THE UNIVERSITY *of* TEXAS
MEDICAL SCHOOL *at* HOUSTON'S

BROWN FOUNDATION INSTITUTE
of MOLECULAR MEDICINE
FOR THE PREVENTION
of HUMAN DISEASES

IMMpact Report

FY 2012

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 on Pressler Street in this cover photo.

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

Editor
Darla Brown, Director, Office of Communications

Contributors:
Darla Brown
IMM Faculty

Design:
Roy Prichard

Photography:
Dwight Andrews
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Terence T. Tang

For information on supporting programs at the UT Medical School at Houston and the IMM, contact James B. Hughes, 713.500.5164, med.uth.tmc.edu

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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 Institute of Molecular Medicine is pleased to provide our latest edition of the IMMpact Report for your review. The last year has been an exciting one for the IMM. We have continued to grow and develop our research programs by recruiting and appointing new faculty.

Successful research programs are built around motivated, creative scientists who increasingly operate as research teams. The IMM is a stand-alone research institute that is embedded within The University of Texas Medical School at Houston. This arrangement provides unique opportunities to forge teams comprised of basic scientists and clinicians, leveraging the best talents that the IMM and the 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. As we go forward, we will continue to link the basic and clinical worlds to enhance translational outcomes that benefit patients.

There is a terrific spirit of cooperation and partnership throughout the IMM that not only helps in crafting highly competitive and innovative grant applications but that importantly provides exciting opportunities for training graduate students to be the next cohort of the world's best scientists.

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 build 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.

It is unfortunate that at time when basic and clinical science can potentially offer so much so much, you are probably aware that the federal government is significantly reducing funding for scientific research. It is a remarkable testament to the quality and creativity of our scientists that the IMM faculty remain 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 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.

John Hancock, M.A., M.B., B.Chir., Ph.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 cause of human diseases at the cellular and molecular levels, using DNA and protein technologies to elucidate disease mechanisms. Its 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 application 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.

Help us cure the diseases of our time within our time

Armed with investigators seeking tomorrow's cures, the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases is making incredible discoveries for the benefit of those who suffer from such debilitating diseases as diabetes, stroke, obesity, and lung disease. We are able to make a difference in patients' lives today through the generous support of our donors.

Gift Planning | Endowed Professorships | Charitable Trusts | Gift Annuities | Bequests
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UTHealth™
The University of Texas
Health Science Center at Houston
Medical School

The University of Texas Medical School at Houston
Office of Development
6431 Fannin, MSB G.150J
Houston, TX 77030-1503
PHONE 713-500-5164
EMAIL James.b.hughes@uth.tmc.edu
WEB www.uthouston.edu/imm

Research Unites Against Stroke

Protecting time for stroke research. Developing new stroke therapies. Pushing the boundaries of stem cell therapies to help stroke patients.

With these goals in mind, the Senator Lloyd and B.A. Bentsen Center for Stroke Research came to life in 2008 as part of the Institute for Molecular Medicine. The center competitively funds investigators from within UTHealth to pursue innovative and collaborative research aimed at new discoveries specific to stroke prevention and rehabilitation.

Currently, seven Bentsen Investigators are working on six research projects using stem cells in three distinct ways.

Qi Lin Cao, M.D.; Ying Liu, M.D., Ph.D.; and Jiaqian Wu, Ph.D.; faculty in the Department of Neurosurgery, are replacing damaged brain cells – both neuron and glial cells destroyed by stroke – with stem cells that can regenerate. Dr. Cao is using patients' own stem skin cells, turning them into glial cells and implanting them into the brain, and Drs. Liu and Wu are using pluripotent cells – which start as one type of cell and then can be reprogrammed as a neuronal cell.

"The benefit of using a patient's own stem cells is that they will not be rejected when they are replanted and start generating healthy new tissue," explains Brian Davis, Ph.D., director of the Center for Stem Cell and Regenerative Medicine of the IMM — the academic and administrative home of the Bentsen Center for Stroke Research.

Scavenger cells selectively destroy harmful material and are being used by Jaroslaw Aronowski, Ph.D., professor in the Department of Neurology. His research is now being carried out in patients to determine if scavenger cells can efficiently remove blood that accumulates following hemorrhagic stroke.

"Excess blood can impede neural function. It is a serious issue for the rejuvenation of tissue," Dr. Davis says.

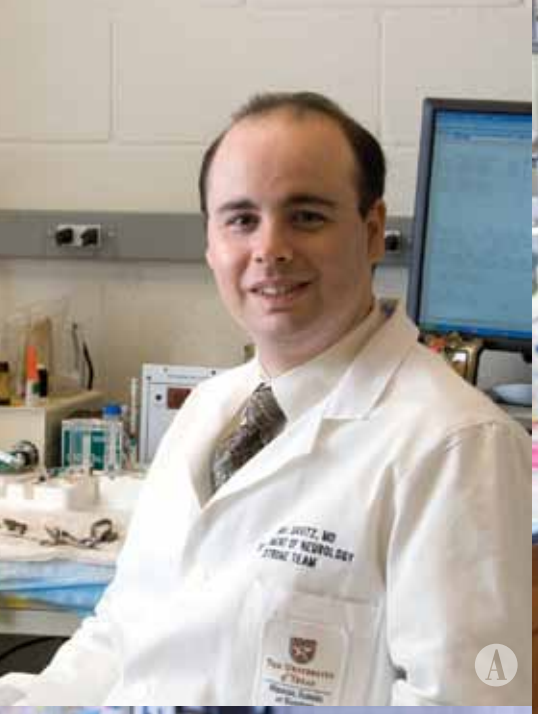
Using stem cells to protect against inflammation and encourage healthy cell growth is the work of Charles Cox, M.D.; Pramod Dash, Ph.D.; and Sean Savitz, M.D. Dr. Cox, professor in the Department of Pediatric Surgery, is using stem cells taken from the amniotic fluid surrounding an embryo known to have a congenital heart defect. Once the baby is born with the abnormal heart and is ready for surgery, the harvested stem cells can be inserted into the baby's bloodstream, helping the body by inducing a protective effect that reduces inflammation.

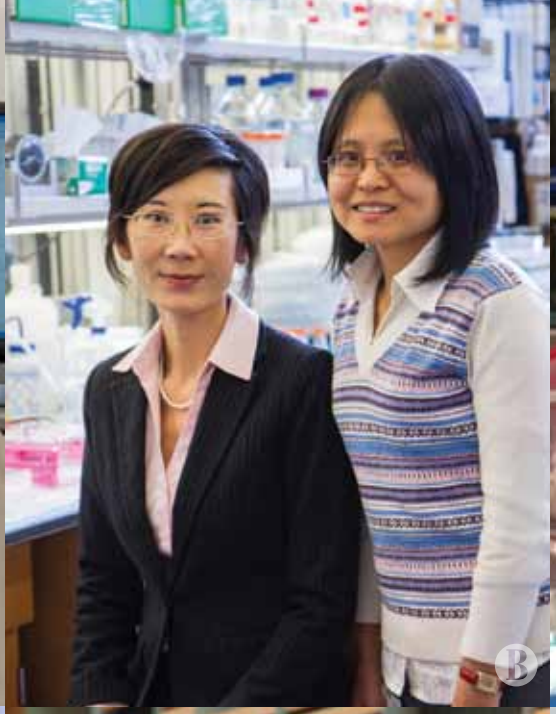
Dr. Savitz, professor in the Department of Neurology, completed the first study in the United States of a patient's own bone marrow stem cells as a potential new treatment for stroke and is studying how stem cells reduce inflammation derived from the spleen after stroke. Dr. Dash, professor and holder of the Nina and Michael Zilkha Distinguished Chair in Neurodegenerative Disease Research in the Department of Neurobiology and Anatomy, is using the protective effects of stem cells found in bone marrow to treat traumatic brain injury patients.

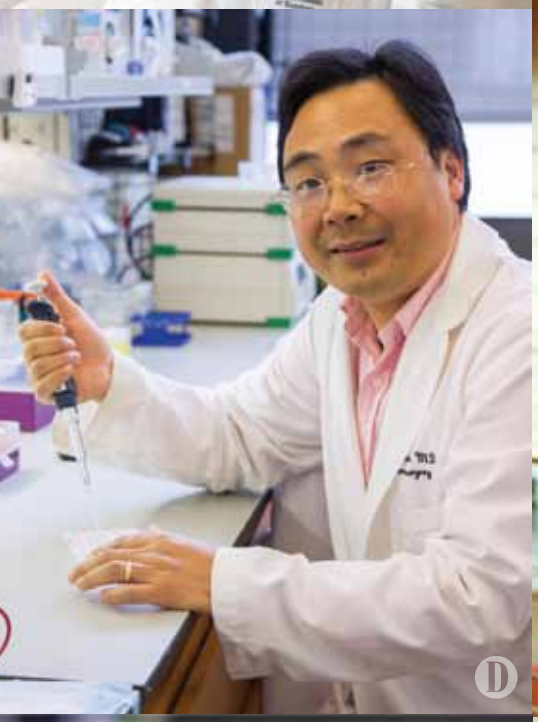
"Dr. Cox is inducing these cells to reduce a patient's symptoms in advance of surgery, while Drs. Savitz and Dash are using the same properties for treatment purposes following injury," Dr. Davis explains.

All of the researchers work collaboratively in an effort to further science for the benefit of all patients.

"This research has a clear pathway to clinical application, and our goal is to move it to translation to patients as quickly as possible," Dr. Davis says.







Senator Lloyd and B.A. Bentsen Center for Stroke Research

A. Sean Savitz, M.D.

B. Ying Liu, M.D., Ph.D. and Jiaqian Wu, Ph.D.

C. Charles Cox, M.D.

D. Qi Lin Cao, M.D.

E. Brian Davis, Ph.D.

F. Pramod Dash, Ph.D.

G. Jaroslaw Aronoski, Ph.D.

Research Focus: An Easier Breath

When you take a breath, do you take it for granted? For those who struggle to catch their breath, or whose lungs are impaired by disease, breathing is anything but an overlooked, automatic function.

Respiratory diseases are a major case of mortality and morbidity worldwide, accounting for approximately 400,000 deaths a year in the United States. Lung diseases are the cause of 20 percent of infant mortality.

Chronic rhinosinusitis is one of the most prevalent chronic illnesses, which translates to 20 million doctor visits a year and an annual cost to the U.S. economy of $3.4 billion.

The causes of pulmonary diseases are many – viral and bacterial infections, environmental toxins, allergies, and genetic mutations – yet the treatments are few, with lung transplantation the only viable option for patients with severe lung disease.

Rick Wetsel, Ph.D., holder of the William S. Kilroy, Sr., Chair in Pulmonary Disease, leads a research team at the Brown Foundation Institute of Molecular Medicine that is committed to improving the lives of those afflicted by respiratory disease.

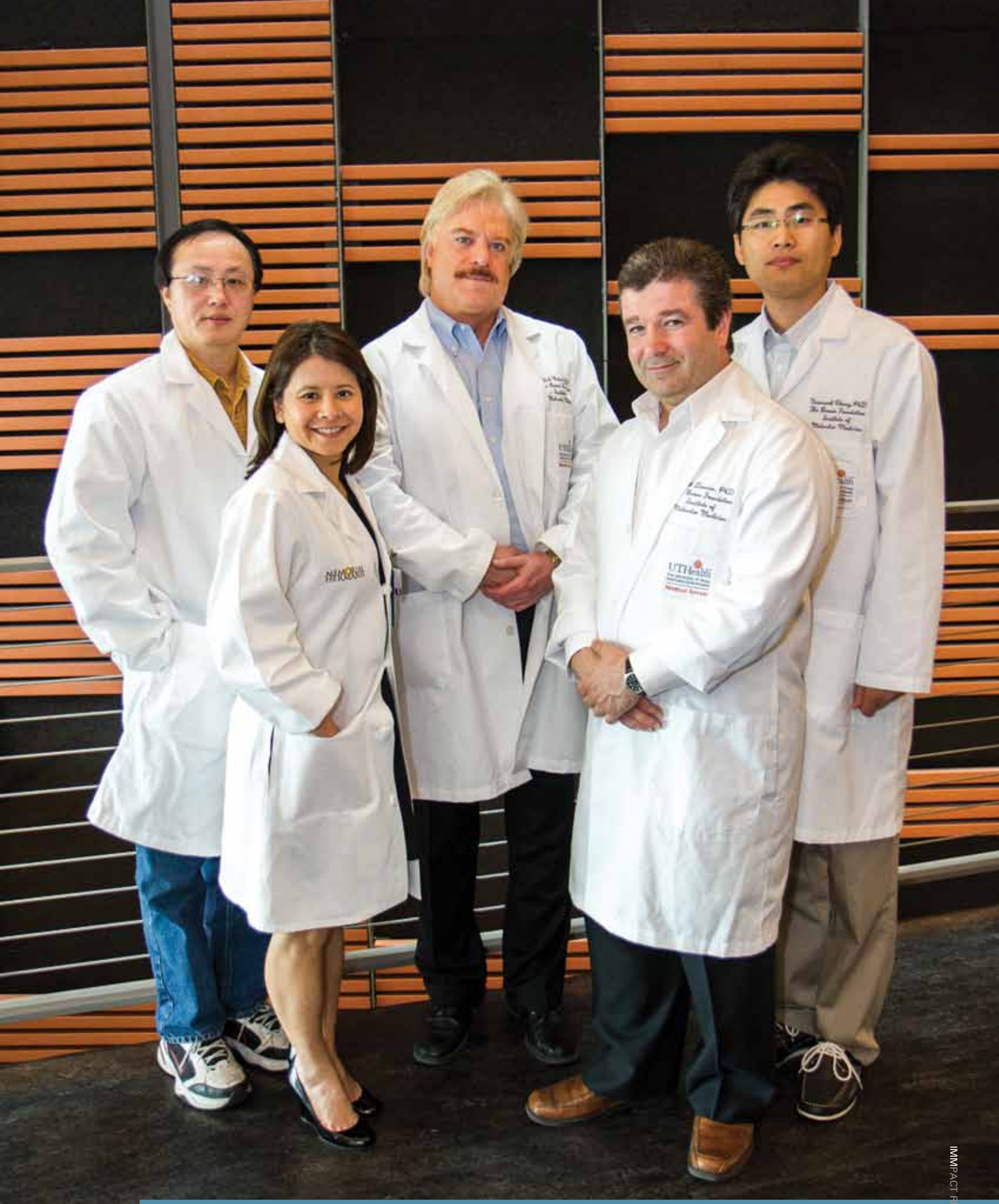
"Robust and well-regulated immune, inflammatory, and cellular repair responses are critical in controlling the development of irreversible respiratory pathologies," Dr. Wetsel says. "However, the paucity of cellular and molecular knowledge regarding respiratory immunity and tissue repair has greatly impaired the development of novel therapeutics that could be used to reverse or repair tissue damage due to respiratory disease."

The collaborative research team is seeking the cause and treatment of lung disease at the cellular level:

- Yeonseok Chung, Ph.D., and his group are investigating the impact of regulatory T-cells on the pathologies of allergic and malignant lung diseases.
- Scott Drouin, Ph.D., and his staff are identifying molecules of the innate immune system that are key to the development of chronic obstructive pulmonary disease and asthma.
- Amber Luong, M.D., Ph.D., who also is assistant professor of otorhinolaryngology, studies the cellular immune responses involved in the pathogenesis of allergic fungal rhinosinusitis.
- The research program of Dachun Wang, M.D., is focused on evaluating the use of embryonic and induced pluriopotent stem cells as sources of lung progenitor cells for the treatment of acute and chronic lung injury.
- Dr. Wetsel, the director of the Hans J. Muller-Eberhard & Irma Gigli Research Center for Immunology, is investigating the pulmonary immune response to bacterial pathogens and fugal stimuli. His laboratory also is developing stem cell-based therapeutics for the regeneration of the lung epithelium destroyed by acute or chronic lung disease and for cell therapy for babies with surfactant protein deficiencies.

The focus of pulmonary research expands beyond UTHealth and the IMM. Dr. Wetsel is forming a consortium of other researchers throughout the Texas Medical Center who have overlapping expertise related to pulmonary regenerative medicine.

"Our goal is to develop a consortium that will be a major leader in pulmonary regenerative medicine both nationally and internationally," Dr. Wetsel says.



Dachun Wang, M.D.; Amber Luong, M.D., Ph.D.; Rick Wetsel, Ph.D.; Scott Drouin, Ph.D.; and Yeonseok Chung, Ph.D.

George Zhao, Ph.D.; Mohit Hulsurkar, and Christine Crumbley, Ph.D., work in the Texas Therapeutics Institute.

Texas Therapeutics Institute

Founded in 2010 to bridge the gap between the worlds of biomedical research and the pharmaceutical companies, the Texas Therapeutics Institute (TTI) is tasked with bringing discoveries closer to commercialization and attracting funding from industry.

Partially funded by the Texas Emerging Technology Fund and The University of Texas System, TTI is a collaborative endeavor that involves multiple University of Texas campuses.

It is led by co-directors Zhiqiang An, Ph.D., who holds the Robert A. Welch Distinguished University Chair in Chemistry, and Qingyun (Jim) Liu, Ph.D., who holds the Janice D. Gordon Distinguished Professorship in Bowel Cancer Research.

TTI has had a successful and busy fiscal year, reporting:

- More than $1 million in new industry grant funding, from such pharmaceutical titans as Johnson and Johnson and Merck.
- Successful funding of an R01 grant from the National Institutes of Health.
- Multiple disclosures, including one patent filing and two U.S. issued patents.
- The growth of its team to include 11 faculty, nine postdoctoral fellows, eight laboratory scientists, and eight graduate students.
- Collaborative ventures on cancer targets with not only scientists from around UTHealth and The University of Texas MD Anderson Cancer Center but also from The University of Texas Southwestern Medical Center at Dallas.
- A discovery of Dr. Jim Liu's lab of a receptor stem cell function was published in *PNAS* and highlighted by *Science* and *Nature*. This discovery resulted in funding at both the federal and state level.
- A new antibody drug-resistant mechanism for breast cancer discovered by TTI researchers resulted in a large-scale collaboration and funding from Johnson and Johnson.
- Drug discoveries targeting the metabolites of cancer are being led by TTI labs and involving researchers from around the state.

Breaking the Link Between Obesity and Cancer

Over the past decade, a link between obesity and cancer has been established.

Today, Mikhail Kolonin, Ph.D., is working to obliterate that link.

"I started my lab here with the goal of answering one question: Can white adipose tissue explain the aggressive nature of cancer?" he recalls.

Dr. Kolonin, the Jerold B. Katz Distinguished Professor in Stem Cell Research, joined the IMM in 2007 because of the opportunity to pursue independent research.

"I saw unlimited potential and resources here as I was embarking upon a new field," Dr. Kolonin says. "The IMM has given me the freedom to do what I wanted."

In these few years, he and his lab have made incredible progress, the results of which have been published in such journals as *Cell Stem Cell* and *Cancer Research* and made international news headlines.

Through research with mouse models at the molecular level, Dr. Kolonin and his lab have proven that white adipose tissue is more than just linked to cancer – it promotes cancer growth.

White adipose tissue is body fat found just below the skin, surrounding internal organs, located in bone marrow, and in breast tissue. Obesity is hallmarked by an overgrowth of white adipose tissue.

"We have found that stem cells from white adipose tissue move to tumors. Signals emitted from these adipose cells activate tumor blood flow and malignant cells, thus increasing the cancer's progression," Dr. Kolonin explains.

Dr. Kolonin and his team carried out research on human specimens and found that, like in mice, these specific fat cells become mobilized in obesity and cancer.

"The next step is to understand the molecules that regulate trafficking of adipose cells, the mechanisms of their effect on cancer, and how we can potentially disrupt these mechanisms," he adds.

Dr. Kolonin and his lab also are investigating the treatment of obesity as a potential complementary treatment of cancer, using targeted therapies to destroy adipose tissue.

"According to published statistics, 15 to 25 percent of cancers-related deaths could have been avoided by preventing obesity. We know that with bariatric surgery, the risk of Type 2 diabetes can be diminished. It is possible that with such weight-loss surgery, or other obesity prevention measures, we can also reduce cancer progression, the risk of cancer recurrence, or even use it as a cancer treatment," Dr. Kolonin says.

Dr. Mikhail Kolonin shows the differences between an obese and normal mouse.

The Runnells: Friends of Science

Whoever heard of a business meeting on Christmas Eve?

Whoever heard of a white Christmas in Houston?

Let's just say, it was a Christmas like no other for Rick Wetsel, Ph.D., professor of molecular medicine, William S. Kilroy, Sr., Chair in Pulmonary Disease, who trudged through the record-breaking and rare Houston snowstorm in 2004 for a meeting called by Clive Runnells, rancher, cable TV pioneer, and executive.

That meeting was the start of what has become not only a long-lasting relationship supporting IMM research – but also a real friendship.

"Before that meeting on Christmas Eve, I read about Rick in the *Houston Chronicle*, and he was crying out for funds," Runnells recalls. "He said the opportunities to do the type of innovative stem cell work he was doing were greater in California than here in Houston."

Determined to stop the H-town brain drain, Runnells asked for a face-to-face meeting immediately.

"He asked me how much it would take to jump start my research, and I told him $100,000," Dr. Wetsel says. "I didn't know if I was asking for too much, or too little. He told me he would have to talk to his wife."

Then Runnells gave him the Christmas gift of his life. In addition to the initial start up, the Runnells family has continued to support Wetsel's stem cell research efforts at the IMM, which has totaled $450,000 during the past 9 years.

"The philanthropic funds have made it possible to develop four of our own stem cell lines – two of which are approved by the National Institutes of Health," Dr. Wetsel explains.

Eva Zsigmond, Ph.D., assistant professor of molecular medicine, and director of the Transgenic and Stem Cell Core, has been instrumental in developing the new stem cell lines.

"These two lines contain fresh cells (early passage), compared with many of the older cell lines, which contain aging and defective cells that are less effective for research," Dr. Wetsel explains.

In addition to Dr. Wetsel's research, these new lines should enhance the research programs of other investigators at UTHealth, including researchers from neuropsychiatry, neurology, cardiology, and pediatrics.

Runnells' unwavering support of embryonic stem cells is personal. He and his wife, Nancy's, son, Pierce, suffered a debilitating back injury due to a skiing accident and died before the promise of stem cell therapy could be realized.

"Pierce's death was a great blow to us," Runnells says. "He was kind, and he loved his parents dearly."

Even though he says he would not choose science as a career, Runnells is passionate about the science of stem cells.

"I am grateful to have someone like Clive who is actively engaged in what we are doing. He understands the science and goes to bat for us in the Legislature and with his colleagues," Dr. Wetsel says.

"Rick, Eva, and I have had a great relationship," Runnells says. "They do great work, and I like to get results."

The Runnells' generosity has engendered the support of new donors and made grants from the federal government possible.

"His dedication, both financially and just knowing his level of commitment to our program, is making a huge difference," Dr. Wetsel says. "We cannot thank Nancy and Clive enough."

Clive and Nancy Runnells, seated, are supporters and friends of researchers Dr. Rick Wetsel and Dr. Eva Zsigmond.

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 Episcopal Hospital, 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 research activities at the center entail human molecular genetic studies, as well as studies in genetic animal models of human heart disease.

Mission: To prevent cardiovascular diseases in humans prior to the development of clinical manifestations and to reverse or attenuate the evolving phenotype in those who already have developed the disease. The focus is on delineating the molecular genetic basis and pathogenesis of cardiovascular diseases in humans and to intervene at genetic and molecular levels to prevent and reverse the disease.

The ongoing basic research in our laboratory encompasses three groups:

I. Human Molecular Genetic Studies

- To identify the causal and modifier genes for various forms of hereditary cardiomyopathies and sporadic forms of heart failure,
- To identify causal and susceptibility alleles for complex cardiovascular traits, and
- To enhance clinical management of patients with genetic cardiovascular diseases by utilizing the genetic and genomic information.

II. Functional Studies

- To delineate the molecular mechanisms involved in the pathogenesis of hereditary cardiomyopathies through *in vitro* and *in vivo* gene transfer studies and in genetically modified animal models, and
- To determine the molecular mechanisms that link the DNA sequence variants to the pathogenesis of common complex cardiovascular phenotypes.

