

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

IMM Impact Report

FISCAL YEAR 2018



ABOUT THE COVER

The stunning interior architecture of McGovern Medical School's Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases.

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DIRECTOR'S MESSAGE



The IMM has two major objectives:

- 1 Discovery is the highest priority for the IMM faculty. This is a major challenge, since diabetes, obesity, cancer, Alzheimer's, and cardiovascular diseases are unsolved medical problems that are not caused by single gene defects. Discoveries lead to new solutions.
- 2 New diagnostics and therapies are derivative of discovery and to the benefit of patients. The IMM focuses on these medical solutions. The IMM has organized talent in the Texas Therapeutics Institute specifically to achieve this goal of patient benefit from discovery.

I'm pleased to introduce the latest annual IMMimpact report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). The IMM is a stand-alone research institute that is embedded within McGovern Medical School. The IMM mission is to deliver translational outcomes from research in molecular medicine that benefits patients. To this end, we have teams of outstanding basic and translational scientists who collaborate closely with our clinical colleagues. Inside the report you will find in-depth articles on some of our faculty and donors plus an account from each IMM faculty member describing their research programs.

This year we have recruited additional outstanding new faculty, who bring with them exciting research ideas and innovative technologies. One of our new recruits, whose story is featured, also secured a prestigious STAR award from The University of Texas System, which are reserved for the recruitment of highly sought-after scientists. The environment for scientific research funding continues to be extremely challenging, especially from the NIH. Despite this, IMM faculty have excelled again. Over the financial year just ended, our new grants and contracts were up some 20 percent over the preceding year, which in turn had seen a considerable increase over the prior year. Indeed, we have now substantially increased our extramural grant funding for each of the last six years! It is a testament to the remarkable quality and creativity of our scientists that the IMM remains so successful in attracting research funds from what is an ever-diminishing national pool. That said, full implementation of our mission remains heavily dependent on attracting support from alternative sources, including research charities and foundations, industry collaborations, and, most importantly, the continuing generosity of our friends and donors.

In addition to advancing science and medicine, we therefore wish to develop our relationships with all in our community who value the aspiration of our mission to translate molecular discoveries into new therapies for human disease. In this regard, we are deeply appreciative of the strong work and dedication of the IMM advisory council, which plays a key role in the continued growth and development of the IMM. If you would like to investigate how you can also be involved, I would be pleased to talk with you personally. Alternatively, I would be delighted to see you at our annual IMM symposium. Last year, 165 guests listened to two talks in the Beth Robertson Auditorium and attended a reception in the James T. Willerson, M.D. Discovery Hall. This year the symposium will be held on May 1, 2019, and will feature talks on obesity and diabetes, answering questions about appetite, why we feel hungry and stop eating (or not!), and how we burn and use the calories that we eat. It will be a very illuminating and entertaining evening. As with last year we will have an extended question time with an expert panel comprising the speakers plus UTHealth physicians. The symposium is an excellent opportunity to hear exciting research stories directly from our faculty, to discuss its implications for the future of medicine and health care, and to have all your questions answered. Full details are in this report; please mark the date in your calendar because it is a great opportunity to visit the IMM.

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



Mission

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

Advances in molecular and cell biology have enormous potential for innovative medical

research and the future practice of medicine with more novel therapies. These approaches have been most successfully used to determine the causes of infectious disorders and genetic diseases.

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

Because the applications of molecular and cell biology

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

As an institute of McGovern Medical School, the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases strives to set the example for research excellence and collaboration locally, nationally, and internationally.

OUR LOCATIONS

FAYEZ S. SAROFIM RESEARCH BUILDING



- Primary home of the IMM's faculty, administration, and support staff.
- Located adjacent to the The University of Texas Health Science Center at Houston (UTHealth) University Center Tower within the Texas Medical Center.
- Opened in 2006, the building encompasses 255,748 gross square feet.

SOUTH CAMPUS RESEARCH BUILDING – 3 (SCRB₃)



- SCR_{B3} is a collaboration between The University of Texas MD Anderson Cancer Center and UTHealth, in cooperation with GE Healthcare and the Texas Enterprise Fund.
- Six-stories, 315,000 square-feet located on the South Campus of the Texas Medical Center.
- Opened in 2009, this facility houses Positron Emission Tomography, Magnetic Resonance Imaging, Optical Imaging Tracers, a Cyclotron, wet labs, and support offices.

THE DENTON A. COOLEY BUILDING – TEXAS HEART INSTITUTE AT ST. LUKE'S EPISCOPAL HOSPITAL



- The IMM occupies a 31,000 square-foot high-tech laboratory.
- Located in the Texas Medical Center.



The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases

IMMpact Symposium

Fayez S. Sarofim Research Building
1825 Pressler Street

Wednesday, May 1, 2019
4:00 - 6:30 pm

SAVE THE DATE

Weighing in on the Science of Staying Slim

To eat or not to eat: It's all in your brain

Dr. Qingchun Tong, Ph.D.
*Associate Professor
Cullen Chair in Molecular Medicine
Center for Metabolic and Degenerative
Disease*

Demand-side economics of blood sugar: Use it to lose it

Dr. Rebecca Berdeaux, Ph.D.
*Associate Professor
Center for Metabolic and Degenerative
Disease*

HARNESSING THE POWER OF PROTEOMICS IN DISEASE RESEARCH

With genetic testing gone mainstream, knowing our ancestry or risk for disease is just a saliva sample or blood draw away.

But did you know that the genetic difference between you and your neighbor is about 0.1 percent? Or that the difference between our genome and that of chimps is just 1.2 percent?

Where the greatest biological differences are expressed are at the protein level, explains Sheng Pan, Ph.D., associate professor in the Center for Precision Biomedicine at The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM) at UTHealth.

“Tissue of muscle is different from the tissue of the lung because the proteome – the entire complement of proteins expressed by that tissue – is different,” says Dr. Pan, director of the Clinical and Translational Proteomics Service Center.

Proteomics – the study of proteins and how they are implicated in disease and health – has flourished as an area of scientific research and discovery over the last 20 years. In fact, a review of the medical literature reveals a 6,000 percent increase in the terms “proteomics and cancer” in published research from 2000 to 2017.

Through the Clinical and Translational Proteomics Service Center, Dr. Pan and his colleagues provide proteomics and mass spectrometry support to researchers at UTHealth and throughout the Houston area.

“Proteomics has become a mainstay in basic, translational, and clinical research,” says Dr. Pan, who earned his doctorate from the University of Washington in Seattle, where he also was on faculty for a decade before joining the IMM in 2017.

Scientists rely upon proteomics technologies to look at the protein expression level and study mutated sequences and post-translational modifications. They also examine proteins for their interactions with proteins or other biomolecules, such as DNA, and their reactions to drugs.

“Proteins are the essential functional biomolecules and participate in all diseases, so they can be biomarkers for diagnosis or potential targets for drug therapies,” Dr. Pan explains.

To study the proteome at a functional level, Dr. Pan and his team extract proteins from various biological and clinical specimens to analyze with the mass spectrometer. Data from there are translated into biologically meaningful results using bioinformatics. “From a typical tissue or cell lysate sample, we get about 1.5 gigabytes of data,” Dr. Pan explains. “It is a big data technology. Cutting-edge mass spectrometry is highly sensitive and accurate – it can determine a molecular mass with one part per million resolution.”

One specific disease that has captured Dr. Pan’s focus is pancreatic cancer. The fourth-leading cause of cancer deaths in the United States, Dr. Pan says that pancreatic cancer is poised

to become the second leading cause of cancer death by 2030.

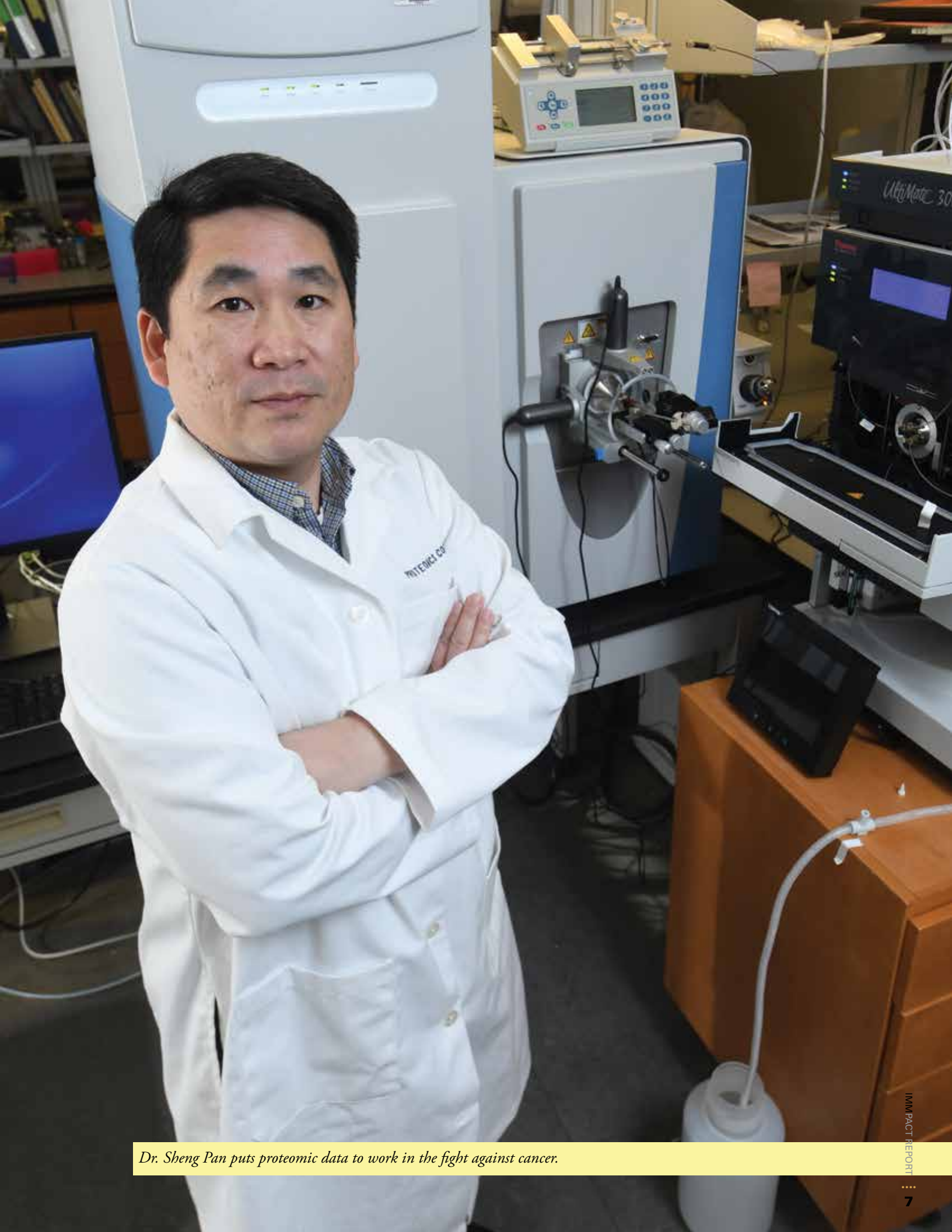
“Most patients are third- or fourth-stage when they are diagnosed because of the difficulties of diagnosis at early stage. Moreover, drug resistance to therapy is a significant challenge in pancreatic cancer treatment,” he explains as to why the death rate is so high.

Looking to improve the diagnosis and therapeutics of pancreatic cancer, Dr. Pan, in collaboration with Baylor College of Medicine and the University of Washington, is working on a National Institutes of Health grant to study early detection of pancreatic cancer in patients with new onset diabetes.

“We know that pancreatic cancer prevalence is higher in this group than in the general population, so we are investigating proteome differences in those who develop cancer and those who do not have cancer to better understand the disease mechanism and to develop biomarkers to assist early cancer detection,” he says.

“We are looking for cancer associated protein abundance changes, protein variances, and post-translational modifications using quantitative proteomics,” he says. “Our goal is to assist in the early detection and intervention of pancreatic cancer.”

In addition to his main research projects on pancreatic cancer and GI-tract diseases, Dr. Pan’s lab also supports proteomics studies of neurological, inflammatory, and other diseases through collaborative efforts.



Dr. Sheng Pan puts proteomic data to work in the fight against cancer.



The University
of Texas
Health Science
Center
at Houston

Fayez S. Sarofim
Research Building

The Brown Foundation
Institute of Molecular
Medicine

1825 Prosser Street

Dr. Kendra Carmon decided to pursue her scientific career at UTHHealth's IMM.

FINDING PHARMA AT HOME

Armed with a degree in chemical engineering, Kendra Carmon had her eyes set on a big pharma career following training at UTHealth.

And why not? The pharmaceutical industry has a massive research and development component attractive to bright young scientists such as Carmon.

But a funny thing happened on the way to the pharma lab.

Carmon completed her doctorate at UTHealth in pharmacology and molecular biology, working in the lab of David Loose, Ph.D., in the Department of Integrative Biology and Pharmacology, where she worked to create assays and run drug screens.

“I intended to move on into pharma after I graduated, but at that time, the Texas Therapeutics Institute and Dr. Jim Liu came to the IMM, and I started learning more about drug discovery right here at UTHealth by becoming a postdoc in his lab,” she explains.

The Texas Therapeutics Institute (TTI) is a joint program of UTHealth, MD Anderson Cancer Center, and the departments of chemistry and biochemistry at The University of Texas at Austin. Its goal is to help biomedical researchers transform their discoveries into novel diagnostics and treatments. It

is co-directed by Zhiqiang An, Ph.D., the Robert A. Welch Distinguished University Chair in Chemistry, and Dr. Liu, the Janice D. Gordon Distinguished Professor in Bowel Cancer Research.

“TTI brings that pharma element I was looking for right here to UTHealth. Working in Jim’s lab provided the expertise I was looking for from industry as he previously was at Merck and Lexicon Genetics,” Dr. Carmon explains.

At this time Dr. Carmon also received high-risk award funding from the Cancer Prevention and Research Institute of Texas (CPRIT), which as authorized by the Legislature, provides funding to Texas-based scientists to expedite innovation in cancer research and product development. The major goal of this project was to generate anti-LGR5 antibody-drug conjugates that target and eradicate LGR5-expressing tumor cells, which are found in cancer stem cells.

After her postdoc training, Dr. Carmon was invited to join the TTI faculty as an instructor in 2012 and was appointed assistant professor in 2015.

She continues to focus her research on cancer – specifically colon cancer.

Colon cancer is the third-most common cancer in the United States, with death rates in 2018 expected to reach about

50,000. Although mortality is declining, new therapies are needed.

“We are trying to identify new drug targets in cancer that are aimed only at the tumor cells, not normal cells in order to decrease side effects,” Dr. Carmon explains. “We are also looking at the functional role of LGR5, which is expressed in cancer stem cells and drives cancer growth.”

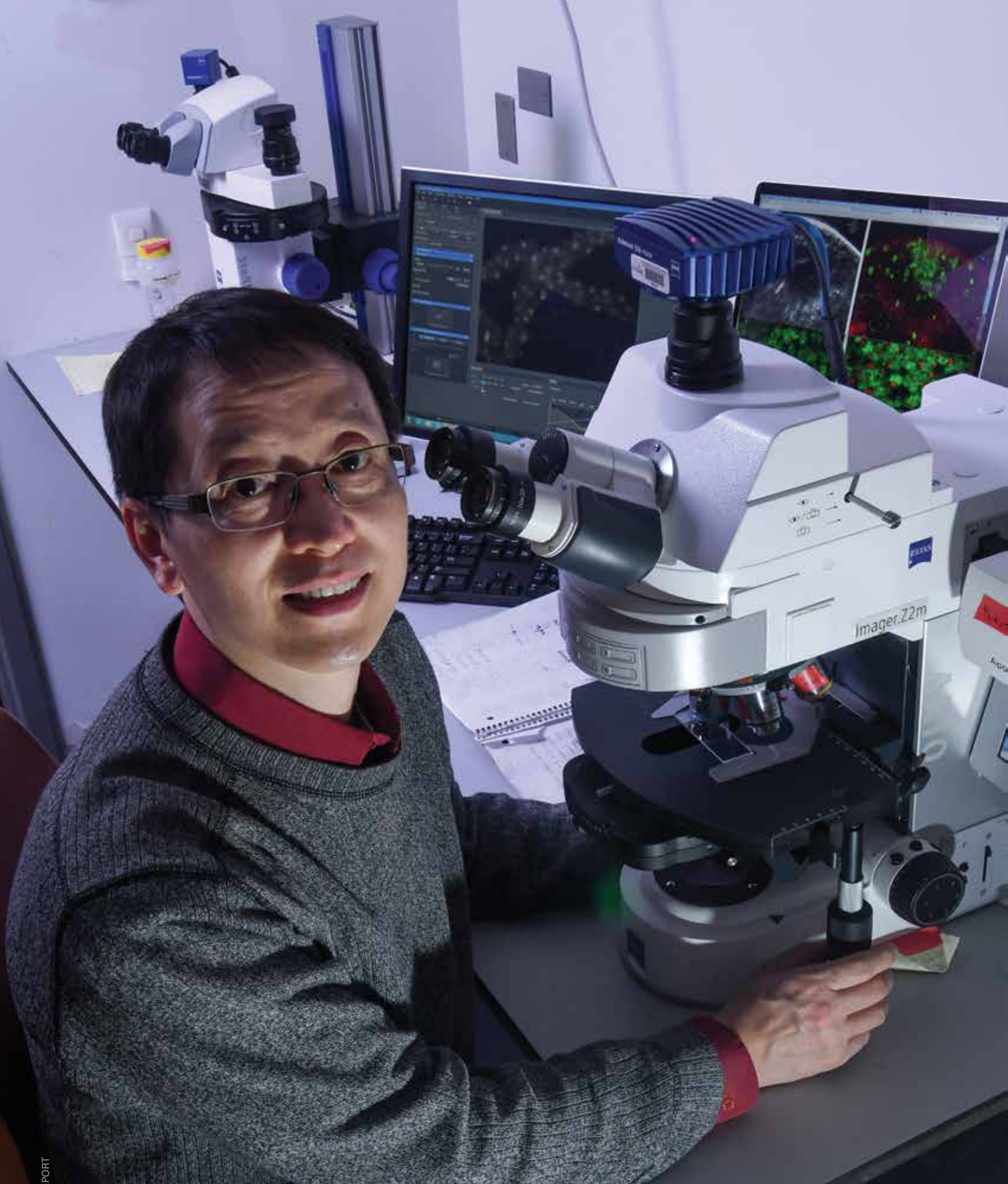
Dr. Carmon’s innovative work has been recognized with a five-year National Institutes of Health (NIH) grant to look at the effect of cancer stem cells in eradicating colon cancer and with a Welch Research Scholar Award to identify novel therapeutic targets and elucidate their role in gastrointestinal cancers.

Dr. Carmon says she was “lucky” to receive her first NIH Research Project Grant Program (R01) so early in her career. However, she also states that “it also takes hard work and innovative ideas.”

A recent study shows that the average age of investigators receiving such grants has climbed to an all-time high: 46.

Getting such a head start means Dr. Carmon is well on her way to her long-term goal of creating “practical, translational” research that will help current and future cancer patients.

“We are trying to identify new drug targets in cancer that are aimed only at the tumor cells, not normal cells in order to decrease side effects.” — Dr. Kendra Carmon



Dr. Sheng Zhang's research could help unlock the key to methods of prevention, and perhaps cures, for neurodegenerative diseases.

UNCOVERING THE MYSTERIES OF NEURODEGENERATIVE DISEASES

Millions of people across the globe struggle every day with debilitating aging-related neurodegenerative diseases. Researchers at the Center for Metabolic and Degenerative Diseases are aiming to give them a fighting chance against these devastating brain disorders by better understanding the culprits responsible for the causes and progress of these diseases and identify methods of prevention and cure.

Dr. Sheng Zhang, assistant professor in the Center, is one of several researchers at the IMM studying neurodegenerative disorders. Dr. Zhang has been particularly interested in Huntington's disease and Parkinson's disease, the two focuses of his study at McGovern Medical School, in addition to his collaborative project on Alzheimer's disease with colleague Dr. Hui Zheng, director of Huffington Center on Aging at neighboring Baylor College of Medicine.

He was first struck by the idea of pursuing neurodegenerative diseases when he was studying cancer while he was a graduate student at Yale University School of Medicine. Through the use of fruit flies, Dr. Zhang found a gene in this small insect that was important for its development and has a highly similar counterpart in humans that, when mutated, causes one of these neurodegenerative disorders.

"Compared to the mountains of information

we already garnered about cancer and their treatment options, even at that time, the knowledge and understanding about neurodegeneration disorders were flatly blank, with hardly any effective treatment available," Dr. Zhang says. "It was very enticing for a young student to plunge into this new uncharted field."

Dr. Zhang has been particularly interested in examining how the abnormal protein clumps develop in the affected brain and how they can be effectively cleared. These clumps are often composed of faulty proteins directly linked to diseases like Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Lou Gherig's disease), however little is known exactly how such clumps form, and why such a common pathological feature can be linked to destruction of different groups of neurons in different parts of the brain.

"If we can know which pathway leads to the disease, there's a chance we can correct them and may be able to slow down, or even cure, the disease," Dr. Zhang says. "What's unique about these neurodegenerative diseases is that patients mostly develop symptoms when they are older, so even just delaying the progress of the disease is more than enough for most patients and their loved ones for the rest of their lifespan."

For example, Huntington's disease is caused by an abnormal expansion of a glutamine tract in the Huntingtin protein.

The faulty Huntingtin protein builds up gradually and forms prominent clumps in the brain, but why this particular abnormality leads to the disease is unclear. Researchers found the removal of the Huntingtin protein from fruit flies weakens them, ages them faster, and they die significantly earlier. The protein was found to be important for the self-maintenance of the cell, as it helps clear cellular waste.

"For the majority of these diseases, there is no clear hereditary pattern or genetic cause," Dr. Zhang says. "But for Huntington's, there is one gene and one mutation, and this mutation is associated with robust formation of pathogenic clumps."

Finding the molecular drivers behind this disease and why this particular mutation causes Huntington's disease is a major passion behind Dr. Zhang's research. While there are still many unknown factors in the pathogenesis of these neurodegenerative diseases left to researchers, Dr. Zhang says it's an exciting time to be in his field as researchers are making substantial progress.

"This is a major challenge for society," Dr. Zhang says. "People have become more aware of this issue, including Congress and many private organizations and regular citizens. It's still too early to say what might happen, but I feel we have moved much closer to an answer, and more likely, answers."

UNDERSTANDING DISEASE RISKS ENCODED WITHIN OUR NON-CODING GENOMES

Dr. Ashish Kapoor, assistant professor, may be relatively new to McGovern Medical School, but he is already looking forward to working with fellow researchers to gain a better understanding of the role non-coding genomes play in the risk of developing disease.

Dr. Kapoor joined the IMM from Johns Hopkins University School of Medicine, where he completed postdoctoral work in the laboratory of Dr. Aravinda Chakravarti. At the IMM, he works to understand the role of variable gene expression driven by non-coding regulatory sequence variation in disease risk.

How much of a gene product is made (expressed) by our cells varies not only across different cell-types within an individual but also across individuals themselves. This natural variability in gene expression plays a major role not only in how we differ from one another but also in how common diseases develop. Dr. Kapoor's lab utilizes electrocardiographic QT interval as a model trait to better understand the mechanisms behind such variances. Much of the risk for common diseases emanates from multiple genes and is due to genetic differences, or variations, outside gene-bodies (non-coding) that likely affect expression of those genes. In other words, how much of a normal gene product is expressed by our cells is tightly controlled and genetic variations

in this regulatory machinery largely underlie common disease risk and trait variation.

“One thing we as a community have learned is that most of the genetic risk/basis for common diseases and traits is not coming from variation in the gene sequences as such or what we call the coding part of the genome,” Dr. Kapoor says. “It's coming from variation in the sequences outside the gene-bodies, or what we call the non-coding genome.”

Among the challenges for researchers is that there isn't a deep understanding of the language(s) used by non-coding genomes.

“Compared to the coding genome, we have very little understanding of how diverse functions are encoded in our non-coding genome and the impact sequence variation has on such functions,” Dr. Kapoor says. “Among several functions, regulation of gene expression is a major function of the non-coding genome.”

Large-scale efforts like the Roadmap Epigenomics, the ENCODE and the GTEx projects are steps in the right direction, but so far such efforts have been applied largely on a tissue level. Dr. Kapoor says the key will be to do similar studies on the level of cell-types and gain a better understanding of the specific molecular components and structure of the regulatory machinery underlying gene expression variation in humans.

