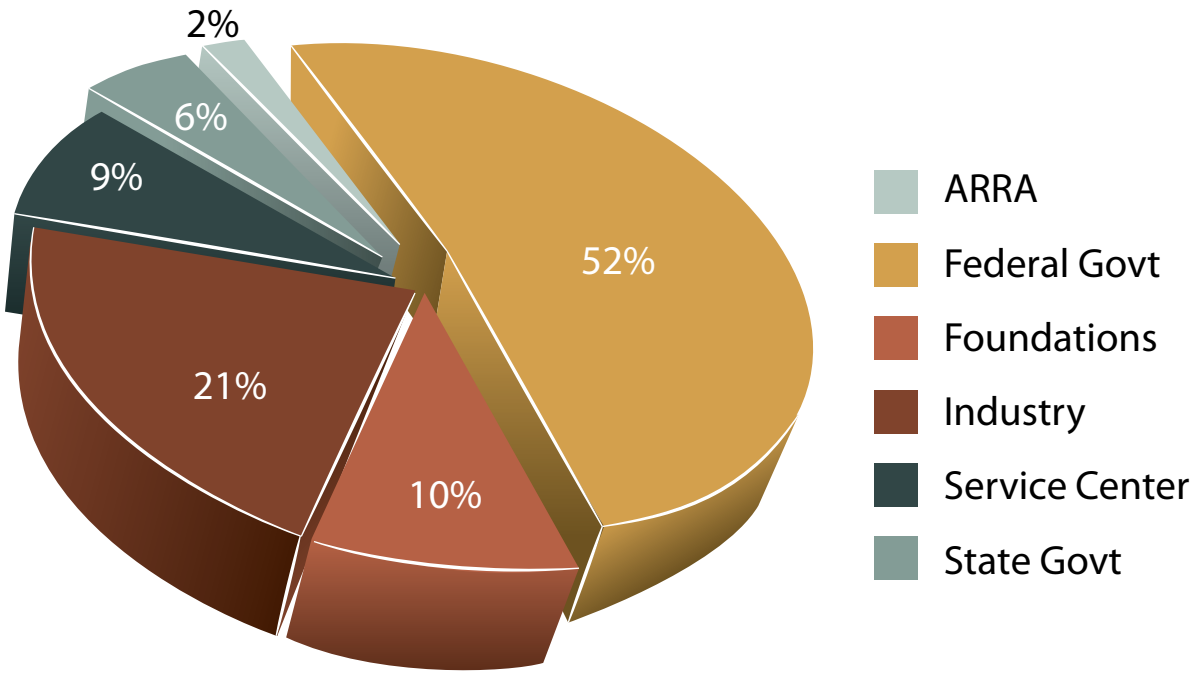
Number of Faculty



Total Expenses Supporting Research



Total Expenses Supporting Research



Gift Report

New Gifts and Bequests
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