III. Clinical Studies

- To investigate potential utility of experimental therapies in human patients with hereditary cardiomyopathies through randomized phase I and II clinical studies,
- Recruitment and phenotyping of individuals and families with various genetic cardiovascular diseases, with primary focus on hereditary cardiomyopathies, and
- Pharmacological intervention to prevent, attenuate and reverse the evolving phenotype in hereditary cardiomyopathies.

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 are currently pursuing active research programs in the three most common forms of hereditary cardiomyopathies, namely; hypertrophic, dilated and arrhythmogenic right ventricular cardiomyopathies. Our research programs entail identification of the causative and modifier genetic variants, complemented by *in vitro* and *in vivo* functional studies in isolated cardiac cells and animal models. 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.

Over the past two decades, we have identified a number of the causal and modifier genes for cardiomyopathies, generated and characterized a number of animal models including transgenic rabbits and lineage tracer mice. Utilizing these tools, we have delineated – in part - the molecular mechanisms that are responsible for the induction of the myopathic phenotypes. Utilizing the information garnered through these studies, we have performed a series of genetic and pharmacological interventions to successfully prevent and attenuate evolving and established phenotypes in animal models of cardiomyopathies. We have extended the findings in the animal models to clinical studies in humans and have tested potential beneficial effects of inhibition of specific pathways involved in the pathogenesis of cardiomyopathies in humans. In accord with the above, we are currently conducting an NIH-sponsored pilot randomized clinical trial to test potential salutary effects of N-acetylcysteine on reversal and attenuation of cardiac phenotype in humans with hypertrophic cardiomyopathy.

RESEARCH PROJECTS

Research activities at the CCG are categorized into two interacting domains:

Clinical Research:

- Clinical trial of testing the effects of medications on reversal and/or attenuation of cardiac hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy
- Recruitment of patients and families with cardiovascular diseases
- Delineation of the genetic causes of Mendelian cardiovascular disorders with an emphasis on hereditary cardiomyopathies

Clinical Trial:

HALT-HCM (Hypertrophy Regression with N-Acetylcysteine)
Status: Active, Enrolling patients
Objective: To assess potential utility of N-Acetylcysteine in reversal of the phenotype in hypertrophic cardiomyopathy

For more information, please contact:
Yanli (Lily) Tan, R.N. at 713 500 2310
AJ Marian, M.D. at 713 500 2350
Center for Cardiovascular Genetics
677 Bertner Street, Suite 900
Texas Heart Institute at St. Luke's Episcopal Hospital
Houston, TX 77030

Basic Research:

- Molecular mechanistic studies and pharmacological interventions in animal models of human hereditary cardiomyopathies

KEY PUBLICATIONS

Rodriguez G, Ueyama T, Ogata T, Czernuszewicz GZ, Tan Y, Dorn II GW, Bogaev RG, Amano K, Oh H, Matsubara H, Willerson JT, Marian AJ. Molecular genetics and functional characterization implicate Muscle-Restricted Coiled-Coil gene (MURC) as a causal gene for familial dilated cardiomyopathy. *Circulation –Cardiovascular Genetics*, 2011, 4: 349-358 PMID: 21642240

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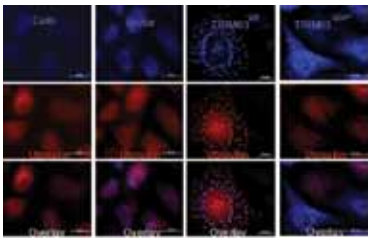
Chen SN, Czernuszewicz GZ, Tan Y, Lombardi R, Jin J, Willerson JT and Marian AJ. Molecular Genetics and Functional Studies Identify TRIM63 As a Novel Gene For Human Hypertrophic Cardiomyopathy. *Circulation Research*: 2012; 111:907-919 PMID: 22821932

Ruggiero R, Chen SN, Lombardi R, Rodriguez G. Marian AJ. Pathogenesis of Hypertrophic Cardiomyopathy Caused by Myozenin 2 Mutations Is Independent of Calcineurin Activity. Cardiovascular Research; 2012; PMID: 22987565

LAB MEMBERS

Research Associate: Grace Czernuszewicz
Faculty – Instructor: Priyatansh Gurha, Ph.D.
Faculty – Assistant Professor: Raffaella Lombardi, M.D., Ph.D.
Post-doctoral Fellows: Suet Nee Chen, Ph.D., Alessandra Ruggiero, Ph.D.



Loss-of-Function TRIM63 Mutation p.Q247*: The p.Q247* mutation – identified in families with hypertrophic cardiomyopathy (HCM) impairs auto-ubiquitination. It causes HCM by shifting the homeostatic balance of cardiac protein-turnover toward hypertrophy.

Center for Human Genetics

The Investigators of the Research Center for Human Genetics focus their work on common cardiovascular diseases, such as heart disease, 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 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 personalized genomic medicine. Genomic medicine is in a remarkable state of rapid progress.

This has grown out of 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 this risk.

We also develop and use a variety of laboratory models of cardiovascular disease, including stroke, atherosclerosis and chronic kidney disease. This allows 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.

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

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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. What is not clear is whether these heritable factors influence risk of diseases that accompany declining renal function, like high blood pressure and diabetes, or whether these diseases are necessary, but insufficient, for 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. Our work indicates that the susceptibility to renal disease is inherited separately from susceptibility to high blood pressure. We have found evidence that genetic variation in important elements of the immune system and the signaling pathways activated by the immune system play a key role in susceptibility to injury. Remarkably, immunosuppressant drugs prevent disease and death in these animals. These observations are leading toward the conclusion that, while high blood pressure or diabetes 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. Our current work is seeking to identify the explicit changes in genes that produce renal disease susceptibility so as to offer the possibility of therapies that target renal disease without producing overall suppressant effect on the immune system. 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 disease
- Non-genomic mechanisms of trans-generational trait sharing

KEY PUBLICATIONS

R.I. Dmitrieva, C.A. Hinojos, E. Boerwinkle, M.C. Braun, M. Fornage and P.A. Doris. HNF1 in hypertensive nephropathy. *Hypertension*. 51:1583-1589, 2008

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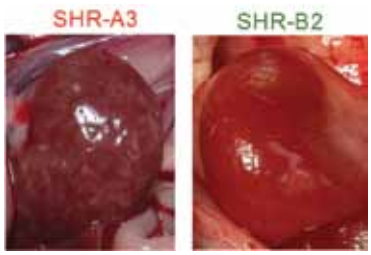
Herring, S.M., N. Gokul, M. Monita, R. Bell, E. Boerwinkle, S.E. Wenderfer, M.C. Braun and P.A. Doris. The rat immunoglobulin locus is associated with serum IgG levels and albuminuria. *J. Amer. Soc. Nephrol.* 22:881-9, 2011

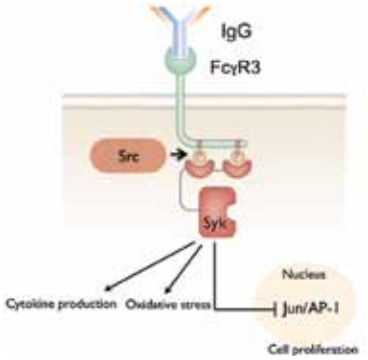
Braun, M.C. and P.A. Doris. Mendelian and transgenerational inheritance in hypertensive renal disease. *Annals Medicine*, 44 Suppl 1:S65-73, 2012.

LAB MEMBERS

Stacy Herring
Teresa Kuncewicz



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. 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 pre-clinical forms in well-characterized populations from young adulthood to old age. Research strategies combine genetic epidemiology and functional genomic approaches using the latest genome resources and technologies. In recent years, I have used the power of genome-wide association studies in collaboration with researchers in the United States and Europe to identify genetic loci influencing risk for stroke and brain aging. 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

- A Genome-wide Association Study of Ischemic Brain Vascular Injury (R01-HL093029)
- A GWAS of longitudinal blood pressure profiles from young adulthood to middle-age (U01-HG004729)
- Genes of the CYP450-Derived Eicosanoids Pathway in Subclinical Atherosclerosis (R01-HL084099)
- Genetic Epidemiology of Causal Variants Across the Life Course (U01-HG004803)
- Collaborative GWAS of Dementia, AD and Related MRI and Cognitive Endophenotypes (R01-AG033193)
- The ARIC Neurocognitive Study (U01-HL096917)
- Genetics of Microangiopathic Brain Injury (R01-NS41558)
- NINDS Ischemic Stroke Genetics Consortium (U01-NS069208)
- A Genome-wide association study in essential hypertension (R01-HL086694)

KEY PUBLICATIONS

kram 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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Wu JHY*, Lemaitre RN*, Manichaikul A*, Guan W*, Tanaka T*, Foy M*, Kabagambe E, Djousse L, Siscovick DS, Fretts AM, Johnson C, King IB, Psaty BM, McKnight B, Rich SS, Chen Y-DI, Nettleton JA, Tang W, Bandellini S, Jacobs Jr DR, Browning BL, Laurie CC, Gu X, Tsai MY**, Steffen LM**, Ferrucci L**, Fornage M**, Mozaffarian D**. Genetic Loci Associated with Plasma Phospholipid Fatty Acids in the De Novo Lipogenesis Pathway: A Meta-Analysis of Genome-wide Association Studies from the CHARGE Consortium. *Circ. Cardiovasc. Genet.* 2012 (accepted) (** denotes equal authors direction)

LAB MEMBERS

Devsmita Das, MD; MPH student
Millennia Foy, PhD; Postdoctoral Fellow
Xiangjun Gu, PhD; Senior statistician
Aron Joon, MS; Statistician
Ping Wang, PhD; Research Associate

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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. *Arterioscler Thromb Vasc Biol* 2012; 32: 1585-1595

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

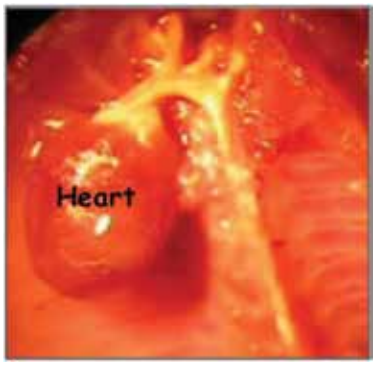
Shumei Zhong, Chichi Liu, David Haviland, Peter A. Doris, and Teng BB: Simultaneous Expression of Apolipoprotein B mRNA Editing Enzyme and Scavenger Receptor BI Mediated by a Therapeutic Gene Expression System. (2006) *Atherosclerosis.* 184: 264-275

Shumei Zhong, Shihua Sun, and Teng BB: The recombinant adeno-associated virus vector (rAAV2)-mediated apolipoprotein B mRNA-specific hammerhead ribozyme: a self-complementary rAAV2 vector improves the gene expression. (2004) *Genetic Vaccines and Therapy* 2: 5.

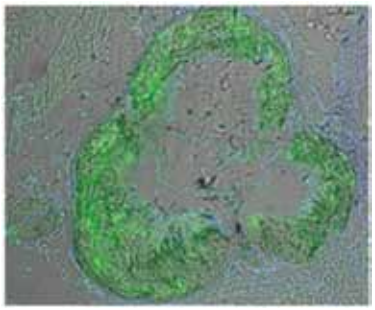
Enjoji M, Wang F, Makato M, Chan L, and Teng BB: Hammerhead ribozyme as a therapeutic agent for hyperlipidemia: Production of truncated apolipoprotein B and hypolipidemic effects in a dyslipidemia murine model. (2000) *Human Gene Therapy.* 11. 2415-2430

LAB MEMBERS

Post Docs: Hua Sun, PhD (Sept. 2012)
PhD Student: Hua Sun
Research Assistants: Hersharan Nischal and Guohua Ji
DeBakey High School Students: Jaqueline Martinez and Johannah Abraham



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 to study the pathogenesis of atherosclerosis.



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

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 has 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 are also actively pursuing the generation of genetically engineered stem cell lines that will avoid immune mediated graft rejection during transplantation procedures.

- 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 anayphylatoxins in the pathogenesis of lung disease. For example, studies using mice in which the C3a receptor has been deleted have demonstrated that C3aR is a important mediator of key hallmarks of asthma, including airway hyperresponsiveness, mucus production, lung cellular inflammation, and the CD4+ Th2 cytokine response.

We also are investigating the therapeutic use of embryonic (ES) and induced pluripotent (iPS) stem cell derived progenitor cells. Part of this program has focused on the development of stem cell therapeutics for the regeneration of lung epithelium destroyed by acute lung injury as well as by chronic lung diseases such as COPD. This research has led to the generation of the first pure population of lung alveolar epithelial type II cells from human ES cells. These cells 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

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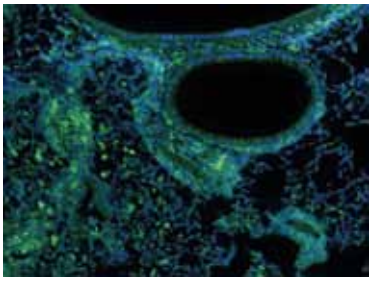
Wang D, Morales JE, Calame DG, Alcorn JL, Wetsel RA. Transplantation of human embryonic stem cell derived alveolar epithelial type II cells abrogates acute lung injury in mice. *Molecular Therapy*. 2010, 18: 625-634.

Mueller-Ortiz SL, Wang D, Morales JE, Li L, Chang JY, Wetsel RA. Targeted disruption of the gene encoding the murine small subunit of carboxypeptidase N (CPN1) causes susceptibility to C5a anaphylatoxin-mediated shock. *J. Immunol.* 2009, 182:6533-6539.

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

LAB MEMBERS

Senior Research Scientist: Dr. Stacey Mueller-Ortiz
MD/PhD Graduate Student: Daniel Calame
Senior Laboratory Technician: John Morales



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 of chronic inflammatory diseases also are associated with aberrant helper T cell responses. Understanding the regulation of helper T cell responses therefore is necessary not only for optimizing protective immunity but also for preventing aberrant inflammatory responses. In this aspect, we are particularly interested in the mutual regulation and contribution of each helper T cell subsets in disease setting, including allergic asthma, autoimmune disorders, and cancers. Among diverse helper T cell subsets, we currently are 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, food. Immune system in these mucosal tissues differs from that of non-mucosal lymphoid tissue. We currently are 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 of 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 actively are 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 fundamental basis for the use of this novel cell population in 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 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 upon exogenous stimuli
- Molecular regulation of follicular regulatory T cells and its application
- Role of metabolic factors in shaping adaptive immunity
- Developing novel vaccine approaches for cancer and infectious agents
- Understanding the biology of IL-10 family cytokines

KEY PUBLICATIONS

Chung Y, Qin H, Kang CY, Kim S, Kwak LW, Dong C. An NKT-mediated autologous vaccine generates CD4+ T cell-dependent potent anti-lymphoma immunity. *Blood.* 2007: 110: 2013

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

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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)

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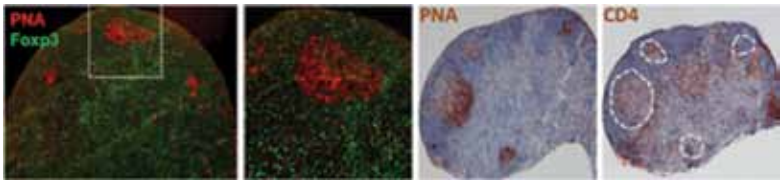
LAB MEMBERS

Post Doc: Hoyong Lim, Ph.D.
PhD Student: Young Uk Kim
Visiting Scientist: Kyoungah Yun, Ph.D.

Subsets of helper and regulatory T cells



Germinal center reaction

Scott Drouin, Ph.D.
Assistant Professor

Molecular mechanisms underlying airway obstruction & inflammatory diseases of the lung

My laboratory studies asthma and chronic obstructive pulmonary disorder (COPD), with a primary interest in the innate immune mechanisms that contribute to these inflammatory lung diseases. These studies are significant when considering that the lung is constantly exposed to an external environment containing a variety of airborne pathogens and pollutants, which could potentially cause damage to this vital organ. To minimize these stresses, tissues of the lung have evolved cellular and molecular mechanisms, which provide barrier and host defense functions yet maintain the ability to transport and facilitate gas exchange with the external environment. This balance is critical. Cells of the lung must be capable of communicating with the immune system in order to defend against external stresses and, at the same time, tightly control and temper these defensive responses in order to prevent damage to the delicate tissues responsible for the transport and exchange of oxygen. When these defense mechanisms don't function properly, a range of disease pathologies can result. Mild pathologies typically result in the reversible airway obstruction that most people experience with asthma or respiratory infections. Severe pathologies such as emphysema or chronic obstructive pulmonary disorder can result in irreversible obstruction and damage to the lung tissue with a gradual loss of a person's ability to breathe. By utilizing rodent models of pulmonary disease and *in vitro* techniques for studying cells of the lung, members of my laboratory and I have primarily focused on understanding the mechanisms that provide defense against the external environment in the hope of gaining insight into the defects that lead to airway obstruction and inflammatory lung disease.

RESEARCH PROJECTS

- Understanding the mechanisms by which environmental stimuli will convert normal epithelial barrier responses into airway obstruction and inflammation.
- Delineating the inflammatory and tolerogenic signals that coordinate myeloid and parenchymal cells of the airway to promote or regulate cells of the adaptive immune response.

KEY PUBLICATIONS

Lim H, Kim YU, Drouin SM, Mueller-Ortiz S, Yun K, Wetsel RA, and Chung Y: Negative regulation of pulmonary Th17 responses by C3a anaphylatoxin during allergic inflammation in mice. *Plos One.* 2012: In press.

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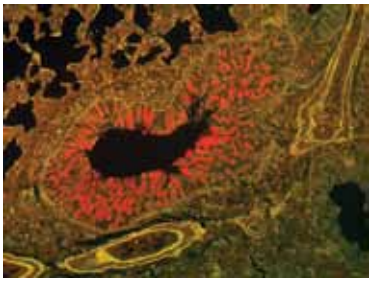
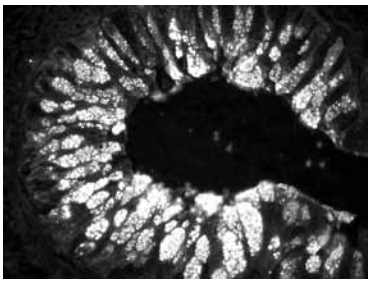
Drouin SM, Corry DB, Kildsgaard J, Wetsel RA: Cutting Edge: The Absence of C3 Demonstrates a Role for Complement in Th2 Effector Functions in a Murine Model of Pulmonary Allergy. *J. Immunol.* 2001: 167: 4141-4145.



Small airway surrounded by inflammatory cells and, more prominently, expressing epithelial mucins (*stained orange*) after exposure to fungal allergens.



Higher magnification of airway showing epithelial cells laden with granules containing Muc5ac, a mucin associated with airway obstruction in asthma.

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

Environmental triggers regulating immunological mechanisms of chronic airway inflammation

Over 40 million Americans suffer from chronic rhinosinusitis (CRS) and CRS represents one of the most prevalent chronic illnesses in the United States. This translates conservatively to 18-22 million physician visits yearly with an annual direct treatment cost of about $3.4 billion. Despite this burden, much remains unknown about its pathophysiology, and current treatment options, which typically involve recurrent surgeries and anti-inflammatory agents, are not curative. My clinical background in management of CRS disease and research interest in the role of environmental triggers in chronic airway inflammation provide unique insight into identifying novel therapeutic targets.

I received my MD/PhD in Molecular Genetics at The University of Texas Southwestern Medical Center at Dallas through the NIH sponsored Medical Scientist Training Program. I obtained my Ph.D. under the Nobel laureates Drs. Michael Brown and Joseph Goldstein for the identification and biochemical characterization of a novel human enzyme, acetyl coA synthetase. I then completed my otorhinolaryngology residency training at UT Southwestern and rhinology fellowship training at the Cleveland Clinic Foundation. It was during my residency training that I began work on a severe subtype of CRS.

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).

I combine my clinical and research interests through studies on CRS subtypes. I am interested in identifying objective molecular means of subcategorizing these various CRS subtypes. In addition, our lab is studying the molecular pathways initiated by various environmental triggers such as fungi to stimulate the innate and adaptive immune response in AFRS as well as other CRS subtypes.

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

Clark DW, Wenaas AE, Luong A, Citardi MJ, and Fakhri S. Chronic Rhinosinusitis with Nasal Polyps: Elevated serum IgE is associated with Staphylococcus aureus on culture. *Int Forum of Allergy Rhinol.* 2011 Nov;1(6):445-50. PMID:22144053

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Pakdaman MN, Corry DB, and Luong A. Fungi Linking the Pathophysiology of Chronic Rhinosinusitis with Nasal Polyps and Allergic Asthma. *Immunol Invest*. 2011;40(7-8):767-85. PMID: 21985305

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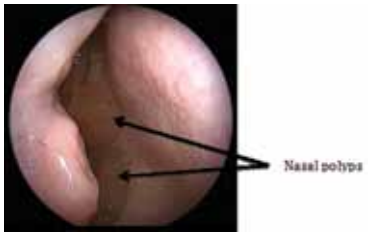
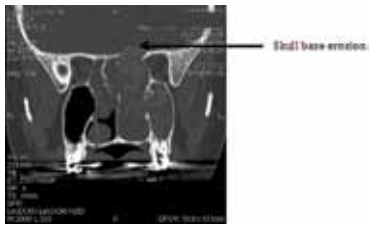
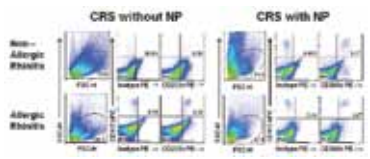
Clark D, Wenaas AE, Luong A, Citardi MJ, and Fakhri S. Staphylococcus aureus Prevalence in Allergic Fungal Rhinosinusitis Versus Other Subsets of Chronic Rhinosinusitis with Nasal Polyps. *International Forum of Allergy and Rhinology,* 2013 Feb;3(2):89-93. PMID:23038642



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



Representative FACS plots showing a population of CD117 / CD203c double positive activated mast cells in inflamed sinonasal mucosa from CRSsNP and CRSwNP patients with and without AR. Mast cell population present in CRSwNP patients irregardless of atopic status.

Dachun Wang, M.D.
Assistant Professor

Lung stem/progenitor cells and tissue regeneration

Lung epithelial stem/progenitor cells are critical for the maintenance of homeostasis of airway and alveolar epithelial cell populations that are constantly exposed to injurious stimuli from the environment. There are at least three stem/progenitor cell types responsible for maintaining distal lung epithelial cell populations: 1) alveolar epithelial type II cells; 2) the transient amplifying bronchiolar Clara cells; and 3) a subset of variant Clara cells located at the bronchioalveolar duct junction and the branch point-associated neuroepithelial bodies. Loss of normal functions of any of these stem/progenitor cells 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 treatment 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 for developing novel therapies to facilitate the regeneration or repair of injuring 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 1) to isolate and characterize human and mouse ES cell derived distal lung stem/progenitor cell types both *in vitro* and *in vivo*; 2) to generate "clinical grade" lung disease-specific iPS cells for studying pulmonary disease processes and for developing cell-based gene therapy strategy for lung tissue regeneration; and 3) to identify and characterize factors or regulatory pathways that control distal lung stem/progenitor cell fate during the diseases 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 regulate 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-I deficient human ES cell line for tissue regeneration

KEY PUBLICATIONS

Wetsel RA, Wang D and Calame DG. Therapeutic Potential of Lung Epithelial Progenitor Cells Derivce from Embryonic and Induced Pluripotent Stem Cells. *Annu. Rev. Med.* 2011. 62:30. 1-30.11

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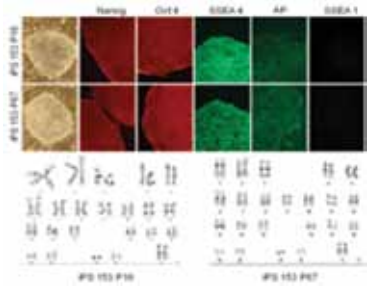
Wang D., Haviland D.L., Burns A.R., Zsigmond E., and Wetsel R.A. A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *PNAS,* Mar 13; 104(11): 4449-54 (2007).

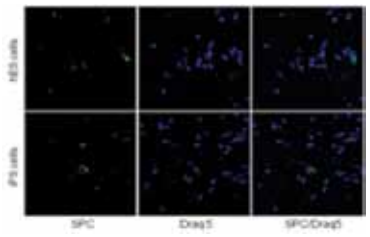
Zhang S., Wang D., Estov Z., Raj S., Willerson JT, and Yeh ET. Both Cell Fusion and Transdifferentiation Account for the Transformation of Human Peripheral Blood CD34-Positivie Cells Into Cardiac myocytes *in Vivo*. *Circulation* Dec. 110 (25):3803-7 (2004).