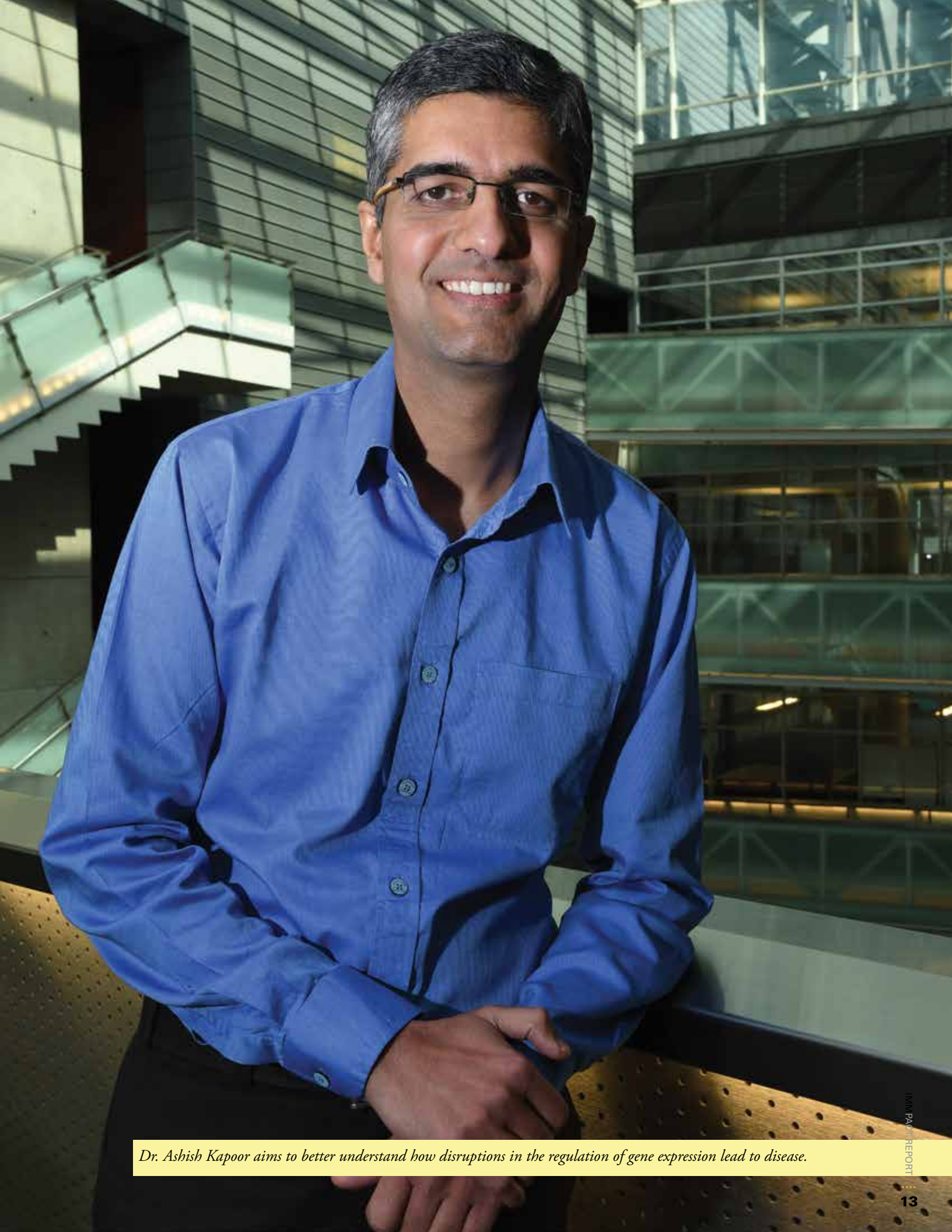
“Tissues are made from a mixture of different cell-types, and when you grind up a tissue, the signals you're seeing, whether epigenomic or transcriptional, are from different cell-types,” Dr. Kapoor says. “I think characterization of the non-coding genome on disease-relevant cell-types is going to be more informative for uncovering the underlying disease mechanisms.”

Despite an uphill battle in understanding the intricacies of the non-coding human genome, Dr. Kapoor says significant discoveries have been made.

His lab is working to identify the pieces of the non-coding genome that regulate gene expression, focusing on those whose functions are altered by disease or trait associated sequence variants. The causal genes, whose expression levels are modulated due to regulatory variants, are then manipulated in model systems (cells and animals) to further assess if they lead to a disease or trait relevant outcome.

Dr. Kapoor says he is excited to continue working with his colleagues and fellow researchers around the Texas Medical Center and begin specific collaborations.

“Given the broad range of skill sets required to carry out this kind of research, I am looking forward to working with fellow researchers at the TMC and beyond with the right expertise,” he says.



Dr. Ashish Kapoor aims to better understand how disruptions in the regulation of gene expression lead to disease.



Shavonnah Roberts Schreiber champions women's health initiatives at the IMM.

A CATALYST FOR CHANGE

Pointing out a problem or a disparity is simple, but taking the personal responsibility to address it and develop a solution takes courage, leadership, determination, and a healthy dash of creativity.

As a business owner, volunteer leader, and philanthropist, Shavonnah Roberts Schreiber dedicates much of her life to solving problems she sees in everyday life and helping others find innovative solutions.

Her fascination with science and resolve to support women's health initiatives blossomed into a natural partnership with The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM) at UTHealth.

"One thing I know to be true," Roberts Schreiber says, "is that there are gender disparities in causes focused on women, including health care. When I dive into issues like this, I always ask myself, 'What am I going to do to become part of the change I want to see?'"

Roberts Schreiber was a member of UTHealth Luminaries, a volunteer leadership group composed of Houston's young community leaders, when she started feeling she could devote even more of her own talents and resources to benefit the university.

Seeking the perfect fit for her passion at UTHealth, she attended an IMM Advisory Council meeting and quickly became interested in the science behind all of the innovative

projects. A naturally inquisitive person, she says she felt destined to become part of the IMM's strong research environment, and it was not long before she joined the IMM Advisory Council.

Again, Roberts Schreiber asked herself what she could do more to bring positive change and began further refining her focus.

"I narrowed it down to women's health because it's so important to me," she says. "I'm always searching for ways to help women become strong, healthy, and whole human beings."

Although Roberts Schreiber says she felt that she was productively contributing her time and talents to the IMM, she felt she could have a greater philanthropic impact and began seeking opportunities to provide more support.

"What really drove it home for me was an IMM tour I took," she says. "I had the opportunity to learn how some critical projects don't get the same level of funding from organizations and have to rely on private donors."

The seed was planted, and she says she knew that an endowment at the IMM could provide perpetual funding for women's health initiatives—thus, the Shavonnah Roberts Schreiber Women's Health Endowment was born.

"My vision is to support physicians and researchers who directly impact women's health and to facilitate outreach events to spread

community awareness," she says. "Ultimately, I hope to raise the endowment to an endowed chair that will provide funding for an exceptional faculty member to champion women's health at the IMM."

As part of the endowment's outreach aim, Roberts Schreiber held an inaugural mixer on March 8, 2018—International Women's Day—to engage nearly 40 members of her own network and inform them of innovative women's health initiatives at the IMM.

"I'm known in my network for being a connector of ideas and people," Roberts Schreiber says. "My goal is also to connect my network to UTHealth. Women's health initiatives may not be each person's philanthropic passion, but there's so much happening here that someone may find what they truly care about."

Two faculty members from the Center for Metabolic and Degenerative Diseases at the IMM—Kristin Eckel Mahan, Ph.D., assistant professor, and Rebecca Berdeaux, Ph.D., associate professor—shared their innovative work and mingled with the group.

As Roberts Schreiber works to empower women through her work and everyday life, her endowment will help bring critical funding and awareness to women's health. Through her philanthropy and determination, she is setting an example that she hopes will inspire others to become part of the positive change they want to see.

The IMM Center for Cardiovascular Genetics, established in 2006, focuses on elucidation of molecular genetics, genomics, and pathogenesis of cardiovascular diseases with the objective of utilizing the discoveries to prevent and treat cardiovascular diseases in humans. The Center provides specialized clinical services to patients with genetic cardiovascular disorders at the Cardiovascular Genetic Clinic. The Center also has a Research Clinic, which is utilized for clinical research activities, including NIH- and industry-sponsored clinical trials.

Mission: To prevent and treat cardiovascular diseases in humans through identification and targeting of the pathogenic genes and pathways.

Faculty: Priyatansh Gurha, Ph.D., assistant professor; AJ Marian, M.D., professor; Raffaella Lombardi, M.D., Ph.D., adjunct assistant professor.

General theme of the research programs: The research programs at the Center start with human molecular genetic studies aimed at identifying the causal genes for human cardiovascular diseases. The focus is primarily on hereditary cardiomyopathies, which are important causes of sudden cardiac death and heart failure. Genetic analysis is performed by whole exome and genome sequencing. Genetic discoveries are then coupled with the genomic studies to identify differentially expressed coding and non-coding transcripts and dysregulated pathways, chromatin remodeling, and DNA methylation in cardiomyopathies. The integrated approach is used to identify the key dysregulated pathogenic pathways for preventive and therapeutic genetic and pharmacological interventions. The findings in the model systems are extended to human patients through pilot randomized placebo-control double-blind studies clinical trials. The findings provide the platform for large-scale, multi-center efficacy clinical trials.

Research Programs:

I. Human molecular genetic studies of cardiomyopathies: We have a repository of several hundred cases and their family members with cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy (ACM). Pathogenic and causal variants are identified by whole exome sequencing in the probands and family members. These studies have



identification of new disease-causing genes and have advanced the genetic causes of heart failure. We are actively recruiting additional probands and family members.

II. Genomics studies of human heart failure and mouse models of cardiomyopathies: The studies predominantly relate to DCM and ACM and included whole transcriptome analysis by RNA-Seq, DNA methylation analysis by RRBS, and chromatin remodeling by ChIP-Seq of specific histones and proteins. The integrated findings are used for preventive and therapeutic targeting.

III. Therapeutic targeting of dysregulated pathways in cardiomyopathies: Dysregulated pathways identified through integrated genomics are targeted through genetic and pharmacological interventions in model organisms and their effects on survival, cardiac function, and clinical outcomes are analyzed. Several active programs are current underway.

IV. Clinical Studies: The Center participates in investigator-initiated, single-center pilot clinical trials as well as industry-sponsored, multi-center clinical trials in hereditary cardiomyopathy. An NIH-sponsored, double-blind randomized pilot study (HALT-HCM) in patients with HCM recently was completed. The Center also participates in industry-sponsored clinical trials in cardiomyopathies.

*AJ Marian, M.D.
Center Director & Professor
James T. Willerson Distinguished
Chair in Cardiovascular Research*



AJ Marian, M.D.

Professor and Director of the Center for Cardiovascular Genetics
James T. Willerson Distinguished Chair in Cardiovascular Research

Molecular genetics, genomics, pathogenesis, and treatment of hereditary cardiomyopathies

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

Arrhythmogenic Cardiomyopathy (ACM): ACM is an enigmatic form of hereditary cardiomyopathies that clinically presents with cardiac arrhythmias, heart failure, and sudden cardiac death, particularly in the young. A unique feature of this disease is a gradual replacement of cardiac myocytes with fibro-adipocytes. There is no effective therapy for ACM.

Hypertrophic Cardiomyopathy (HCM): HCM is the most common form of hereditary cardiomyopathies, affecting ~ 1 in every 500 individuals in the general population. The affected individuals are typically asymptomatic and sudden cardiac death is often the first manifestation of this disease. HCM is the most common cause of sudden cardiac death in the young. While there are effective therapies to alleviate the patient's symptoms, there is no effective therapy to prevent or reverse the disease process.

Dilated Cardiomyopathy (DCM): DCM is genetically the most heterogeneous form of hereditary cardiomyopathies and a major cause of heart failure and heart transplantation in the young. The affected individuals often present with symptoms of heart failure, cardiac arrhythmias, and sometimes sudden cardiac death. There are a number of effective pharmacological and non-pharmacological therapies for DCM but currently there is no cure for DCM.

The overall approach entails an integrated approach that includes human molecular genetic studies through high throughput whole exome and genome sequencing to identify the causal genes and mutations, followed by genomic studies, including transcriptomics and epigenetics to define molecular remodeling of chromatin in the presence of causal mutations. The aim

is to link the causal mutations to genomic remodeling and to the pathogenic pathways. The responsible molecular mechanisms are identified through molecular mechanistic studies in genetically modified animal models and cultured cells. The mechanistic discoveries are then utilized to intervene in model organisms, utilizing genetic and pharmacological approaches that target the pathogenic pathways, in order to prevent the evolving phenotype and reverse or attenuate the established phenotype. These findings in the model organisms are extended to human studies through pilot randomized placebo-controlled, double-blind clinical trials. The findings, if favorable, are pursued through collaborative large-scale clinical trials.

RESEARCH PROJECTS

- Identification of causal genes for heart failure and sudden cardiac death
- Identification and characterization of epigenetic and transcriptomic changes, including non-coding RNAs and histone modifications in hereditary cardiomyopathies
- Identification and characterization of the molecular pathways that link the genetic mutations to the clinical phenotype in patients with cardiomyopathies, including, delineation of the mechanical signaling pathways regulated at the intercalated discs
- HALT-HCM (Hypertrophic Regression with N-Acetylcysteine in Hypertrophic Cardiomyopathy) clinical trial (ClinicalTrials.org NCT01537926)
- Maverick study: An industry-sponsored clinical trial to test efficacy of an ATPase modulator

on improving symptoms and exercise tolerance in patients with obstructive hypertrophic cardiomyopathy

KEY PUBLICATIONS

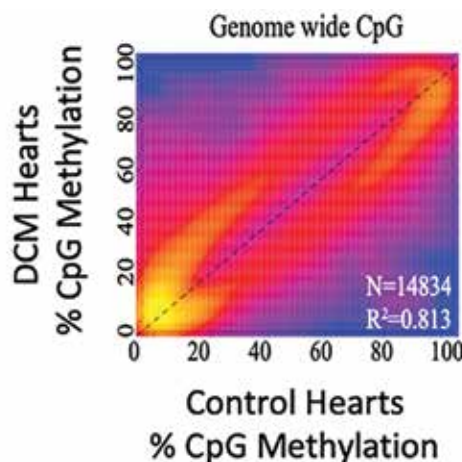
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A Distinct Cellular Basis for Early Cardiac Arrhythmias, The Cardinal Manifestation of Arrhythmogenic Cardiomyopathy, and the Skin Phenotype of Cardiocutaneous Syndromes. Karmouch J, Zhou QQ, Miyake CY, Lombardi R, Kretzschmar K, Bannier-Hélaouët M, Clevers H, Wehrens XH, Willerson JT, Marian AJ. *Circ Res.* 2017;121: 1346-1359. PMID: 29018034

LAB MEMBERS

Post-doctoral fellows: Gaëlle Auguste, Ph.D.; Sirisha C Marreddy; Leila Rouhgharabaei, Ph.D. Visiting scholars: Ping Yuan, Jinzhu Hu, M.D. Research associate: Grace Czernuszewicz, M.S. Research and clinical nurse: Yanli Tan, RN



Differential CpG methylation in the intergenic region human dilated cardiomyopathy



Priyatansh Gurha, Ph.D.
Assistant Professor

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

The broad goal of my research is to better understand the role of gene regulatory mechanisms involved in the pathogenesis of cardiomyopathies and heart failure. It is now clear that non-coding RNAs not only play a role in proper heart function but also are involved in the pathogenesis of cardiomyopathies and heart failure. Previously, we identified a pathological role of miR-22, one of the most abundant miRNA in the heart. We demonstrated that miR-22 is a key regulator of cardiac hypertrophy and fibrosis. Building on these studies, our research identified several dysregulated miRNAs in Arrhythmogenic Cardiomyopathy (ACM), a primary disease of the myocardium that clinically manifests with cardiac arrhythmias, heart failure, and sudden death. One of the key pathogenic features of ACM is the gradual replacement of myocytes by fibro-adipocytes. Using genomic approaches along with LOF and GOF studies we implicated miR-184 in the pathogenesis of ACM. Specifically, we showed that miR-184 was predominantly expressed in cardiac mesenchymal progenitor cells, and in ACM an epigenetic network encompassing E2F1 pathway and CpG DNA methylation transcriptionally suppress miR-184 expression in the heart. We showed that suppression of miR-184 leads to enhanced adipogenesis and overexpression of this miRNA partially rescue the adipogenic phenotype in ACM.

Recently, we have begun to investigate the regulatory role of Lamin (LMNA) in reprogramming the epigenetic code that governs gene transcription ensuing cardiac phenotype in Laminopathies. The objective of this study is to identify and characterize molecular component and mechanistic details that lead to tissue-specific disease phenotypes in laminopathies. By studying the human heart with an LMNA mutation, an LMNA-deficient (*Lmna*^{-/-}) mouse model, and isolated cardiac myocyte transcriptomic analysis, we identified a diverse set of the differentially expressed gene in laminopathies and specifically identified Lysine demethylase 5 (KDM5A and B) as a most induced upstream

regulator of gene dysregulation. KDM5 is a histone demethylase that removes tri- and di-methylation of lysine 4 of histone H3 (H3K4me3), often leading to suppression of gene expression. The role of KDM5 in heart and in laminopathies has not been documented so far. To determine the causal relation of KDM5 in laminopathies we re-expressed *Lmna*^{WT} in *Lmna*^{-/-} mouse (by AAV9) and found that this was associated with rescue of the KDM5 network and decreased apoptosis and increased overall survival. Currently using a wide array of genomic approaches, and *in vivo*, RNAi approached we are investigating the tissue and cell type-specific contribution of KDM5 in laminopathies to ascertain the elusive role of KDM5 in heart and determine if induction of KDM5 is pathogenic in heart failure in general.

RESEARCH PROJECTS

- Role of lncRNAs in the pathogenesis of Cardiomyopathies
- Identification and characterization of molecular mechanisms and functions of Lysine demethylase KDM5 in cardiomyopathies and heart failure.

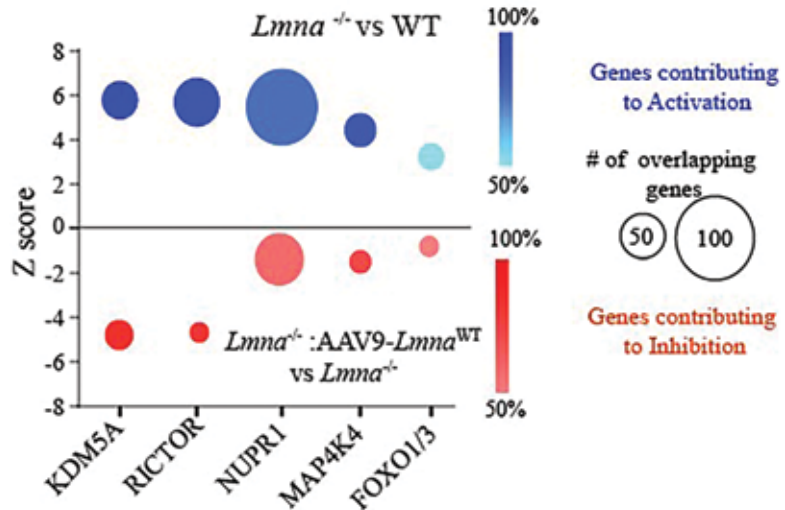
KEY PUBLICATIONS

Gurha P 1*, Chen X*, Lombardi R, Willerson JT, Marian AJ 1. Knockdown of Plakophilin 2 Down-regulates miR-184 Through CpG Hypermethylation and Suppression of the E2F1 Pathway and Leads to Enhanced Adipogenesis *In Vitro*. *Authors contributed equally *Circ Res*. 2016 Sep 2; 119(6):731-50. (Corresponding authors).

Auguste G, Gurha P, Lombardi R, Coarfa C, Willerson JT, Marian AJ. Suppression of Activated FOXO Transcription Factors in the Heart Prolongs Survival in a Mouse Model of Laminopathies. *Circ Res*. 2018 Mar 2; 122(5):678-692

LAB MEMBERS

Research assistant: Jordi Coste Pradas

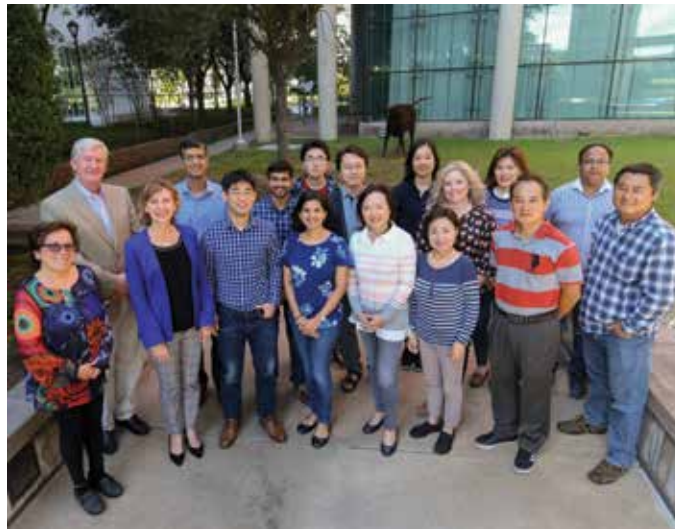


IPA analysis of Upstream regulators that are upregulated (blue) and downregulated (red) in *Lmna*^{-/-} hearts and there corresponding enrichment profile upon AAV9-*Lmna*^{WT} treatment (FDR<0.05).

The Center for Human Genetics works to generate new understanding about genetic risk for common cardiovascular diseases and to use that information to identify effective therapies for these diseases. High blood pressure is an amplifying element that drives cardiovascular disease risk from stroke, heart, and kidney disease. These diseases emerge in middle and later life and so are interlinked with the normal processes of aging. The genetic variation that makes us unique individuals and that has been passed to us from our parents impacts our risk of these diseases. Our work targets the identification of genes that contribute to cardiovascular diseases and the mechanisms by which variation in these genes reshape the biological pathways in which disease emerges.

An emerging concept developing in our laboratories is that an important element of chronic disease of the cardiovascular system is that these diseases involve a persistent state of inflammation. For example, in atherosclerosis, the blood vessel wall is invaded by immune cells and the danger posed in atherosclerotic plaques may reflect the ongoing level of inflammation in them. We need a better understanding of these processes of “sterile inflammation” in which our immune systems become activated in response to the emergence of damage to our tissues. We need greater understanding of the genetic variants that determine whether these inflammatory responses subside or remain active or even advance. The challenge of identifying these genetic variants is made more complex by the fact that there is a lot of genetic variation affecting in our immune responses. In order to be able to adapt to the continuous and rapid mutation of pathogens like viruses and bacteria, our immune systems harbor extensive genetic variation. Such variation can provide us a head-start in responding to new or evolving pathogens. But it can also create risk of disease later in life. As our living standards have increased and our lives have lengthened, the advantages provided earlier in life can turn into threats to our health by increasing our risk of chronic cardiovascular disease.

Progress in the laboratories of our investigators continues to yield exciting and important insights. Our human population geneticists, working under the direction of Dr. Myriam Fornage, are global leaders in their field, and are making



notable progress in the study of susceptibility to stroke and age-related decline in cognitive function. A significant fraction of sudden cardiac death results from rhythm disruptions that arise in genetic variation in the proteins processing the electrical activity within the heart. Our newest faculty member, Dr. Ashish Kapoor, is an emerging leader in this field. We have shown that kidney injury associated with increased blood pressure results from the emergence of auto-antibodies that damage tissues. This unexpected finding from Dr. Doris’ lab points to a role of immune system genetic variation in creating disease risk. Dr. Ba-bie Teng continues to advance understanding of susceptibility to atherosclerosis and the interplay between new drug targets, such as PCSK9, and lipoprotein uptake by cells. As our understanding of the complexity of information storage and retrieval in the genome expands, our colleague Dr. Sidney Wang is addressing approaches to assess, extract, and exploit new levels of genomic complexity that will inform work in this field.

All of us have had, or will have, one of our close relationships in life disrupted by common cardiovascular disease. In the Center for Human Genetics we have the opportunity to work for change, pushing forward the knowledge from which current medicine draws towards new insights and new opportunities for disease prevention.

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



Peter A. Doris, Ph.D.

Professor/Center Director

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

Genetics of cardiovascular end organ injury

High blood pressure is a common disease and its impact on public health arises largely because it leads to secondary pathologies, for example, stroke and progressive loss of kidney function. It is these “end organ” diseases that are the principal health cost of high blood pressure. Risk of these diseases is not equal among patients with high blood pressure as genetic susceptibility to end-organ disease varies. For example, risk of dialysis resulting from loss of renal function in a hypertensive patient is best predicted by whether relatives have experienced serious renal disease. Knowledge of the genes that produce genetic susceptibility may indicate the currently unknown path from high blood pressure to end-organ damage. In turn, this may point to opportunities to prevent disease. We have applied genetic and genomic methods to study hypertensive end-organ disease. We have discovered that disease results from genetic variation that affects antibody function in the system that we study. We have evidence that the likely source of antigen stimulating this antigen formation is the bacteria that live in the gut. They share a protein in common with mammals that pathogenic antibodies target. This protein has an important protective function. Antibodies may impede this function, leading to damage to organs and the blood vessels supplying them.

RESEARCH PROJECTS

Gut bacteria in induction of hypertensive renal disease. High blood pressure appears to disturb the normal separation of the gut bacteria from the host. We investigate whether hypertensive animals prone to renal disease experience greater gut barrier dysfunction than hypertensive animals resistant to disease. We determine whether differences in antibody genetics alters the gut bacteria and constitutes another component of the defective gut barrier. We are examining which bacteria are successful in breaching the gut barrier and whether passive or active immunization against these bacteria can modulate disease. Finally, translocation of gut bacteria into the host

is stressful for the bacteria, we are studying whether there are strategies to place additional stress on the gut bacteria that will limit their capacity to enter the host and interact with host immune mechanisms.