LAB MEMBERS

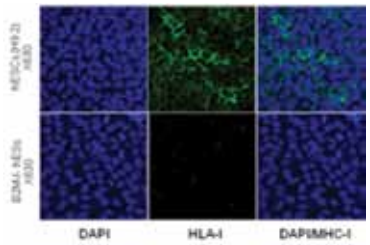
Postdoctoral Fellow: Dr. huanhuan Sun
Research assistant: Dr. Yuan Quan
Visiting scientist: Dr. Zhengyun Zou



Lung disease-specific iPS ells



Lung disease-specific iPS cell-derived alveolar type II cells



HLA-I deficient human ES cell type

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, as well as for cell 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 mice
- Microinjection of ES cells for the production of 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.

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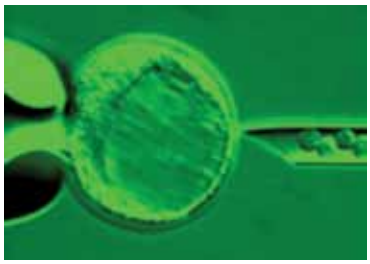
Wetsel, R.A., Kildsgaard, J., Zsigmond, E., Wei L. and Chan, L.: Genetic deficiency of Acylation Stimulating Protein (ASP/C3ades Arg) does not cause hyperapobetalipo- proteinemia in mice. *J. Biol. Chem.* 274: 19429-19433, 1999.

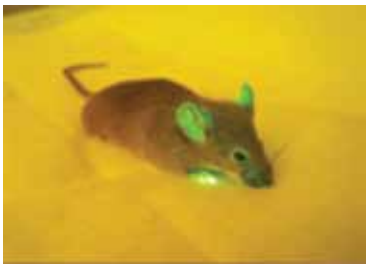
Kildsgaard, J., Zsigmond, E., Chan, L. and Wetsel, R. A.: A critical evaluation of the putative role of C3adesArg, ASP in lipid metabolism and hyperapobetalipoproteinemia. *Molec. Immunol.* 36: 869-876,1999.

LAB MEMBERS

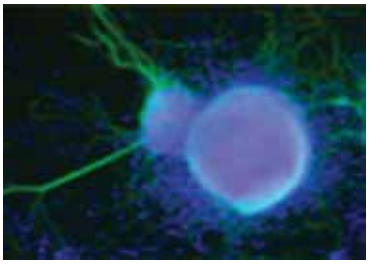
Manager: Aleksey Domozhirov
Research Assistant: Jing Yang



Microinjection of targeted mouse ES cells into blastocysts for the production of knock-out mice



GFP-expressing transgenic mouse



Mouse ES cells undergoing neural differentiation

Center for Metabolic and Degenerative Diseases

The Center for Metabolic and Degenerative Diseases takes an integrative and collaborative approach to tackling some of the most pressing health challenges of our time: diabetes, obesity, and aging-related neurological diseases such as Alzheimer's and Parkinson's. These different health conditions involve defects in multiple related cell signaling pathways and processes. The guiding vision for the center has been to recruit investigators who focus on complementary aspects of energy, metabolism, cell signaling, or muscle and neuronal degeneration and who use a variety of methods and technologies.

Key questions being addressed by the center's faculty include the following:

- How do cells regulate their storage and use of fat?
- Why does obesity increase the risk for certain cancers?
- How does the brain control the body's energy balance and influence glucose metabolism?
- Can medicines be used to mimic exercise and, thereby, produce its beneficial effects for those who cannot exercise or have muscle degeneration?
- How does the attachment of glucose to certain proteins influence normal organ development and function?
- What signaling pathways control the actions of steroid hormones on breast tissue development and on breast cancer initiation and progression?
- How does abnormal processing of cellular proteins cause metabolic and neuronal degeneration?
- 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?

To address these questions, the center employs state-of-the-art methods and diverse model organisms, such as the fruit fly and mouse. Strong collaboration among the center's laboratories promotes research synergy, thereby increasing productivity and innovation. The center's faculty members also collaborate with epidemiologists, biochemists, geneticists, and clinical scientists to speed the translation of their discoveries for the benefit of patients with metabolic and degenerative diseases. The upcoming year will see a series of new faculty recruitments to the center, including the appointment of a new center director.

John Hancock, M.A., M.B., B.Chir., Ph.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

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

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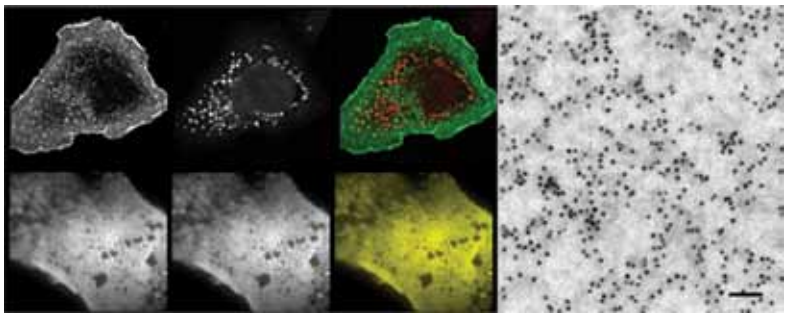
Zhou Y, Cho KJ, Plowman SJ, Hancock JF. (2012) Nonsteroidal anti-inflammatory drugs alter the spatiotemporal organization of Ras proteins on the plasma membrane. *J Biol Chem.* 287, 16586-16595

LAB MEMBERS

Instructor: Yong Zhou, PhD
Post Docs: Kwang-jin Cho, PhD, Dharini van der Hoeven, PhD, Travis Rodkey, PhD
Technicians: Xiaping Ma, Wei Chen, Hong Liang Jin-Hee Park, Sravanthi Chigurupati



Ras localization imaged by confocal, TIRF and electron microscopy

Vihang Narkar, Ph.D.
Assistant Professor

Transcriptional aerobics in skeletal muscle diseases

Skeletal muscle is a remarkably plastic tissue that adapts to environmental cues by undergoing changes in its metabolic and contractile properties. For example, endurance training (or exercise) increases slow-twitch myofibers that are rich in mitochondria, fat oxidizing enzymes and fatigue-resistant contractile proteins. This, in turn, leads to improved aerobic capacity and energy efficiency at the physiological level. Conversely, loss of these myofibers is commonly linked to the pathology associated with physical immobilization, aging, diabetes, and even certain type of muscular dystrophies, underscoring the importance of muscular aerobic capacity in health. Despite the known benefits of increasing aerobic muscles, gene regulatory pathways that encode this fiber type remain unclear. Discovery of these pathways will have important therapeutic implications in metabolic and muscle degenerative diseases.

In our laboratory, we are particularly interested in understanding how nuclear receptors – that are hormone or drug-activated transcriptional factors – regulate metabolic and contractile properties of the skeletal muscle. Recently, we identified a molecular interaction between serine/threonine kinase AMPK and nuclear receptor PPARδ that can be pharmacologically targeted to activate genes linked to mitochondrial biogenesis, fatty acid oxidation, and slow-twitch contractile myofibers in skeletal muscles and improve exercise endurance. These findings reveal that exercise-activated kinases and nuclear receptors are key components of myocellular transcriptional machinery controlling metabolism and fatigue. 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 oxidative slow-twitch 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 fatigue. Furthermore, we are exploring the potential role of ERRγ in ameliorating diabetes and related muscle microangiopathy as well as muscular dystrophy.

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

Chao LC, Wroblewski K, Ilkayeva OR, Stevens RD, Bain J, Meyer GA, Schenk S, Martinez L, Vergnes L, Narkar VA, Drew BG, Hong C, Boyadjian R, Hevener AL, Evans RM, Reue K, Spencer MJ, Newgard CB, Tontonoz P (2012) Skeletal muscle Nur77 expression enhances oxidative metabolism and substrate utilization. *J Lipid Res*. 53(12): 2610-9.

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

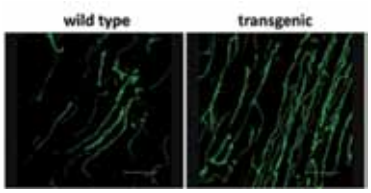
Matsakas A, Macharia R, Otto A, Elashry MI, Mouisel E, Romanello V, Sartori R, Amthor H, Sandri M, Narkar V, Patel K (2012) Exercise training attenuates the hypermuscular phenotype and restores skeletal muscle function in the myostatin null mouse. *Exp Physiol.* 97(1): 125-40.

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

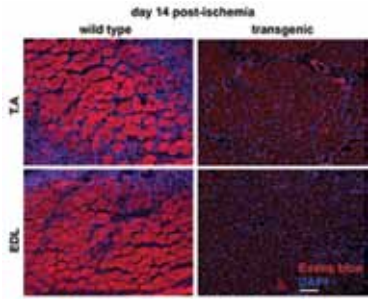
Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, Evans RM. (2008) AMPK and PPARdelta agonists are exercise mimetics. *Cell.* 134(3): 405-15.

LAB MEMBERS

Post-docs:
Antonios Matsakas
Vikas Yadav
Technicians: Sabina Lorca



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.

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 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 also will 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

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.

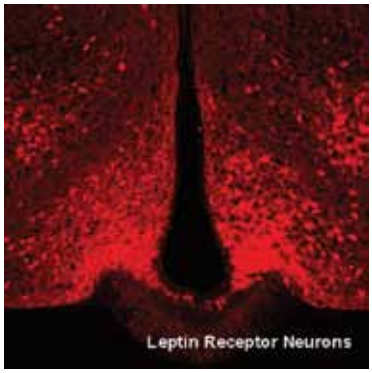
Xu Y, O'Brien W, Lee C-C, Myers MG, and Tong Q. Role of GABA release from leptin-recpeor-expressing neurons in body weight regulation. *Endocrinology,* 2012, 153(5): 2223-2233, PMID: 22334723. Corresponding Author.

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.

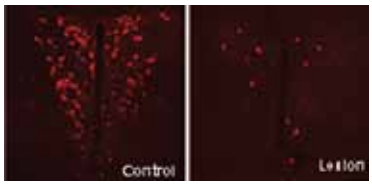
Tong Q, Ye C, Jones JE, Elmquist JK and Lowell BB. Synaptic Release of GABA by AgRP Neurons is Required for Normal Regulation of Energy Balance. *Nature Neuroscience* 11(9): 998-1000, 2008. PMID: 18690230.

LAB MEMBERS

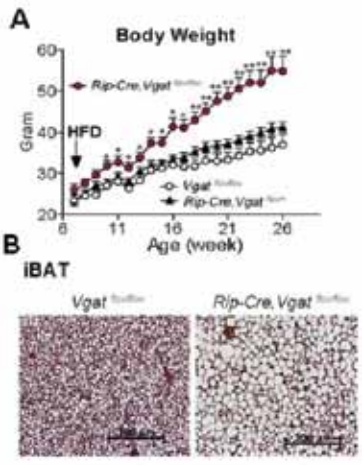
Post Docs: Yuanzhong Xu, Zhaofei Wu, Eun Ran Kim
Staff: Yaming Zhu
Visiting Scientist: Yi Wang



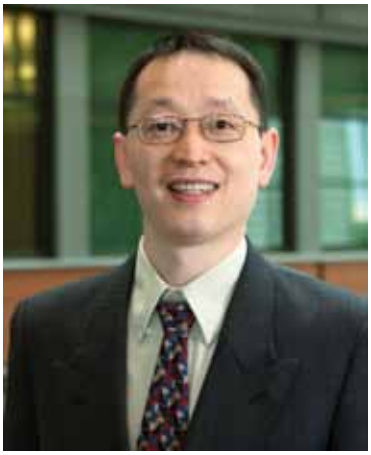
Leptin receptor expressing neurons illustrated by the expression of red-fluorescent protein.



Specific lesion of oxytocin neurons using mouse genetics.



Deletion of GABA release from Rip-Cre neurons leads to massive obesity on high fat diet (A) associated with altered function of brown fat adispose tissues (B).



Sheng Zhang, Ph.D.
Assistant Professor

Molecular mechanisms of human brain degenerative diseases

While our society is enjoying an unprecedented longer life expectancy, it also is facing a pressing challenge from aging-related brain degenerative diseases, such as Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD). To tackle these mysterious yet devastating disorders, we need to address some basic biological questions: (1) what do these disease genes normally do in the cell? (2) why do mutations in these genes almost invariably link to formation of protein aggregates (e.g., plaques or tangles in AD) in the brains? (3) how do these mutations affect neuron's normal physiological activity and survival?

Our laboratory is studying brain diseases by using *in vitro* mammalian cell culture system together with *in vivo* animal model Drosophila, commonly known as the fruit fly. The fly, small and simple, bears many remarkable similarities to humans, such as the existence of most human disease genes' counterparts in its genome, and its simple yet well-conserved nervous system. Many other features in the fly, such as easy to raise, its powerful genetics and the availability of abundant experimental tools, make it a favorite model organisms in basic biology and diseases studies.

Our laboratory is focusing on the following projects:

Genetically, HD is a simple disease, caused by a unique mutation (abnormal expansion of a polyglutamine tract) in a single gene called Huntingtin. Disruption of Huntingtin's normal functions has been implicated in the disease pathogenesis. We have knocked out the fly Huntingtin (dhtt) from the fly genome and are characterizing the mutant animals' phenotypes (Figure 1), in order to elucidate the still unknown cellular functions of this enigmatic gene.

Protein aggregates, the common pathological feature of brain degenerative disorders, is believed to be a contributing pathogenic factor. Their formation is likely a dynamic process involving multiple intermediate species of different sizes and conformations (e.g., oligomers, proto- and pre-fibrils, fibrils), with their cellular effects varying from toxic to protective. Thus, understanding their regulation will be important in finding effective therapies. We have established cell- and animal-based assays to analyze protein aggregation in the fly (Figure 2). Together with tools such as genome-wide RNA interference (RNAi) screens, we are systematically studying the molecular networks regulating aggregates formation and neuronal toxicity.

PD is caused by the progressive loss of dopaminergic neurons in the brain. We are developing assays to study the *in vivo* regulation of intracellular handling of neurotransmitter dopamine using Drosophila, which has a remarkably conserved dopaminergic system (Figure 3).

RESEARCH PROJECTS

- Understanding the normal cellular functions of Huntington's disease gene Huntingtin
- Studying the regulation of intracellular formation of protein aggregates associated with different brain degenerative diseases by genome-wide RNAi screens and Drosophila-based models.
- Regulations of intracellular handling of neurotransmitter dopamine in dopaminergic neurons and in Parkinson's disease models

KEY PUBLICATIONS

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).

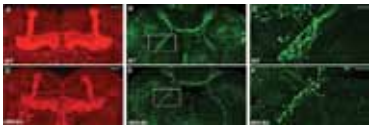
Zhang S, Xu L, Lee J, Xu T. (2002). Drosophila Atrophin homolog functions as a transcriptional co-repressor in multiple developmental processes. *Cell,* 108 (1): 45-56.

Tao WF, Zhang S, Turenchalk GS, Stewart RA, St John MA, Chen WL, Xu T. (1999). Human homologue of the Drosophila melanogaster lats tumor suppressor modulates CDC2 activity. *Nature Genetics,* 21(2):177-81.

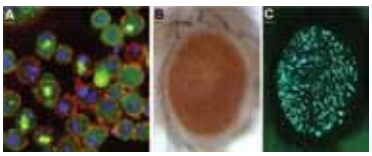
5Hu G, Zhang S, Vidal M, Baer JL, Xu T, Fearon ER. (1997). Mammalian homologs of seven in absentia (sina) regulate DCC via the ubiquitin-proteasome pathway. Genes & Development. 11(20):2701-14.

LAB MEMBERS

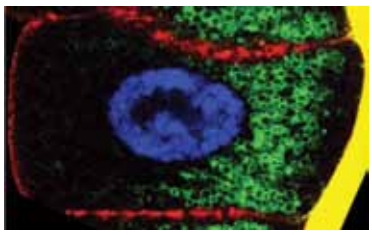
Post Docs: Dr. Zhen Xu, Dr. Yanning Rui, Dr. Dongsheng Chen
Students: Antonio Tito, Michael McCarthy, Ryan Singer
Technicians: Lili Ye, B.A., Research Assistant I, Zhihua Chen, Ph.D., Research Associate



Drosophila Huntingtin mutants (*dhtt-ko*) show abnormal brain organization and neuronal structures (D-F) as compared to wild-type (WT) controls (A-C).



Development of protein aggregates (green puncta) in *Drosophila* cells (A) and in adult fly eyes (B and C).



Dynamic subcellular localization of a dopamine regulator protein (labeled in green) in a *Drosophila* cell (cell morphology outline in red and its nucleus stained in blue)

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 with accurate imaged phenotypes 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. The CMI is one of four centers in the United States comprising the National Cancer Institute's Network for Translational Research.

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

- 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, Cullen Chair of Molecular Medicine, & Center Director
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease 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." The CMI is one of four Centers in the United States comprising the National Cancer Institute's Network for Translational Research.

RESEARCH PROJECTS

- Developing, building, and translating NIR fluorescence imaging instrumentation and algorithms for multi-modality molecular imaging in preclinical and clinical studies
- Developing and applying tomographic algorithms for NIR tomography for small animal and human imaging
- 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

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," *PNAS*, 2013, May 6, PMID: 23650393.

Robinson HA, Kwon, S., Hall, M.A., Rasmussen, J.C., Aldrich, M.B. and E.M. Sevick-Muraca, "Non-invasive optical imaging of the lymphatic vasculature of a mouse," *J Vis Exp*, 8(73): doi 10.3791/4326, 2013.

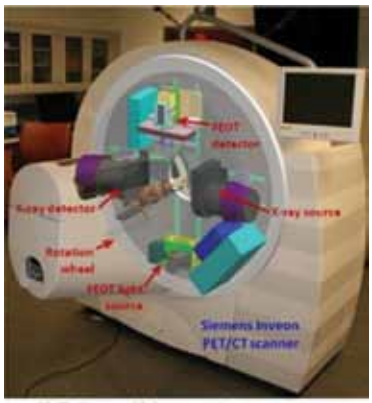
Zhu, B., Wu, G., Robinson, H., Wilganowski, N., Hall, M.A., Ghosh, S.C., Pinkston, K.L., Azhdarinia, A., Harvey, B.R., and E.M. Sevick-Muraca, "Tumor margin detection using quantitative NIRF molecular imaging targeting EpCAM calidated by far red gene reporter iRFP," *Mol., Imaging, Biol.*, April 26, 2013; PMID: 231619897.

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

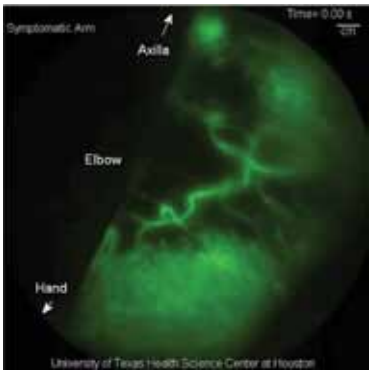
Darne, C.D., Lu, Y., Tan, I.C., Zhu, B., Rasmussen, J.C., Smith, A.M., Yan, S., and E.M. Sevick-Muraca, "A compact frequency-domain photon migration system for integration into commercial hybrid small animal imaging scanners for fluorescence tomography," *Phys Med Biol*, 57(24): 8135-52, 2012. PMID: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

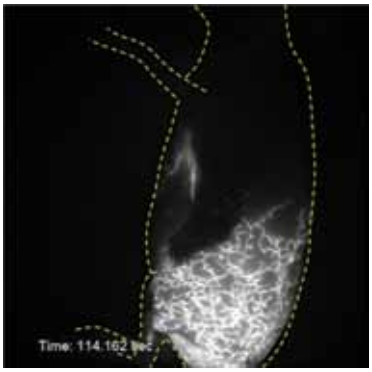
LAB MEMBERS

Research of Flow Cytometry: Amy Hazen
Chief Histology Technician: Sarah Amra
Research Engineers: Dr. I-Chih Tan, Banghe Zhu, Yujie Lu
Research Scientists: Dr. Melissa Aldrich, Dr. Mary Hall, Pradip Ghosh, Dr. Sukhen Ghosh
Research Coordinators: Holly Robinson, Nathaniel Wilganowski, Karen Gore, Grace Wu
Chief Histology Technician: Sarah Amra
Research Assistants: Gabriel Dickinson
Admin Assistants: Jessica Nollkamper, Dana White, Fei Li
Postdoctoral Fellow: Dr. Chinmay Darne
Students: Germaine Agollah, Pier-Anne Lachance, Cynthia Davies-Venn



I-Chih Tan, Chinmay Darne, Anne Smith (Siemens),





Melissa B. Aldrich, Ph.D.
Assistant Professor

Program for imaging in immunology

I bring a combination of expertise in translational science and immunology to lead the program of lymphatic imaging, the circulatory system which is critical to immune surveillance and response. NIRF imaging promises to deliver 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 the side of 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 also has worked in other 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 validation core that authored a consensus paper 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, a suggestion evidenced in collaborative work with Dr. Manuel Gonzalez-Garay.

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, 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.

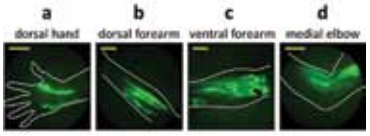
Aldrich MB, Wang XJ, Hart A, Kwon SK, Sampath L, Marshall MV, and Sevick-Muraca, E. Assessment of free dye in solutions of dual-labeled antibody conjugates for in vivo molecular imaging. 2010. *Molecular Imaging and Biology* 13:32-42.

Aldrich MB, Davies-Venn C, Angermiller B, Robinson H, Chan W, Kwon K, Sevick-Muraca EM. Concentration of indocyanine green does not affect lymphatic function. 2012. *Lymphatic Research and Biology* 10(1):1-5.

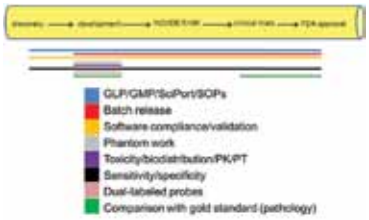
Aldrich MB, Sanders D, Lapteva N, Huang, XF, and Chen SY. SOCS1 downregulation in dendritic cells promotes memory T cell responses. 2008. *Vaccine* 26(8):1128-35.

LAB MEMBERS

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



Normal arm lymphatic vessel architecture



Translation "pipeline"

Ali Azhdarinia, Ph.D.
Assistant Professor

Program for dual-labeled 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 and other diseases. Currently, I serve 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. Our work utilizes radioactive and near-infrared fluorescent (NIRF) contrast agents, which can be used for whole-body and intraoperative imaging, respectively. The combination of both modalities into a single agent is a key area where we have focused our efforts through synthesis of a panel 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. Our lab conducts full pharmacological characterization of lead compounds to determine suitability for clinical translation. As part of the CMI, I have participated in establishing a dedicated clean room for production of probes under Current Good Manufacturing Practices (cGMP) to facilitate translational research. To complement the existing application of nuclear imaging, our lab is developing new NIRF dyes with enhanced optical properties and compatibility with common radiolabeling processes. The addition of a NIRF dye onto a radiotracer permits image-guidance in the operating room and may potentially improve surgical outcome while minimizing morbidities associated with current methods. Our lab is 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
- Development of multimodal probes for theranostics applications
- Pharmacological evaluation of probes targeting tumors and other molecular processes

KEY PUBLICATIONS

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

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. PMID:23214723.

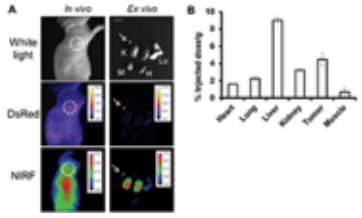
Moss, J.A., Vâvere, A.L., Azhdarinia, A. Design of Peptide Imaging Agents for Whole-body and Intraoperative Molecular Imaging. *Curr Med Chem.* 1;19(20):3255-65, 2012. PMID:22664243.

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.

Azhdarinia, A., Ghosh, P., Ghosh, S., Wilganowski, N., Sevick-Muraca, E.M. Dual-labeling Strategies for Nuclear and Fluorescence Molecular Imaging: a Review and Analysis. *Mol Imaging Biol.* 14(3):261-76, 2012. PMID:22160875.

LAB MEMBERS

Research Scientist: Dr. Sukhen Ghosh
Research Coordinator: Nathaniel Wilganowski
Research Assistant: Otis Hall



Representative multimodality images in a tumor-bearing mouse 40 h postinjection of a dual-labeled anti-EpCAM antibody (64Cu-7) (A). The 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 64Cu-7 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 et al., J Med Chem, 2013).

Chemical structure of a multimodal MMP-2/9-targeting peptide. The peptide (black) is conjugated to a NIRF dye (green) and a bifunctional chelating agent (blue) that sequesters a radiometal (M).

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

Bioinformatics analysis of whole genome sequencing for discovery and diagnosis of human disorders

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

I foresee a day in the near future when getting your genome sequenced and interpreted will be standard practice. To get to this point, we need to develop tools to analyze the whole genome sequence, interpret the information and detect markers that will allow physicians to develop personalized treatment for every patient.

Our laboratory focuses on the use of NGS to detect and associate genetic markers (variations) with genetic disorders. One main project is in collaboration with the Center for Molecular Imaging to identify genetic markers associated with lymphedema. Lymphedema is a condition of irreversible tissue swelling caused by a compromised lymphatic system. The disease affects both males and females, with onset occurring at birth, puberty, or adulthood, and with variable phenotype. Our approach consists of studying families with multiple affected individuals. The lymphatic phenotype of family members is non-invasively imaged using near-infrared fluorescence (NIRF) to directly visualize lymph pumping in the arms and legs and to detect lymphatic vascular anomalies. The results are then used to match the genotype to the phenotype to delineate the molecular and genetic bases of lymphedema, identify novel genetic and molecular diagnostic markers, as well as potential therapeutic targets and individualization of therapy (pharmacogenetics).

Another project is directed to demonstrate the clinical utility of NGS to predict clinical significant risk in a cohort of healthy adults, (YPO project). For this project we recruited ~100 non-related individuals from the Houston area and sequenced all the volunteers using whole exome sequencing. Every individual in the group provided detailed medical and family histories. Our results indicate a strong utility of NGS, and recommend guidelines for the bioinformatics analysis of genomes.

Finally, our team is working with several other clinicians to identify genetic markers associated with genetic disorders like schizophrenia, Tuberous Sclerosis Complex, Dercum's disease, Adiposis dolorosa and Madelung's disease.

RESEARCH PROJECTS

- Genome and Bioinformatics Analysis of patients with Lymphedema.
- Genetic diagnostics 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.
- Identification of genetic causes for hemo- and lympho-vascular diseases. Collaborator of Patricia E. Burrows, Wisconsin Medical College

KEY PUBLICATIONS

The Human Chromosome 12 Group. 2006. The finished DNA sequence of human chromosome 12. *Nature* 440:346-51. PMID: 16541075

The Human Chromosome 3 Group. 2006. The DNA sequence, annotation and analysis of human chromosome 3. *Nature* 440:1194-8. PMID: 16641997

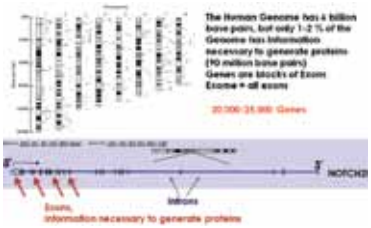
Sea Urchin Genome Sequencing Consortium. 2006. The genome of the sea urchin Strongylocentrotus purpuratus. *Science* 314:941-52. PMID: 17095691

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

Sr. Research Coordinator: Otis Hall, Jr.
Research Coordinator: Karen Gore
Co-advised: Germaine Agollah



Exon Capture and Exomes.

Basic steps in our variant analysis pipeline.

Mary A. Hall, MBA, Ph.D.

Assistant Professor, Center for Molecular Imaging - Lymphatic Vascular Biology & Immune Mediators; Molecular Imaging Probe Development

Lymphatic vascular biology & immune mediators; molecular imaging probe development

My research involves multimodal imaging methods and agent development for detecting metastatic lymph nodes (LNs) in preclinical models of cancer; imaging lymphatic vasculature during injury repair; and investigating immune parameters that may be involved in LN metastasis, lymphangiogenesis, and wound healing. More specifically, I am interested in the mechanisms by which immunocompetent cells, the lymphatics, cytokines/chemokines and growth factors contribute to or prevent cancer cell dissemination and injury repair. Recently, I have had the opportunity to play a significant role in the development of a hybrid positron emission tomography/computed tomography/near-infrared (NIR) fluorescence (PET/CT/NIRF) imaging method and dual-labeled probe to detect metastatic cancer cells in a preclinical model of human prostate cancer, as well as implementation of NIR flow cytometry for validation of NIRF-labeled imaging agent biological activity. In addition, I have been investigating early detection of lymphangiogenesis via NIRF imaging and cytokine/chemokine involvement during healing in a mouse full-dermis thickness wound model with and without administration of exogenous growth factor. My research within the CMI laboratory involves utilization of interdisciplinary skills within the fields of molecular imaging, microbiology, molecular biology, and immunology to investigate the processes of cancer metastasis and wound healing, and is aimed at development of strategies to arrest cancer metastasis and enhance wound healing, as well as optimization of NIRF imaging for clinical applications.

RESEARCH PROJECTS

- Multimodal imaging method and agent development for preclinical studies aimed at detecting cancer metastasis to LNs and subsequent clinical translation for NIRF intraoperative guidance during nodal resection.
- Implementing and validating NIR flow cytometry for assessing the immunoreactivity of NIR fluorophore-labeled antibody prior to use during molecular imaging for detection of cancer cells in vivo, in situ, and ex vivo.
- Implementing a mouse model of wound healing, and studying lymphangiogenesis and the wound-healing process via molecular imaging and immunological techniques.

KEY PUBLICATIONS

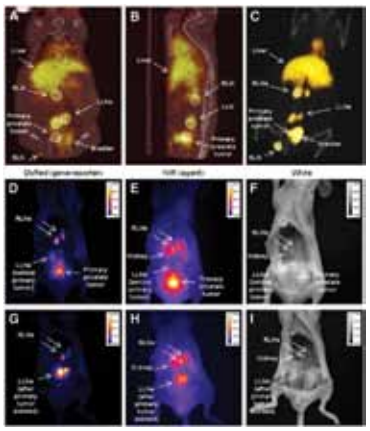
Hall MA, Pinkston KL, Wilganowski N, Robinson H, Ghosh P, Azhdarinia A, Vazquez-Arreguin K, Kolonin AM, Harvey BR, Sevick-Muraca EM: Comparison of mAbs targeting epithelial cell adhesion molecule for the detection of prostate cancer lymph node metastases with multimodal contrast agents: quantitative small-animal PET/CT and NIRF. *J Nucl Med.* 2012 Sep; 53(9):1427-37. Doi 10.2967/jnumed.112.106302. Epub 2012 Aug 7. PMID: 22872743 [PubMed – indexed for MEDLINE]

Hall MA, Aldrich MB, Azhdarinia A, Lachance, PA, Robinson H, Hazen AL, Haviland DL, Sevick-Muraca EM: Quantifying multimodal contrast agent biological activity using near-infrared flow cytometry. *Contrast Media Mol Imaging*. 2012 May-Jun; 7(3):338-45. Doi 10.1002/cmmi.502. PMID: 22539404 [PubMed – indexed for MEDLINE]

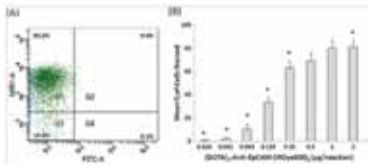
Hall MA, Kwon S, Robinson H, Lachance PA, Azhdarinia A, Randanathan R, Price RE, Chan W, Sevick-Muraca EM: Imaging prostate cancer lymph node metastases with a multimodality contrast agent. *Prostate.* 2012 Feb 1;72(2):129-46. Doi 10.1002/pros.21413. Epub 2011 May 2. PMID: 21538422 [PubMed – indexed for MEDLINE]

Olabisi RM, Lazard ZW, Franco CL, Hall MA, Kwon SK, Sevick-Muraca EM, Hipp JA, Davis AR, Olmsted-Davis EA, West JL: Hydrogel microsphere encapsulation of a cell-based gene therapy system increases cell survival of injected cells, transgene expression and bone volume in a model of heterotopic ossification. *Tissue Eng Part A.* 2010 Dec; 16(12):3727-36. Doi 10.1089/ten.TEA.2010.0234. Epub 2010 Sep1. PMID: 20673027 [PubMed – indexed for MEDLINE] PMCID: PMC3120095 Free PMC article

Sampath L, Kwon S, Hall MA, Price R, Sevick-Muraca EM: Detection of cancer metastases with a dual-labeled near-infrared/positron emission tomography imaging agent. *Transl Oncol*. 2010 Oct 1; 3(5):307-17. Doi 10.1593/tlo.10139. PMID: 20885893 [PubMed] PMCID: PMC2935634 Free PMC article



Detection of lymph node (LN) metastases via multimodality imaging using a dual-labeled monoclonal antibody-based imaging agent targeting epithelial cell adhesion molecule (EpCAM) in a mouse model of human prostate cancer. Signals from the primary tumor and metastatic LNs were detected via non-invasive PET/CT (A-C) and in situ NIRF imaging (E and H) after intravenous administration of the imaging agent. Fluorescence was also detected via DsRed gene reporter imaging in situ (D and G), confirming the presence of cancer cells in the same tissue. White light photographs (F and I) depict the anatomic location of imaged tissue within the mouse. [From Hall et al., J Nucl Med, 2012.]



Near-infrared flow cytometry (NIR FC). [From Hall et al., Contrast Media Mol Imaging, 2012.]

Barrett Rowland Harvey, Ph.D.
Assistant Professor

Therapeutic and diagnostic antibody development

Technological achievements in antibody engineering have made antibody therapeutics 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 goals: 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 for the engineering of therapeutic or diagnostic antibodies. Coupled with molecular imaging, agent development can be monitored using *in vivo* models to predict efficacy, specificity and for target validation prior to the clinic. This line of research allows our laboratory to venture into a number of diverse biological fields, with current projects currently focused in oncology and infectious disease.

RESEARCH PROJECTS

- Role of TGF-β Superfamily Proteins in Cancer Metastasis.
- Molecular Imaging for Nodal Staging of Cancer.
- Virulence Factor Regulation Governing Enterococcal Infection

KEY PUBLICATIONS

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

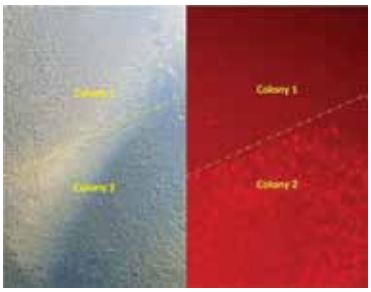
Gao P, Pinkston KL, Nallapareddy SR, van Hoof A, Murray BE, Harvey BR. "The Enterococcus faecalis rnjB is required for pilin gene expression and biofilm formation." *Journal of Bacteriology,* 192(20): 5489-98, 2010. PMID: 20729365

Harvey BR, Shanafelt A, Baburina I, Hui R, Vitone S, Iverson BL, and G Georgiou, "Engineering of Recombinant Antibody Fragments to Methamphetamine by Anchored Periplasmic Expression (APEx)," *Journal of Immunological Methods,* 308(1-2): 43-52, 2006. PMID: 16337958

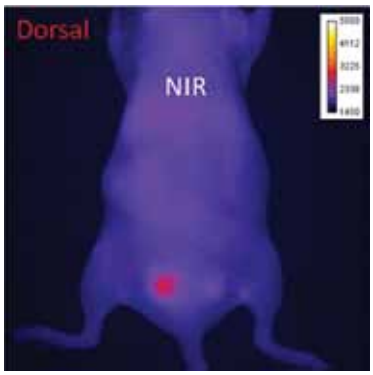
Harvey BR, Georgiou G, Hayhurst A, Iverson BL, and GK Rogers, "Anchored Periplasmic Expression (APEx), a Versatile Technology for the Isolation of High Affinity Antibodies from E.coli Expressed Libraries," *Proc Natl Acad Sci U S A,* 101(25): 9193-8, 2004. PMID: 15197275

LAB MEMBERS

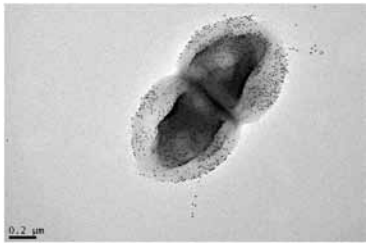
Kenneth L. Pinkston – Research Coordinator II
Dr. Peng Gao – Research Instructor
Emily Stinemetz – Graduate Student



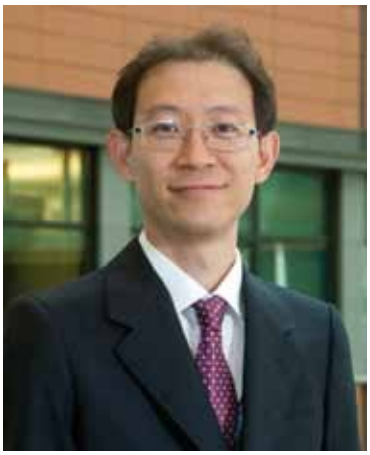
Evaluation of colonies from tetracarcinoma cell line for ligand induced DsRed signaling.



In vivo monitoring of cancer metastasis to sciatic lymph node using selected anti-EpCAM mAb–near infrared fluorophore conjugate.



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

Program for innovation in small animal imaging

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. The alteration of lymphatic function can cause obesity, edema, diabetes, as well as other diseases. 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 non-invasive NIRF imaging with sufficient temporal resolution and sensitivity to quantitatively assess different patterns of dynamic contractile function of the murine intestine resulting from the secretion of fluorescent bile after injection of ICG. Moreover, our group non-invasively imaged 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 his scientific interests revolve around multi-modality molecular imaging. The Center for Molecular Imaging is developing and translating imaging agents, which are dual-labeled with a PET/SPECT radiotracer and a NIR fluorescent dye. We are currently conducting molecular imaging of cancer and LN metastasis and inflammation in different animal models of disease.

RESEARCH PROJECTS

- Non-invasive characterization of lymphatic function in mice with lymphedema-like phenotypes, hypertension, cancer, and inflammation and tracking response to therapeutic agents.
- Investigation of physical, neural and humoral factors that can influence lymphatic function in normal physiology.
- Elucidating the molecular mechanisms which regulate lymphatic function.
- Non-invasive imaging of gastrointestinal motility using a fluorescence optical imaging technique.
- Multi-model molecular imaging.

KEY PUBLICATIONS

Lapinski P. E., Kwon S., Lubeck B. A., Wilkinson J. E., Srinivasan R. S., Sevick-Muraca E. M., and King P. D., RASA1 maintains the lymphatic vasculature in a quiescent functional state in mice. *Journal of Clinical Investigation*, 2012. 122: 733-47.

Kwon S., Agollah G. D., and Sevick-Muraca E. M., Altered lymphatic function and architecture in salt-induced hypertension assessed by near-infrared fluorescence imaging. *Journal of Biomedical Optics*, 2012. 17: 80504-1

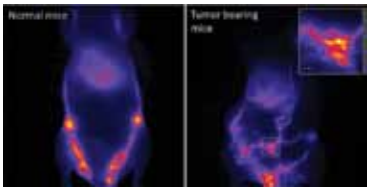
Kwon S., Davies-Venn C., and Sevick-Muraca E. M., In vivo dynamic imaging of intestinal motions using diet-related autofluorescence. *Neurogastroenterololgy and Motility,* 2012. 24:494-497.

Kwon S. and Sevick-Muraca E. M., Non-invasive, dynamic imaging of murine intestinal motility. *Neurogastroenterology and Motility,* 2011. 23: 881-e344.

Kwon S. and Sevick-Muraca E. M., Mouse phenotyping with near-infrared fluorescence lymphatic imaging. *Biomedical Optics Express,* 2011. 2: 1403-1411.

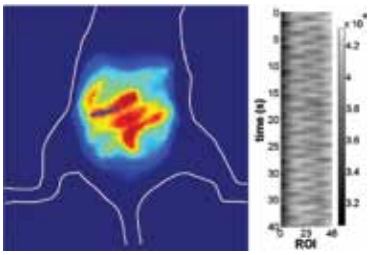
LAB MEMBERS

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

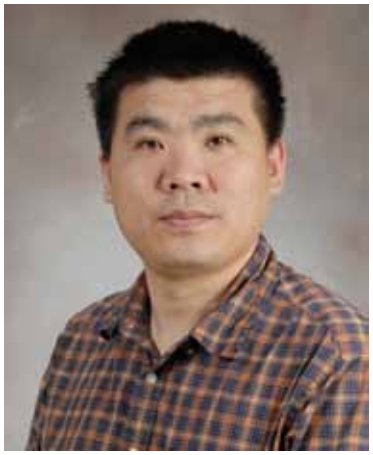


Fluorescent images in the ventral view of mouse showing abnormal tortuous and leaky lymphatic vessels.

Extensive lymphatic vessel hyperplasia in induced RASA1 deficient mice.



Fluorescent images showing autofluorescence in the intestines induced by standard murine diet and 3-D plot of fluorescent intensity as a function of time and ROI showing segmental motion.



Yujie Lu, Ph.D.
Assistant Professor

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Darne, C.D.*, Lu, Y. *, 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)

Zhang, X., Lu, Y., and Chan, T., "A Novel Sparsity Reconstruction Method from Poisson Data for 3D Bioluminescence Tomography," *Journal of Scientific Computing,* 50(3), 519-535 (2012).

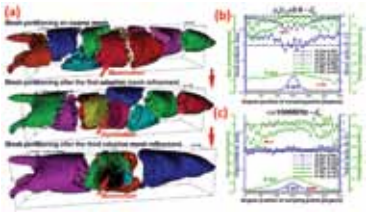
Lu, Y., Machado, H.B., Bao, Q., Stout, D., Herschman, H., and Chatziioannou, A.F., " In vivo Mouse Bioluminescence Tomography with Radionuclide-Based Imaging Validation," *Molecular Imaging and Biology,* 13:53-58, 2011.

Lu, Y., Zhu, B., Darne, C., Tan, I., Rasmussen, J.C., and Sevick-Muraca, E.M., "Improvement of Fluorescence-enhanced Optical Tomography with Improved Optical Filtering and Accurate Model-based Reconstruction Algorithms," *Journal of Biomedical Optics*, 16:126002, 2011.

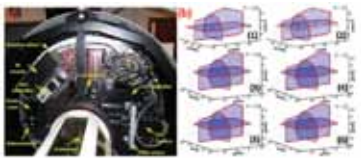
Lu, Y., Zhu, B., Shen, H., Rasmussen, J.C., Wang, G., and Sevick-Muraca, E.M., "A Parallel Adaptive Finite Element Simplified Spherical Harmonics Approximation Solver for Frequency Domain Fluorescence Molecular Imaging," *Physics in Medicine and Biology,* 55:4625-4645, 2010. (Featured Article and Highlights of 2010)

LAB MEMBERS

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



A fully parallel adaptive finite element radiative-transfer-based frequency-domain fluorescent photon immigration simulator. (a) is dynamic mesh partitioning and evolution using the digital mouse (MOBY) in the proposed simulator. Comparisons of the exiting fluorescent partial current J_m^+ between the diffusion equation (DA), Monte Carlo (MC) method and SP¬N approximations in the cylindrical homogeneous phantom when the ratio of the reduce scattering coefficient to the absorption coefficient ((μ_s^')/μ_a) and the modulation frequency (ω) are 2.0 and 100MHz (b) and 10.0 and 1000MHz (c) respectively. 'A * to MC' and 'P * to MC' denote the normalized amplitude ratios and the errors of the absolute phase shift between SPN and MC. Two dash-dot lines are 0 degree (top) and the ratio of '1' (bottom). J_m^+ was acquired along with the circle at the center position of the surface of the cylindrical phantom.



The developed multimodal (Optical/μPET/μCT) fluorescence tomography imaging system and relevant evaluation. (a) is a photograph showing the frequency-domain fluorescence imaging components installed within the Siemens Inveon CT scanner (in conjunction with I-Chih Tan) and (b) is tomographic reconstructions of mouse-shaped phantom with (b)(1)-(2) two-projections benchtop, (b)(3)-(4) two-projections and (b)(5)-(6) four-projections gantry installed configurations. For each configuration, reconstructions with N = 128 and N = 32 datasets are displayed to compare the reconstruction accuracy and the associated artifacts. Cross sections with blue (thin) and red (thick) boundaries are the center position of the actual and reconstructed targets, respectively. The volumetric mesh denotes the top 80% of the contour levels for the reconstructed fluorophore distribution.

John Rasmussen, Ph.D.
Assistant Professor

Program for instrumentation for translational optical 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 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 its application to new answer 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 TMC to (i) study the growth and reorganization of the lymphatics, termed lymphangiogenesis, (ii) elucidate its role in the development of lymphovascular diseases, such as lymphedema and cancer metastasis, as well as (ii) discover the various roles of the lymphatics in rare adipose disorders that may have a lymphovascular component. My expertise involves the application of NIRF imaging instrumentation and development of software for clinical applications. Specific projects focus on the development of analytical tools to facilitate lymphatic image processing and analysis.

RESEARCH PROJECTS

- Nodal staging of melanoma using non-invasive NIRF imaging
- Etiology of cancer related lymphedema
- Development of automated NIRF image analytical algorithms
- Application driven enhancement of NIRF imaging systems

KEY PUBLICATIONS

Rasmussen, J.C., P.E. Burrows, M.L. Gonzalez-Garay, 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. DOI: 10.1073/pnas.1222722110, ePub May 6, 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.

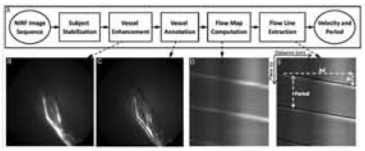
Rasmussen, J.C., Kwon, S., Sevick-Muraca, E.M., and J.N. Cormier, The Role of Lymphatics in Cancer as Assessed by Near-Infrared Fluorescence Imaging, *Annals of Biomedical Engineering,* 40(2):408-421, 2012 (Invited, Cover).

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.

Rasmussen, J.C., Tan, I.C., Marshall, M.V., Fife, C.E., and E.M. Sevick-Muraca, "Lymphatic Imaging in humans with near-infrared fluorescence," *Current Opinion in Biotechnology,* 20: 74-82, 2009.

LAB MEMBERS

Co-advisement of Germaine Agollah, student
Co-advisement of Chinmay Darne



(A) Analysis workflow of ALFIA. (B) An aggregated image is generated to facilitate vessel identification. (C) The lymphatic vessels are manually identified and annotated. (D) A flow map of fluorescent intensity as a function of distance (d) and time (t) is generated and (E) the flow lines of lymph propagation are manually annotated and automatically adjusted to select the maximal intensity value near the ends. The velocity (Δd/Δt) and propulsion periods are then calculated and exported to a spreadsheet. Reproduced from Zhang, J., et al., BOE, 3(7):1713-1723, 2012.

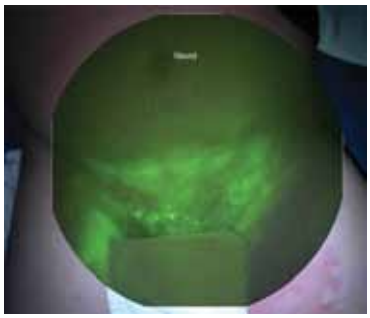
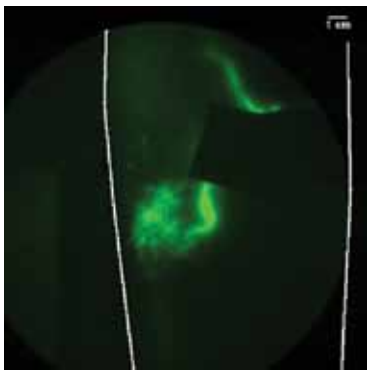


Image of abnormal lymphatic drainage in the groin and pelvic region of a man with Parkes-Weber syndrome and a confirmed mutation on the RASA1 gene. Reproduced from Rasmussen, J.C., et al. PNAS, 2013.



Images illustrating the lymphatic response to sentinel lymph node biopsy (SLNB) in a subject with melanoma. (Unpublished data)

I-Chih Tan, Ph.D.
Assistant Professor

Instrumentation for NIRF lymphatic imaging and optical tomography

My research program focuses upon the application-specific development of technologies for unmet clinical needs, as well as broad-based development of technologies for basic science investigation.