Antibody-mediated hypertensive renal disease. We are using hypertensive animals that lack the ability to form antibodies to prove the role of antibodies in disease pathogenesis. We have applied a new protein-based array technology to identify the targets that autoantibodies bind to in order to create disease. We are developing antibodies from cells cloned from animals with disease to discover if these antibodies alone are sufficient to create disease.

Genetic pathway of hypertensive renal disease. In this project we assess the role of three genes that we have identified that carry variation that we have implicated in disease pathogenesis. We are interested in how these genes lead to altered antibody function. Antibody formation is a random process that is honed to targets by interactions between T cells and B cells that remove potentially harmful antibodies that are self-reactive. Antibodies that target bacterial proteins that resemble host proteins are refined by these T and B cell interactions so that they can recognize their bacterial target without interrupting host protein function. The three genes we are studying are expressed in B cells, the unique cellular type that expresses and develops antibodies. The repertoire of antibodies encoded by the genome is highly variable among individuals, and we are examining whether this can influence the reactivities that antibodies develop. This requires applying novel genome sequencing and assembly methods to acquire a full representation of the antibody-encoding genes.

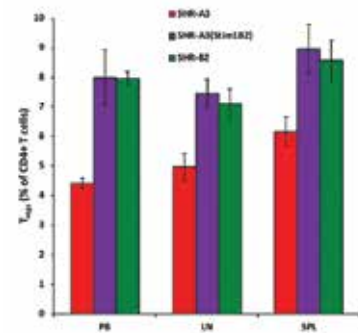
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LAB MEMBERS

Post-doctoral fellow: Isha S Dhande, Ph.D.
 Research assistants: Yaming Zhu, M.D., Aniket Joshi, B.S.



Hypertensive SHR-A3 rats experience renal injury, while similar SHR-B2 rats do not. We discovered a single nucleotide mutation in the gene *Stim1* in SHR-A3. This causes a reduction in the T regulatory lymphocytes which assist in specific antibody maturation. Replacing the single erroneous nucleotide in *Stim1* in SHR-A3 restores T regulatory cell counts to those in SHR-B2. B. The same mutation replacement also results in reduction in kidney damage in SHR-A3 to the much lower levels seen in SHR-B2.



Myriam Fornage, Ph.D.

Professor

The Laurence and Johanna Favrot Distinguished Professorship in Cardiology

Molecular epidemiology of the aging brain

These discoveries may yield new insights into disease mechanisms and lead to the development of new therapeutics to prevent or slow disease progression.

RESEARCH PROJECTS

- Discovering DNA sequence variants influencing ventricular enlargement on MRI, a key feature of several neurological and psychiatric diseases
- Discovering novel epigenetic (DNA methylation) variants that influence risk for brain small vessel disease and its related neurocognitive outcomes
- Discovering novel genetic variants for high blood pressure using gene-lifestyle interactions and pathway analysis. In particular, discovering how depression and anxiety affects genetic risk of hypertension.
- Investigating the distribution of the APOE4 mutation, a major risk factor for cognitive decline and Alzheimer's disease, in diverse Hispanics/Latinos

KEY PUBLICATIONS

Vojinovic D, Adams HH, Jian X, Yang Q, Smith AV, Bis JC, Teumer A, et al., Longstreth WT, van Duijn CM, Launer LJ, Seshadri S, Ikram MA, Fornage

M. Genome-wide association study of 23,500 individuals identifies 7 loci associated with brain ventricular volume. *Nat Commun.* 2018; 9:3945.

Bis JC, Jian X, Kunkle BW, Chen Y, Hamilton-Nelson KL, et al., Schellenberg GD, Seshadri S, Naj AC, Fornage M, Farrer LA. Whole exome sequencing study identifies novel rare and common Alzheimer's-Associated variants involved in immune response and transcriptional regulation. *Mol Psychiatry.* 2018 (in press)

Story Jovanova O, Nedeljkovic I, Spieler D, Walker RM, Liu C, et al., Hou L, Eriksson JG, Fornage M, Deary IJ, Baccarelli A, Tiemeier H, Amin N. DNA Methylation Signatures of Depressive Symptoms in Middle-aged and Elderly Persons: Meta-analysis of Multiethnic Epigenome-wide Studies. *JAMA Psychiatry;* 75:949-959

LAB MEMBERS

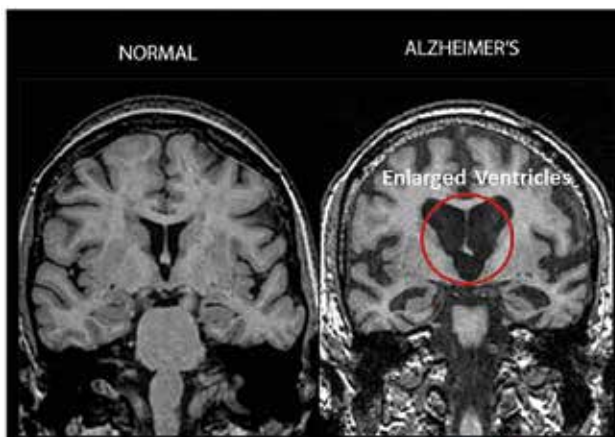
Post-doctoral fellow: Xueqiu Jian, Ph.D.

Graduate students: Daokun Sun, M.D., M.P.H.; Yunju Yang, Ph.D.

Research assistants: Rui Xia, Ph.D., biostatistician; Ping Wang, Ph.D., research associate

Diseases of the aging brain, such as stroke and dementia, are among the most significant public health problems of our time. Stroke is the fifth-leading cause of death in the United States and is a major cause of serious long-term disability for adults. Alzheimer's disease is now the sixth-leading cause of death and more than 5 million Americans are living with the disease. By 2050, this number is projected to reach 16 million. There is growing evidence that these disorders begin years, if not over decades, before manifestation of symptoms or clinical diagnosis. Indeed, neuroimaging techniques, such as magnetic resonance imaging (MRI), have consistently detected brain abnormalities beginning in middle age, which are associated with an increased risk of stroke, cognitive and functional impairment, dementia, and death. We investigate the genetics and genomics of vascular and neurodegenerative disease of the brain both in its clinical and pre-clinical forms in large population samples from young adults to elderly subjects.

We use powerful genome technologies to discover novel genes influencing the risk for stroke, Alzheimer's disease, and brain MRI abnormalities. In collaboration with researchers in the United States and Europe, we apply genome sequencing technologies to identify variants in the DNA sequence that influence risk for these disorders. We also study the links between DNA methylation and these diseases. DNA methylation is an epigenetic mechanism used by cells to control gene expression. Unlike DNA sequence variants, DNA methylation marks are not fixed at birth. Some of them can change throughout the lifetime and in response to environmental influences and aging. For example, we have used DNA methylation as a marker of a person's biological aging and compared it to his/her chronological age. We have shown that individuals whose biological age estimated from their DNA methylation profile is significantly older than their chronological age are at increased risk of developing brain abnormalities and cognitive function impairment.



Investigating the genome for genetic variations that influence Alzheimer's disease and its neuroimaging features provides new clues for unraveling disease mechanisms.



Ashish Kapoor, Ph.D.
Assistant Professor

Role of non-coding cis-regulatory sequence variation in cardiac arrhythmias and sudden death risk

Despite the progress in the prevention and treatment of cardiovascular diseases in general, sudden cardiac death (SCD) remains a major public health problem. SCD, defined as a sudden and an unexpected pulseless condition due to a cardiac arrhythmia (when heart beats out of rhythm) without evidence of a non-cardiac cause, is the leading cause of deaths in US (~500,000 each year) and accounts for ~15% of all-cause deaths and ~50% of deaths from cardiovascular diseases. Moreover, in almost half the cases, SCD is the first sign of an underlying cardiovascular condition. Although many forms of heart disease can lead to SCD, the most common process underlying SCD is ventricular fibrillation (VF), an irregular and uncoordinated contraction of cardiac muscles of ventricles (lower chambers of heart) due to disorganized electrical signals. VF is usually fatal if not reversed by defibrillation immediately. Most of the existing cardiovascular risk factors are poor at predicting SCD, even in those individuals with a history of heart disease, clearly showing that other environmental and/or genetic factors are likely to play a role in developing VF and SCD. Indeed, from population- and family-level studies, there is evidence for genetic susceptibility to SCD. However, studies to identify genetic factors underlying susceptibility to SCD directly have had limited success due to pooling of the very diverse forms of heart diseases leading to SCD into one group. Instead, we focus on the electrocardiographic QT interval, an intermediate observable characteristic/trait (phenotype) that predisposes to SCD. Electrocardiography, also known as ECG, measures the electrical activity of heart chambers, and the QT interval in an electrocardiogram corresponds to the time taken by ventricles to depolarize (activated state) and repolarize (resting state) in every heart beat. In the general population, QT interval varies across individuals and is a useful clinical marker as both prolongations and shortenings of the QT interval have been known to be associated with increased risk of cardiac arrhythmias and

SCD. We are interested in identifying the genes that underlie this variation with the aim that understanding the genetic factors for QT interval variation will potentially impact our understanding of SCD risk and its management. Our studies have the prospect to identify the genetic causes for QT interval variation, some of which in turn could serve as potential therapeutic (drug) targets or potential biomarkers (genes and gene products) to identify individuals at high risk for SCD. What we as a community have learned so far is that many genes together contribute to QT interval variation and that majority of DNA changes leading to QT interval variation do so not by altering the form of the gene product but rather by altering the amount of the gene product made by our heart cells. Starting with known genetic associations between DNA sequence variants and the QT interval in the general population, our work involves pinpointing the causes behind these associations to identify the underlying gene defects and how they impact QT interval.

RESEARCH PROJECTS

- Molecular characterization of QT interval GWAS signals to identify the underlying causal variants, genes and their mechanisms.

- Evaluation of constitutive and heart-restricted *Nos1ap* null mice to understand its role in cardiac electrophysiology.
- Functional genomic approaches to understand cardiac gene expression regulation.

KEY PUBLICATIONS

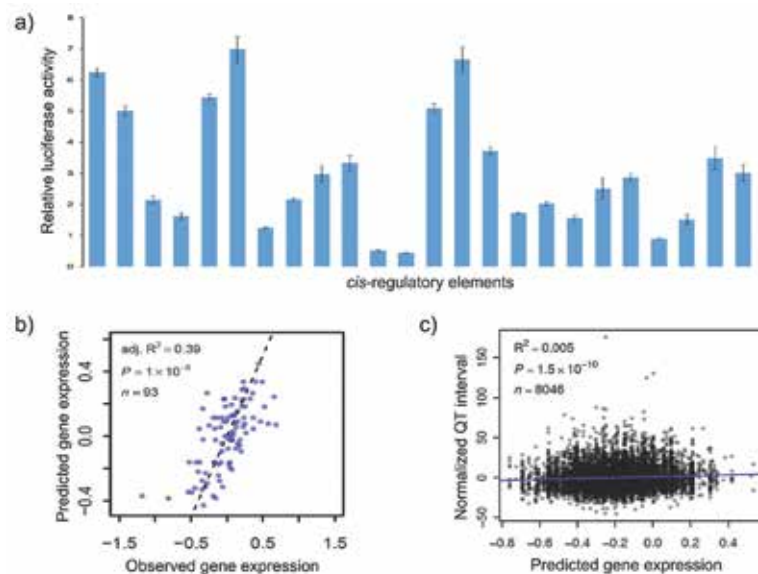
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LAB MEMBERS

Research assistant: Morgan Johnson, B.S.



We identify the causal *cis*-regulatory variants using reporter assays (a) and the causal gene by the impact these *cis*-regulatory variants have on gene expression (b), which in turn is evaluated for its impact on QT interval (c).



Ba-Bie Teng, Ph.D., FAHA

Professor

The Jerry and Maury Rubenstein Foundation

Distinguished Professorship in Heart Disease Research

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

Atherosclerosis is an inflammatory disease in the aorta that increases its severity as we age. The disease includes imbalance lipid metabolism that leads to hyperlipidemia and maladaptive immune responses that affect the arterial vasculature. Our research focuses on understanding the development of atherosclerosis and to elucidate the cross-regulation between atherosclerosis and immunity. We have generated a mouse model that mimics humans with hyperlipidemia by deleting both LDL receptor (LDLR) and RNA editing enzyme (Apoec1) genes (*LDb=Ldlr/-Apoec1/-*). These mice develop atherosclerosis as they age. Feeding on a Western high-fat diet accelerates their atherosclerosis development. Moreover, male mice develop atherosclerosis faster and more severely than females.

PCSK9 (proprotein convertase subtilisin/kexin type 9) is a newly identified causative gene for hyperlipidemia. Patients with elevated PCSK9 levels have increased plasma cholesterol and premature coronary artery disease. We delete PCSK9 gene from *LDb* mice (*LTP=Ldlr/-Apoec1/-Pcsk9/-*), showing decreased atherosclerosis and apolipoprotein B levels with improved function of endothelial cell. PCSK9 modulates autophagy signaling pathway by inhibiting the degradation of molecules and organelles via autophagosome/lysosome. PCSK9 modulates SREBP-1c lipogenesis, producing more atherogenic LDL containing elevated levels of cholesteryl ester and phospholipids, resulting in increased atherosclerosis. Furthermore, the presence of PCSK9 down regulates the protection role of TGF-β in endothelial cells. Thus, PCSK9 contributes to atherosclerosis through its multiple effects on autophagy, hepatic lipid metabolism and cellular immune function in endothelial cells.

The participation of various helper T cells in the development of atherosclerosis is complex and controversial. We show that *LDb* atherogenic mice exhibit increased plasma interleukin-17 (IL-17), which is associated with increased numbers of T helper 17 cells (Th17).

By deleting PCSK9 from *LDb* mice, these triple knockout *LTP* mice show opposite effect with decreased IL-17 and reduced Th17 cells. We hypothesize that PCSK9 might regulate the function of Th17 cells, which are critical for the pathogenesis of atherosclerosis.

Taken together, our laboratory is using current technologies including RNA-Seq, ATAC-Seq, RPPA and CRISPR/Cas9 to define the cellular and molecular mechanisms by which proatherogenic factors modulate disease development. Our discovery will provide insight into the understanding of physiological and pathological of disease process. It will provide a basis to develop efficient therapeutic approaches to combat the progression of diseases.

RESEARCH PROJECTS

- The role of PCSK9 in autophagy, inflammation, and atherosclerosis.
- Using CRISPR/Cas9 technique to generate IL-17 RC triple knockout mice to study its effect on atherosclerosis.
- Using genetic tools and proteomics to identify genes associated with atherogenesis and to develop genetic therapy for the treatment of atherosclerotic vascular diseases.

KEY PUBLICATIONS

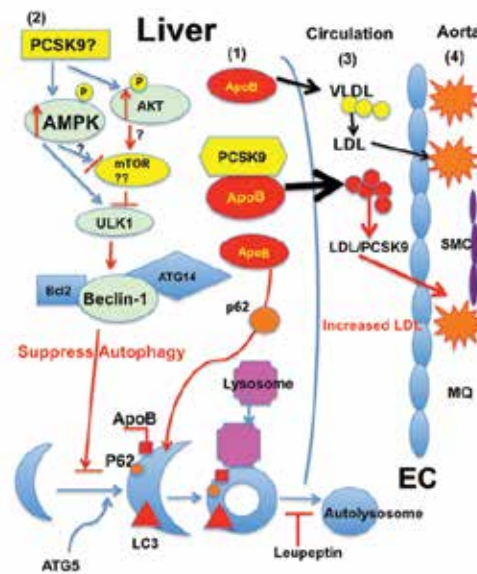
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PCSK9 Deficiency Reduces Atherosclerosis, Apolipoprotein B Secretion and Endothelial Dysfunction: Hua Sun, Ronald M. Krauss, Jeffrey T. Chang, and Ba-Bie Teng. *J Lipid Res* 59: 207-223 (2018). PMID: 29180444*. (This article was selected as the Cover for J. Lipid Research as shown below).

Non-native conformational isomers of PCSK9 induce immune response, reduce lipids and increase LDL receptor levels: Chuantao Jiang, Hua Sun, Hersharon Nischal, Ying Cao, Peng Wei, Jui-Yoa Chang, and Ba-Bie Teng. *International J. of Molecular Sciences* 19, 640 (2018). doi10.3390/ijms19020640

LAB MEMBERS

Research scientist: Hua Sun, Ph.D.
Visiting scientist: Yunlong Wang, Ph.D.
Research assistant: Xin Li



PCSK9 interacts with ApoB, modulates autophagy, alters LDL composition and affects atherogenesis. PCSK9 interacts with ApoB, modulates the autophagy degradation pathway by increasing the production of VLDL and altering the LDL compositions (1 and 3). The consequence of differences in LDL compositions induces different reaction on endothelial cells, affecting atherogenesis (4). PCSK9 regulates autophagy signaling pathway; it increases p-AKT and p-AMPK and suppresses the autophagy (2).



Sidney Wang, Ph.D.
Assistant Professor

Deciphering the regulatory code: A functional genomics approach to protein translation

Regulation of gene expression is fundamental to a wide range of biological processes. From cell fate determination during development to malignant transformation during tumorigenesis, precise control of gene expression forms the basis of these processes. Our current understanding of gene regulation is, however, far from complete. Most published studies that profile gene expression are transcript-centric (i.e. they focus on measuring mRNA levels and levels of transcription factor binding). While these efforts revealed intricate networks of cooperativity amongst transcription factors in shaping complex biological processes, much of the post-transcriptional regulation are left unexplored. It remains unclear whether the process of protein translation is regulated by a network of factors to an extent of complexity similar to transcription regulation. We ask questions such as “Do sequence specific RNA binding proteins (RBP) cooperate in controlling translation?” “Are there translational regulatory networks that orchestrate critical biological processes?” Our research program focuses on addressing these questions in biological contexts that are relevant to human health. Our immediate goals are to develop novel tools to systematically study RBP binding; to investigate regulatory functions of upstream Open Reading Frames (uORFs); and to integrate these functional genomics annotations with results from genetic studies in order to fine map the regulatory variants and to provide mechanistic understanding for disease associated variants.

RESEARCH PROJECTS

- Regulation of protein translation by uORF in stress response. Translation regulation by uORF has long been hypothesized based on supports from studies of a handful of uORFs. We have reported a systemic survey of uORF impact on protein translation and identified genetic variants associated with this impact. We are further expanding this line of research in the context of stress response, where global scale changes in translational regulation are expected.
- Using RNA binding protein footprint sequencing to investigate translational regulation of protein synthesis. RNA binding proteins are known to regulate protein translation. We aim to develop a general and effective tool to facilitate research in this area.
- Identification of functional novel coding regions across multiple tissues. We have previously identified 7,273 novel coding regions from a single cell type using ribosome profiling data. While we provided evidence of active translation at these loci, the biological function and importance of these loci remains unknown. We are following up on this line of research by designing knockout screens to identify loci that are essential for cell survival. We also are expanding our efforts

in identifying novel coding regions through performing ribosome profiling experiments in additional cell types and tissues.

- Gene expression buffering at the post-translational level. Gene expression at the transcript level are often assumed to propagate to the protein level. In a series of studies, we have demonstrated that, in our cell line model system, the variations observed at the transcript level is often buffered at the protein level through post-translational processes. In order to evaluate how general this observation is, we are now expanding our analysis to other tissue types and species.

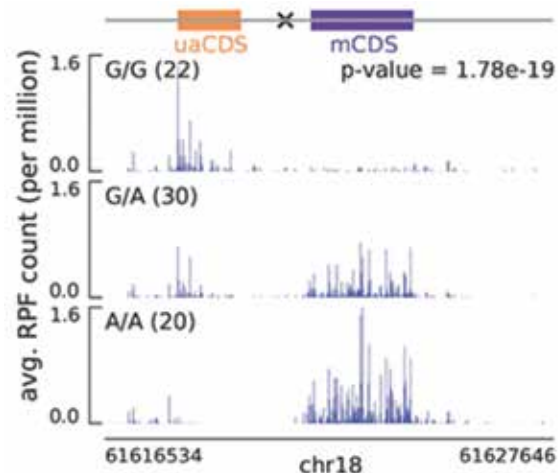
KEY PUBLICATIONS

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Wang SH, Hsiao CJ, Khan Z and Pritchard JK. Post-translational buffering leads to convergent protein expression levels between primates. *Genome Bio.* 2018 Jun 27;19(1):83.

LAB MEMBERS

Post-doctoral fellow: Sandeep Bansal
Research assistant: Zhen Zuo



Genotype of a genetic variant is associated with uORF regulation of protein translation at HMSD locus in HapMap LCL. Negative correlation in the levels of protein translation between the two Open Reading Frames at HMSD locus is clearly shown through stratifying ribosome profiling data by genotype.



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

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

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

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

erythematosus.

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

Research interests include:

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

Rick Wetsel, Ph.D.

Center Director & Professor

Hans J. Müller-Eberhard, M.D., Ph.D. and Irma Gigli, M.D. Distinguished Chair in Immunology



Rick Wetsel, Ph.D.

Professor and Director of the Center for Immunology and Autoimmune Diseases
 Hans J. Muller-Eberhard, M.D., Ph.D. and Irma Gigli, M.D. Distinguished Chair in Immunology

Innate immunology, inflammation, infectious diseases, and stem cell therapeutics for diseases of the lung and eye

Chronic diseases of the lung and eye are often the result of dysregulation of the immune and inflammatory response to pathogenic or toxic substances, resulting in the destruction of healthy tissue, establishment of debilitating pathologies due to fibrosis, and impairment of normal tissue repair mechanisms. However, the paucity of cellular and molecular knowledge regarding lung and eye immunity, inflammation, and repair processes has slowed the development of novel therapeutics that could be used for the effective treatment of chronic diseases of the lung and eye. Accordingly, our laboratory has for the past several years focused on delineating the key molecules that mediate the inflammatory and immune responses in the lung and eye during both normal and pathological conditions. Much of this research has involved studies of the complement system. The complement system is a major arm of the innate immune system and is well known for being the first line of defense against bacterial and viral pathogens. It is comprised of over 30 plasma proteins and cellular receptors. It has become evident in the past decade that the complement system is very important in other biological functions other than killing bacteria and viruses. These other functions include tissue regeneration, polarization of immune cells including T-cells, and normal development of the central nervous system. In addition to these novel complement biological functions, dysregulation of the complement system has been discovered as a major cause of AMD and a major contributor to lung diseases such as asthma and COPD. To determine the overall importance and biological functions of complement, we have generated numerous “knock-out” mice in which the genes encoding specific complement proteins, regulators, and cell receptors have been selectively ablated by gene targeting and homologous recombination using mouse embryonic stem cells. The generation of these mice has facilitated the discovery of numerous biological roles of complement in the pathogenesis of various disease pathologies.

For example, in studies using mice in which the C3a receptor was deleted, we discovered that the complement anaphylatoxin peptide C3a is an important mediator of key hallmarks of asthma, including airway hyperresponsiveness, and therefore may prove to be an excellent therapeutic target for the treatment of asthma. As part of this overall research program, we are investigating the therapeutic use of embryonic (hES) and induced pluripotent (iPS) stem cell derived cells for repair of damaged retina in AMD, for regeneration of the damaged lung epithelium in acute lung injury, and for cell-based gene therapy for newborns born with genetic deficiency of surfactant protein B.

RESEARCH PROJECTS

- Determine how the function of vascular and lymphatic endothelial cells are impacted by complement during the immune response
- Generate “universal donor” embryonic stem cell lines that can be differentiated into transplantable cells that will not be rejected after transplantation
- Evaluate the therapeutic potential of gene corrected iPS cell-derived lung cells for surfactant protein deficiencies
- Develop hES-retinal pigment epithelial cells therapeutics for treatment of AMD

KEY PUBLICATIONS

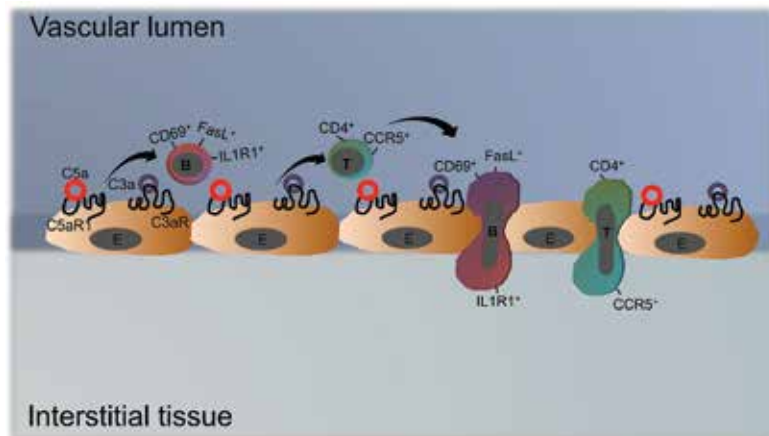
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LAB MEMBERS

Senior research scientist: Stacey Mueller-Ortiz, Ph.D.
 Research scientist: Ken Simmons, Ph.D.
 Post-doctoral fellow: John L. Mazzilli, M.D.
 Instructor: Pooja Shivshankar, Ph.D.



Model illustrating how the vascular endothelium on stimulation by the complement anaphylatoxin peptides (C3a and C5a) activates B-cells and polarizes T-cells during an immune response. Endothelial cells shown in brown with letter E. T-cells and B-cells shown in green and purple, respectively. The elongated cells depict activated B-cells and polarized T-cells as they transigrate through the endothelium.



Michael R. Blackburn, Ph.D.

Dean and John P. McGovern Distinguished Professor of Biomedical Sciences
 The University of Texas Graduate School of Biomedical Sciences at Houston
 Professor and Vice Chairman Department of Biochemistry and Molecular Biology
 William S. Kilroy Sr., Chair in Pulmonary Disease
 Executive Vice President and Chief Academic Officer, UTHealth

Adenosine signaling and the regulation of chronic lung disease

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

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

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

RESEARCH PROJECTS

- Examining the role of A2B adenosine receptor expression on pulmonary macrophages during the progression of pulmonary fibrosis
- Investigation of adenosine transport in acute and chronic lung injury

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

KEY PUBLICATIONS

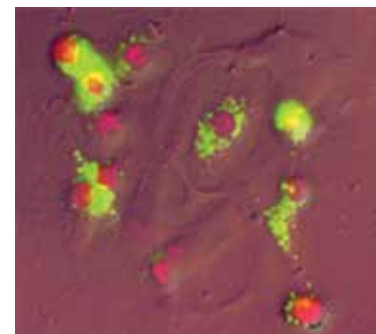
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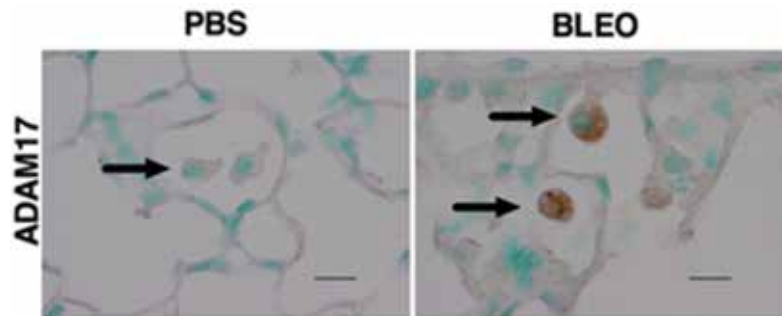
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LAB MEMBERS

Assistant professor: Tingting Weng, Ph.D.
 Senior research scientist: Kelly Volcik, Ph.D.
 Research associate: Ning-Yuan Chen
 Research scientist: Jose Molina, Sr.
 Graduate student: Josh Ko, Ph.D.



Primary type II alveolar epithelial cells isolated from genetically modified mice.



Increased expression (brown color) of proteinases in pulmonary macrophages in mice with pulmonary fibrosis (BLEO).



Amber Luong, M.D., Ph.D.

Associate Professor, Center for Immunology and Autoimmune Diseases and Department of Otorhinolaryngology – Head and Neck Surgery

Environmental triggers regulating innate immune responses in chronic airway inflammation

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

CRS is clinically classified into two groups defined by the absence or presence of nasal polyps. This clinical classification has been supported generally by immunologic profiles of the inflamed sinus tissue. CRS without nasal polyps is characterized by predominance of neutrophils and elevated T helper cell type 1 (Th1) cytokines, while CRS with nasal polyps (CRSwNP) has high presence of eosinophils, mast cells, and basophils and expression of type 2 cytokines such as IL-4, IL-5, and IL-13. However, recent study by our labs using cluster analysis of genetic information has identified endotypes within these clinical phenotypes, allowing for possible personalized treatment.

Allergic fungal rhinosinusitis (AFRS) is a clinical subtype of CRSwNP that is associated with an accumulation of thick entrapped mucus laden with fungal hyphae and eosinophils between the nasal polyps and within sinus cavities. This trapped mucus can cause expansion of sinus cavities and ultimately erosion of bone separating the sinuses from the intracranial and orbital cavities which can result in intracranial

complications and blindness, respectively.

Epithelial cells

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

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

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

In addition, my lab is interested in the molecular characterization of fungi-mediated signaling pathway(s) and the fungal component responsible for signaling in the inflammatory response in some CRS subtypes. This has led us to our recent interest in CRS representing a disease at the crossroads of coagulation and inflammation. studies are focusing on the elucidating the specifics of the pathways that intersect these two entities as it relates to CRS.

RESEARCH PROJECTS

- Characterization of immunologic and molecular defects contributing to pathophysiology of allergic fungal rhinosinusitis
- Molecular signaling through respiratory epithelial cells of fungi alone and with other environmental triggers responsible for initiating and/or maintaining the characteristic Th2 immune response
- Clinical characterization and identification of biomarkers for CRS subtypes

KEY PUBLICATIONS

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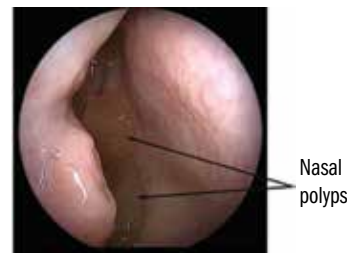
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LAB MEMBERS

Hua Sun, Ph.D.
Yi-Dong Li



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



Dachun Wang, M.D.

Assistant Professor

Lung stem/progenitor cells and diseases

Lung epithelial stem/progenitor cells are critical for the maintenance of homeostasis of airway and alveolar epithelial cell populations that are constantly exposed to injurious stimuli from the environment. There are at least three stem/progenitor cell types responsible for maintaining distal lung epithelial cell populations: 1) alveolar epithelial type II cells; 2) the transient amplifying bronchiolar Clara cells; and 3) a subset of variant Clara cells located at the bronchioalveolar duct junction and the branch point-associated neuroepithelial bodies. Loss of normal functions of any of these stem/progenitor cell types due to injuries or genetic deficiencies is thought to play an important role in the development of chronic or severe pulmonary diseases, including pulmonary fibrosis, asthma, COPD, cystic fibrosis, neonatal respiratory distress syndrome (RDS) and cancer. However little is known regarding the pathogenesis of these pulmonary diseases as well as the corresponding repair mechanisms, since there is no reliable biomedical research model available for studying the biological and disease processes both *in vivo* and *in vitro*. In addition, currently available treatments for those pulmonary diseases at best release symptoms and improve life quality within a limited time range, and the long-term outcome is unfortunately not positive. There is an imperative need for developing of novel therapies to facilitate the treatment of lung diseases. Without doubt, the distal lung stem/progenitor cells represent the key targets for exploring the mechanisms underlying pathogenesis of lung diseases. During the past few years, considerable interest has developed in the therapeutic 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 provide a good model to study lung disease processes. My research efforts are focused on 1) to isolate

and characterize human and mouse ES cell derived distal lung stem/progenitor cell types both *in vitro* and *in vivo*; 2) to generate "clinical grade" lung disease-specific iPS cells for studying pulmonary disease processes and for developing cell-based gene therapeutic strategy for lung tissue regeneration; 3) to identify and characterize factors or regulatory pathways that control distal lung stem/progenitor cell fate during the disease processes for developing a novel strategy for targeted activation of endogenous stem/progenitor cells for lung tissue repair; and 4) to explore lung cancer stem cell-derived exosome miRNA pathways.

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

KEY PUBLICATIONS

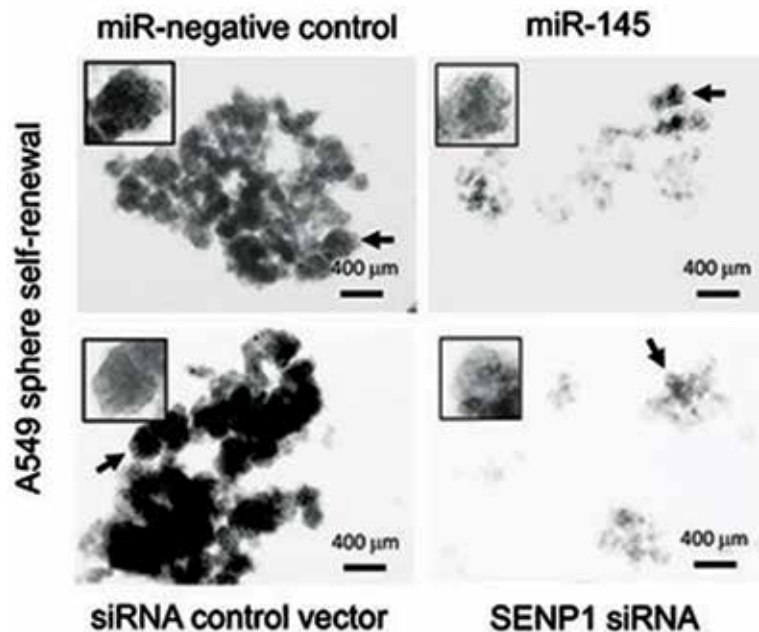
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RESEARCH PROJECTS

- Isolation and characterization of ES/iPS cell derived distal lung stem/progenitor cells

LAB MEMBERS

Research associate: Dr. Yuan Quan



Exosome miRNA-145 suppresses self-renewal capacity of lung cancer stem cells using SENP1 as a key target.



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

Transgenic and stem cells core facility

The Transgenic and Stem Cells Core Facility provides a unique service to the UTHealth scientific community by generating animal models of specific human diseases in order to develop novel treatments. The laboratory was established in 1998 and since that time, it has generated more than 800 new transgenic, knock-out and knock-in animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions.

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

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

Our laboratory has also generated human ES cells with stable deletion of an X-chromosome. This cell line represents a pluripotent stem cell model to study the disease mechanisms of Turner's syndrome, one of the most common genetic abnormalities seen in female embryos.

RESEARCH SERVICES

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

Accomplishments:

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

RESEARCH PROJECTS

- Stem Cell Therapy for Age-Related Macular

Degeneration

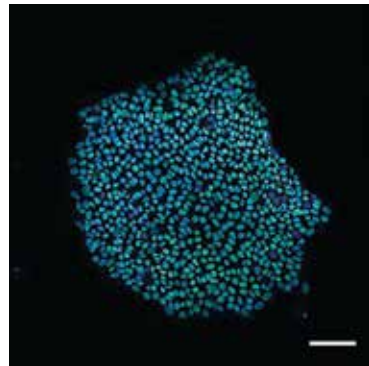
- Patient-derived tumor xenograft implantation models

KEY PUBLICATIONS

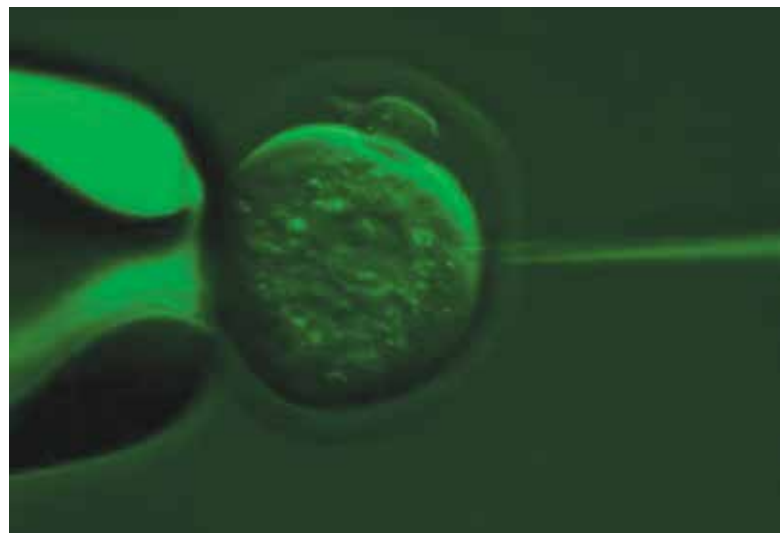
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LAB MEMBERS

Senior research associate: Aleksey Domozhirev
Post-doctoral fellow: John Mazzilli, M.D.



NMR-1 human ES cells: A pluripotent stem cell model of Turner's syndrome



CRISPR/Cas9-mediated gene editing: microinjection of mouse zygotes to produce genetically-engineered mice with specific genetic mutations



The Center for Metabolic and Degenerative Diseases integrates eight laboratories investigating aging-associated diseases, including muscle wasting, neurodegeneration, type-2 diabetes, and cancer. Aging, stress, and obesity-associated changes in brain activity, energy metabolism, cell signaling, protein homeostasis, and cell fate determination that lead to physiological abnormalities are being interrogated in animal models and studies on clinical specimens. The specific questions being addressed by the Center's faculty include the following:

- How do progenitor cells in adipose tissue commit to white and brown adipogenesis?
- How does dysfunction of adipose cells promote progression of diseases and aging?
- How does fibrosis and inflammation in adipose tissue affect insulin sensitivity?
- How is angiogenesis implicated in adipose and muscle tissue remodeling?
- Can cells in adipose tissue be targeted for therapeutic purposes?
- How do stress hormones regulate muscle energy utilization in type 2 diabetes?
- What gene expression pathways can be targeted to treat muscle diseases?
- How does the brain and circadian clock

control the body's energy balance?

- How does the circadian clock protect against liver disease and cancer?
- How does the brain control glucose homeostasis in type 1 and type 2 diabetes?
- What are the functions of the genes mutated in neurodegenerative diseases?
- How does disruption of cellular homeostasis networks, including chaperones and autophagy, cause neurodegeneration in Huntington's and Parkinson's disease?
- How does stress impact the pathogenesis of Alzheimer's disease and post-traumatic stress disorder?

Collaboration among the Center's laboratories promotes research synergy, thereby increasing productivity and innovation. The Center's members collaborate with pathologists, epidemiologists, and clinicians to translate their discoveries for the benefit of patients with metabolic and degenerative diseases.

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



Mikhail Kolonin, Ph.D.

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

Adipocyte progenitor cells: Dysfunction in disease and aging

My group has a broad interest in aging-related diseases, including type 2 diabetes, degenerative diseases, and cancer. We are focusing on the role of adipose tissue and stem cells in the context of these pathologies. Healthy aging relies on the function of fat (adipose) tissue that coordinates the metabolism of other organs. Lipids, the molecules serving the body as an energy source, can cause metabolic disease if fat tissue stops acting like a sponge by absorbing lipids. There are two types of fat cells (adipocytes) that use lipids differently. White adipocytes store lipids and release them in times of energy scarcity, while brown adipocytes burn lipids off to keep the body warm. In obesity, overgrown white fat becomes inefficient in holding lipids, hence causing diabetes, cardiovascular diseases, and cancer. In contrast, active brown fat can prevent the onset of metabolic disease. Both white and brown adipocytes are continuously replaced as we age, and their pools in fat tissue are maintained by adipose stem cells (ASCs). In obesity, increased numbers of white fat ASCs are generated. Our laboratory has discovered that tumors recruit these ASCs that fuel cancer progression. Currently, no drugs targeting cells in fat tissue for obesity or cancer indications are available.

Taking advantage of our expertise in targeted therapeutics, we have developed the first experimental drug (D-WAT) targeting ASCs. Our publications demonstrate that D-WAT prevents obesity and suppresses tumor growth in mice. In collaboration with bariatric surgeons, we recently showed that D-WAT targets human ASCs. Our reports indicate that D-WAT treatment spares brown fat ASCs, leads to generation of brown adipocytes, and enables a short-term metabolic benefit. The future focus of our work is on investigating the role of ASCs in healthy aging. As we age, fat cell numbers decrease and the deficient fat tissue fails to effectively absorb lipids, which start spilling into other organs. This can cause inflammation and metabolic disorders accounting for cancer and organ failure in the elderly. We hypothesize that adipocytes run out because ASCs become 'exhausted' with age and lose replicative potential and that obesity leads to premature exhaustion of ASCs because it involves excessive fat cell number expansion. This hypothesis is now being tested in animal models. To ensure safety of ASC targeting, we are performing studies in mouse models to establish the consequences of D-WAT treatment in aging. Understanding the roles of transient and chronic adipose tissue inflammatory signaling in integrative biology and insulin resistance in type 2 diabetes is our most recent pursuit.

RESEARCH PROJECTS

- Adipocyte progenitors: lineages, function in health, and targeting in disease
- Aging-associated exhaustion of adipocyte progenitors and its role in metabolic disease
- Cancer-associated adipocytes as the source of lipids driving cancer progression

KEY PUBLICATIONS

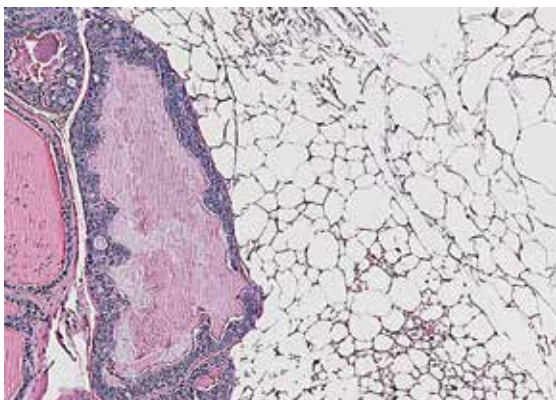
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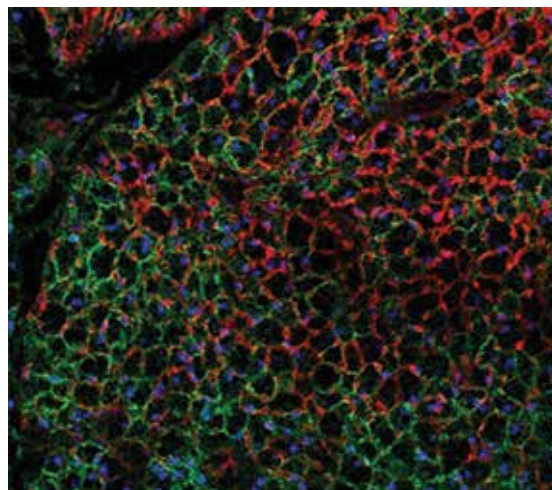
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LAB MEMBERS

Senior research scientists: Alexis Daquinag, Zhanguo Gao
 Research scientist: Fei Su
 Graduate student: Shraddha Subramanian
 Research assistant: Cale Fussell



A prostate section stained with hematoxylin/eosin showing a tumor (left) and adjacent adipose tissue (right).



Distinct lineages of adipocytes identified in mouse brown fat tissue through lineage tracing of progenitor cells by using green and red reporter genes. Blue: nuclei.



Rebecca Berdeaux, Ph.D.

Associate Professor

Regulation of muscle metabolism and regeneration by cellular signaling pathways

Skeletal muscle comprises approximately 40% of body mass in healthy individuals. In addition to the major function of skeletal muscle to contract and perform work such as lifting objects and escaping predators, skeletal muscle is important for utilization and storage of energy, maintenance of spine stability and mobility, and generation of heat to help maintain body temperature. In fact, skeletal muscle is responsible for ~85% of insulin-induced uptake of sugar from the blood after a meal. Muscle insulin resistance is a major contributing factor to high blood sugar levels in people with type 2 diabetes. During aging, muscle mass declines, causing both disruptions in mobility as well as metabolism. In aging individuals, loss of muscle stem cell activity is thought to be partly responsible for the loss of muscle mass.

Our lab studies hormone-activated pathways in mature skeletal muscle and in muscle stem cells in hopes of identifying novel therapeutic targets to improve muscle metabolism and muscle stem cell function. The overall goal is to ameliorate insulin resistance and promote healthy aging by maintaining healthy muscle metabolism and promoting the capacity of muscle stem cells to be activated. We found that intracellular pathways activated by hormones like adrenaline stimulate muscle stem cell activity, and we aim to identify how that occurs at the molecular level. One of the proteins induced by hormone activity in muscle is an enzyme called SIK1. One of our major aims is to understand how SIK1 contributes to impaired muscle energy utilization in type 2 diabetes and whether SIK1 might be a useful drug target to treat insulin resistance and type 2 diabetes.

RESEARCH PROJECTS

- Identification of the roles of SIK1 in muscle glucose metabolism in obesity
- Study of how cAMP signaling regulates skeletal muscle stem cell activity in aging
- Understand how different hormonal pathways collaborate to regulate energy utilization and storage in skeletal muscle in times of feast and famine

KEY PUBLICATIONS

Akhmedov D, Mendoza-Rodriguez MG, Rajendran K, Rossi M, Wess J, Berdeaux R. Gs-DREADD knock-in mice for tissue-specific, temporal stimulation of cyclic AMP signaling. *Mol Cell Biol.* 2017 37(9): e00584-16.

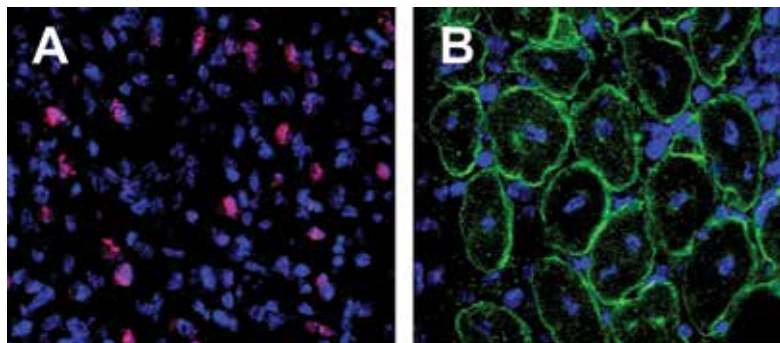
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LAB MEMBERS

Research scientist: Dmitry Akhmedov
 Research assistants: Alexander Ambrocik, Mahmoud Farhan
 Medical student: Victor Gonzalez



Characterization of regenerating skeletal muscle. A) Three days after muscle injury, muscle stem cells become activated to grow and divide. The red stain recognizes the proliferating cells within the damaged muscle. Blue shows all cells in the tissue section. B) Two days later, new muscles are formed and regrow. In this experimental model, we expressed a receptor (stained in green) in the newly formed muscle cells. This receptor allows us to stimulate the same hormonal pathways with a drug that does not act on any other receptors normally expressed in the muscle. In this way, we can mimic hormone-activated pathways and study the effects of those pathways on muscle regeneration and regrowth of new muscle fibers.



Kristin Eckel-Mahan, Ph.D.

Assistant Professor

Circadian clocks in health and disease

The goals of my lab center on the role of the circadian clock in health and disease. Circadian rhythms, which are endogenous, self-perpetuating oscillations of 24-hour periodicity, are present in all cells of the body. While our sleep/wake cycle, food intake, internal body temperature, hormone secretion, etc. adapt to and are aligned with the 24-hour rotation of the earth on its axis, modern technology has provided ample ways to disrupt this alignment. Examples include travel across time zones (jet-lag), working a night shift or rotating shifts, and light contamination by white and blue light sources. In addition, some clock gene mutations lead to sleep disorders. When the circadian clock is disrupted genetically or environmentally, several deleterious outcomes result, including accelerated aging, cancer, and metabolic disease. We are trying to understand why circadian disruption produces these effects.

While the central clock (or “pacemaker”) of the brain is predominantly controlled by light, circadian oscillations in peripheral organs are heavily influenced by other *zeitgebers* (“time-givers”), such as food. When circadian clocks across the body are misaligned, risk for metabolic disease increases. Our current experiments include those designed to reveal which *zeitgebers* are most important for tissue-specific clock function and the mechanisms underlying their *zeitgeber* properties. We are also interested in how disrupted peripheral clocks communicate back to the brain and affect the function of the central pacemaker, the suprachiasmatic nucleus.

Our lab and others have shown that nutrient timing and quality have a strong influence on peripheral circadian clocks, such as the clocks of the liver, muscle, and adipose tissue. Over-consumption of an unhealthy, high-fat diet can cause substantial disruption of the circadian clock in normally highly insulin-sensitive tissues that become insulin resistant under these feeding conditions. Thus, we hypothesize that a high-fat diet induces weight gain in part by “misaligning” the circadian rhythms of normally

highly insulin-sensitive peripheral tissues, vs. the brain clock. Clock misalignment is thought to be involved with several metabolic disorders, including type 2 diabetes.

In addition to metabolic diseases, such as obesity and diabetes, circadian disruption also has been linked to cancer. We have identified that a nuclear receptor with circadian activity gets altered in the context of specific liver cancers. Attempts to restore the circadian function in these cells causes cell death and impairs tumor growth. We are using this information to determine whether these subsets of liver cancers might be responsive to particular circadian manipulations not previously thought of as being applicable to hepatic tumorigenesis.

RESEARCH PROJECTS

- Mechanisms by which circadian disruption leads to metabolic disease
- Mechanisms linking circadian disruption to liver cancer
- Elucidating the role of the circadian clock in human adipose tissue

KEY PUBLICATIONS

“Incompatibility of the circadian protein BMAL1 and HNF4 α in hepatocellular carcinoma” Baharan Fekry, Aleix Ribas-Latre, Corrine Baumgartner, Jonathan R. Deans, Christopher Kwok, Pooja Patel, Loning Fu, Rebecca Berdeaux, Kai Sun, Mikhail G. Kolonin, Sidney H. Wang, Seung-Hee Yoo, Frances M. Sladek, and Kristin Eckel-Mahan *Nature Communications* 2018; 9: 4349.