In the first research arena, I work with clinicians to apply measurements of lymphatic function to understand the etiology of disease. So far our understanding of the lymphatic architecture and function and its role in many diseases in limited due to the lack of a suitable imaging technique that has sufficient spatial and/or temporal resolutions. Recently, we developed and translated lymphatic imaging technology using near-infrared fluorescence (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.

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

Another focus of our 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
- Using NIRF lymphatic imaging to understand the molecular genetics of disease
- Nodal staging in cancers using non-invasive 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.

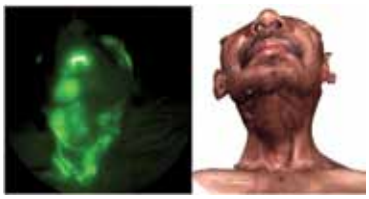
B. Zhu, I. C. Tan, J. C. Rasmussen, and E. M. Sevick-Muraca, "Validating the sensitivity and performance of near-infrared fluorescence imaging and tomography devices using a novel solid phantom and measurement approach," *Technol Cancer Res Treat,* vol. 11, pp. 95-104, 2012.

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.

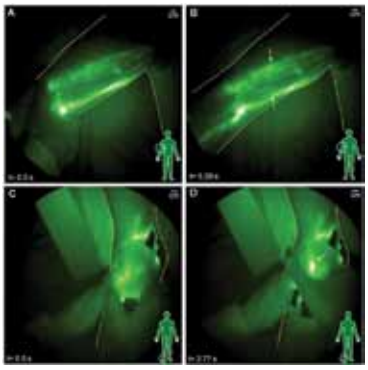
J. C. Rasmussen, I. C. Tan, M. V. Marshall, C. E. Fife, and E. M. Sevick-Muraca, "Lymphatic imaging in humans with near-infrared fluorescence," *Curr Opin Biotechnol,* vol. 20, pp. 74-82, 2009.

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 in validation of devices/drugs for translational fluorescence molecular imaging

My program focuses upon developing the novel validation steps required to translate "first-in-humans," molecularly targeting, NIR fluorescence (NIRF) imaging agents using calibrated devices designed for optimal sensitivity. Since NIRF imaging is comprised of a combinational drug/device product, it first requires device validation for detecting fluorescence from agents targeting disease markers at pico- to femto-molar concentrations. My current research interest focuses on developing and deploying National Institute of Standards and Technology (NIST) traceable phantoms that can be used to develop specifications for platforms of NIRF molecular imaging devices. The phantom is employed in our own NIRF camera systems (qualified under FDA-approved microdosing studies), for intraoperative guidance based upon a NIRF targeted molecular imaging agents.

In addition, I have expanded the utility of fluorescence imaging into far red gene reporters. With collaborators at BCM, I am using imaging to longitudinally track *B. anthracis* infection as well as prostate cancer progression using far-red gene reporters (IFP1.4 and iRFP). The collaborative work is made possible by a custom ICCD camera based fluorescence imaging device installed with optimized filter combinations to improve measurement sensitivity.

RESEARCH PROJECTS

- Developing and optimizing NIRF imaging systems with optimized filter combination and a high sensitive ICCD camera for molecular imaging and tomography.
- Imaging prostate tumor progression and B. anthracis infection with far-red gene reporters iRFP and IFP1.4 and validating NIR fluorophore-labeled mAbs for targeting cancer in preclinical model.
- Working with NIST to develop a traceable fluorescent solid phantom.

KEY PUBLICATIONS

Sevick-Muraca, E.M. and B. Zhu, "The need for performance standards in clnical translation and adoption of fluorescence molecular imaging," *Med Phys,* 40(4):040402, 2013. PMID:23556867.

Zhu, B., Tan, I.C., Rasmussen, J.C., 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,* 11(1): 95-104, 2012. PMID: 22181335

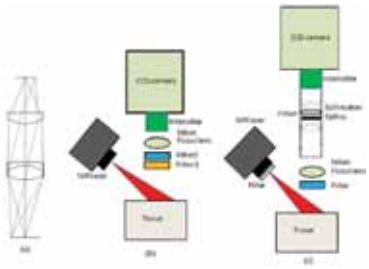
Zhu, B. and E.M. Sevick-Muraca, "Reconstruction of sectional images in frequency-domain based photoacoustic imaging," *Opt Express,* 19(23): 23286-97, 2011. PMID: 22109207

Lu, Y., Zhu, B., Darne, C., Tan, I.C., Rasmussen, J.C., and E.M. Sevick-Muraca, "Improvement of fluorescence-enhanced optical tomography with improved optical filtering and accurate model-based reconstruction algorithms," *J Biomed Opt*, 16(12): 126002, 2011. PMID: 22191919; PMCID: PMC36264420

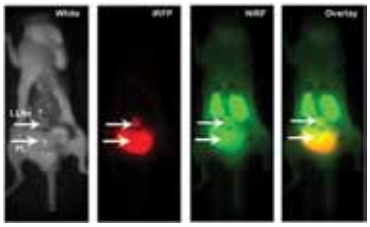
Zhu, B., Rasmussen, J.C., Lu, Y., and E.M. Sevick-Muraca, "Reduction of excitation light leakage to improve near-infrared fluorescence imaging for tissue surface and deep tissue imaging," *Med Phys,* 37(11): 5961-70, 2010. PMID: 21158309; PMCID: PMC2988832

LAB MEMBERS

Coadvised: Grace Wu, Nathaniel Wilganowsk, Holly Robinson



Schematic of the collimation optics (a) and the ICCD NIRF imaging system before (b) and after (c) integration of filtering and collimation schemes.



Overlay of iRFP fluorescence and NIRF images shows that IRDye 800 labeled mAb7.4 targets the primary lesion (PL) and metastasized lumbar LNs (LLNs) from a representative mouse.

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 of 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 to the UTHealth 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 within the Center

- Protein Chemistry
- Proteomics
- Systems Biology
- Proteomics Core Laboratory of the Center for Clinical and Translational Sciences
- 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).

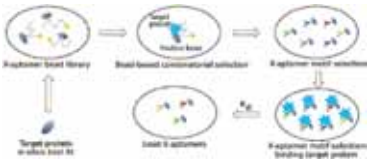
Xianbin Yang, Li Na, David G. Gorenstein, Strategies for the discovery of therapeutic aptamers, *Expert Opinion in Drug Discovery*, Volume 6, Number 1, January 2011, pp. 75-87(13). PMID: 21359096; PMCID: PMC3045091. doi: 10.1517/17460441.2011.537321

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).

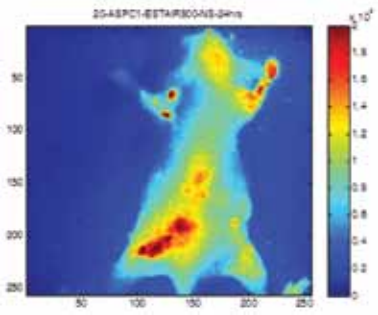
Weiguo He, Miguel-Angel Elizondo-Riojas, Xin Li, Ganesh Lakshmana Rao Lokesh, Anoma Somasunderam, Varatharasa Thiviyanathan, David E. Volk, Ross H. Durland, Johnnie Englehardt, Claudio N. Cavasotto, and David G. Gorenstein "X-Aptamers: A bead-based selection method for random incorporation of drug-like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

LAB MEMBERS

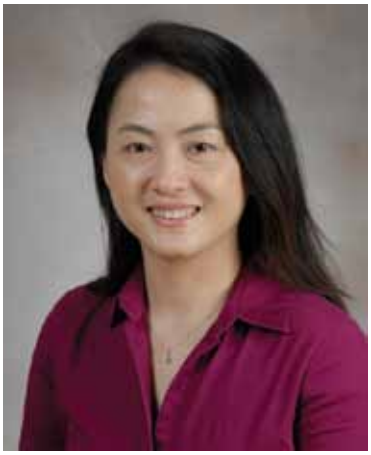
Research Scientists: Lokesh Rao, PhD, Hongyu Wang, PhD, Li Li, PhD
Research Assoc.: Xin Li, MS
Post Docs: Miguel-Angel Elizondo-Riojas, Weiguo He, Sai Gandham
Medical Students: Angela Sung, Max Polansky
Graduate Student: Kurtis Anderson



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 diagnosis is an emerging field because it allows researchers and physicians to use light as therapeutic and diagnostic tools in a fast and non-invasive manner. Our research focuses on the development and application of various optical techniques, especially Raman spectroscopy (RS), for disease diagnosis, therapy response evaluation as well as guidance of surgery. RS probes molecular structure and 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 Raman reporters and targeting agents to gold nanoparticles, such SERS methods can detect biomarkers in body fluid in up to femtomole 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
- Nanoparticles for multifunctional imaging
- Raman imaging for pathogenesis

KEY PUBLICATIONS

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).

X. Bi, C.A. Patil, C.C. Lynch, G.M. Pharr, A. Mahadevan-Jansen, and J.S. Nyman. Raman and mechanical properties correlate at whole bone- and tissue-levels in a genetic mouse model. 2011, *Journal of Biomechanics*. 44: 297-303 (2011).

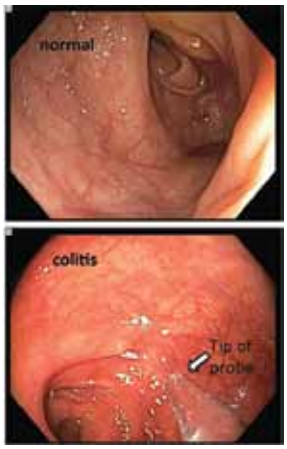
X. Bi, A. Walsh, A. Mahadevan-Jansen and A. Herline, Development of spectral markers for the discrimination of ulcerative colitis and Crohn's disease using Ramans spectroscopy, 2011, *Disease of the Colon and Rectum*, 54(1), 48-53 (2011).

S. Nyman, C.C. Lynch, D.S. Perrien, S. Thiolloy, E.C. O'Quinn, C.A. Patil, X. Bi, G.M. Pharr, A. Mahadevan-Jansen, and G.R. Mundy. Differential effects between the loss of MMP-2 and MMP-9 on structural and tissue-level properties of bone. 2011, *Journal of Bone and Mineral Research*, 26(6), 1252-60 (2011)

JS Nyman, AJ Makowski, CA Patil, TP Masui, EC O'Quinn, X Bi, SA Guelcher, DP Nicollela, A Mahadevan-Jansen, Measuring Differences in Compositional Properties of Bone Tissue by Confocal Raman Spectroscopy, 2011, *Calcified Tissue International,* 89(2): 111-22 (2011).

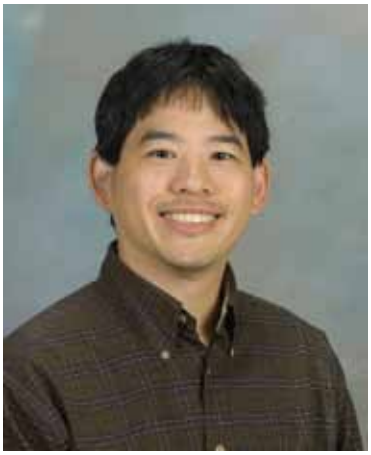
LAB MEMBERS

Postdoc: Hao Ding, Rameshwar Rao Tatavarty
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 and osteolytic lesions are show in representative 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 from the tibial metaphysis. A mixture of osteolytic lesions (yellow arrow heads) and osteoblastic lesions (red arrows) are easily distinguishable in both the radiograph and microCT images which were used to characterize the predominance of these lesion types in each specimen. C) Mean Raman spectra from the tumor-bearing tibiae (dashed line) and the contralateral controls (solid line). Selective Raman bands were marked with biochemical assignments. D) Illustration of Raman spectra collection spots (red dots) on the cortex of mouse tibia.



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 four areas of focus:

1. Targeted cancer therapies. Over the last 15 years, therapies have been developed to target the genetic aberrations that drive the development of cancer. While there have been some clear successes, many of the drugs show low response rates, even with carefully selected patient populations. Evidence is now accumulating that this is due in part to crosstalk and complexity within the signaling pathways. To better able to select therapies, we are developing approaches to elucidate the structure of signaling pathways and associate them with response to targeted therapies.

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. Transcriptional regulatory programs. We are also decoding combinatorial transcriptional regulatory programs. Here, we focus on E2F, a family of transcription factors that regulate a range of activities through interactions with cofactors. E2F1 has a unique ability to regulate both cell cycle progression and apoptosis, processes whose decoupling is a fundamental step in the development of cancer. To better understand this, we are investigating the combinatorial interactions that underlie this transcriptional program, and how alterations can lead to the uncontrolled proliferation seen in cancer.

4. 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

- Cell signaling networks and sensitivity to targeted cancer therapies.
- Alterations of drug sensitivity profiles in cancer stem cells.
- Genetic perturbations of Ras signaling.
- Transcriptional regulatory programs of E2F1-driven apoptosis.
- Modeling cell signaling networks with Bayesian statistics.
- Automated planning of genomic data analyses pipelines with expert systems.

KEY PUBLICATIONS

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Chang JT, Gatza ML, Lucas JE, Barry WT, and Nevins JR. A Software Platform for Gene Expression Signature Analysis. *BMC Bioinformatics* 12(443), 2

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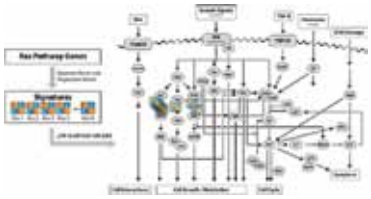
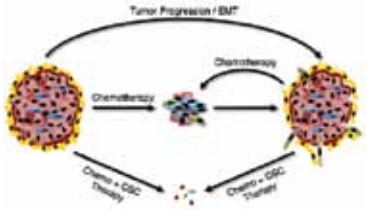
Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, Olson JA, Marks JR, Dressman HK, West M, and Nevins JR. Oncogenic pathway signatures in human cancers as a guide to targeted therapies. *Nature* 439(7074): 353-357, 2005.

LAB MEMBERS

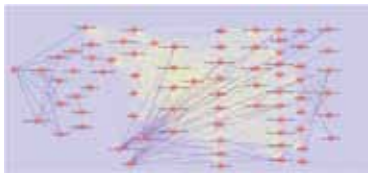
Postdocs: Shiyun Ling, Ph.D., Weina Zhao, Ph.D., Sarah Prjic, Ph.D.
Graduate Student: Jialu Li
Undergraduate Student, Lin Li
Bioinformaticians: Xiaoling Chen, Ph.D., Emily Lu
Technicians: Jessie Sjol, Mike Tisza



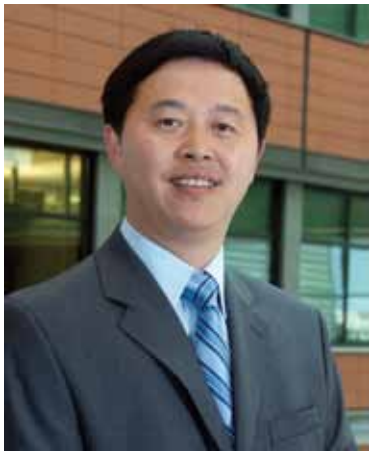
Gene expression signatures predict pathway activation.



The plasticity of cellular phenotypes complicates cancer treatments.



Network representation of gene expression analyses.



Chuantao Jiang, M.D., Ph.D.
Assistant Professor
John S. Dunn Research Scholar

Alpha-synuclein for Parkinson's disease

My research focuses on the functional roles of α-synuclein (α-syn) in the survival of Dopaminergic (DA) neurons and the pathogenesis of Parkinson's disease (PD) so as to uncover a new path in α-syn based therapeutic strategies.

PD and other synucleinopathies are defined by the manifestation of insoluble intracellular aggregates consisting, to varying degrees, of the protein α-syn. α-Syn is a presynaptic protein that is implicated as causative in both familial and sporadic forms of the disease; consequently, tremendous effort has aimed at removing this protein as a therapeutic strategy in PD. Our previous PD immunotherapy studies focus on immunization of human α-syn transgenic mice with non-native α-syn conformations, which showed higher immunogenicity as compared to the native α-syn. The immunization resulted in a two-fold increase of α-syn antibodies and correspondingly, lower α-syn levels in brains of PD mice. Unexpectedly, we did not observe the demonstrable behavioral and pathological improvement in the immunized mice. In another collaborative study, we explored the use of recombinant adeno-associated virus (AAV)-mediated targeted knockdown of α-syn expression as a potential therapeutic intervention for PD. We observed a significant loss of α-syn in mature *substantia nigra pars compacta* (SNc) DA neurons and substantial DA neuronal loss. Importantly, this neuronal loss could be rescued by co-expression of rat α-syn, demonstrating that neuronal loss was explicitly owing to a toxic loss-of-function (LOF) of α-syn. As combined with both studies, we envision that the expression of α-syn is important for the survival and function of DA neurons and the strategy of eliminating α-syn is not protective, but instead is toxic to DA neurons. If our central hypothesis is proven true, the findings will not only further our understanding of the importance of this PD-linked protein in the neurodegeneration, but also uncover a new path in α-syn based therapeutic strategies aimed at preserving rather than removing this crucial protein. Though this hypothesis is under examination using PD models, the findings will benefit the much broader field of synucleinopathies.

We are also interested in how α-syn and its antibodies can be used to diagnose PD at early stage and/or to monitor disease progression. We have observed increased α-syn antibodies in certain PD patients and we will sought to identify some epitope specific α-syn antibody(ies) as better biomarker for early diagnosis/staging of PD.

RESEARCH PROJECTS

- Biomarkers for early diagnosis of Parkinson's disease (PD)
- The functional roles of α-synuclein in DA neurons and PD pathogenesis

KEY PUBLICATIONS

Jiang CT, Wan X, He Y, Pan T, Jankovic J, Le W. (2005) Aging-dependent dopamine dysfunction in Nurr1 Knock-out Mice. *Exp Neurol.* 191: 154-162.

Jiang CT and Chang JY. (2007). Isomers of human alpha-synuclein stabilized by disulfide bonds exhibit distinct structural and aggregative properties. *Biochemistry.* 46:602-609.

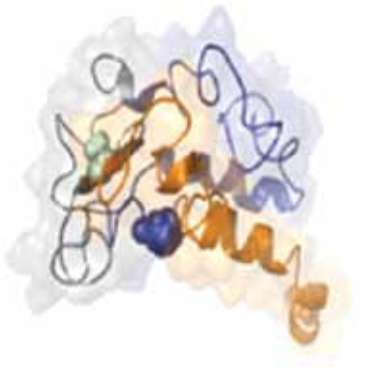
Jiang CT, Xiong W, Lu BY, Gonda MA, Chang JY. (2010) Synthesis and Immune Response of Non-native Isomers of Vascular Endothelial Growth Factor. *Biochemistry.* 49:6550-6556.

Hong DP, Xiong W, Chang JY, Jiang CT*. (2011) The role of the C-terminus of Human alpha-Synuclein: Intra Disulfide Bonds between the C-terminus and Other Regions Stabilize Non-Fibrillar Monomeric Isomers. *FEBS Letters.* 585:561-566.

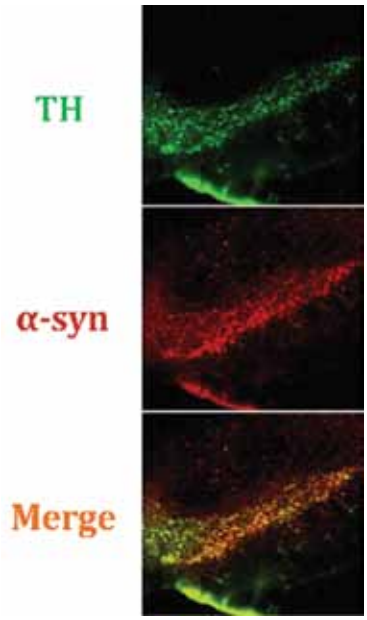
Xiong W, Hong DP, Chang JY, Mandredsson FP, Jiang CT*. (2012) Increased alpha-synuclein antibodies reduce brain alpha-synuclein levels without improving behaviors and pathology (In preparation)

LAB MEMBERS

Research Associates: Wei Xiong (Currently appointed in Dr. Qingyun Liu's laboratory at TTI)
Dong-Pyo Hong (Currently appointed as Assistant Professor at University of South Florida)



Alpha-synuclein Protein



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

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

One of our extraordinary scientific achievements from basic research to innovative human application was the success in hand-made assembly of the International Space Station in the most hostile environment that man ever had to face (spatial void, absence of gravity, extreme temperatures). During the preparation (O_2 prebreathe) for extra-vehicular activities (EVAs), the decompression from sea-level pressure to its third may lead to the presence of bona fide nano-, micronuclei of gases or microbubbles in blood, brain or other tissues forming and growing in situ by cavitation or tribonucleation. This extensive collaborative effort between NASA and several North American institutions led to products and procedures that were delivered to NASA such as the decrease from 24-72 hours EVA preparation down to two hours. Members of the team received several awards from NASA for those achievements. Special skeletal muscle exercise prevents potential adverse events (neurological, pain,...) to occur. Non-invasive near infrared spectroscopy (NIRS) allowed observation of instantaneous variations of total, oxygenated and deoxygenated hemoglobin/myoglobin concentrations in microcirculatory networks of active limbs during the dynamic exercise that was used to for the successful two-hour O_2-prebreathe. Our conclusion was that besides N_2 tissue washout another unknown exercise-induced effect may have further enhanced the protection possibly mediated via the anti -inflammatory effect of exercise, gas micronuclei reduction, NO pathways or other molecular mechanisms. Experience of microbubbles also leads to other applications. NanoMedicine, in a non-detrimental way, uses encapsulated gas microbubbles as drug-loaded liposomes to target tissues (tumors, ...); cavitation-induced of encapsulated microbubbles has an effect on drug release. New challenges will be to develop biomarkers (X-aptamers) or protein discovery to elucidate exercise-induced and O_2-induced neurogenesis and their implications for cognitive plasticity, and characterize the molecular mechanisms that regulate O_2-induced neurogenesis, neural differentiation in human neural stem cells & proliferation of cancer stem cells.

RESEARCH PROJECTS

- Understand the role of gases (O_2, CO_2) on neuronal oxygen consumption (effects on cerebral circulation and vigilance) using functional MRI, and development of "stress-aptamers".
- Develop biomarkers (X-aptamers) or protein discovery to elucidate exercise-induced and O_2-induced neurogenesis and their implications for cognitive plasticity.
- Characterize the molecular mechanisms that regulate O_2-induced neurogenesis, neural differentiation in human neural stem cells.
- Study the O_2-induced molecular mechanisms that regulate cancer stem cells.

KEY PUBLICATIONS

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

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

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

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

Foster PP, Rosenblatt KP, Kuljiš RO. Exercise-induced cognitive plasticity, implications for mild cognitive impairment and Alzheimer's disease. *Front Neurol* 2: 28, 2011.

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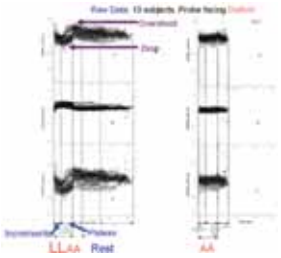

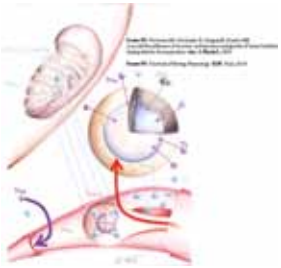

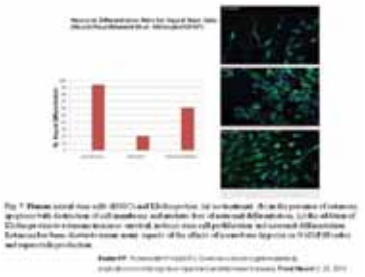

Near infrared spectroscopy (deltoid muscle). Coated bubbles & system of transport-diffusion-delivery of gases. Functional MRI, Human neural stem cells, cognitive plasticity & biomarkers.

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-κB activation pathway. *Journal of Biological Chemistry* 286: 37187-37195. PMID: 21900239.

Fisher, W.G., Lucas, J.E., Mehdi, U., Qunibi, D.W., Garner, H.R., Rosenblatt, K.P., and Toto, R.D. (2011) A Method for Isolation and Identification of Urinary Biomarkers in Patients with Diabetic Nephropathy. *Proteomics-Clinical Applications* 5: 603-612 (Co-Senior Author; Epub Sept. 28, 2011).