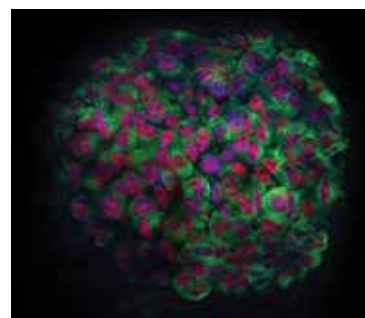
“Rosiglitazone Reverses High Fat Diet-Induced Changes in BMAL1 Function in Muscle, Fat, and

Liver Tissue in Mice” Ribas Latre, A.; Fekry, B.; Kwok, C.; Bamgarner, C.; Shivshankar, S.; Sun, K.; Chen, Z.; and Eckel-Mahan, K. *International Journal of Obesity* 2018 May 24

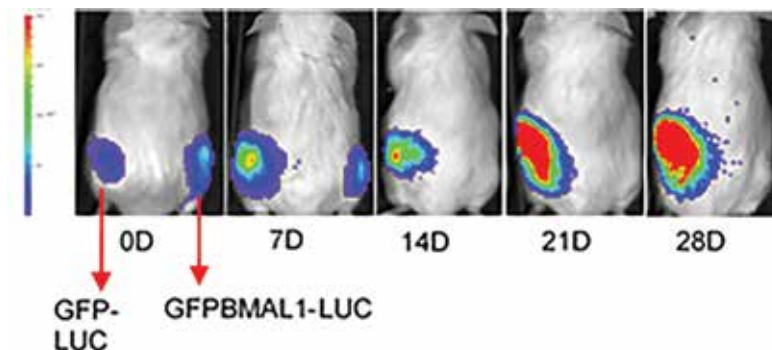
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LAB MEMBERS

Post-doctoral fellows: Baharan Fekry and Aleix Ribas Latre
 Research assistants: Corrine Baumgartner and Alaa Tamim



HNF4 α -positive tumor cells grown as a spheroid. These cells are deficient in the circadian protein BMAL1. (Blue= nuclear stain, Green=tubulin, Red= HNF4 α).



Impaired growth of HNF4 α -positive liver cancer cells ectopically expressing the circadian protein BMAL1 (right flank) compared to control cells (left flank). Tumor growth was monitored over the course of four weeks.



Nicholas Justice, Ph.D.

Assistant Professor

Stress signaling in psychiatric and degenerative disease

High stress causes anxiety- and depression-related diseases that are among the most common suffered by our population. In addition, the consequences of chronic stress, including elevations in the stress hormone, cortisol, can negatively impact other seemingly unrelated conditions, from metabolic diseases like diabetes, to age-dependent degeneration of neurons that occurs in Alzheimer's disease.

Our lab aims to identify mechanisms by which stress in our lives changes brain function to alter our bodies' physiology and negatively impact health. The identification of specific stress-related mechanisms that drive disease will not only demonstrate the preventative power of controlling stress in our own lives, it also will offer drug targets in the context of specific diseases where manipulation of stress circuits could benefit patients. Our long-term goal remains preventing the onset and progression of metabolic and degenerative diseases, in part through the understanding and manipulation of the neural and hormonal circuits that respond to stress.

I obtained a B.A. from the University of California at Berkeley, and a Ph.D. in Neuroscience at the University of California, San Francisco (UCSF), where I studied in the lab of Yuh Nung Jan. I moved to the Salk Institute in San Diego for post-doctoral training, where I studied under Wylie Vale, my first Houstonian mentor. At the Salk Institute, I learned the molecular mechanisms by which the body responds to stress. I moved to Houston as an instructor at Baylor College of Medicine studying the influence of stress in Alzheimer's disease with Hui Zheng at the Huffington Center on Aging, before accepting a faculty position at the IMM.

RESEARCH PROJECTS

- Defining the anatomy of neuronal circuits that respond to stress
- Understanding a novel influence of stress hormones on behavioral control
- Defining a local circuit that both controls and

- coordinates stress hormone release
- Searching for biomarkers that predict PTSD in recently traumatized patient populations
- Understanding how chronic stress-related disease influences Alzheimer's disease progression.
- Identification of stress circuits that are perturbed by ongoing neurodegenerative disease

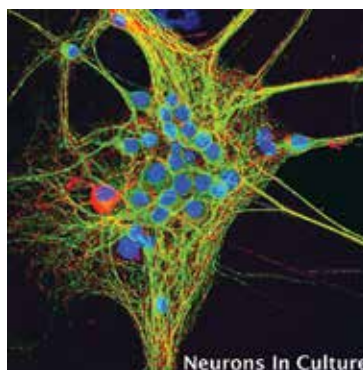
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Hunt, A.J., Dasgupta, R., Rajamanickam, S., Jiang, Z., Beierlein, M., Chan, C.S., and Justice, N.J. (2018). Paraventricular hypothalamic and amygdalar CRF neurons synapse in the external globus pallidus. *Brain Struct. Funct*

Jiang, Z., Rajamanickam, S., and Justice, N.J. (2018). Local Corticotropin-Releasing Factor Signaling in the Hypothalamic Paraventricular Nucleus. *J. Neurosci.* 38, 1874-1890.

Ramot A, Jiang Z, Tian JB, Nahum T, Kuperman Y, Justice N*, Chen A*. Hypothalamic CRFR1 is essential for HPA axis regulation following chronic stress. *Nat Neurosci.* 2017 Mar;20(3):385-388. doi: 10.1038/nn.4491. Epub 2017 Jan 30. (* - co-senior)



Neurons in Culture: Cultured primary neurons form networks. Excitatory neurons (green) and inhibitory neurons (red) signal to each other to maintain steady neural activity. The nucleus of each neuron is labeled in blue.

Justice NJ*, Huang L, Tian JB, Cole A, Pruski M, Hunt AJ Jr, Flores R, Zhu MX, Arenkiel BR, Zheng H. Posttraumatic Stress Disorder-Like Induction Elevates β -Amyloid Levels, Which Directly Activates Corticotropin-Releasing Factor Neurons to Exacerbate Stress Responses. *J Neurosci.* 2015 Feb 11;35(6):2612-23. (*-Corresponding author)

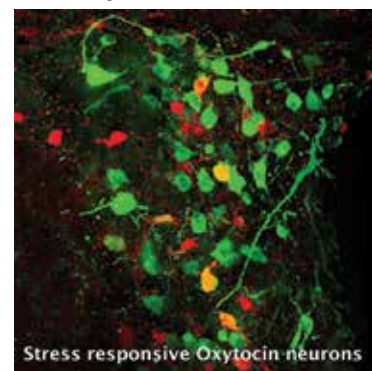
Justice NJ, Blount AL, Pelosi E, Vale W, Bilezikjian LM. Impaired FSH β expression in Foxl2Mutant Pituitaries. *Mol Endocrinol.* 2011 Aug;25(8):1404-15. Sztainberg, Y, Kuperman,

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

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LAB MEMBERS

Post-doctoral fellows: Shivakumar Rajamanickam, Ph.D.; Zhiying Jiang, Ph.D. Student: Jing Cai



Stress responsive Oxytocin Neurons: We recently discovered a subpopulation of neurons that make Oxytocin (green) - a hormone that promotes milk production and maternal care, and is also purported to improve emotional and social behavior - that are sensitive to the stress neuropeptide, CRF (CRF receptor cells are red). These stress sensitive Oxytocin neurons (yellow) express CRF receptors only in females that have given birth to a litter, suggesting a link between stress signaling and raising offspring that is likely a key to postpartum depression.



Vihang Narkar, Ph.D.
Associate Professor

Nuclear receptor & co-activator signaling in skeletal muscle

LAB MEMBERS

Post-doctoral fellows: Danesh Sopariwala, Neah Likhite
Research assistants: Meghna Seth, Nitya Narayana

We are investigating the role of nuclear receptors and their co-regulator proteins in skeletal muscle function and diseases. Specifically, we are interested in how these factors control skeletal muscle endurance, size, and regenerative capacity through regulation of gene expression. Our work has therapeutic implications in sports medicine, diabetes, and orphan diseases, such as muscular dystrophies, where muscle function and structure is commonly compromised.

RESEARCH PROJECTS

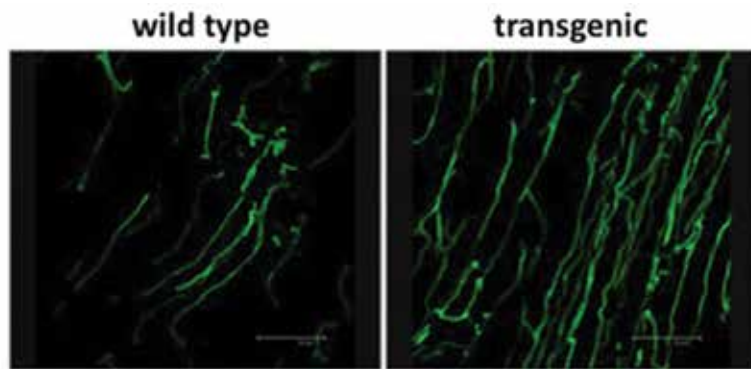
- Regulation of muscle metabolism, vascularization, and endurance by ERR-alpha/gamma.
- Regulation of apoptosis, autophagy, and muscle mass by PGC1-beta.
- Therapeutic role of ERR's in ischemic muscle and Duchenne Muscular Dystrophy.
- Activation of muscle stem cells by nuclear receptors.

KEY PUBLICATIONS

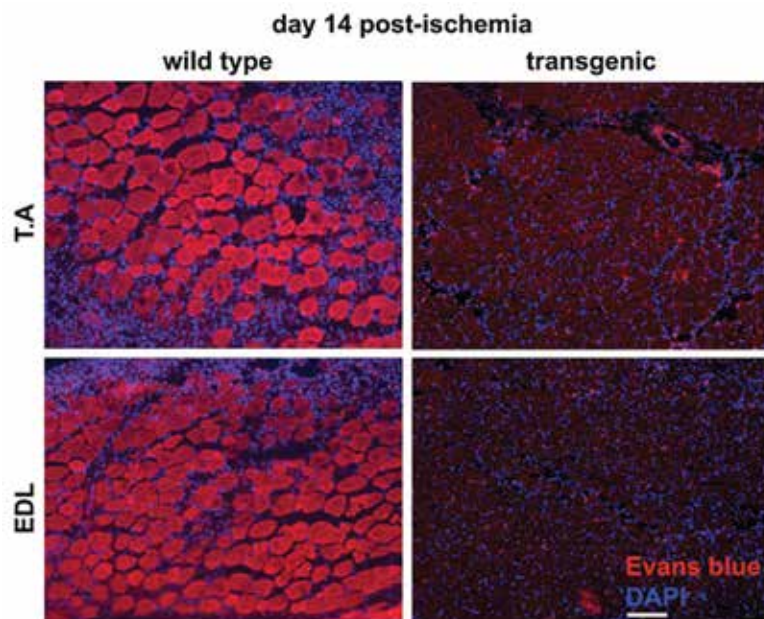
Bhat RR, Yadav P, Sahay D, Bhargava DK, Creighton CJ, Yazdanfard S, Al-Rawi A, Yadav V, Qin L, Nanda S, Sethunath V, Fu X, De Angelis C, Narkar VA, Osborne CK, Schiff R, Trivedi MV. GPCRs profiling and identification of GPR110 as a potential new target in HER2+ breast cancer. *Breast Cancer Res Treat.* 170(2): 279-292, 2018

Narkar VA. PGC1 α Promoter Methylation and Nucleosome Repositioning: Insights Into Exercise and Metabolic Regulation in Skeletal Muscle. *Endocrinology.* 158(7): 2084-2085, 2017

Sopariwala DH, Yadav V, Badin PM, Likhite N, Sheth M, Lorca S, Vila IK, Kim ER, Tong Q, Song MS, Rodney GG, Narkar VA. Long-term PGC1 β overexpression leads to apoptosis, autophagy and muscle wasting. *Sci Rep.* 7(1): 10237, 2017



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



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



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

Assistant Professor

Targeting adipose tissue for treatment of obesity and diabetes

Research in our laboratory examines the essential contributions of adipocyte-derived factors to the dynamics of adipose tissue remodeling during obesity development and pinpoints them as critical factors with clinical significance in human obesity and insulin resistance.

In the past years, we discovered that obese fat pads are frequently hypoxic and HIF1 α induction is the initial step which ultimately leads to local fibrosis and inflammation in adipose tissue. More importantly, we further demonstrated that VEGF-A induced angiogenesis in white adipose tissue could be dichotomous and metabolic context dependent: at the early stage of obesity development, angiogenesis is metabolically beneficial by improving vascularization and inducing a “browning” phenotype in white adipocytes. In contrast, in pathologically expanded adipose tissue, antiangiogenic action leads to improvements in metabolism by ablating dysfunctional adipocytes.

We further explored the fine-tuned regulation of adipose tissue remodeling at other levels in obese and diabetic animal models. Indeed, we found fibrosis is the hallmark in the metabolically dysfunctional adipose tissue and MT1-MMP (MMP14) plays a critical role in regulation of the levels of extracellular matrix (ECM). Of note, our recent research suggests that the regulation of ECM flexibility by MT1-MMP is also metabolic context dependent: On the one hand, at early stages of obesity, MT1-MMP cleaves collagenous proteins and stimulates angiogenesis in combination with VEGF-A and leptin, thus relieving the pathological conditions caused by hypoxia. On the other hand, in the context of pre-existing unhealthy adipose tissue, it digests collagen 6 α 3 and produces endotrophin which accelerates fibrosis and inflammation, ultimately leading a highly unfavorable microenvironment to sustain metabolic flexibility.

Most recently, we purified lipid droplet proteins from the brown fat tissues and analyzed them by Mass Spectrometry. Excitingly, we found many novel proteins that target lipid droplets upon sympathetic activation. Ces3 is one of the

candidates. We found that lipid-targeting Ces3 has lipase activity which digests lipid droplets. More importantly we discovered that Ces3 is involved in thermogenesis during cold exposure by regulating the thermogenic molecule UCP-1. We are now investigate the mechanism(s) by which Ces3 up-regulates UCP-1.

RESEARCH PROJECTS

- Hypoxia induced pathological changes in adipose tissue.
- VEGF-A stimulated metabolic benefits during adipose tissue healthy expansion.
- Reversibility of adipose tissue fibrosis by novel anti-fibrotic therapies.
- Essential role of Ces3 in thermogenesis in brown adipose tissue.

KEY PUBLICATIONS

Zhao YS, Gu X, Zhang NY, Kolonin MK, An ZQ, Sun K. Divergent Functions of Endotrophin on Different Cell Populations in Adipose Tissue.

AJP-Endo & Metab. 2016 Dec 1;311(6):E952-E963.

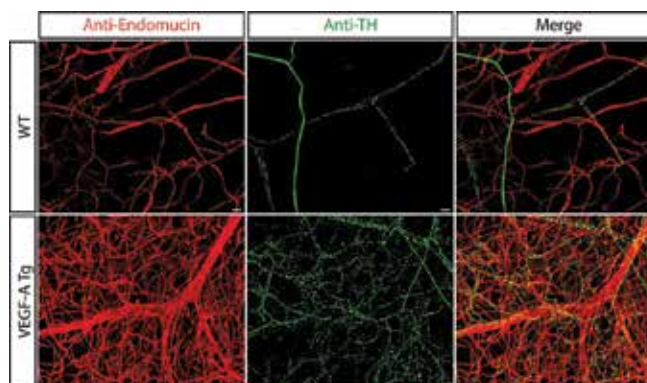
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Zhao YS, Li X, Yang L, Eckel-Mahan K, Tong Q, Gu X, Kolonin MG, Sun K. Transient Overexpression of VEGF-A in Adipose Tissue Promotes Energy Expenditure via Activation of the Sympathetic Nervous System. *Mol Cell Biol.* 2018 Aug; 20. doi: 10.1128/MCB.00242-18.

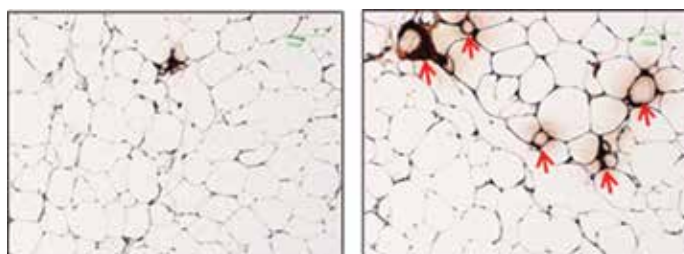
LAB MEMBERS

Post-doctoral fellows: Xin Li, M.D., Ph.D.; Li Yang, Ph.D.

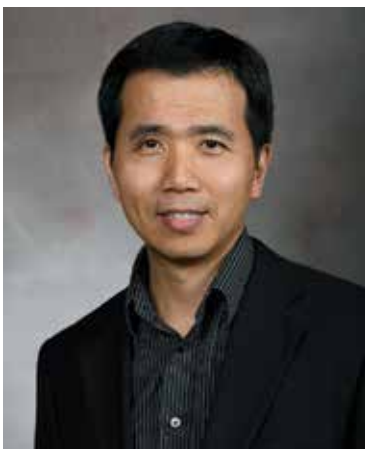
Visiting scientist: Leya He, M.D.



Adipose tissue-derived VEGF-A induces angiogenesis and nerve innervation: Whole mount immunofluorescent staining by anti-endomucin antibody (red, the marker of blood vessels) and anti-tyrosine hydroxylase (TH) antibody (green, the marker of nerve fibers) in subcutaneous white adipose tissue of VEGF-A transgenic mouse (bottom panel) and its littermate control (up panel). Scale bar = 20 μ m.



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



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

Brain control of feeding, body weight, and glucose metabolism

The current obesity epidemic and its associated metabolic syndrome have imposed unprecedented challenges to society and medicine but with no apparent effective therapeutics. Our research is directed to understand the fundamental mechanistic insights on key driving causes for defective feeding and body weight regulation, therefore providing conceptual and effective targets for prevention and treatment of obesity and its associated diabetes.

Toward our goals, we employ various animal models in combination with the state-of-the-art techniques including electrophysiology, optogenetics, chemogenetics, and *in vivo* live imaging. Cre-lox P mouse genetics is used to achieve neuron-specific manipulations in the brain. Also various adenoassociated viral vectors (AAV) harboring genes that exhibit Cre-dependent expression or inactivation will be stereotaxically delivered to specific brain regions of Cre-expressing neurons, achieving neuron-expression or inactivation of foreign tool genes. Example foreign genes include specific channels that either activate or inhibit neurons. In addition, virus based tracing is used to map specific neural projections and their implications in physiology and behaviors. We are also exploring CRISPR/Cas9 technology to achieve neuron-specific gene deletion in adult mice. These advanced techniques ensure our studies are effective and conclusions are insightful.

One major direction in the lab is to identify and map novel neurocircuits underlying emotion control of feeding. Emerging evidence suggests that feeding abnormalities are associated with defects in control of emotion and clinical drugs that reduce symptoms of psychiatric disorders cause obesity development. Using unique animal models coupled with behavioral analysis and optogenetics, we aim to delineate important neurons and neural pathways that underscore interactive regulation of feeding and emotion. This line of research is highly significant to current clinical treatments for obesity, psychiatric patients, and eating disorders.

RESEARCH PROJECTS

- Novel neurons and neural pathways for feeding regulation and its relation with emotional states
- Brain efferent pathways controlling peripheral metabolism
- Brain mechanisms mediating blood hormone action on energy and glucose, and their involvement in obesity and diabetes pathogenesis
- Chronic stress and obesity development

KEY PUBLICATIONS

Xu Y, Lu Y, Xu P, Mangieri LR, Isingrini E, Xu Y, Giros B and Tong Q. Dopamine Release from Midbrain Leptin Receptor Neurons in High-fat Diet Feeding Regulation. *eNeuro*, 2017 May 26;4(3). pii: ENEURO.0083-17.2017.

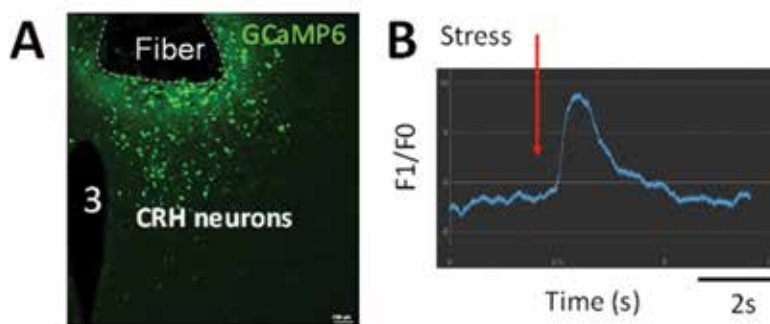
Kim ER, Fan S, Akhmedov D, Sun K, Lim H, O'Brien W, Xu Y, Mangieri LR, Zhu Y, Lee CC,

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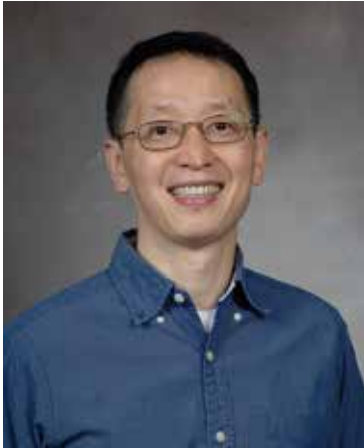
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LAB MEMBERS

Post-doctoral fellows: Wei Wei (visiting)
Graduate students: Ryan Cassidy, Canjun Zhu (visiting)
Instructor: Yuanzhong Xu, M.D., Ph.D.
Undergraduate student: Katie Lobodzinski (Rice)



Corticotropin-releasing hormone neurons (CRH) neurons in the paraventricular hypothalamus (PVH) are demonstrated to respond to stress by increasing neuron activity using *in vivo* photometry in live behaving mice. A: Specific expression of GCaMP6 (green), an indicator of Ca²⁺ by fluorescence changes, in PVH CRH neurons by injecting AAV-FLEX-GCaMP6 to CRH-Cre mice. An optic fiber was implanted to target CRH neurons for detecting changes in fluorescence. B: Changes in fluorescence of PVH CRH neurons in response to a stressful event (water spray).



Sheng Zhang, Ph.D.

Assistant Professor

Becker Family Foundation Professor in Diabetes Research

Molecular mechanisms of neurodegenerative diseases

With an unprecedented longer life expectancy, more people are being affected by aging-related neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Lacking effective prevention and therapy avenues, these incapacitating maladies inflict unbearably high emotional and financial toll to patients and their families, posing a pressing threat to the well-being of society. Our lab studies the molecular machineries that normally operate inside neurons to maintain their health throughout life but become disrupted in affected brains. Findings from these studies should help discover effective treatment strategies against these brain diseases.

Neurodegenerative disorders arise from significant loss of neurons and their functional connections that underlie our senses, reasoning, and responses. Unlike other cell types, such as skin cells that are constantly dividing and being replenished each day, neurons face one particularly unique challenge: once they are born and mature into interconnected functional units, they mostly lose their ability to reproduce and no longer get replaced for the rest of life. To maintain longevity, these long-lived neurons normally harbor robust internal self-clearance machineries to stay health and ward off internal crisis and external insults for decades to come.

Indeed, one common pathological hallmark of almost all the neurodegenerative diseases is the presence of abnormal protein deposits, often known as tangles and plaques, in the affected brains. Cells normally operate multiple robust self-maintenance machines, including *chaperones* that help proteins to stay in shape, *autophagy* (meaning "self-eating" in Greek), and *lysosomal* systems that clean up and recycle worn-out or toxic cellular materials. In neurodegenerative diseases, these self-protective mechanisms often become inefficient or nonfunctional, leading to the gradual formation of pathogenic deposits and neuronal loss in the brain.

Using genetic, biochemical, and cell biology tools in both model organism *Drosophila* and

mammalian systems, we study how these self-maintenance machines operate in the cell to recognize and efficiently clear away toxins while spare and protect normal cellular constituents. Our goal is to find ways to command these internal protection machineries to fight against neurodegenerative diseases.

Protein folding, aggregation and clearance on neuronal survival

Chaperone Hsp110 is one of the most abundant proteins in the brain and is also a major component of *disaggregase*, a potent molecular machine capable of dismantling large and tightly packed protein deposits. We are studying how this chaperone machine works inside neurons to preserve neuronal health.

Huntington's disease (HD), a devastating fatal brain disorder, is caused by a unique mutation (polyglutamine expansion) in Huntingtin protein. Due to its clear genetic cause, HD has been a favor model to study the general principles underlying neurodegeneration. We found that Huntingtin itself plays an important role in *selective autophagy*, a subtype of autophagy. This raises an intriguing possibility that the disease-causing mutations in HD can interfere with a self-protective mechanism, while correction of such abnormality might offer an effective therapeutic approach.

Biogenesis of specialized cellular organelles, and their dysfunction in brain diseases

In neurons, specialized cellular organelles, such as synaptic vesicles (SVs), autophagosomes, and lysosome-related organelles (LROs) control diverse aspects of cellular functions, and their disruption leads to a spectrum of disorders including AD, PD, HD, and schizophrenia.

We are studying the formation and regulation of these specialized cellular organelles and their links to brain diseases.

RESEARCH PROJECTS

- Mechanisms of protein folding and clearance pathways in brain degenerative disorders
- Endogenous functions of Huntingtin and its perturbation in Huntington's disease
- Biogenesis of autophagosomes and lysosome-related organelles

KEY PUBLICATIONS

Gabriela David-Morrison, Zhen Xu, Yan-Ning Rui, Wu-Lin Chang, Manish Jaiswa, Shinya Yamamoto, Bo Xiong, Ke Zhang, Hector Sandoval, Lita Duraine, Sheng Zhang*, Hugo J. Bellen*. (2016) "WAC Regulates mTOR Activity by Acting as an Adaptor for the TTT and Pontin/Reptin Complexes." *Dev Cell*. 36(2):139-51. (*corresponding authors)

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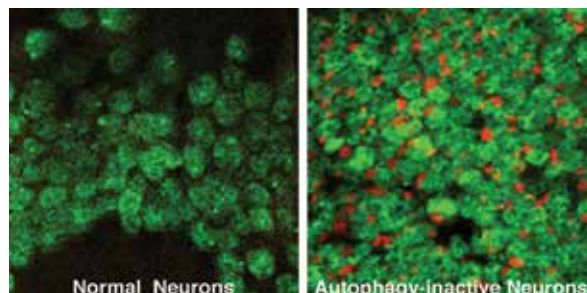
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LAB MEMBERS

Instructor: Shiyu Xu, Ph.D.