Zhu, Q.–S., Rosenblatt, K.P., Lahat, G., Brobey, R., Bolshakov, S., Nguyen, T., Lazar, A., Dicker, A., Mills, G.B., Hung, M.-C., and Lev, D. (2011) Vimentin is a novel AKT1 downstream target in soft-tissue sarcomas. *Oncogene* 30: 457-470. PMID: 20856200

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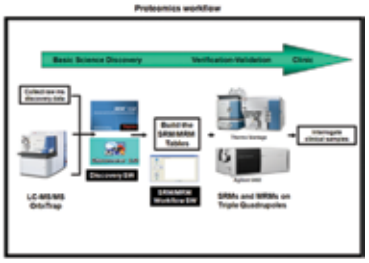
Rosenblatt, K.P., Huebschman, M.L., and Garner, H.R. (2012) Construction and Hyperspectral Imaging of Quantum Dot Lysate Arrays. *In Methods of Molecular Biology: Individualized Molecular Medicine.* Espina, V. and Liotta, L.A., eds. (New York: Humana Press, Inc.), pp 311-324.

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

LAB MEMBERS

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



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.

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 are also 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 from biobank samples
- Develop targeting DNA molecules for drug delivery and imaging of tumors
- Structural studies of non-steroidal anti-inflammatory drug complexes
- Development of next-generation X-aptamers (DNA)

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.* DOI: 10.1021/bi300471d.

1H Nuclear Magnetic Resonance (NMR) Metabolomic Study of Chronic Organophosphate Exposure in Rats, T. Alam, M. Neerthilingam, K. Alam, D. E. Volk, S. Sarkar, S. Ansari, & B. Luxon, B., *Metabolites,* 2(3):479-495 2012.

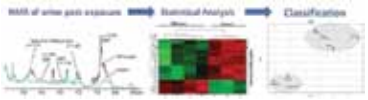
Insight into NSAID-induced membrane alterations, pathogenesis and therapeutics: characterization of interaction of NSAIDs with phosphatidylcholine. L.M. Lichtenberger, V. Jayaraman, J.R. Doyen, R.G. O'Neil, E.J. Dial, Y. Zhou, D.E. Volk, D.G. Gorenstein, U. Marathi, M.B. Boggara and R. Krishnamoorti,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

NMR Structure of human thymosin alpha-1 M.-A. Elizondo-Riojas, S.M. Chamow, C.W. Tuthill, D.G. Gorenstein and D.E. Volk, *Biochem & BioPhys. Res. Comm.* 416:356-361, 2011

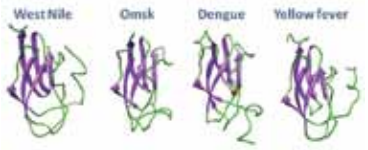
Thioaptamer Conjugated Liposomes for Tumor Vasculature Targeting. A.P. Mann, R.C. Bhavane, A. Somasunderam, B.L. Montalvo-Ortiz, K.B. Ghaghada, D. Volk, R. Nieves-Alicea, K.S. Suh, M. Ferrari, A. Annapragada, D.G. Gorenstein, T. Tanaka, *Oncotarget* 2(4), 298-304, 2011.

LAB MEMBERS

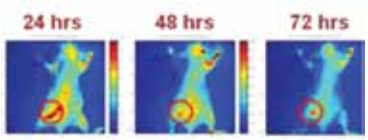
Bioinformatician: Emily Lu, PhD
Research Scientists: Lokesh Rao, PhD, Hongyu Wang, PhD
Research Assoc.: Xin Li, MS, Li Li, PhD
Post Docs: Miguel-Angel Elizondo-Riojas, PhD, Weiguo He, PhD
Medical Students: Angela Sung, Max Polansky



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 diseases and trauma. Regenerative medicine is the process of replacing or regenerating human cells, tissues, or organs to restore or establish normal function. This field holds the promise of regenerating damaged tissues and organs in the body by replacing damaged tissue and/or by stimulating the body's own repair mechanisms to heal previously irreparable tissues or organs. Implicit in the successful design, implementation, and application of regenerative medicine approaches to the repair of a damaged tissue or organ is the reliance on the unique biological properties of specialized cells: stem cells.

The mission statement of the Center for Stem Cell and Regenerative Medicine at the IMM 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. While it is therefore implicit that any such program would span basic-translational-clinical research, it is essential that such an endeavor is ultimately underpinned by excellence in fundamental stem cell research. The center has successfully recruited a multidisciplinary faculty with the appropriate breadth of expertise, innovation, and scientific rigor in the discipline of stem cell biology with the dual intention to promote the excellence and innovation of research within the center and secondly to ensure the quality and appropriateness of stem cell based translational research initiatives emanating from the center.

At present, center faculty with primary appointments in the IMM, Neurosurgery, and Pediatric Surgery are pursuing research for therapeutic application focused on five organ systems (Central Nervous System: Spinal Cord Injury, Stroke, Traumatic Brain Injury; Hematopoietic System: Sickle Cell Anemia, Wiskott-Aldrich Syndrome, Mantle Cell Lymphoma; Adipose System: Cancer, Diabetes, Obesity; Musculo-Skeletal System: Muscular Dystrophy, Osteoarthritis, Sports Injury; and Respiratory System: Cystic Fibrosis, Surfactant Protein B Deficiency, Alpha 1 Anti-Trypsin Deficiency). By interfacing effectively with other programs and institutions within UTHealth, the center also acts as a focus to stimulate the development and implementation of novel cellular therapies for a range of diseases and disorders.

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

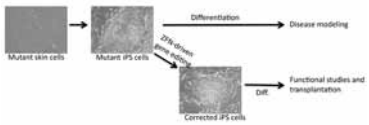
Brian Davis, Ph.D.
Associate Professor
Director of the Center for Stem Cell and Regenerative Medicine
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

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

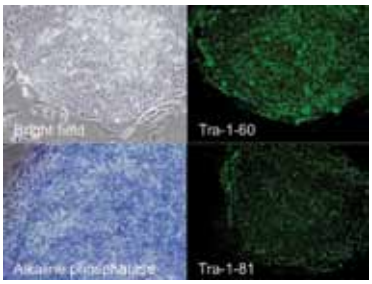
My laboratory has as its primary objective the sequence-specific genetic correction of mutations in the chromosomal DNA of induced pluripotent stem (iPS) cells derived from patients with inherited disorders affecting the lung or blood system, with the ultimate goal of developing stem/progenitor cell-based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

RESEARCH PROJECTS

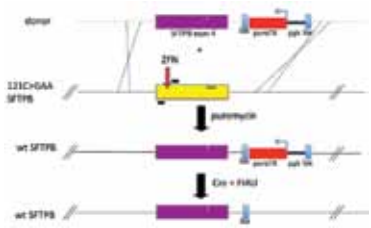
- Correction and Lung Differentiation of iPS cells from Inherited Lung Diseases (Cystic Fibrosis, Surfactant Protein-B Deficiency, Alpha 1 Anti-Trypsin Deficiency)
- Correction and Blood Differentiation of iPS cells from Inherited Blood Disorders (Wiskott-Aldrich Syndrome, Sickle Cell Anemia)
- Characterization of Spontaneous Gene Mutation Resulting in Correction of Inherited Wiskott-Aldrich Syndrome Defects



Application of Mutant and Corrected Induced Pluripotent Stem Cells



Characterization of Induced Pluripotent Stem Cells



Molecular Strategy for Correction of Surfactant Protein B Deficiency Mutation in Induced Pluripotent Stem Cells

KEY PUBLICATIONS

Goncz KK., Prokopishyn NL., Abdolmohammadi A., Bedayat B., Maurisse R., Davis BR.,Gruenert DC. SFHR-Mediated Modification of Genomic β-Globin Sequences in Human Hematopoietic Stem/Progenitor Cells. *Oligonucleotides* 16:213-24, 2006.

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.

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LAB MEMBERS

Research Scientist: Dr. Ana M. Crane
Postdoctoral Fellows: Dr. Philipp Kramer, Dr. Xuan Shirley Li, Dr. Rasoul Pourebrahamin
Ph.D. Students: Jacquelin Bui, Zita Garate (visiting student from Spain), Tamara Laskowski
Technician: Wei Liao

Qi Lin Cao, Ph.D.
Associate Professor

Stem cells for neurological diseases

Despite extensive research, the central nervous system has little ability for repair with no therapeutic approach current available to restore the functions after many neurological diseases. Stem cells have shown great therapeutic potential for neurological disorders and may represent an effective novel therapy for these devastating diseases. Grafted stem cells can replace the lost neural cells, such as neurons, oligodendrocytes, or astrocytes. They also can facilitate host repair by suppressing secondary injury to preserve more neural tissue and enhance plasticity of spared neuronal circuits in the host. Human embryonic stem cells (ESCs) may be an ideal source of neural stem and progenitor cells for transplantation to treat neurological diseases. They can expand readily without depletion and differentiate into neural stem and progenitor cells to derive various neural cell types. Inducible pluripotent stem cells (iPSC), which are newly developed remarkable pluripotent, ESC-like cells reprogrammed from somatic cells, 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. However, several critical issues need to be resolved in order to realize the full therapeutic potential of human ESC or iPSC for neurological diseases. It still remains very challenging but is essential to direct human ESC or iPSC to differentiate into desired neural stem or precursor cells *in vitro* and then purify these cells before transplantation without the contamination of undifferentiated ESC or iPSC since undifferentiated ESC or iPSC will form teratoma *in vivo* after transplantation. It also remains to be determined the ideal grafting cell types to restore functions for different neurological diseases. Importantly, the mechanisms by which NSC or NPC grafting might enhance the functional recovery are unknown. My laboratory is studying the molecular mechanisms to regulate the neural differentiation of human ESCs and iPSCs 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 human ESCs- and iPSC-derived neural stem or precursor cells 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 to regulate neural differentiation of human ESCs and iPSCs.
- The long-term therapeutic efficacy and safety of human ESCs and iPSC-derived neural stem or precursor cells for spinal cord injury and stroke.
- Identification and characterization of key regulators for oligodendrocyte differentiation and remyelination after spinal cord injury.
- The molecular mechanisms to regulate astrogliosis and the functions of astrogliosis after spinal cord injury, traumatic brain injury, or stroke using conditioned knockout mice models.
- Screening and identification of novel neuroprotection agents for spinal cord injury.

KEY PUBLICATIONS

Cheng XX, Wang YP, He Q, Qiu MS, Whittemore SR and Cao QL (2007) BMP signaling and olig1/2 interact to regulate the differentiation and maturation of adult oligodendrocyte precursor cells. *Stem Cells:* 25: 3204-3214.

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.

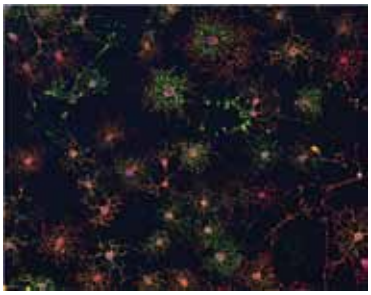
Wang YP, Cheng XX, He Q, Kim DH, Whittemore SR and Cao QL (2011) Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult OPCs by increasing the expression of bone morphogenetic proteins. *J Neurosci* 31(16):6053– 6058.

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

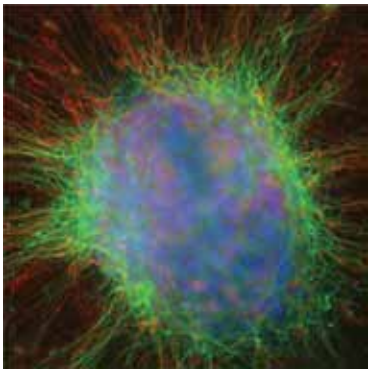
Fan CL, Zheng YY, Cheng XX, Qi XB, Bu P, Luo XG, Kim DH and Cao QL (2013) Transplantation of D15A-expressing glial-restricted-precursor-derived astrocytes improves anatomical and locomotor recovery after spinal cord injury. *Int J Biol Sci.* 2013;9(1):78-93.

LAB MEMBERS

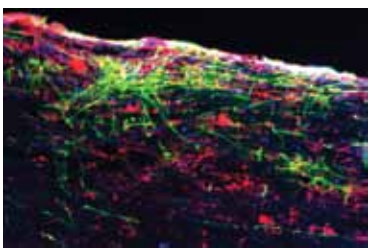
Postdoc Research Associate: Hezou Lu, Shaohui Wang, Yiyan Zheng
Research Assistant: Io Long Chan
Visiting Scholar: Xue Xu



Oligodendrocyte precursor cells in culture



Neuronal differentiation of human ESCs *in vitro*



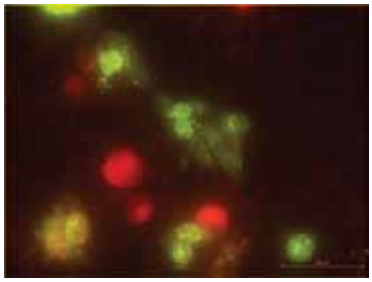
The survival and differentiation of grafted neural stem cells after spinal cord injury

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.



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

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.

Walker P, Harting MT, Jimenez F, Pati S, Dash PK, Cox CS. Direct intrathecal implantation of mesenchymal stromal cells leads to enhanced neuroprotection via an NFκβ mediated increase in IL-6 production. *Stem Cells Dev* .19: 867-876, 2010.

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

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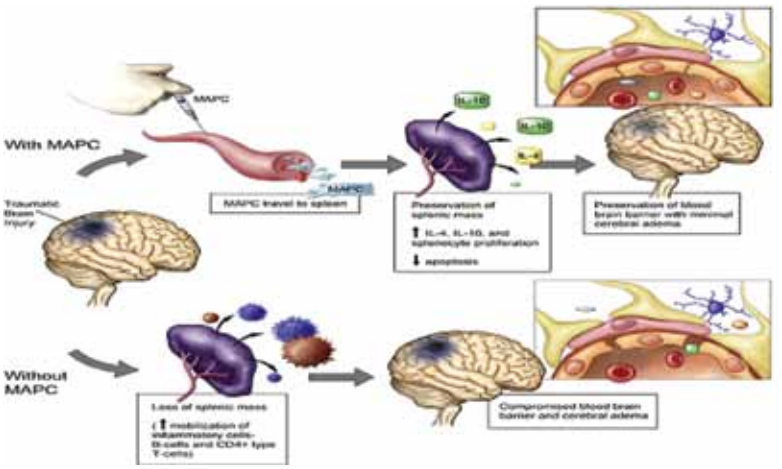
LAB MEMBERS

Supinder Bedi, Ph.D- Instructor
Robert Hetz, M.D.-Brown Foundation Post-Doctoral Fellow
Phillipa Smith, M.S.-Flow Cytometry Technician
Chelsea Thomas, B.S.-Medical Student.
Hasan Xue, M.D.-Research Scientist
Fabio Triolo, Ph.D.-GMP center director
Andrew Haven, Ph.D-GMP QA director



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.

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 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 base 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.

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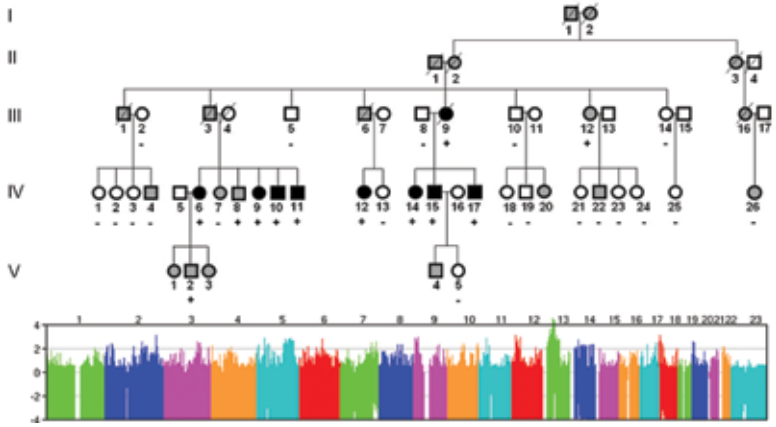
M., Khan, N., Grange, D. K., Mendoza-Londono, R., Bradley, T. J., Olney, A. H., Adès, L., Maher, J. F., Guo, D., Buja, L. M., Kim, D., Hyland, J. C. and Regalado, E. S. (2010), De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction. *American Journal of Medical Genetics 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

The role of adipose stem cells in obesity and cancer

Research in my laboratory encompasses biology of cancer, stem cells, and adipose tissue. Both cancer and obesity progression rely on recruitment of stromal and progenitor cells, as well as on angiogenic blood vessel formation. We discovered the phenomenon of adipose stromal cell (ASC) mobilization in obesity and cancer based on the analysis of clinical specimens. By using animal transplantation models, we have demonstrated trafficking of adipose cells to tumors where they differentiate into pericytes and adipocytes and engage as components of tumor microenvironment, stimulating vascular patency, desmoplasia, and malignant cell survival/proliferation. These findings, now reproduced by other groups, have provided a new insight on the linking between obesity and cancer progression. In collaboration with MD Anderson, we have established a bank of human prostate cancer patient adipose tissue specimens for mechanistic studies. We have performed high-throughput screens for candidate chemokines/receptors mediating adipose cell migration to tumors and are currently in the process of validating individual cytokine signaling pathways in genetic mouse models. Based on our collaboration with N3D Biosciences, we are developing a 3D levitation co-culture system simulating tumor microenvironment based on human cancer cells and primary stromal/vascular cells from patient adipose tissue as the microenvironment component (SBIR grant submitted).

The laboratory has extensive experience in combinatorial peptide library screening, which we perform in live animals to isolate molecules binding to surface receptors differentially expressed on specific cell types. Through this approach, we discovered prohibitin (Phb) as a protein marking adipose endothelium and adipocytes and annexin 2 (Anx2) as its cell surface interactor. We are now characterizing the functions of the Anx2/Phb complex in molecular transport, adipogenesis, and angiogenesis. We have demonstrated binding of the matricellular protein SPARC to alpha5/beta1 integrin on ASC surface. We are analyzing the function of adipose tissue-specific SPARC isoforms (which we discovered) in signaling through alpha5/beta1 and in ASC migration.

Based on combinatorial screens, we recently identified an isoform of decorin (delta-DCN) as the first specific surface marker of adipose progenitor cells. A cell-ablating peptide targeting adipose progenitor cells through binding to delta-DCN has been designed by my group. Our collaborative studies in mice (submitted) show that targeted adipocyte progenitor cytoablation results in compromised WAT expansion despite increased food consumption, thus introducing new prospects for pharmacological obesity prevention. Based on the recently appreciated role of adipose progenitors in tumor growth, we also are testing this compound in mouse cancer models.

RESEARCH PROJECTS

- Tumor microenvironment: the role of adipose tissue cells
- Development of experimental drugs targeting cell populations in obesity and cancer
- Adipose tissue markers and mechanisms of intercellular communication
- Development of imaging compounds targeting brown adipose tissue
- Investigating the role of adipose progenitors in liposarcoma
- Exploring the role of adipose tissue in leukemia progression
- Identification of prostate cancer stem cell markers

KEY PUBLICATIONS

Zhang Y, Daquinag A., Traktuev D., Amaya F., Simmons P. J., March K.L., Pasqualini R., Arap W. and Kolonin M. G. Mouse white adipose tissue is a source of cells that are recruited by tumors and promote cancer progression, *Cancer Research*. 15 (69) 5259-5266, 2009.

Daquinag A., Zhang Y., Amaya F., Simmons P. and Kolonin M.G. Cleavage Fragment of Decorin Interacts with Adiponectin and Resistin on the Surface of Adipose Stromal Cells, *Cell Stem Cell.* 9 (1):74-86, 2011.

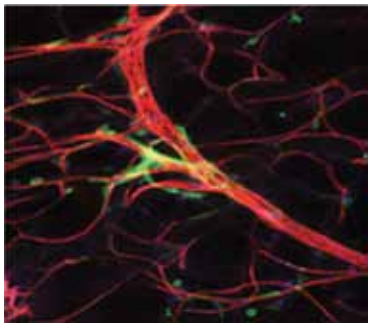
Klopp A.H., Zhang Y., et al., and Kolonin M.G. Omental adipose tissue-derived stromal cells promote vascularization and growth of endometrial tumors. *Clinical Cancer Research.* 18(3):771-782, 2012.

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

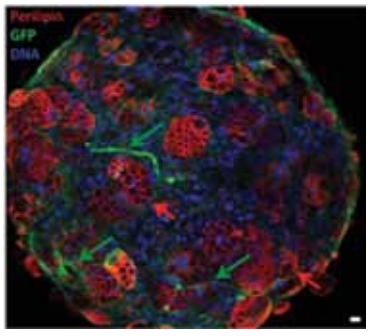
Daquinag A., Souza G. and Kolonin M.G. Adipose tissue engineering in three-dimensional levitation tissue culture system based on magnetic nanoparticles, *Tissue Engineering Part C Methods.* 2012.

LAB MEMBERS

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



Adipose Tissue Vessels (red) with Perivascular Adipose Stem Cells (green).



A spheroid of Adipose Tissue Grown in 3D Culture Displaying Vascular Structures (green) and Adipocytes (red).

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

Enlarge stem cell pool for regenerative medicine

This research team has developed several novel techniques for molecular, cellular and animal-based studies to focus on three major areas of study: 1) exploring the properties of the dedifferentiation/transformation of terminally differentiated cells into various stem cells for regenerative medicine and tissue engineering applications; 2) studying the processes involved with fibrous scar formation and prevention in the injured and diseased tissues of the neuron and musculoskeletal system; and 3) use of 3D printer or updated bioengineering techniques to build 3D soft tissues to repair wound defects with scarless healing. The laboratory also is interested in translational study and clinical application of stem cells and engineered tissue for treating congenital diseases and traumatic injuries. The laboratory has set up a classic tissue or 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 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. We are also building 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 stem cell pool without genetic modification as a cell source for regenerative medicine.
- Adult Embryonic Potential Stem Cells and Application: Obtain natural embryonic potential stem cells from adult tissue for utilization in tissue engineering and regenerative medicine, such as central neurologic disorder and disease.
- Fibrosis and Prevention Studies: Investigate the mechanism behind the fibrosis process after injuries and diseases, and seek methods for prevention 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.

KEY PUBLICATIONS

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.

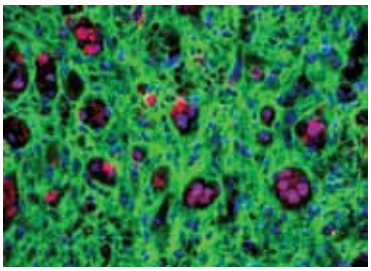
Bellayr I, Holden K, Mu XD, Li Y. Matrix Metalloproteinase inhibition negative affects muscle stem cell behavior. *Int J Clin Exp Pathol* 2013;6(2):124-141.

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* (2012, accepted).

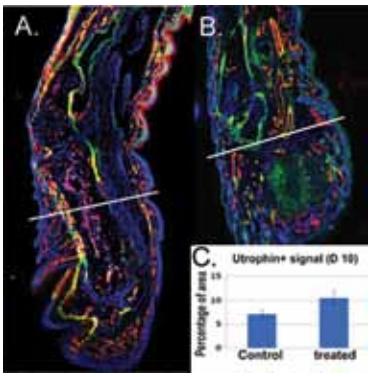
Tang YD*, Xu W*, Pan HY, Li Y. Benefits of dedifferentiated stem cells for neural regeneration. *Stem Cell and Discovery* 2012;2(3):108-121.*First two authors equally contributed to this paper.