Post-doctoral fellows: Boli Hu, Ph.D.; Gang Li, Ph.D.; Antonio Tito, Ph.D.

Research assistant: Lili Ye



Abnormal protein aggregates (red dots), similar as that found in human disease brains, accumulate in neurons (green) in a *Drosophila* brain that are defective in autophagy (right panel), while such aggregates are completely absent in a brain with active autophagy (left panel).



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 to 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 fluorescence (NIRF) to enable new understandings of disease and chronic conditions. Sponsored industry, philanthropic, and federal research funding focuses upon autoimmune disorders, neuroinflammation, cancer metastases, hemo- and lymph-vascular diseases, and lymphedema. The team has experts in instrumentation, imaging agent development, antibody engineering, animal models of human disease, and translational science that effectively moves inventions and discoveries “bench to bedside” and when discoveries are made in the clinic, from “bedside back to bench.”

A highlight of the CMI is the basic science/clinical translational team that engages clinicians at UTHHealth and at partnering institutions in the Texas Medical Center and in the Houston suburbs. These FDA-approved clinical studies

enable visualization of the lymphatic system using photonics technologies for better diagnosis and directed treatments. Conditions such as vascular anomalies, congenital heart disease, peripheral vascular disease, breast cancer, and head and neck cancer are under investigation using our investigational imaging technologies. Translational activities further explore visualization of brain function in neonates, in preclinical models of human disease, CSF outflow into the lymphatics, and intraoperative detection of lymph node metastases and tumor margins. Our team focuses upon translating new NIRF Molecular imaging agents using validated standards that can be applied across different photonics device platforms.

In addition to having an assembly of faculty-driven independent basic science and clinical research projects, the center synergistically operates a “collaboration” center where clinicians and researchers partner to effectively apply imaging diagnostics to investigate and translate novel therapeutics.

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



Eva Marie Sevick-Muraca, Ph.D.

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

Diagnostic imaging and delivery of therapeutics directed by NIRF imaging

The Center for Molecular Imaging (CMI) consists of an interdisciplinary team of scientists and engineers who focus upon multi-modality molecular imaging including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and our specialty, near-infrared fluorescence (NIRF) to enable new understandings in several disease states. Between 2005-2007, my team was the first to dual label imaging agents with nuclear and NIR fluorescence agents to demonstrate the potential for pre-surgical PET imaging and intraoperative NIRF imaging for reduced tumor burden in surgical oncology. In addition, my team adapted NIRF tomography within the CT gantry of a PET/CT device to demonstrate hybrid small animal PET/CT/NIRF tomography. Finally, we translated into the clinical setting unique NIRF instrumentation and trace dose of NIRF Imaging agent to demonstrate for the first time to visualize the active “pumping” of the lymphatic vasculature, which mediates immune response and maintains fluid homeostasis. We actively collaborate with clinical scientists in Pediatrics, Interventional Radiology, the UTHealth Vascular Anomalies Clinic, Pathology, and Otorhinolaryngology, as well as engineers and scientists at Rice, Baylor College of Medicine, and the Methodist Hospital. Our team effectively translates new NIR imaging technologies literally from “bench-to bedside” and back again in order to make discoveries in translational NIR. Fluorescence lymphatic imaging studies conducted by the CMI team include identifying key signaling pathways and regulators associated with aberrant processes of lymphangiogenesis and lymphatic stasis in human diseases and in animal models of human disease.

RESEARCH PROJECTS

- Imaging chylo- and lymphothorax in children with congenital heart defects
- Molecular Imaging of MMP-targeted viral gene delivery vectors for treatment of heart disease
- Lymphatic delivery of therapeutics targeting the immune system

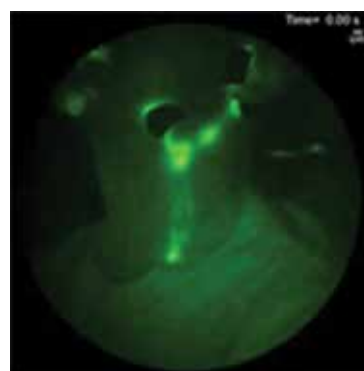
- Imaging cancer-positive lymph nodes with a cancer-specific near-infrared fluorescent molecular imaging agent to guide intraoperative lymph node dissection
- Imaging lymphatic responses to progressive rheumatoid arthritis and its treatment with biologics
- Imaging lymphatic responses to radiation and surgery in head and neck cancer
- Assessing CSF outflow into the lymphatics under microgravity conditions
- Small animal imaging and tomography

KEY PUBLICATIONS

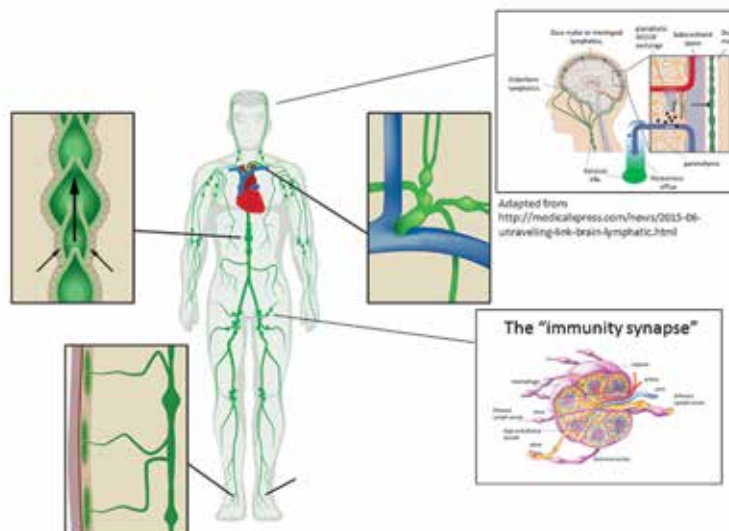
O'Donnell, T.F., Rasmussen, J.C., and E.M. Sevick-Muraca, “New diagnostic modalities in the evaluation of lymphedema,” *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, Mar; 5(2): 261-273, 2017 PMID: 28214496.

Aldrich, M.A., Velasquez, F.C., Kwon, S., Azhdarinia, A., Pinkston, K., Harvey, B.R., Chan, W., Rasmussen, J.C., Ross, R.F., and E.M. Sevick-Muraca, “Lymphatic delivery of etanercept via nanotopography improves response to collagen-induced arthritis,” *Arthritis Research And Treatment*, 19(1): 116, 2017 PMID: 28566090.

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Lymphatic outflow imaged in head and neck cancer. Reproduced from Rasmussen, J.C., Tan, I.C., Naqvi, S., Aldrich, M.B., Maus, E.A., Blanco, A.I., Karni, R.J., and E.M. Sevick-Muraca, “Longitudinal monitoring of head and neck lymphatics in response to surgery and radiation,” *Head and Neck*, 39(6): 1177-1188, 2017 PMID: 28263428.



Adapted from O'Donnell, Rasmussen, Sevick-Muraca, *J. Vasc. Surg.*, 2017

The lymphatics in health and disease. Reproduced in part from O'Donnell, T.F., Rasmussen, J.C., and E.M. Sevick-Muraca, “New diagnostic modalities in the evaluation of lymphedema,” *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, Mar; 5(2): 261-273, 2017 PMID: 28214496.



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

Imaging in immunology

Cancer survivors face the possibility of developing a devastating side effect of cancer treatment: lymphedema, which manifests as a permanently swollen arm, leg, neck, or trunk. Lymphedema requires constant compression garment wear, meticulous skin care, and specialized massage. Lymphedema patients suffer discomfort, depression, cellulitis bouts, and there is no cure—only palliative treatment. Studies have shown that, if caught early in development, lymphedema treatment can reverse the disease. Near-infrared fluorescence lymphatic imaging (NIRFLI) imaging delivers high-resolution, low-cost images of lymphatic vessel architecture and pumping. In disease states such as lymphedema, NIRFLI imaging can provide information for early diagnosis and evaluation of treatment efficacy. I lead a five-year prospective and longitudinal study using NIRFLI surveillance of breast cancer patients to identify early lymphedema development and biomarkers that could suggest pharmacological treatment.

Delivery of pharmacological therapeutics directly to the site of disease activity could reduce the amount of pharmaceutical required, and minimize off-target toxicities. In a rat model of rheumatoid arthritis, NIRFLI revealed that delivery of a tumor necrosis factor-alpha (TNF-alpha) blocker directly through lymphatic vessels to lymph nodes resulted in significantly reduced disease activity, as evidenced by improved lymphatic pumping.

NIRFLI studies of patients with lipedema, a fat disorder that affects ~11% of women, revealed that leg lymphatic vessels are dilated and slow-pumping, suggesting the disease is an inflammatory disorder. Compression garment wear to promote leg lymph movement and anti-inflammatory dietary practices have improved outcomes for these patients. I am a member of the Center for Molecular Imaging (CMI) team that participates with a national coalition of researchers to investigate lipedema.

Chylothorax occasionally affects neonatal heart surgery patients. I and my colleagues here

at CMI and Memorial Hermann Hospital have used NIRFLI to help visualize the source of pleural effusion in babies with chylothorax. We have also imaged numerous pediatric patients with lymphovenous anomalies to help physicians direct optimal care.

RESEARCH PROJECTS

- Longitudinal study of breast cancer-related lymphedema
- Imaging of lymphatics in lipedema
- Imaging of neonatal chylothorax and pediatric lymphovenous anomalies

KEY PUBLICATIONS

Aldrich M.B., Velasquez F.C., Kwon S., Azhdarinia A., Pinkston K., Harvey B., Chan W., Rasmussen J.C., Ross R.F., Fife C.E., Sevick-Muraca, E.M. Lymphatic delivery of etanercept via nanotop-

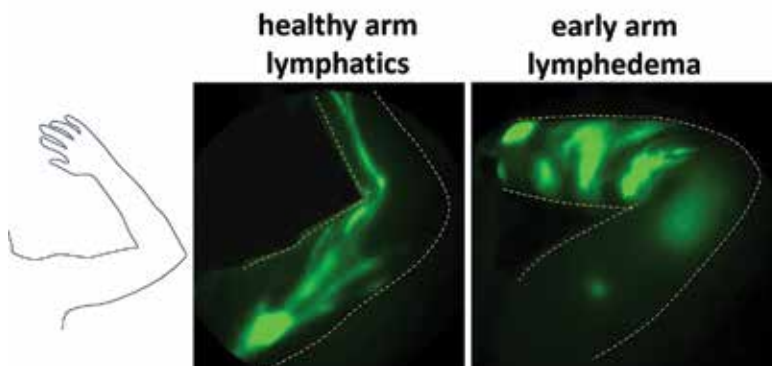
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Schaverien M.V., Aldrich M.B. New and emerging treatments for lymphedema. *Semin Plast Surg* 32:48-52, 2018.

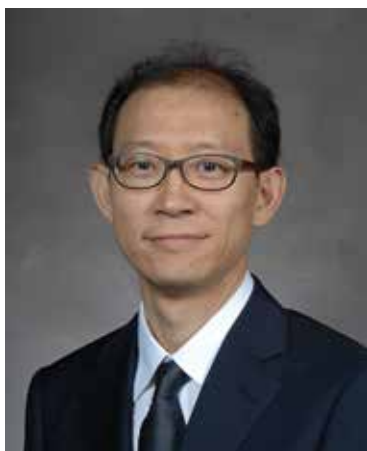
Aldrich M.B., Gross D., Morrow J.R., Fife C.E., Rasmussen, J.C. Effect of pneumatic compression therapy on lymph movement in lymphedema-affected extremities, as assessed by near-infrared fluorescence lymphatic imaging. *J Innov Optical Health Sciences*, Vol. 10, No. 02, 1650049 doi: 10.1142/S1793545816500498, March 2017.

LAB MEMBERS

Medical student: Kay Pham



Near-infrared fluorescence lymphatic imaging (NIRFLI) of healthy arm lymphatics and early breast cancer-related lymphedema. Green fluorescent dye is visualized through the skin, pumping through vessels (in a healthy arm) or pooling as backflow (in lymphedema).



Sun Kuk Kwon, Ph.D.

Assistant Professor

Carolyn Frost Keenan Professor in Cardiovascular Disease Research

Lymphatic function in health and disease

I lead the development and application of small animal imaging techniques to address biological questions. My main research interest focuses on investigating the microcirculatory movement of fluid and macromolecules, particularly in the lymphatic system. Recently, we developed non-invasive, dynamic near-infrared fluorescence (NIRF) imaging methods for imaging and quantifying lymphatic function in health and disease. Using this novel technique, we showed abnormal lymphatic function and drainage patterns in animal models of lymph node metastasis, hypertension, and inflammation.

Recently, CMI demonstrated that direct infusion of an immune-attenuating biologic into the lymphatics can result in improved local and systemic responses when compared to conventional routes of administration in an arthritis rat model. Recently, I used the novel lymphatic infusion device for immunotherapy to maximize drug exposure to tumor-draining lymph nodes and reduce toxicity by localizing immune stimulation to the regional lymphatics for systemic anti-tumor immunity. I demonstrated that when the repeated dose of anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) was successfully delivered via the lymphatic system, tumor shrinkage occurred when compared to the untreated cohort.

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

AD pharmacological intervention.

Other directions of my scientific interests revolve around multi-modality molecular imaging. The Center for Molecular Imaging is developing and translating imaging agents. I am currently conducting molecular imaging of cancer and lymph node metastasis, inflammation, and myocardial infarct in mice.

RESEARCH PROJECTS

- Non-invasive dynamic lymphatic imaging to show lymphatic remodeling in a mouse model of melanoma and restoration of normal lymphatic vessel integrity after cancer therapy.
- Characterizing impaired cerebrospinal fluid (CSF) drainage into peripheral lymph nodes in Alzheimer disease animal models in collaboration with Dr. Claudio Soto at the medical school.
- Developing PET/CT methodology for quantifying infarct size in gene therapy trials of myocardial infarct in mice in collaboration with Dr. Jung Suh at Rice University.
- Assessing the normal lymphatic drainage in the lower extremities as well as in the cervical region after intradermal and intraoral administration of ICG, under conditions of supine position and head down tilt (HDT), the latter of which mimics aspects of microgravity.
- Using non-invasive lymphatic imaging to show

efficacy of drug delivery with SOFUSA™ into the lymph nodes in mice and successfully translated to humans supported by Kimberly Clark Coop (Currently Sorrento Therapeutics).

KEY PUBLICATIONS

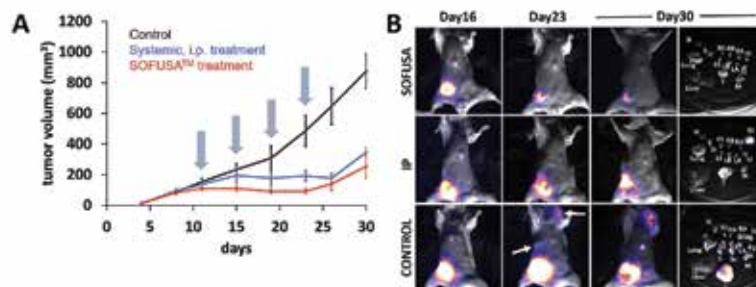
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Sunkuk Kwon, Fred Christian, Velasquez, and Eva M. Sevick-Muraca, “Near-infrared fluorescence lymphatic imaging in vascular endothelial growth factor-C overexpressing murine melanoma,” *Biomedical Optics Express*, 9; 4631-4637. 2018.

LAB MEMBERS

Research assistants: C.J. Vaelasquez, Amanda Pinal (summer pre- Baccalaureate student)



A. Growth rates of orthotopic 4T1-Luc in Balb/c mice treated with control antibody (black) or with CTLA-4 administered i.p., (blue) or with SOFUSA™ delivered CTLA-4 (red). Grey arrows indicate treatment days. The mean tumor volumes ± SE (bars) are shown at the times that tumor measurements were made. B. Bioluminescence imaging of orthotopic 4T1-Luc in Balb/c mice 16, 23, and 30 days after tumor inoculation and tissues after euthanasia. CTLA-4 was treated i.p. or via SOFUSA™ at 11, 15, 19, and 23 days post implantation. H, heart. RS/LS, right/left submandibular LN, RB/LB, right/left brachial LN. RA/LA, right/left axillary LN. RI/LI, right/left inguinal LN. T, tumor. M, muscle. S, stratum. Sc, Scapula.



John Rasmussen, Ph.D.

Assistant Professor

Device translation for lymphatic imaging

The lymphatic system is a vital, yet poorly understood, component of the circulatory system. As blood flows through the arteries and veins, water leaks from the vessels entering the small gaps between the tissue cells. As the water moves through the tissues, it picks up cell waste, foreign contaminants, proteins, etc., and the resulting solution is taken up by the lymphatics, processed for immune response, and is ultimately returned to the veins. In addition, the lymphatics provide a pathway for the absorption of nutrients from the gut. However, because the lymphatics are typically small and primarily transport clear fluids, they are difficult to distinguish from the surrounding tissues, either with our eyes or using traditional clinical imaging modalities such as scintigraphy, X-ray, MRI, and ultrasound. Over the past few years, my research has focused upon the development and translation of near-infrared fluorescence (NIRF) optical imaging as a way to noninvasively image and characterize human lymphatics and quantify their contractile function in health and disease using microdose amounts of a fluorescent contrast agent.

One of our primary focuses is the relationship between the lymphatics and the blood circulatory system. It has been known for many years that patients with advanced chronic venous disease often co-develop lymphedema, a condition of chronic swelling with fibrotic tissue changes and poor immune response. We recently imaged a group of patients with active venous leg ulcers and demonstrated abnormal lymphatics in all the legs with advanced disease. However, what was most surprising was that we also observed lymphatic abnormalities in all the contralateral legs, including those with no external sign of venous disease. We are currently imaging additional subjects with early venous or arterial disease to determine at what stage of disease the abnormal lymphatic anatomy and function appear, and whether these lymphatics changes are a result of or contribute to the development of the vascular disease. A better understanding of the role of the lymphatics in early vascular

disease may enable the development of more efficacious therapeutic approaches. To date, we have observed abnormal lymphatic anatomy and/or function in all subjects with early disease. Additional studies focus on the relationship between the lymphatics and vascular malformations.

We are also using NIRF imaging to assess the recovery, or lack thereof, of the lymphatics after cancer treatment. We are particularly interested in the head and neck cancer population as it has been reported that 75% of head and neck cancer survivors will develop lymphedema. Our imaging has shown the development of abnormal lymphatics in this population, with the extent of abnormal lymphatics generally increasing with time (months) after the end of radiation treatment. This past year we used NIRF imaging to assess the lymphatic response to a new advanced pneumatic compression device developed specifically for subjects with head and neck lymphedema. In this study we observed a reduction in the extent of abnormal lymphatics with as little as two weeks of intervention in 75% of subjects.

We continue the development of this technology including improving device sensitivity, automating different aspects of the hardware, and developing analytical tools to facilitate lymphatic image processing and analysis with the ultimate goal of answering new biological and clinical questions not addressed by other

technologies.

RESEARCH PROJECTS

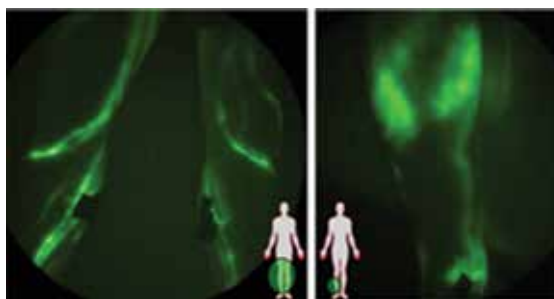
- Understanding the role of lymphatics in the development of peripheral venous and arterial disease
- Assessing the development of cancer-related lymphedema and its response to intervention
- Translation of lymphatic imaging to the pediatric population

KEY PUBLICATIONS

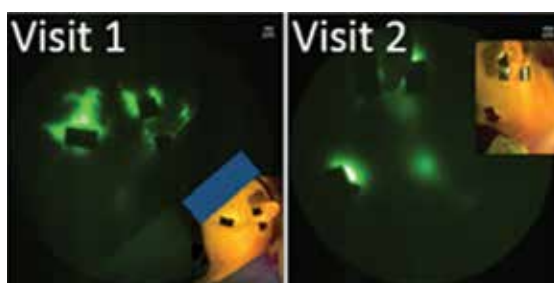
Rasmussen, J.C.,* Tan, I.,* Naqvi, S., Aldrich, M.B., Maus, E.A., Blanco, A.I., Karni, R.J., and Sevick-Muraca, E.M., "Longitudinal monitoring of the head and neck lymphatics in response to surgery and radiation," *Head and Neck*, 39(6):1177-1188, 2017 (PMID: 28263428).

Rasmussen, J.C.,* Zvavanjanja, R.C.,* Aldrich, M.B., Greives, M.R., and Sevick-Muraca, E.M., "Near-infrared fluorescence lymphatic imaging of Klippel-Trénaunay syndrome," *Journal of vascular surgery: Venous and lymphatic disorders*, 5(4):533-537, 2017 (PMID: 28623992).

Greives, M.R., Aldrich, M.B., Sevick-Muraca, E.M., and Rasmussen, J.C., "Near-infrared fluorescence lymphatic imaging of a toddler with congenital lymphedema," *Pediatrics*, 139(4): e20154456, 2017 (PMID: 28356336).



Normal lymphatic vessels in a healthy volunteer (left) and abnormal lymphatics in a patient with early peripheral arterial disease (right)



Reduction of abnormal lymphatics after two weeks of advance pneumatic compression therapy in a patient with head and neck lymphedema (Adapted from Rasmussen, et al., OthNS, 2018).



Banghe Zhu, Ph.D.

Assistant Professor

High-resolution diffuse optical tomography for brain functional imaging in children with brain tumor and high sensitive near-infrared fluorescence imaging of lymphatic function for underwater microgravity simulation studies

High-resolution diffuse optical tomography for brain functional mapping in children with brain tumor: Brain functions, including cognitive functions, are frequently disturbed in brain tumor patients. These disturbances may result from the tumor itself, but also from the treatment directed against the tumor. Surgery, radiotherapy, and chemotherapy all may affect cerebral functioning, both in a positive as well as in a negative way. Childhood cancers, such as leukemia, also have evidence of cognitive decline after treatment. Childhood brain tumors represent an anatomically and biologically diverse group of neoplasms that can present with both common and unusual symptoms; chemotherapy and radiation treatment are made on basis of optimizing tumor treatment and minimizing brain network dysfunction side effects. Currently, we are developing a high resolution, multi-pixel technique based Functional Near Infrared Spectroscopy - Diffuse Optical Tomography (fNIRS-DOT) imaging system with a computationally-efficient tomographic algorithm to assess brain network dysfunction in children with brain tumors.

High sensitive near-infrared fluorescence imaging of lymphatic function for underwater microgravity simulation studies: The objective of this work is to build a near-infrared fluorescence (NIRF) imaging system that can image through water simulating microgravity in space to sensitively detect lymph motion non-invasively and test its capacity for imaging in water before employing within clinical studies. NIRF imaging system consists of an intensified scientific CMOS (sCMOS) camera, a laser source operating at 780 nm wavelength for excitation, and a color camera for white light imaging. To validate the measurement performance of developed NIRF imager for underwater imaging, an ICG based solid phantom is constructed and its radiance is calibrated using a NIST-certified photodiode and transfer camera and lamp set up. The developed sCMOS camera imager has been validated on the ICG phantom immersed in water, demonstrating its ability for imaging

lymphatic function in the future studies of head-out-immersion.

RESEARCH PROJECTS

- Develop high-resolution fNIRS-DOT imaging system
- Develop underwater NIRF imaging system
- Perform peripheral vascular disease clinical studies
- Perform head & neck surgery clinical studies

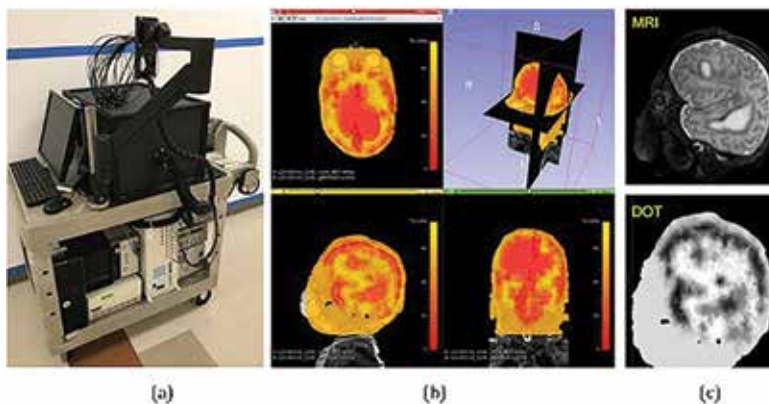
KEY PUBLICATIONS

Alvarez-Urena, P., Zhu, B., Sonnet, C., Henslee, G., West, J., Sevick, E. M., Davis, A., Olmsted D. E., "Development of a Cell-based Gene Therapy Approach to Selectively Turn off Bone Formation". *J. Cell. Biochem.* 9999: 1-8, 2017.