LAB MEMBERS

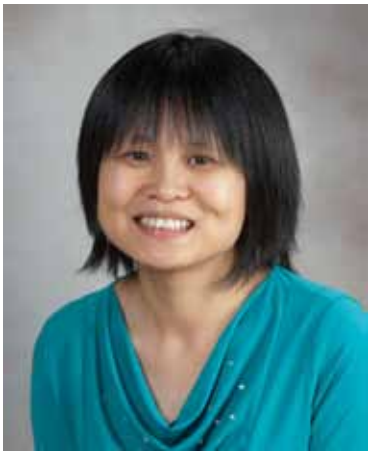
Administrator: Stephanie Baca
Lab senior technician/manager: Haiying Pan
Postdoc research fellow: Dr. Yohan Choi, Dr. Kinga Vojnits
Medical student: Chen Fu
Volunteer student: Lydia Liu



Fibrosis formation and potential mechanisms



Finger regrowth after amputation injury



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 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 reporters by gene targeting in hESCs and hiPSCs for purification and transplantation tracing
- Identification of optimal neural lineage progenitors for cell replacement therapy in spinal cord injury and stroke
- Analysis of ALS patient-specific iPSCs and their neural derivatives
- Characterization of transcriptional regulatory network of OLIG genes

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)

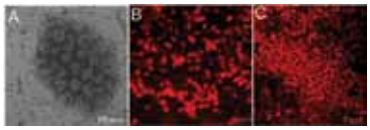
Liu, Y., Rao, M. (2011) Gene targeting in human pluripotent stem cells. *Methods Mol Biol.* 767:355-367.

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

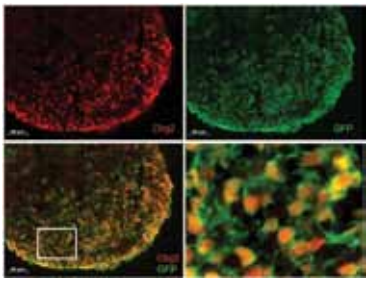
Liu, Y., Thyagarajan, B., Lakshmipathy, U., Xue, H., Lieu, P., Fontes, A., MacArthur, C.C., Scheyhing, K., Rao, M.S., and Chesnut, J.D. (2009). Generation of a platform human embryonic stem cell line that allows efficient targeting at a predetermined genomic location. *Stem Cells Dev* 18, 1459-1472

LAB MEMBERS

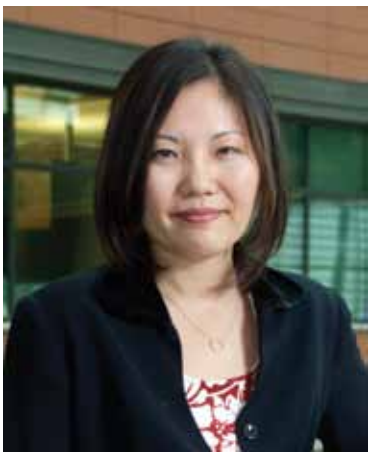
Postdoctoral Fellow: Jianbo Wu
Research Associate: Haipeng Xue
Research Assistant: Jianhu Zhang, Shenglan Li



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



Human embryonic stem cell (hESC) 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 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 also are 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

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

Jung, H-J., Zheng, C., and McCarty, N. (2011) Stem-like tumor cells confer drug resistant properties to mantle cell lymphoma. *Leukemia and Lymphoma.* 52:1066-1079.

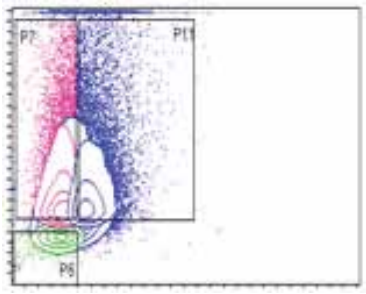
Jung, H-J., Chen. Z., and McCarty, N. (2011) Drug resistant stem-like tumor cells. *Clinical Lymphoma, Myeloma and Leukemia.* 11:131.

Zheng, C., Ayala, P., Wang, M., Fayad, L., Katz, R., Romaguera, J.E., Caraway, N., Neelapu, S.S., Kwak, L., Simmons, P.J., and McCarty, N. (2010) Identification of clonogenic mantle cell lymphoma initiating cells. *Stem Cell Research* 5:212-225.

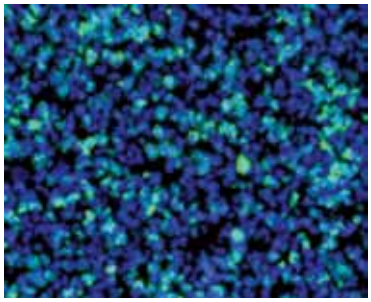
Alvarez Arias, D.A, McCarty, N*., Lu, L., Maldonado, R., Shinohara, M.L., and Cantor, H. (2010) Unexpected role of clathrin adaptor, AP-1 in MHC-dependent positive selection of T cells. *Proc. Natl. Acad. Sci.* 107:2556-2561 *Co-first author.

LAB MEMBERS

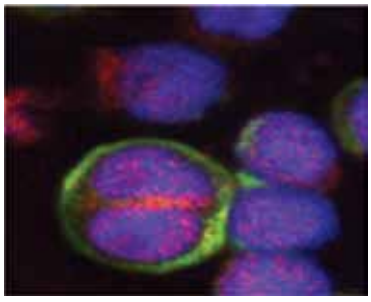
Research Associate: Judy Chen
Research Assistant: Eric Pittman
Graduate student: Albert Teo



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-types *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 human. 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 developed and purified from hPS cells paraxial mesoderm and neural crest progeny with the capacity to expand and differentiate into chondroprogenitor cells. We have also established a condition where these progeny generate hyaline-like cartilage particles that are stably maintained in an ectopic transplantation model. We are currently addressing a question whether under such conditions, the hypothetical human joint progenitor cells, which are supposed to be the precursor of synovial joint components including articular and meniscal chondrocytes and ligaments, have been generated from PS cells.

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 us to generate and purify hemogenic as well as non-hemogenic endothelial progeny from hPS cells, the former of which display no to 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 endothelial cells (e.g. those generated from hPS cells, or isolated from human placenta/cord artery and vein, mouse dorsal aorta, etc.).

RESEARCH PROJECTS

- Specification, prospective isolation and expansion of three embryonic chondroprogenitors (sclerotome, limb mesenchyme and ectomesenchyme) from hPS cells through their corresponding developmental intermediates (i.e. paraxial mesoderm, lateral plate mesoderm and neural crest, respectively).
- Generation, detection and isolation of joint progenitor cells from hPS cells.
- 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

Umeda, K., Davis, B.R., and Nakayama, N. (2012) "Stable expansion chondrogenic activity using human pluripotent stem cell-derived ectomesenchymes" In review.

Umeda, K., Zhao, J., Simmons, P., Stanley, E., Elefanty, A., and Nakayama, N. (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.

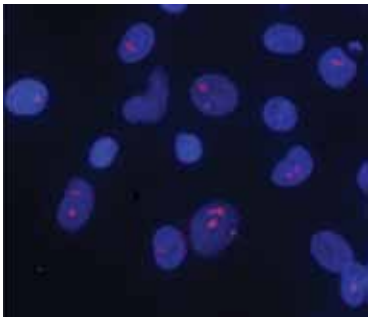
Tanaka, M., Jokubaitis, V., Wood, C., Wang, Y., Brouard, N., Pera, M., Hearn, M., Simmons, P., and Nakayama, N. (2009) "BMP inhibition stimulates WNT-dependent generation of chondrogenic mesoderm from embryonic stem cells". *Stem Cell Res.*, 3:126-141.

Nakayama, N., Duryea, D., Manoukian, R., Chow, G., and Han, C.-Y.E. (2003) "Macroscopic cartilage formation with embryonic stem cell-derived mesodermal progenitor cells". *J. Cell Sci.* 116, 2015-2028.

Nakayama, N., Lee, J., and Chiu, L. (2000) "Vascular endothelial growth factor synergistically enhances bone morphogenetic protein-4-dependent lymphohematopoietic cell generation from embryonic stem cells in vitro" *Blood* 95, 2275-2283.

LAB MEMBERS

Research Associate: Qing Yan, PhD
Research Technician: Suprita Trilok
Animal Specialist: Nadine Matthias, DVM



Paraxial mesoderm derived from human PS cells: human MEOX1 protein in nucleoli (stained pink)

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 friction and stretching, impact function, development, specification, and expansion of stem cells and their precursors.

One arm of our research is designed to address how biomechanical force activates the embryonic hematopoietic program and how we might use this information in the laboratory to expand improved sources of hematopoietic cells that can be used for patients in the clinic. 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 biomechanics, microfluidics, pharmacology, embryonic stem cell modeling, 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 in patients who are treated with cellular therapies. 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 anti-inflammatory programs. We utilize culture-based assays, cellular phenotyping, and mesenchymal stem cell-based therapy models of stroke and traumatic brain injury as readouts of response to mechanical stimuli.

RESEARCH PROJECTS

- Mechanobiology of blood development
- Biomechanical modulation of anti-inflammatory genetic programs in mesenchymal stem cells

KEY PUBLICATIONS

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.

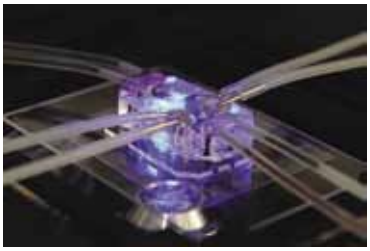
Naveiras, O., Nardi, V.*, Wenzel, P.L.*, Hauschka, P.V., Fahey, F., and Daley, G.Q. (2009). Bone marrow adipocytes as negative regulators of the hematopoietic microenvironment. *Nature* 460: 259-263. *Equal contribution.

Wenzel, P.L.*, Wu, L.*, de Bruin, A., Chong, J.L., Chen, W.Y., Dureska, G., Sites, E., Pan, T., Sharma, A., Huang, K., Ridgway, R., Mosaliganti, K., Sharp, R., Machiraju, R., Saltz, J., Yamamoto, H., Cross, J.C., Robinson, M.L., and Leone, G. (2007). Rb is critical in a mammalian tissue stem cell population. *Genes & Development* 21(1): 85-97. *Equal contribution.

LAB MEMBERS

Research Assistant: Siobahn Evans
Research Associate: Miguel Diaz
Administrative Assistant: Stephanie Baca (Pediatric Surgery)

Blood flow in the embryonic aorta promotes specification and enhances function of stem cells by generation of three distinct mechanical forces



Microfluidic application of frictional and stretching type forces allows us to simulate the natural hematopoietic environment and promises to inform the design of scalable tools for generation and expansion of transplantable cells used in cellular therapies

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 earl stages of hematopoietic precursor self-renewal and differentiation. This study can serve as a model for the analysis of cell self-renewal and differentiation in general and provide insight for efficient expanding and manipulating hematopoietic precursor and stem cells, including reprogramming partially differentiated cells to return them to a self-renewing state.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Wu, J. Q., 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. and Snyder, M. (2008). RNA polymerase II promoter proximal stalling: Loading at the start line prepares genes for a sprint. *Genome Biology.* 9:220

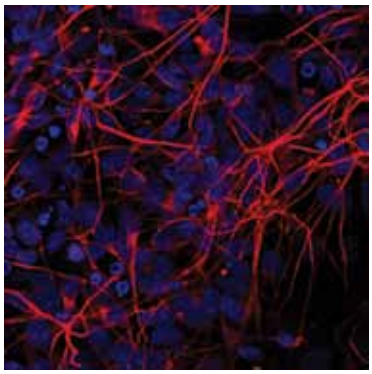
Wu, J. Q., Habegger, L., Noisa, P., Szekely, A., Qiu, C., Hutchison, S., Raha, D., Lin, H., Egholm, M., Weissman, S., Cui, W., Gerstein, M., and Snyder, M. (2010). Dynamic Transcriptomes during Neural Differentiation of Human Embryonic Stem Cells Revealed by Integrating Short, Long, and Paired-end Sequencing.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

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

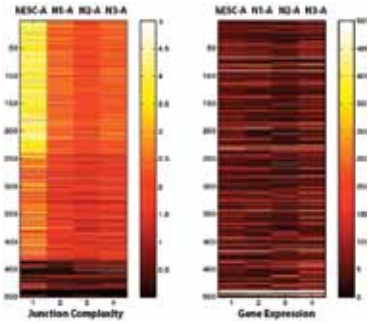
Wu, J. Q., Seay, M., Schulz, V., Hariharan, M., Tuck, D., Lian, J., Du, J., Shi, M., Ye, Z. J., Gerstein, M., Snyder, M., and Weissman, S. (2012). Tcf7 is a key regulator of the self-renewal and differentiation switch in a multipotential hematopoietic cell line. *PLoS Genet* 8(3): e1002565.

LAB MEMBERS

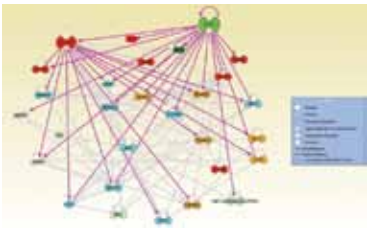
Postdoctoral Fellow: Kenian Chen
Postdoctoral Fellow: Guodong Yang
Research Assistant: Shuyun Deng



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

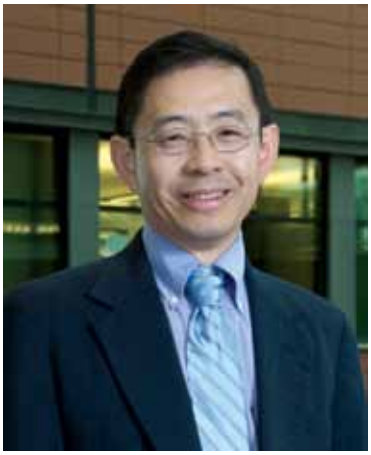
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston for the discovery, development, and 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 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, and the Cancer Prevention and Research Institute of Texas, and have made significant scientific discoveries in the areas of cancer biology and biologics drug development.

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

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.

Supported by a grant from the Texas Emerging Technology Fund and as part of the Texas Therapeutics Institute, the 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
- Therapeutic antibody discovery and development

KEY PUBLICATIONS

Fan X, Brezski RJ, Fa M, Deng H, Oberholtzer A, Gonzalez A, Dubinsky WP, Strohl WR, Jordan RE, Zhang N, and An Z. 2012. A single proteolytic cleavage within the lower hinge of trastuzumab reduces immune effector function and in vivo efficacy. *Breast Cancer Res*. Aug 8;14(4):R116.

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

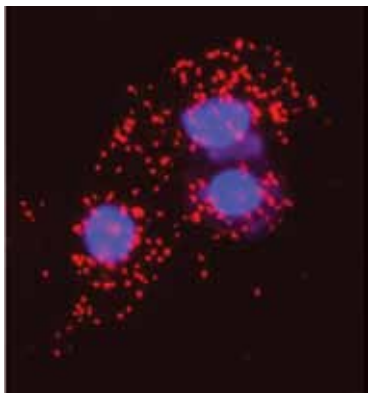
Choi B-K, Fan X, Deng H, Zhang N, and An Z. 2012. ERBB3 (HER3) is a key sensor in the regulation of ERBB-mediated signaling in both low and high ERBB2 (HER2) expressing cancer cells. *Cancer Medicine* 1(1):28-38.

Yang E, Xu L, Yang Y, Zhang X, Xiang M, Wang C, and An Z, and Xingzhong Liu. 2012. Origin and evolution of carnivorism in the Ascomycota (fungi). *PNAS* 109 (2):10960-10965.

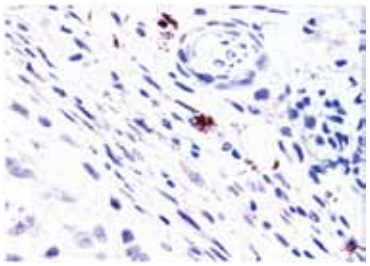
Glantschnig H, Scott K, Hampton R, Wei N, McCracken P, Nantermet P, Zhao J, Vitelli S, Huang L, Haytko P, Lu P, Fisher J, Sandhu P, Cook J, Williams D, Strohl W, Flores O, Kimmel D, Wang F, Z. An Z. 2011. A rate-limiting role for DKK1 in bone formation and the remediation of bone loss in mouse and primate models of postmenopausal osteoporosis by an experimental therapeutic antibody. *J Pharmacol Exp Ther.* 338(2):568-578.

LAB MEMBERS

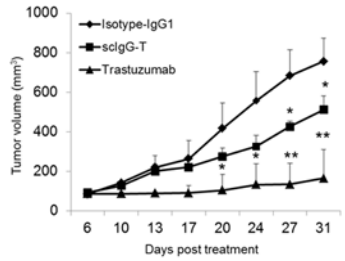
Post Docs: Zhao (George) Huang, Yun Shi, Weixu (Ella) Meng, Qi Tang
Students: Pooja M. Dhupkar, Seema Mukherjee, Lin Xia
Scientists/Research Associates: Byung-Kwon Choi, Hui Deng, Xuejun Fan, Ming Fa



EGFR/HER3 dimerization in breast cancer cells. Cancer Medicine 1(1):28-38, 2012.



Trastuzumab induced immune cell infiltratrion in xenograft breast tumors. Breast Cancer Res. 8:14(4):R116, 2012.



Protease cleavage of trastuzumab compromises its anti-tumor efficacy in xenograft breast tumor model. Breast Cancer Res. 8:14(4):R116, 2012.

Gerald Bills
Professor

Genome mining, biosynthesis and discovery of microbial metabolites for infectious diseases and cancer therapies

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

We are using 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-non-ribosomsal 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 with the goal of building a microbial chemical collection focused on metabolites appropriate for intervention in cancer biology, modulation of human molecular signaling pathways, and in other human therapies. The collection will emphasize Texas-based natural microbial resources, will be promoted among Texas-based screening centers and will result in new chemicals for human therapy.

RESEARCH PROJECTS

- Biosynthesis and pathway engineering of the pneumocandin lipopeptides for improved antifungals.
- 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., 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.

de la Cruz, M., J. Martín, V. González-Menéndez, I. Pérez-Victoria, C. Moreno, J.R. Tormo, N. El Aouad, J. Guarro, F. Vicente, F. Reyes & G.F. Bills. 2012. Chemical and physical modulation of antibiotic activity in Emericella species. *Chemistry & Biodiversity* 9:1095-1113.

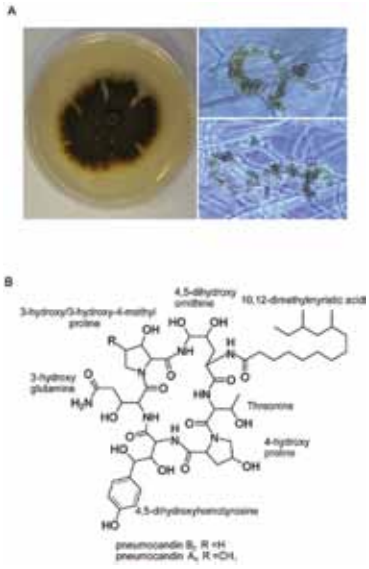
Bills, G.F., A.W. Dombrowski & M.A. Goetz. 2012. The "FERMEX" method for metabolite-enriched fungal extracts. *Methods in Molecular Biology.* Fungal Secondary Metabolism. N.P. Keller & G. Turner. Eds. 944:79-96.

Xu, D, J. Ondeyka, G.H. Harris, D. Zink, J. Nielsen-Kahn, H. Wang, G. Bills, G. Platas, W. Wang, A.A. Szewczak, P. Liberator, T. Roemer & S.B Singh. 2011. Isolation, structure and biological activities of fellutamides C and D from an undescribed Metulocladosporiella (Chaetothyriales) using the genome-wide Candida albicans fitness test. *Journal of Natural Products* 74:1721-1730.

118. Roemer, T., D. Xu, S.B. Singh, C.A. Parish, G. Harris, H. Wang, J.E. Davies & G.F. Bills. 2011. Confronting the challenge of natural product-based antifungal discovery. *Chemistry & Biology* 18:148-164.

LAB MEMBERS

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



The morphology of Glarea lozoyensis in culture and its conidia. B. The structure of the pneumocandin lipopeptide antifungals. BMC Genomics (in review). 2013.

The FERMEX method for producing complex fungal metabolite mixtures. 1. Coarse particle vermiculite. 2. Roller bottles (2 l) on roller bottle machine. 3. Autoclaved vermiculite bottle (1 l) before inoculation and growth of Emericella parvathecia (14 d, 22 °C). 4. Mycelium and vermiculite matrix formed by Trichoderma virens on roller bottle wall. 5. Bottom view of roller bottles showing hollow center. Emericella navahoensis (left), E. parvathecia (right). Methods in Molecular Biology. 944:79-96. 2012.

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.

I have recently been issued 2 U.S. patents (8,298,589 & 8,303,995) based on our discoveries and have seven more pending worldwide. We also have been successful at commercializing these discoveries. Through the formation of Neogenis Labs, Inc., we have been 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

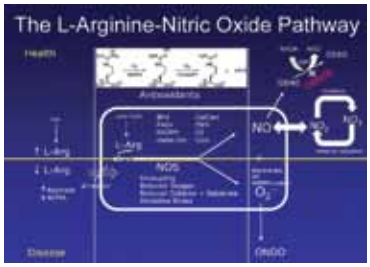
Hord NG, Ghannam J, Garg HK, Berens PD, Bryan NS: Nitrate and nitrite content of human, formula, bovine and soy milks: implications for dietary nitrite and nitrate recommendations *Breastfeeding Medicine* 2010 Oct 19.

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

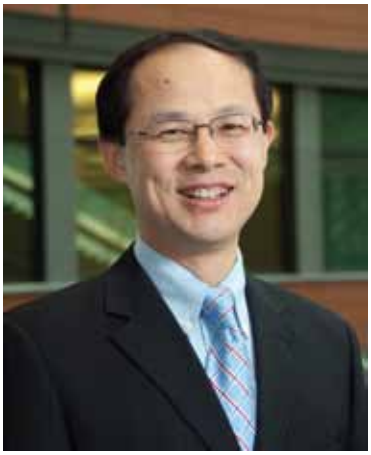
Bryan NS and Loscalzo J (Editors) Nitrite and Nitrate in Human Health and Disease – Springer Humana Press New York ISBN: 978-1-60761-615-3, May 2011

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 studies novel molecular mechanisms of metastasis with the goal of identifying new biomarkers and drug targets for the development of better therapeutics for human cancers.

Metastasis 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 is identifying human genes that may play important but previously unknown roles in cancer metastasis. Signaling pathways and molecular mechanisms of these interesting candidates are under investigation with molecular, cellular, biochemical, genomic, proteomic approaches, and mouse models.

Another exciting research program in our lab is involved in identifying and studying human genes (kinases in particular) as novel regulators of epithelial-mesenchymal transition (EMT) and stem cell phenotypes. 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. The literature search indicated that the majority of >700 kinases in human kinome are still poorly studied. Our lab is employing unbiased functional screens against human kinome to identify kinases as novel regulators of EMT and linking them to stem cell phenotypes and metastasis. Investigation of the molecular mechanisms of these kinases will have a significant impact in expanding our knowledge in the crossroad of exciting and critical areas, such as development, stem cell, drug resistance, and metastasis. These kinases may become new biomarkers and cancer drug targets for the development of novel therapeutics for human cancer.

RESEARCH PROJECTS

- Novel regulators for cancer metastasis
- Epithelial-mesenchymal transition (EMT)
- Cancer stem cell phenotypes and mechanisms
- Acquired resistance to cancer therapeutics.

KEY PUBLICATIONS

Li W*, Bhattacharya N, Ai N, Vrbanac V, Collins M, Signoretti S, Hu Y, Boyce FM, Harlow E, Watnick RS. Identification of human kinases that are essential for proliferation of metastatic cells and promote prostate tumor progression (under review). *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.

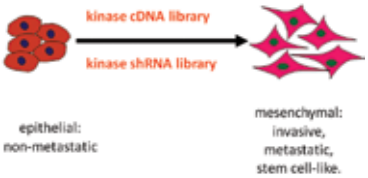
Baldwin A, Li W, Grace M, Harlow E, Münger K and Grueneberg DA. II. Genetic Interaction Screens Identify Alterations in Kinase Requirements Following HPV16 E7 Expression in Cancer Cells. *Proceedings of the National Academy of Sciences USA* (PNAS). 2008 Oct 28;105(43):16478-83.

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)

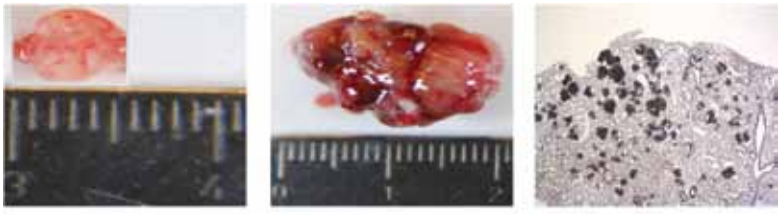
Note: These 4 PNAS papers I-IV were selected as Signaling Breakthroughs of 2008 (the most exciting advances in signaling transduction research in 2008) in the popular annual Editorial Guide of journal *Science Signaling* (formerly *Science STKE*), a Science family journal.