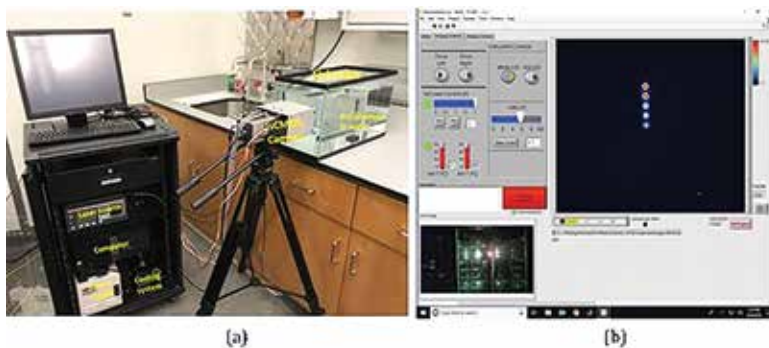
Zhu, B., Rasmussen, J. C., Litorja, M., and Sevick-Muraca, E. M., "Determining the Performance of Fluorescence Molecular Imaging Devices Using Traceable Working Standards with SI Units of Radiance", *IEEE Transactions on Medical Imaging*, 35(3), 802-811 (2016).

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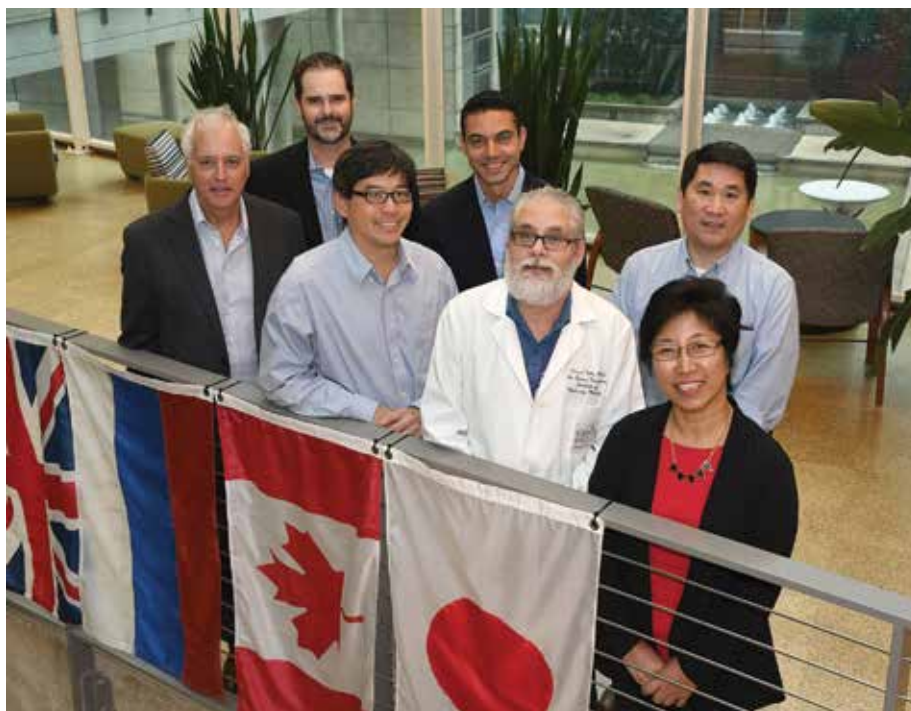
(a) fNIRS-DOT imaging system. (b) 3-D, Sagittal, Coronal and Axial cross-sections of the reconstructed HbT using fNIRS-DOT optical data overlaid onto MIR image. (c) The cross selection of an MRI image (top), and a DOT image (bottom), demonstrating the consistence in mapping brain activation.



(a) A photo of the NIRF imager designed to image through the window of an immersion tank or therapy pool; (b) The phantom image acquired using the NIRF imager, as shown on the main window.

The Center for Precision Biomedicine focuses on developing the mathematical, analytical, and translational technologies that will precisely deliver medication to the correct tissues at the correct time. This is accomplished by understanding the underlying physiological problems of disease and the associated proteomic and genomic biomarkers, developing molecules for selective targeting of tissues or toxins, applying targeted contrast agents for disease visualization, and mechanistic mathematical models of tissue and vasculature to predict and overcome biological barriers to tissue penetration.

These efforts connect us with collaborators across UTHealth, institutions within the Texas Medical Center, such as Baylor, Methodist and MD Anderson, and across Texas through interactions with the Centers/programs for Clinical and Translational Science, nanomedicine researchers, and faculties studying disease mechanisms using proteomics, genomics, and bioinformatics. At the IMM, we have state-of-the-art mass spectrometers, providing in-depth proteomic analysis of cells, tissues, or biological fluids, leading to the discovery of novel targets for drug development and biomarkers for early detection and personalized precision medicine. We also have an active probe development program that includes the development of new aptamers and multi-functional nanoparticle therapeutics for targeting pathological tissues, such as cancer. In addition, we have expertise in the development and application of novel biologics, namely antibody-based agents, that have imaging and therapeutic implications in cancer as well as infectious disease. Furthermore, the Center specializes in the development of multifunctional peptides that combine radioactive and fluorescent contrast to enable tumor



identification before, during, and after surgery, thus introducing a precision surgery approach. Using custom-built NIRS/Ramen spectrometers, we provide novel ways to investigate the structures of diseased bones and ulcerative colitis. We also have large-scale, multi-color, high resolution state-of-the-art 3D printers for both fast prototypes and finished production level models of new surgical tools and instruments or patient-specific organ models. We provide multiscale mathematical and computational modeling to aid current prospective clinical trials focusing on understanding drug penetration barriers in tumors and improving tumor response and patient outcomes.

Hubs of research collaboration with the Center include:

- Clinical and Translational Proteomics Core Laboratory
- Nanochemistry/3D-printing Service Center
- NCI Programs in Computational Cancer Biology and Nanomedicine
- UT System-wide Proteomics Core Facility Network
- UTHealth / MDACC Clinical and Translational Center for Translational Technologies

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



Ali Azhdarinia, M.S., Ph.D.

Assistant Professor
John S. Dunn Research Scholar III

Molecular imaging probe development

My laboratory is at the interface of chemistry and biology and is focused on developing molecules for the visualization and treatment of disease. Using novel chemistry platforms, we have the ability to produce molecules with multiple labels and thus, multiple applications. For example, the addition of radioactive and fluorescent labels onto tumor-seeking agents has allowed us to develop new approaches to specifically identify cancer by whole-body and intraoperative imaging, respectively. This could potentially provide surgeons with real-time intraoperative images that will distinguish cancer from normal tissue, minimize removal of healthy tissues, and identify small tumors that would otherwise be missed by the naked eye. In cases where cancer has spread and surgery is not possible, we aim to use our chemistry platform to specifically deliver toxins to tumors and visualize the effects to personalize treatment protocols. Importantly, our fundamental expertise in chemistry, imaging, and drug characterization has allowed us to establish diverse collaborations in areas beyond cancer, such as imaging of “good” fat tissue, characterization of novel nanomaterials for biomedical use, and assessing the effectiveness of emerging antibody-based cancer treatments. Common to each project is our focus on translation of discoveries and technologies into the clinic to improve human health.

RESEARCH PROJECTS

- Development of contrast agents for real-time surgical guidance
- Receptor-targeted delivery of chemotherapy agents for treatment of cancer

KEY PUBLICATIONS

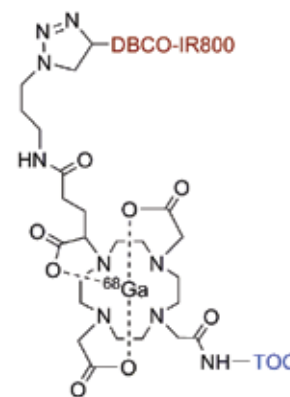
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Ghosh, S.C., Hernandez Vargas, S., Rodriguez, M., Kossatz, S., Voss, J., Carmon, K.S., Reiner, T., Schonbrunn, A., Azhdarinia, A.* Synthesis of a Fluorescently Labeled ⁶⁸Ga-DOTA-TOC Analog for Somatostatin Receptor Targeting. *ACS Med Chem Lett.* 6;8(7):720-725, 2017. PMID:28740605.

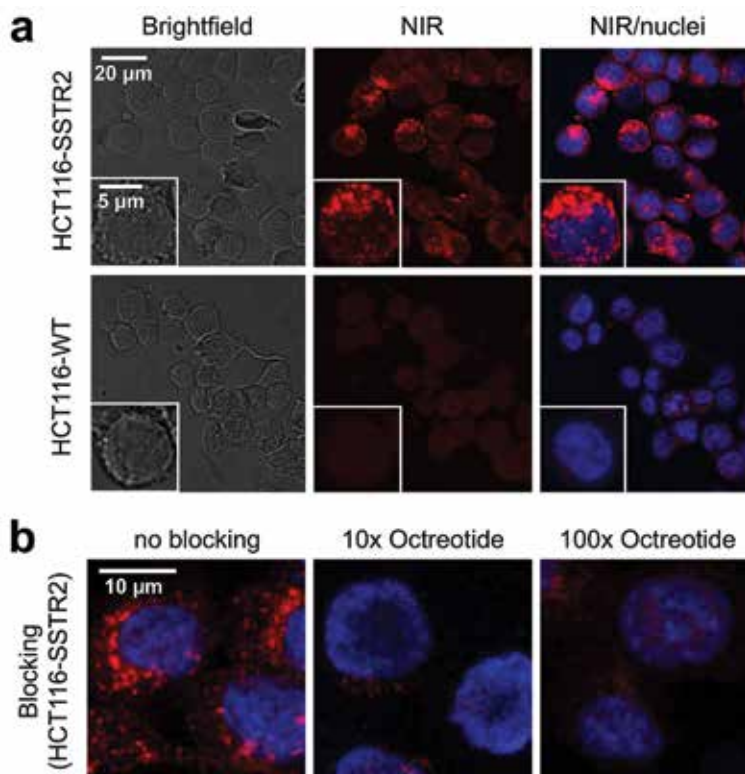
LAB MEMBERS

Graduate students: Servando Hernandez Vargas
Research assistant: Julie Voss
Research scientist: Sukhen Ghosh

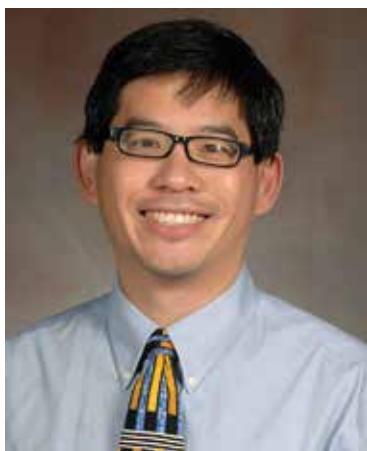


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⁶⁸Ga-MMC(IR800)-TOC

Chemical structure of a hybrid somaostatin analog containing radioactive and fluorescent labels for multimodality imaging.



Confocal microscopy showing (a) uptake and internalization and (b) blocking of MMC(IRDye® 800CW) TOC in HCT116-SSTR2 and wild type HCT116 cells. (Red: Ga-MMC(IRDye® 800CW)-TOC, blue: Hoechst 33342 nuclear stain.



Jeffrey Chang, Ph.D.
Associate Professor
CPRIT Scholar in Cancer Research

Deciphering the signaling programs underlying cancer metastasis

Our lab is focused on understanding the signaling programs underlying cancer progression and developing therapeutic strategies to prevent or treat the metastatic state. We wish to understand the events that lead tumor cells to acquire a metastatic state, whether through acquired mutations or epigenetic mechanisms. Our ultimate goal is to translate these findings into the clinic through the development of genomic biomarkers and repositioning of drugs. To do this, we use a range of approaches encompassing genomics, cell biology, and biochemistry, and use models, including cell culture, mouse models, and clinical samples.

Our research program encompasses two broad and complementary areas of emphasis:

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

2. Artificial intelligence for genomic analysis. Many of our projects requires the integration with bioinformatics to mine public data sets, develop hypotheses, or analyze results. To amplify our ability to do bioinformatics, we have developed an artificial intelligence, BETSY, that can automatically plan and execute these tasks, presenting us with finished results. It is a backwards-chaining expert system that leverages a knowledge base containing descriptions of common bioinformatics algorithms.

RESEARCH PROJECTS

- The role of cholesterol trafficking in cancer stem cell differentiation, the epithelial-to-mesenchymal transition, and cancer

- metastasis.
- Heterogeneity and progression of metastatic cancers.
- Intelligent computational pipelines for bioinformatic analysis.

KEY PUBLICATIONS

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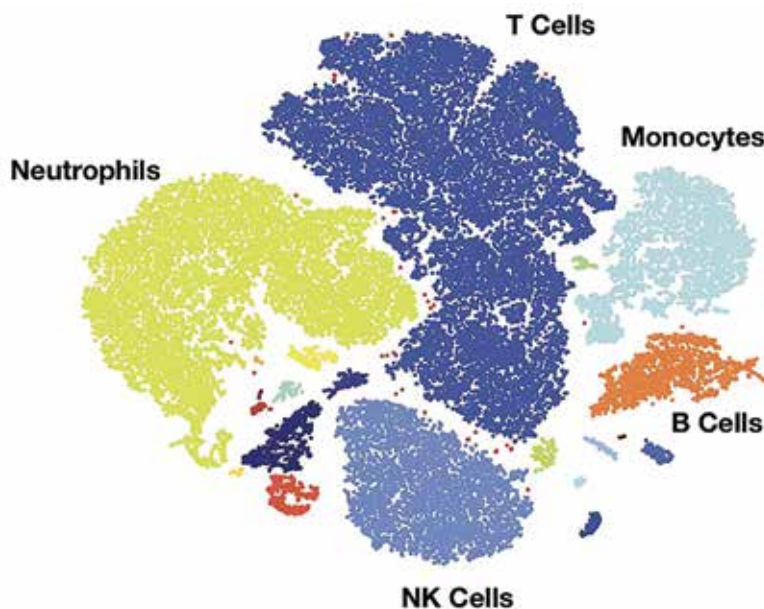
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* Co-Corresponding Authors

Chen X and Chang J.T.: Planning bioinformatics workflows using an expert system. *Bioinformatics* 33(8), 2017.

LAB MEMBERS

Instructor: Weina Zhao, Ph.D.
Research scientist: Xuan Liu, Ph.D., Neena Leggett
Visiting scientist: Jing Li
Research assistant: Aurnab Baidya



We are using genomics to profile the tumor microenvironment and identify signaling programs that promote cancer metastasis. This is a recent analysis of the immune cells from cancer patients. We have profiled thousands of cells with single-cell RNA-Seq technology to identify components that are associated with tumor outcomes.



Barrett R. Harvey, Ph.D.

Assistant Professor
John S. Dunn Research Scholar I

Development of diagnostic imaging agents for cancer and infectious disease

Engineered to recognize and specifically target disease, antibodies are powerful tools for both basic and translational research. As basic research tools, antibodies designed by our lab have been instrumental in helping to improve our understanding of bacterial physiology, and the factors that govern infection. Taking advantage of antibody specificity and technological achievements in antibody engineering, our lab also develops antibody-based imaging agents with focused efforts in imaging cancer and bacterial infection. Combined with modern imaging equipment, these agents have direct clinical applications to help guide physicians with clinical diagnosis or surgeons in the removal of disease.

RESEARCH PROJECTS

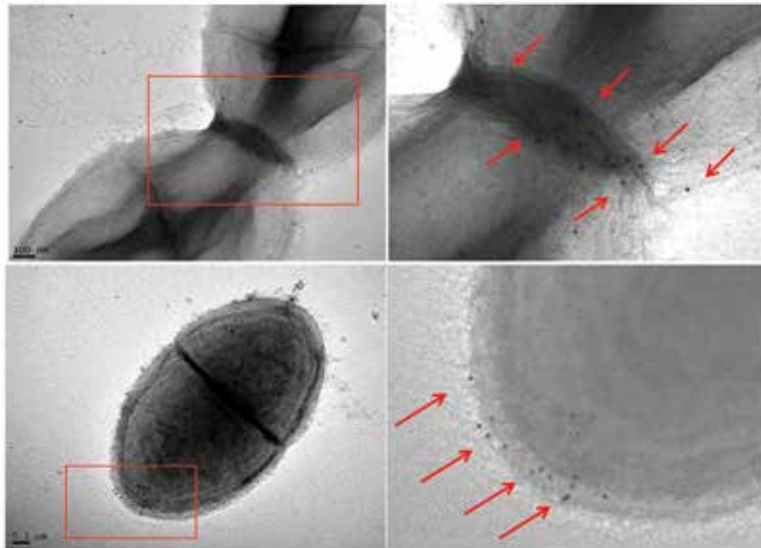
- Targeted treatment of infection with an antibody-antibiotic conjugate.
- Molecular Targeting of Nodal in Aggressive Breast Cancer.
- Virulence factor regulation governing bacterial infection.

KEY PUBLICATIONS

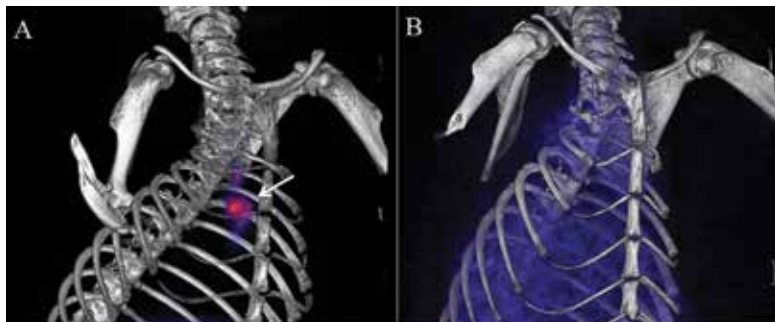
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Functional studies of *E. faecalis* RNase J2 and its role in virulence and fitness. P, Pinkston KL, Bourgogne A, Murray BE, van Hoof A, Harvey BR. PLoS One. 2017 Apr 6;12(4):e0175212. doi: 10.1371/journal.pone.0175212. eCollection 2017. PMID: 28384222



Bacterial chaining, thought to encourage colonization and infection is regulated by AtIA, an enzyme important in cell wall cleavage. Beads (designated by red arrows) denote AtIA localization at poles and septum of dividing bacterial chains.



Live animal imaging to diagnose enterococcal endocarditis infection within the heart valve of a rat.



Sheng Pan, Ph.D.

Associate Professor

Deciphering proteome alterations associated with diseases

Proteins are essential functional biomolecules that are involved in all sorts of cellular physiologic activities and have been important targets for drug development and early detection of diseases. Proteomics, especially quantitative proteomics, has been a vital tool in basic, translational, and clinical research, providing a unique avenue to investigate disease-associated molecular alterations at a functional level. Proteome alterations that are associated with diseases may include changes in protein expression, sequence, post-translational modifications (PTMs), and protein interactions with proteins and other biomolecules, which may all lead to malfunction of cellular processes. In our lab, mass spectrometry-based proteomics technologies are applied to study cancer and other diseases. These studies are carried out with various goals, aiming to better understand the molecular mechanisms underlying tumorigenesis, to investigate changes in PTM status associated with diseases, and to identify cancer associated protein biomarkers to improve diagnosis or therapeutic treatment. The samples analyzed include a variety of disease and control specimens, including tumor tissues, blood and other bodily fluids, as well as isolated cells from various clinical specimens. Currently, our main disease interests are pancreatic cancer and other GI-tract malignancies. In addition, through collaborative efforts, our lab also supports proteomics investigation of neurological diseases, chronic inflammatory diseases, degenerative diseases, infectious diseases, and therapeutic drug development. Bioinformatics and systems biology are important components in our study for analysis of protein interaction networks and regulatory pathways associated with disease mechanisms.

RESEARCH PROJECTS

- Discovery of protein biomarkers and drug targets in pancreatic ductal adenocarcinoma (PDAC), especially proteome alterations associated with early malignant signals in PDAC precursors.
- Investigation of protein glycosylation, glycation and other PTMs in malignances, diabetes, chronic inflammation and degenerative diseases.
- Innovation of proteomics technologies for basic, translational and clinical applications

KEY PUBLICATIONS

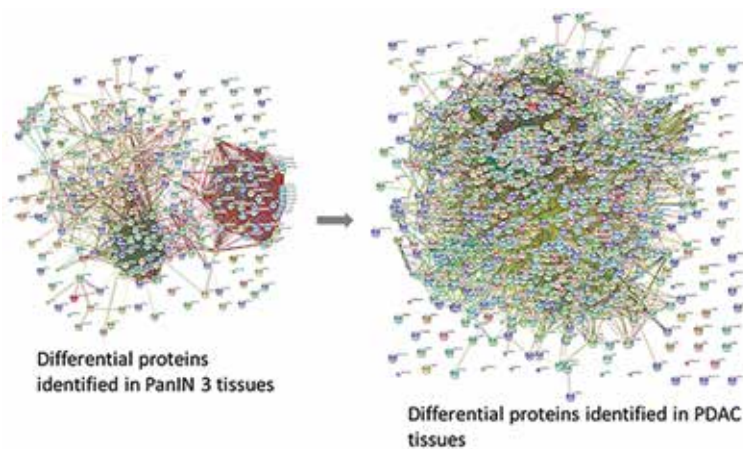
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Chen R, Lai LA, Sullivan Y, Wong M, Wang L, Riddell J, Jung L, Pillarisetty VG, Brentnall TA, Pan S, "Disrupting glutamine metabolic pathways to sensitize gemcitabine-resistant pancreatic cancer", *Scientific Reports*, 2017, 7(1):7950.

Nigeh EN, Chen R, Brand RE, Petersen GM, Chari ST, von Haller PD, Eng JK, Feng Z, Yan Q, Brentnall TA, Pan S, "Quantitative proteomics based on optimized data-independent-acquisition in plasma analysis", *Journal of Proteome Research*. 2017, 16(2):665-676.

LAB MEMBERS

Post-doctoral fellow: Hong Peng, Ph.D.
 Research coordinator: Li Li



The proteome of pancreatic tissue alters as the disease progresses - differential proteins identified in PanIN 3 lesions and PDAC.



David Volk, Ph.D.
Assistant Professor

Targeting cancer with X-aptamers and nanoparticles

The focus of my lab is to develop targeting agents and smart particles that attack cancer or infectious organisms, such as tuberculosis. Current treatments are often ineffective or create harsh side effects for patients. We use modified DNA joined to drug-like or protein-like attachments (X-aptamers). X-aptamers can be used alone or as complex particles containing anti-cancer agents to act as a one-two punch. Such particles also can be loaded into larger silicon particles for a sustained release of the disease-fighting particles.

Aptamer Development - In recent years we have developed DNA aptamers targeting breast and ovarian cancer. Such DNA can greatly reduce cancer in a dose-dependent manner. However, DNA aptamers are even more effective when used in combination therapy together with chemotherapeutic agents such as siRNA or drugs like Paclitaxel. We have shown that our aptamer targeted approach reduces tumor size and, more importantly, the spread of metastatic cancer. Furthermore, we also have shown our method is safe in preclinical testing. Our recent aptamer-related research has shown the following.

- ESTA1 multistage particles directed anti-cancer siRNA to the bone marrow, reducing breast cancer metastasis and leading to increased survival rates.
- Our Annexin A2 (Mangala et al., 2016)

aptamer directed delivery of siRNA improves vascular maturation to enhance anti-tumor effects in ovarian cancer.

- Our AXL aptamer (Kanlikilicer et al., 2017) can reduce cancer alone and enhances anti-tumor effects in combinatorial therapy.
- Developed aptamers (Liu et al. 2018) targeting the endothelium of lymphoma in bone marrow.
- X-aptamers can be used to develop biomarkers in schizophrenia (Walss-Bass et al, in press, 2018)

Other projects target infectious agents such as Dengue 2 virus, *C. difficile* infections, and tuberculosis. We recently (Leonard et al. 2017) showed that our ESTA1 and CD44 aptamers deliver mesoporous silicon particles to macrophages infected with *M. tuberculosis*, thereby enhancing the immune system and reducing the *M. tuberculosis* (Tb) burden.

Software Development - Another focus of the lab is to provide bioinformatics support and to develop novel software for the analysis of next-generation sequencing (NGS) data. We therefore developed Aptaligner (Lu et al. 2014), a completely automated program with easy-to-use graphical user interfaces. We are currently working on software for the analysis of DNA sequence changes during recombination events in *B. burgdorferi* infections, the cause of Lyme disease. Such antigenic variation is thought to cause long-term Lyme disease infection and post-infection deficits.

RESEARCH PROJECTS

- Development of smart particles to attack breast and ovarian cancers

- Developing new X-aptamers targeting other diseases
- Software to analyze how Lyme disease escapes host immune systems

KEY PUBLICATIONS

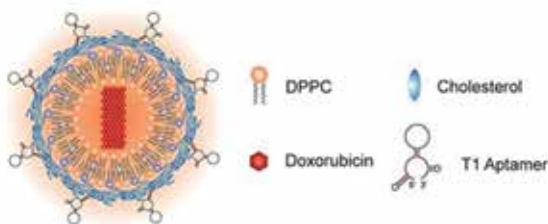
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Thioaptamer Targeted Discoidal Microparticles Increase Self Immunity and Reduce Mycobacterium Tuberculosis Burden in Mice. Leonard, F, Ha, NP, Sule, P, Alexander, JF, Volk, DE, Lokesh, GLR, Liu, X, Cirillo, JD, Gorenstein, DG, Yuan, J, Chatterjee, S, Graviss, EA, Godina, B. *Molecules* (2017) 22:1355.