LAB MEMBERS

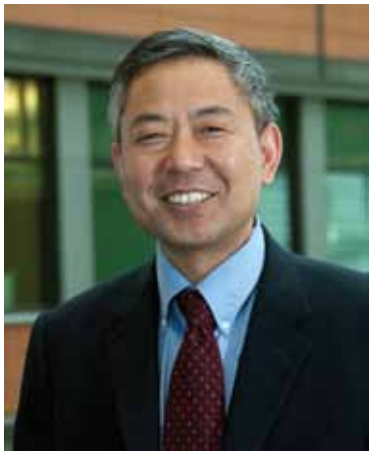
Postdoc Fellows: Linna Li, Haiping Song
PhD student: Mohit Hulsurkar



In search for novel regulators for epithelial-mesenchymal transition and cancer metastasis.



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

Our research is focused on delineating the mechanisms of a group of cell surface receptors that are essential for 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 as potential drug targets and identification of lead molecular as potential anticancer therapeutics. We modulate the activity of these receptors in normal and cancer cells using a variety of techniques and measure their effect on the survival and growth of the cells. Most recently, we successfully identified the factors 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 that these receptors have critical roles in the survival and metastasis of cancer cells and are now in the process of generating leads as potential cancer treatments.

RESEARCH PROJECTS

- Identification of signaling mechanisms of stem cell receptors.
- Determination of the function and mechanism of the receptors in the growth and differentiation of normal and cancer stem cells.
- Validation of the receptors as potential drug targets for regenerative medicine and cancer treatment
- Identification of lead molecules for the discovery and development of novel anticancer therapeutics.

KEY PUBLICATIONS

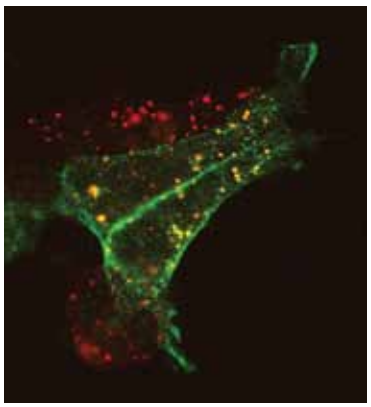
Carmon KC, Lin Q, Gong X, Thomas A, and Liu Q (2012). LGR5 Interacts and Cointernalizes with Wnt Receptors To Modulate Wnt/beta-Catenin Signaling. *Mol Cell Biol* 32:2054-2064.

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.

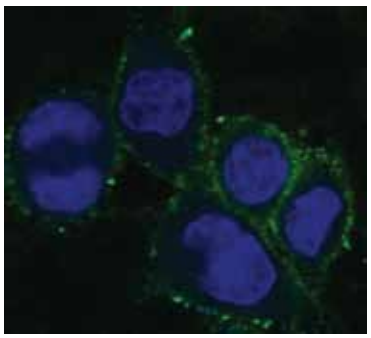
Carmon, K.S., Gong, X, Lin, Q., Thomas, A., and Liu, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A,* 108:11452-11457 (2011).

LAB MEMBERS

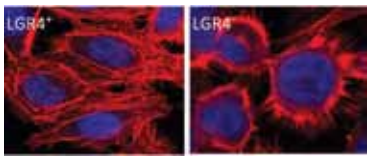
Instructors: Kendra Carmon and Xing Gong
Postdoctoral fellows: Christine Crumbley and Jing Yi
Research Associate: Wei Xiong
Technicians: Anthony Thomas



Co-localization (yellow) of stem cell receptor LGR5 (red) with LRP6 (green) following receptor activation in normal cells.



Localization of stem cell receptor LGR4 (green) on the cell surface of breast cancer cells. Blue staining represents cell nuclei.



Deletion of LGR4 in cancer cells led to changes in cytoskeletal structure.

Kalpana Mujoo, Ph.D.
Assistant Professor

HER3 signaling in prostate cancer and NO-cGMP pathway in stem cells

Prostate cancer is one of the most common cancers reported in Western countries. Approximately 242,000 new cases of prostate cancer were reported in the United States in 2012 and out of that ~ 28,000 will die of the disease. The primary treatment of prostate cancer is prostatectomy and radiation therapy. Patients who fail their primary treatment undergo 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. Therefore, we are interested in studying the role of novel HER-3 interacting partners in regulation of HER-3 signaling using human prostate cancer as a model. Furthermore, we propose to evaluate the efficacy of novel HER-3 antibodies in cell culture (2D and 3D) and human xenograft models of prostate cancer. These studies are being conducted in collaboration with Dr. Zhiqiang An and Dr. Ningyan Zhang of Texas Therapeutics Institute at IMM. We are also collaborating with Dr. Robert Amato of Division of Oncology to validate our novel HER-3 interacting partners such as NEDD4 (an E3 Ubiquitin ligase), DJ-1(known oncogene with multiple functions) and CRKII (family of adapter proteins) as biomarkers for HER3-driven prostate cancers.

The nitric oxide-cyclic GMP (NO-cGMP) pathway mediates important physiological functions associated with various integrative body systems including the cardiovascular and nervous systems. We are interested in understanding if manipulation of the NO-cGMP pathway will regulate stem cell differentiation. Our previous studies with human and mouse ES cells indicate differential expression and function of various nitric oxide signaling components in stem and differentiated cells. Furthermore, we have shown that NO donors and NO receptor soluble guanylyl cyclase (sGC) activators combined show enhanced differentiation of stem cells into myocardial cells with robust increase in cGMP production.

Our studies with induced pluripotent cells demonstrate aberration in cyclic GMP signaling downstream of NO receptor soluble guanylyl cyclase. Therefore, we are interested in elucidating the underlying molecular mechanisms involved in such aberration by focusing on events downstream of NO receptor sGC.

RESEARCH PROJECTS

- HER3/ERB3 signaling in prostate cancer
- Nitric oxide-cyclic GMP signaling in embryonic and induced pluripotent stem cells

KEY PUBLICATIONS

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.

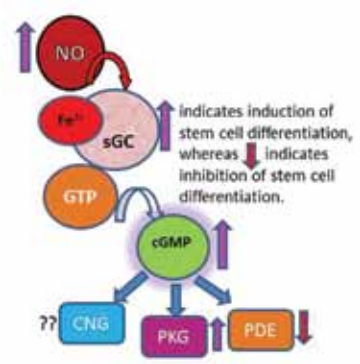
Mujoo, K.*, Sharin, V.G., Bryan, N., Krumenacker, J.S., Sloan, C., Parveen, S., Kots, A., Murad, F*. (2008). Role of nitric oxide signaling components in differentiation of embryonic stem cells into myocardial cells. *Proc Natl Acad Sci. USA,* 105: 18924-18929.

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.

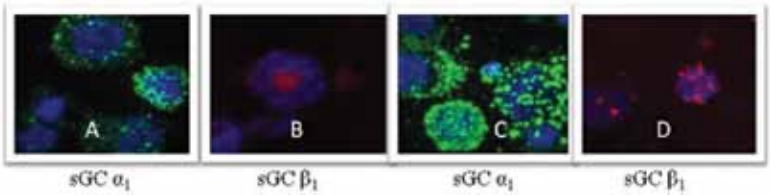
*Corresponding author

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

Trastuzumab resistance mechanisms in cancer

Antibodies are rapidly becoming a major drug modality for cancer treatment, and they are among the most efficacious targeted therapies available today. This trend is continuing as about 50% of the new drugs in various stages of clinical development are antibodies, and cancer therapeutic antibodies constitute a majority. 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 both EGFR and HER2 are proven oncogenes and have been successfully targeted using several monoclonal antibodies for treatment of various types of cancers, including non-small cell lung cancer, colon cancer and breast cancer. Trastuzumab is a humanized anti-HER2 IgG1 antibody and has shown clinical success for treatment of breast cancer patients with HER2 over-expression at both adjuvant and neoadjuvant settings. However, mechanisms of action are still not fully understood and multiple mechanisms have been proposed including inhibition of HER2 signaling, HER2 receptor downregulation, prevention of HER2 extracellular domain shedding, and antibody dependent cell cytotoxicity (ADCC) mediated through antibody Fc interaction with immune effector cells. Both innate and acquired resistance to trastuzumab have been widely reported, which presents significant challenges in the clinic.

My research interest is centered on mechanisms of action of therapeutic antibodies targeting the EGFR family of receptors and the resistance mechanisms to those therapies, including the HER2 targeting antibody trastuzumab. We employ a wide array of experimental approaches from *in vitro* cell culture and mouse models to clinical samples from cancer patients. State-of-the-art technologies are used in our studies such as high content fluorescence imaging, mass spectrometry, surface plasmon resonance (SPR) based kinetic binding analysis, and high throughput screening methods. Current research projects focus on trastuzumab interaction with cancer cells and immune cells in the tumor microenvironment. Type of interactions results in cancer cell escape from inhibition and leads to drug resistance. We also are studying the functional role of matrix matalloproteinases (MMPs) in cancer cell resistance to trastuzumab.

RESEARCH PROJECTS

- Role of immune evasion by cancer cells in trastuzumab resistance
- Effect of proteolytic cleavage of trastuzumab on its engagement with immune effector cells

KEY PUBLICATIONS

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.

M. Fa, K. Hoch, X. Fan, W. P. Dubinsky, Z. An, N Zhang. 2013. Novel approach for quantitative measurement of matrix metalloprotease-1 (MMP1) in human breast cancer cells using mass spectrometry. *Journal of Analytical Sciences, Methods and Instrumentation.* 3:54-61.

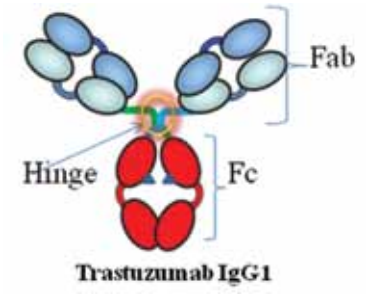
Choi B-K, Cai X, Yuan Y, Huang Z, Fan X, Deng H, Zhang N, An Z. (2012). HER3 intracellular domains play a crucial role in HER3/HER2 dimerization and activation of downstream signaling pathways. *Protein & Cell* 3(10):781-789.

Fan X, Brezski RJ, Fa M, Deng H, Oberholtzer A, Gonzalez A, Dubinsky WP, Strohl WR, Jordan RE, Zhang N, An Z. A single proteolytic cleavage within the lower hinge of trastuzumab reduces immune effector function and *in vivo* efficacy. *Breast Cancer Res*. 2012 Aug 8;14(4):R116.

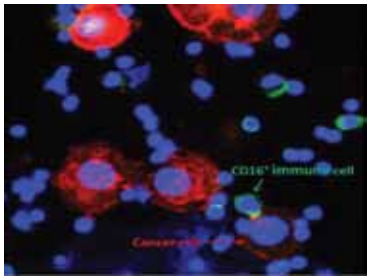
Choi B, Fan X, Deng H, N Zhang, Z An. 2012. ERBB3 (HER3) is a key sensor in the regulation of ERBB-mediated signaling in both low and high ERBB2 (HER2) expressing cancer cells. *Cancer Medicine* 1(1):28-38.

LAB MEMBERS

Joined team with Dr. Zhiqiang An's laboratory, see list of members in Dr. An's page



Schematic diagram of antibody



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

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 the 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.

Contact: KEVIN ROSENBLATT, MD, PHD
Associate Professor, Center for Proteomics and Systems Biology
Levit Family Chair in the Neurosciences
713-500-3611

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 scientists to evaluate a large number of samples in a short time frame and gather information on very rare populations of cells. The service center provides training, instrumentation, and technical expertise for both analysis and cell sorting.

Director: EVA M. SEVICK, PHD
Professor & Director
Center for Molecular Imaging
Contact: AMY HAZEN, PHD
Co-Director, 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 and sectioning
- Frozen tissue embedding and sectioning
- Blood smear stain
- Immunohistochemistry and special stain

Director: EVA M. SEVICK, PHD
Professor & Director
Center for Molecular Imaging
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research
Contact: SARAH AMRA, BS, HT (ASCP)
Chief Histology Technician
713-500-3386

Microscopy Service Center

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

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

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

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.

Contact: KEVIN ROSENBLATT, MD, PHD
Associate Professor, Center for Proteomics and Systems Biology
Levit Family Chair in the Neurosciences
713-500-3611

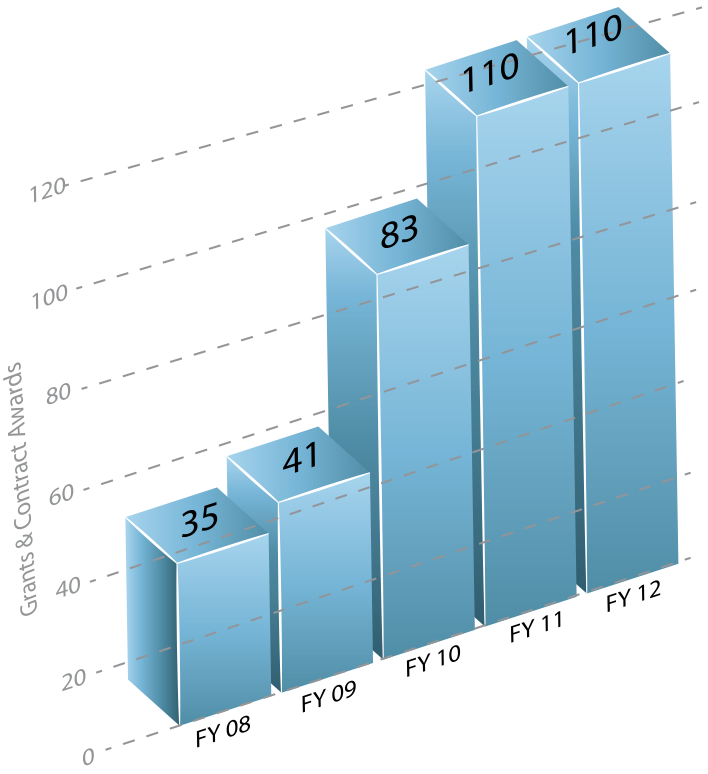
Transgenic and Stem Cell Services

Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cell service center, which was established in 1998. It has generated over 700 new transgenic and knock-out mouse animal models for all research investigators from UTHealth and external organizations on a fee-for-service basis.

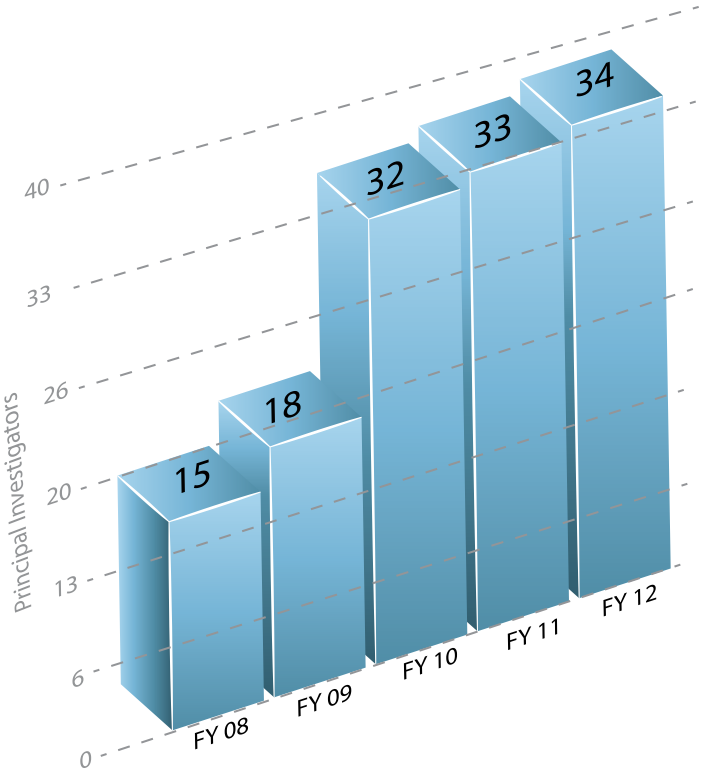
The stem cell lines that have been derived in the laboratory are highly effective for the generation of knock-out/knock-in mice and for cell differentiation studies. In addition to the production, cryopreservation, and re-derivation of genetically-engineered mice and rats, the services of the facility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

Contact: EVA M. ZSIGMOND, PHD
Assistant Professor, Center for Immunology and Autoimmune Diseases
Director, Transgenic and Stem Cells Service Unit
713-500-2453

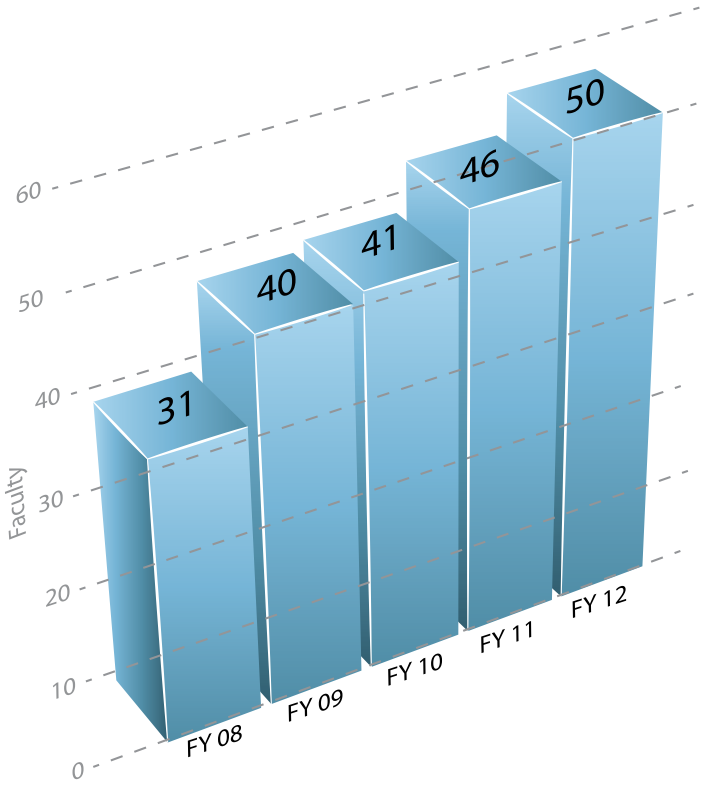
Number of Grant and Contract Awards



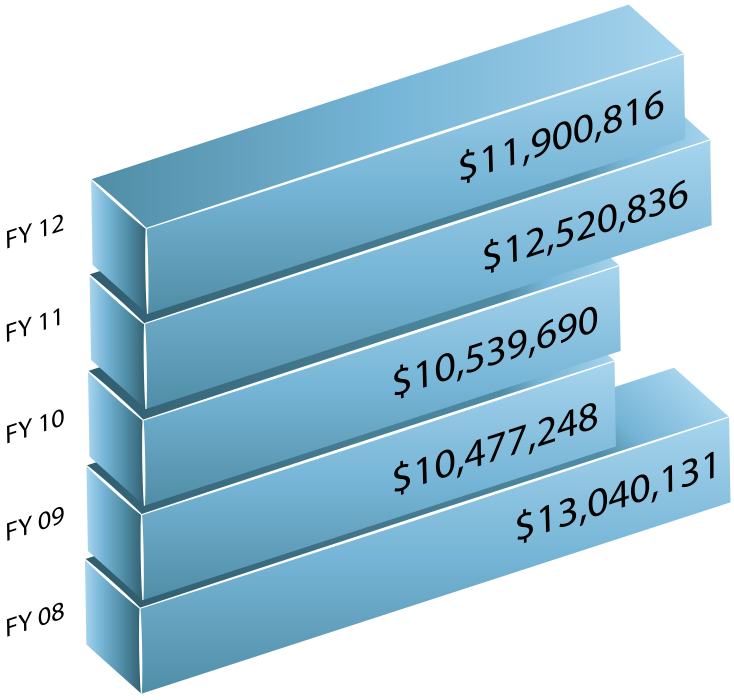
Number of Principal Investigators



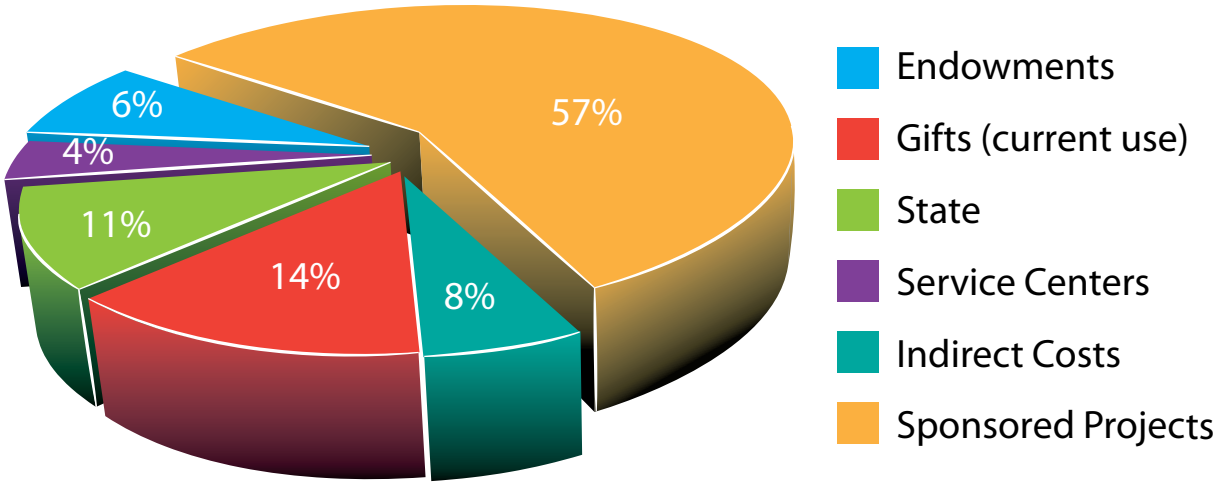
Number of Faculty



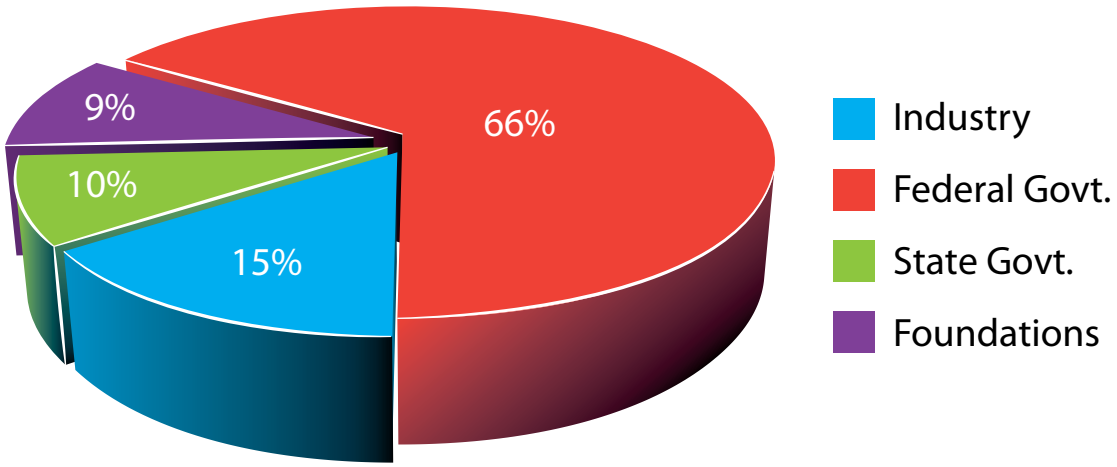
Research Expenditures



Total Expenditures FY2012 by Source



Research Expenditures FY2012 by Sponsor



Gift Report

New Gifts and Bequests
Fiscal Year 2012

(September 1, 2011 through August 31, 2012)

$100,000 TO $1 MILLION
Brad McWilliams
Jasper L. and Jack Denton Wilson Foundation

$25,000 TO $99,999
Janice and Steve Gordon
George and Mary Josephine Hamman Foundation
Marjorie and Herbert Poyner, Jr.
Nancy and Clive Runnells

$5,000 TO $24,999
Communique, Inc.
Gary Mercer
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United Soybean Board

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Office of Communications
6431 Fannin Street
MSB B.340
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