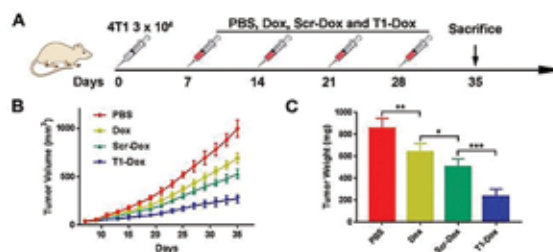
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LAB MEMBERS

Research associates: Xin Li, M.S.
Medical students: Andrea Costello, Brenda Saucedo



Aptamer coated liposome particles containing doxorubicin.



Weekly dose of T1-coated liposomal doxorubicin reduces tumors.



Hongyu Wang, M.D., Ph.D.

Assistant Professor

Cancer biomarker discovery and targeted therapy

Biomarker discovery and targeted therapy are important parts of precision medicine. Aptamer mediated biomarker discovery and targeted therapy are attractive approaches for precision cancer treatment. Aptamers are oligonucleic acid or peptide molecules that can bind to a specific target with high affinity and specificity. DNA aptamers have many significant advantages over monoclonal antibodies in terms of feasibility, low cost, non-immunogenicity, and facile modification for various applications.

We created a systematic biology approach that combines a bead-based modified aptamer library with flow cytometry sorting and mass spectrometry to identify proteomics biomarkers. Patient's plasma were incubated with beads-X-aptamer library and sorted by flow cytometry based on fluorescence intensity (Figure1). Using this approach, we can select a panel of prognostic biomarkers for hepatocellular carcinoma (HCC) patients under Lipiodol-based transarterial chemoembolization (TACE) treatment. Our goal is to develop clinically applicable prognostic biomarkers to help clinicians predict the course of HCC post-treatment in order to personalize therapeutic strategies.

Aptamer mediated targeted therapy offers a unique opportunity for selective delivery of therapeutic siRNA or drugs. Several modified aptamers have been identified in our lab for further targeted studies. Using Morph-X-Select method, we have successfully identified and characterized a Vimentin-specific thio-aptamer that has shown specific binding to tumor vasculature of human ovarian tissue. Specific binding motifs of the Vimentin-specific thio-aptamer also have been identified by MFold analysis. Immune checkpoint aptamers (XA-PD1 and XA-PDL1) have been identified by beads-based X-aptamer selection. Specific binding of XA-PDL1 has been validated by PDL-1 expression cell lines, including triple negative breast cancer cell line MDA-MB-231 and human pancreatic ductal adenocarcinoma cell line Capan-2. Our future studies will focus on developing a novel drug-delivery system using aptamer guided

nanoparticles (ie, nanoliposomes) for selective delivery of therapeutic siRNA or drugs to tumor cells. It will increase the therapeutic efficacy of anticancer agents and reduce their side effects.

RESEARCH PROJECTS

- Proteomics biomarker discovery for hepatocellular carcinoma
- Targeted cancer therapy with aptamer mediated nanoparticle-drug delivery

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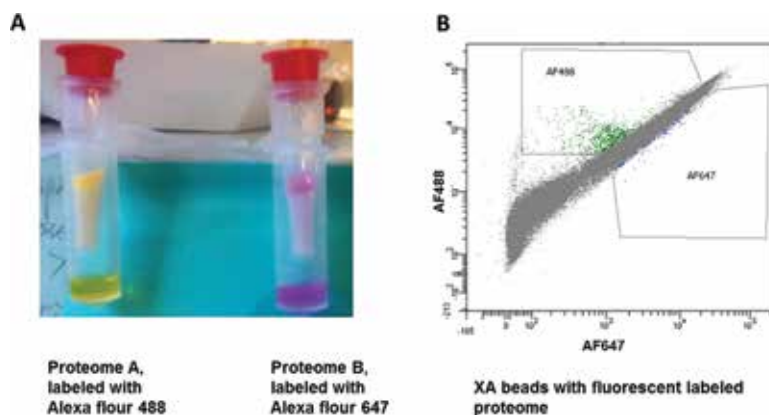
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LAB MEMBERS

Research associate: Xin Li
Medical student: Andrea Costello



X-aptamer based proteome selection. Patient plasma and health donor plasma cells were labeled with Alexa Fluor 488 or Alexa Fluor 647 respectively (A), mixed and then incubated with X-aptamer beads library (10⁸ different X-aptamers). Proteome bound to bead-X-aptamer were sorted based on fluorescence intensity at each channel by a two-color BD FACS ARIA II flow cytometer (B).

The faculty, research staff, and trainees of the Center for Stem Cell and Regenerative Medicine (CSCRM) are focused on experimental studies of the biological properties of stem cells in both



health and disease. The interest in healthy (or normal) stem cells is motivated by their essential role in both normal development – from the fertilized egg to fully developed organism – as well as in maintenance of tissues and organs throughout life. One of the hopes of regenerative medicine is that this fundamental understanding of stem cells may be effectively translated into therapies in which healthy stem cells, or their derivatives, can be employed to replace cells and tissues lost as a consequence of normal aging, injury, or disease.

There are at least two distinct classes of stem cells under active investigation within the Center for such therapeutic applications. The first of these are tissue-resident stem cells; such cells present throughout life in various organs, such as bone marrow, intestine, and lung, are involved in active regeneration of cells and tissues lost due to normal cell turn-over, aging, injury, or disease. A second class of stem cells of significant therapeutic interest to Center investigators is induced pluripotent stem cells (iPSCs). iPSCs are patient-specific stem cells that can be generated from easily obtained cells from any individual and, in principle, may be specifically guided into the various cell types and tissues present within the human body. Faculty within the Center are seeking to develop efficient and robust methodologies to convert iPSCs into various cells/tissues of therapeutic interest, including neural, blood, lung, muscle, and cartilage – as well as how to best deliver and maintain such cells/tissues for therapeutic benefit.

For patients presenting with genetically inherited disease, Center faculty are utilizing recently developed gene editing technologies to correct the disease-causing mutations in either

tissue-resident stem cells or iPSCs. The goal of these studies is development of therapies that include correcting the mutations in a patient's own stem cells, then delivering either the corrected stem cells or cells/tissues derived from them back into the same patient.

Finally, there is increasing evidence for the presence within cancers of cells having specific properties typically associated with stem cells. Center faculty are interrogating the role of such cells in the initiation and maintenance of cancers such as ovarian cancer and lymphoma.

In the pages following you will find several examples of Center faculty exploring the potential therapeutic value of stem cells for repairing tissues such as spinal cord, brain, muscle, cartilage, lung, and blood, as well as elucidating the role of stem cells in cancer. Competitive grant funding for these studies has been received from various sources, including the National Institutes of Health, Department of Defense, Cancer Prevention Research Institute of Texas, Cystic Fibrosis Foundation, University of Texas Rising STARS Program, and Mission Connect. Importantly, philanthropic funds made available to Center investigators, either in the form of endowed chairs, gifts, or pilot grants, have been and continue to be essential in seeding the early stage advances required for demonstrating proof of principle and eventual external grant funding.

If I may provide any additional information, please do not hesitate to contact me.

Brian R. Davis, Ph.D.

Associate Professor and Center Director

The C. Harold and Lorine G. Wallace Distinguished University Chair



Brian R. Davis, Ph.D.

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

Genetic correction of stem cells for treatment of inherited lung and blood 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 and/or tissue-specific stem cells derived from patients with inherited disorders affecting the lung or blood system. This is being pursued with the ultimate goal of developing stem/progenitor cell-based therapeutic approaches. We have utilized DNA sequence-specific nuclease-mediated homology directed repair to correct the most common genetic mutations in iPS cell lines derived from patients with Cystic Fibrosis – and have demonstrated genetic and functional correction in lung epithelial cells derived from these corrected iPS cells. We have recently reported introduction of lung-specific fluorescent reporters into iPS cells and utilized them to specifically isolate early lung progenitors for purposes of molecular and functional characterization. One of our objectives is to employ CF patient-specific iPS cell-derived lung epithelium for testing sensitivity to specific CF drugs – in order to facilitate a personalized therapeutic approach. We also are presently utilizing the fore-mentioned gene correction methodologies to correct the CF mutations in tissue-specific stem cells directly obtained from CF patients. The second major project in the laboratory focuses on the site-specific correction of gene mutations responsible for inherited blood disorders, such as the Wiskott-Aldrich Syndrome (WAS), a primary immune deficiency. Again, we are seeking to correct the disease-causing mutations in patient-specific blood stem cells or iPS cells with subsequent differentiation to blood stem cells for transplantation. In both the CF and WAS projects, the ultimate objective is the delivery back to patients of their own lung or blood stem cells, only differing from the original stem cells by the genetic correction of the relevant mutation. The third laboratory project focuses on “natural gene correction,” 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 WAS patients.

RESEARCH PROJECTS

- Correction of iPS and airway stem cells from Cystic Fibrosis patients
- Correction of iPS and blood stem cells from Wiskott-Aldrich Syndrome patients
- Characterization of Spontaneous Gene Mutations Resulting in Correction of Inherited Wiskott-Aldrich Syndrome Defects

KEY PUBLICATIONS

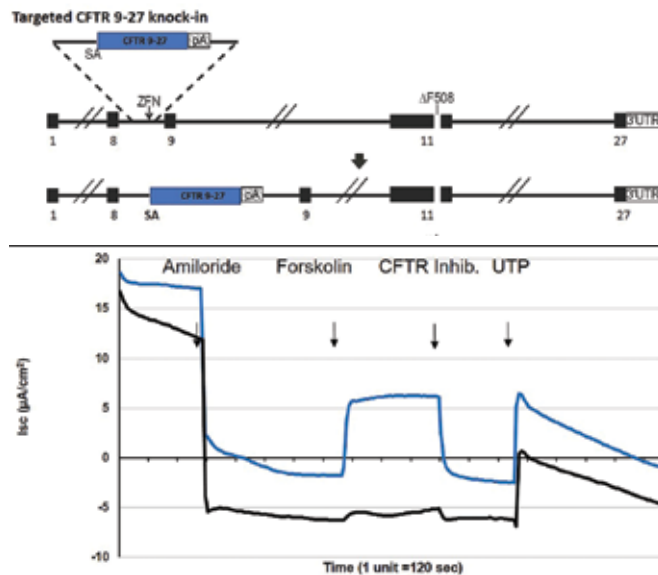
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LAB MEMBERS

Post-doctoral fellows: Dr. John M. Avila, Dr. Shingo Suzuki
 Graduate students: Varada Anirudhan
 Research staff: Dr. Ana M. Crane, Dr. Nadine Matthias



One approach to fix Cystic Fibrosis mutations is to insert a correcting partial CFTR gene (shown in blue in the upper panel) directly into the defective CFTR gene (shown in black) in patient’s stem cells. When the corrected stem cells are turned into airway tissue, they exhibit restored CFTR function (shown in blue in the lower panel), in contrast to the defective tissue (shown in black).



Laura A. Smith Callahan, Ph.D.

Assistant Professor

Tissue engineering approaches for the treatment of CNS injuries

The research in my laboratory focuses on developing biomaterials to be used in clinical treatments for spinal cord injury, traumatic brain injury, and stroke. The laboratory uses an interdisciplinary approach involving techniques from cell, molecular, and stem cell biology, chemistry, and material science. Utilizing engineering approaches, the laboratory seeks to optimize scaffold design for the expansion of clinically relevant cell sources for use in stem cell therapy and to support the cells after implantation into patients.

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

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

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

RESEARCH PROJECTS

- Optimization of substrates and matrices to direct human induced pluripotent stem cells to neural progenitor cells to therapeutic lineages using combinatorial approaches.
- Modulation of cellular environment *in vivo* to promote cell therapy survival, integration with the host and maturation toward functional mature cell types after central nervous system injury.

KEY PUBLICATIONS

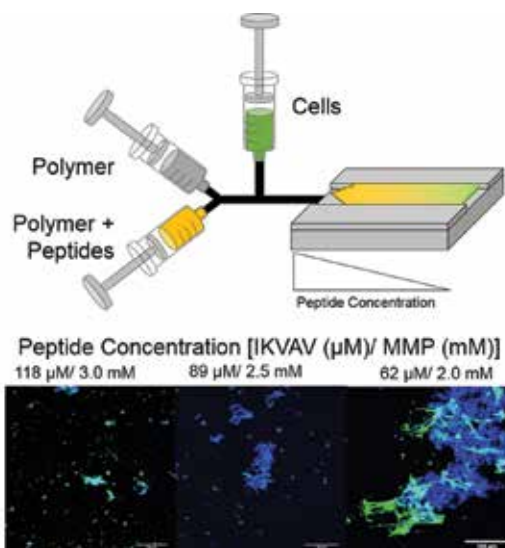
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LAB MEMBERS

Post-doctoral fellows: T. Hiran Perera, Xi Lu



Schematic of hydrogel with a continuous gradient in two peptide concentrations (IKVAV and MMP) and response of human induced pluripotent stem cell derived neural stem cell cytoskeletal organization (green) encapsulated in the hydrogel for 1 week undergoing neural differentiation. Nuclear staining is blue. Scale bars= 100µm.



Qi Lin Cao, M.D.
Professor

Stem cells for neurological diseases

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

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

RESEARCH PROJECTS

- *In vivo* reprogramming of reactive astrocyte and chemogenetic approach for SCI repair
- Treating neuropathic pain by *in vivo* reprogramming of astrocytes after SCI
- Combinatorial approaches to promote axonal regeneration and functional recovery after SCI
- Human iPSC-derived neural stem or precursor cells for spinal cord injury and stroke

KEY PUBLICATIONS

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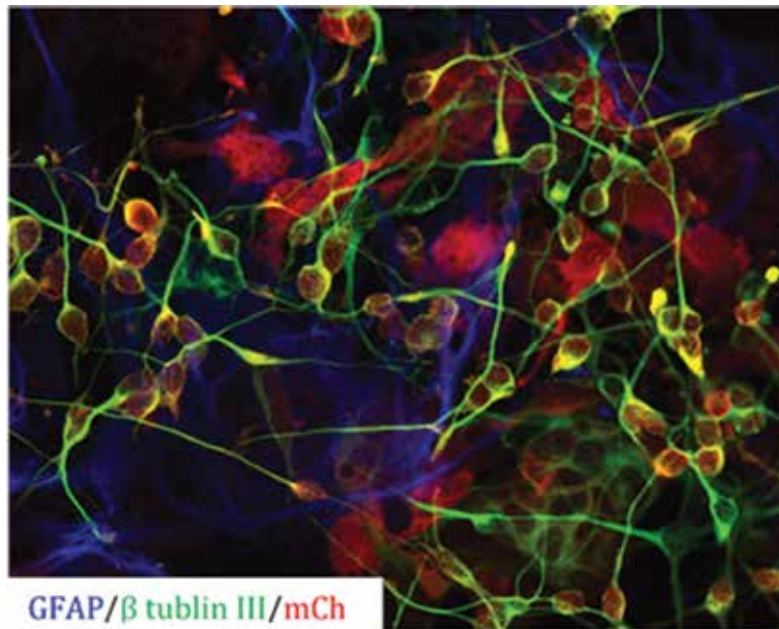
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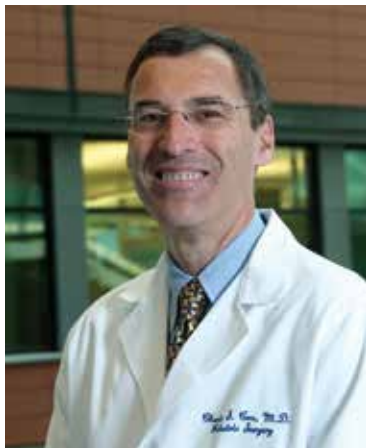
Cheng XX, Zheng YY, Bu P, Qi XB, Fan CL, Li FQ, Kim DH and Cao QL (2018). Apolipoprotein E as a novel therapeutic neuroprotection target after traumatic spinal cord injury. *Experimental Neurology* 299: 97-108.

LAB MEMBERS

Post-doctoral fellows: Yiyan Zheng, Xiuquan He
Graduate student: Chrystine Gallegos
Research associate: Haipeng Xue



Overexpression of transcription factors, sox2 and mash1, converts astrocytes into neurons *in vitro*.



Charles Cox, Jr., M.D.

Professor

George and Cynthia Mitchell Distinguished University Chair

Cellular therapies for neurological injury

Our current research program focuses on the use of cellular therapies for neurological injuries, principally traumatic brain injury, or TBI. We have been interested in the modulation of the innate immune response to TBI and how cellular therapies have been successful without significant engraftment in the brain long term. Cell-cell interactions in the peripheral reticuloendothelial system have resulted in Treg upregulation and modulation of the microglia/macrophage phenotype in the brain. We use these types of data to help us determine dosing regimens (number of cells, type and route of delivery as well as timing), which may be very specific to the pathophysiology in question. We use *in vivo* models of injury and *in vitro* test beds.

Our team directs the Griffin Stem Cell Laboratory and the Hoffberger Stem Cell Laboratory, which are cGMP and cGTP cell processing facilities that enable us to translate discovery into treatments. These facilities allow clinical grade cell production for use in our clinical protocols.

RESEARCH PROJECTS

- Development of Phase 1 and 2 Clinical Trials using non-ESC stem/progenitor cells for traumatic brain injury
- IND-enabling studies using APCs for traumatic brain injury
- Amniotic fluid derived MSCs for the treatment of neurological injury associated with congenital heart disease and cardiopulmonary bypass/hypothermic circulatory arrest
- Novel delivery systems for stem cells in neurological injury
- Imaging of microglial activation *in vivo*

KEY PUBLICATIONS

Jackson ML, Srivastava A, Cox CS. Pre-clinical progenitor cell therapy in traumatic brain injury: a Meta-Analysis. *J Surg Res* 214:38-48, 2017. PMID: 28624058

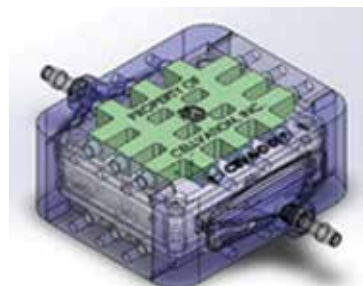
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LAB MEMBERS

Steven Kosmach, MSN, RN, CCRC-TBI clinical
 Joiya Arrington, MSN, RN, TBI clinical
 Yidao Ca, -programmer analyst
 Akshita 'Jade' Kumar, M.D., TBI clinical and cell therapy
 Louis Carrillo, M.D., TBI clinical and cell therapy
 Mitchell George, M.D., TBI clinical and cell therapy
 Scott Olson, Ph.D., assistant professor
 Katherine Ruppert, Ph.D., Sr research associate
 Karthik Prabhakara, Sr research assistant
 Cecilia Martin, Ph.D., research associate
 Supinder Bedi, Ph.D., assistant professor
 Amit Srivastava, Ph.D., assistant professor
 Naama Toledo-Furman, Ph.D., flow cytometry/innate immunity
 Hasen Xue, M.D., research associate

Fabio Triolo, Ph.D., GMP center director
 Sufira Kiran, GMP, QA director
 Romina Gipson-Love, quality improvement coordinator
 Deepa Bhattarai, Sr research associate
 Matteo Costantini, research assistant
 Kevin Aroom, scientific programmer
 Tushar Sharma, scientific programmer
 Max Skibber, research assistant
 Christina Willingham, program manager
 Stephanie Baca, program manager



Development of a novel bio-reactor for stem cell production.



Radbod Darabi M.D., Ph.D.

Assistant Professor

Using human pluripotent stem cells to treat common skeletal muscle disorders

Skeletal muscle disorders are among major health problems in our society and worldwide. Most common types of muscle disorders include gradual muscle loss during normal aging (sarcopenia) or after systemic disorders (such as patients with heart or kidney failure, or in patients with cancer), as well as muscle loss injuries after accidents or combat injuries. In addition, genetic disorders of the muscle, such as muscular dystrophies, are also a very common health problem with no definitive cure. All of these disorders might eventually lead to progressive muscle weakness and patients' confinement to the wheelchair. Unfortunately, in severe cases, these disorders are fatal due to the involvement of heart or breathing muscles.

Our lab's main interest is to use pluripotent stem cells (PSCs) for the treatment of these common disorders. During the last few years, our lab has developed novel methods for myogenic differentiation of induced pluripotent stem cells (iPSCs) as cell therapy in different skeletal muscle disorders, such as Duchenne muscular dystrophy (DMD) and muscle loss injuries.

Here at IMM, by using cutting-edge gene targeting/editing technologies (such as CRISPR/Cas9) our lab has successfully generated knock-in human stem cell lines for early myogenic genes, such as PAX7 and MYF5. This allows us to study the emergence of early myogenic progenitors from human PSCs, a crucial step to identify and isolate myogenic progenitors useful for stem cell therapy in muscle disorders. These cells are being tested for the engraftment potential in the mice models for Duchenne muscular dystrophy, which so far have demonstrated superior engraftment and regeneration potential in the treated muscles.

Other major goals of our lab include using high throughput screening (HTS) to identify important pathways for myogenesis, evaluation of the engraftment potential of human PSCs in mice models for muscle disorders, as well as identification of novel regulators of myogenic program using genome-wide gRNA library screen. Our research team also works on

derivation of iPSCs from patients with new types of muscular dystrophies, gene correction of patient-derived iPSCs, as well as enhancing cell delivery and engraftment in the skeletal muscle. Our research program is currently funded by two NIH grant awards from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) to support these exciting and novel projects.

RESEARCH PROJECTS

- Generation of knock-in human ESC reporter cell lines for early myogenic genes (PAX7, MYF5)
- Developing efficient myogenic induction and purification methods for human pluripotent stem cells (hPSCs) to generate early myogenic progenitors useful for disease modeling as well as stem cell therapy in muscle disorders
- Evaluation of the engraftment potential of hPSCs in the mice models for Duchenne muscular dystrophy (DMD) and muscle mass loss injury.
- Using bio-scaffolds along with stem cells for skeletal muscle repair
- Gene correction of patient-derived iPSCs using CRISPR/Cas9 system
- Genome-wide gRNA library screen to identify

novel upstream regulators of skeletal myogenesis

KEY PUBLICATIONS

Wu J, Hunt SD, Xue H, Liu Y, Darabi R. Generation and Characterization of a MYF5 Reporter Human iPS Cell Line Using CRISPR/Cas9 Mediated Homologous Recombination. *Scientific Reports*. 2016 Jan 5; 6:18759. doi: 10.1038/srep18759.

Wu J, Hunt SD, Matthias N, Servián-Morilla E, Lo J, Jafar-Nejad H, Paradas C, Darabi R. Generation of an induced pluripotent stem cell line (CSCRMi001-A) from a patient with a new type of limb-girdle muscular dystrophy (LGMD) due to a missense mutation in POGGLUT1 (Rumi). *Stem Cell Research*, 2017 Sep; 24: 102-105.

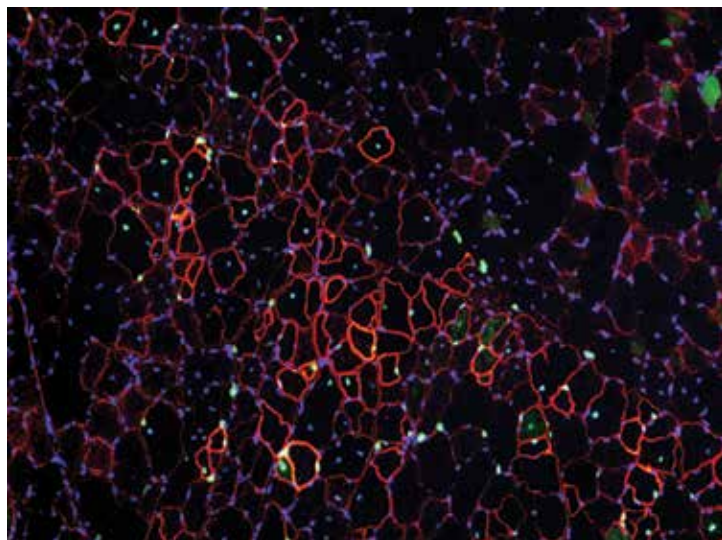
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LAB MEMBERS

Instructor: Jianbo Wu

Research associate: Nadine Matthias

Master student (GSBS): Jose L. Ortiz-Vitali



Engraftment of human iPSC - derived myogenic progenitors in the mouse muscle, one month after transplantation. Red and green colors mark new human stem cell-derived muscle fibers (cross-sections) stained for human-specific markers (human dystrophin in red and human lamin A/C in green) in a mouse muscle one month after transplantation



Pramod Dash, Ph.D.

Professor

Nina and Michael Zilkha Distinguished Chair, Neurodegenerative Disease Research

Traumatic brain injury and neurodegenerative diseases

Dementia has emerged as a major cause of disability among the elderly that interferes with daily activities and compromises quality of life. While early stage dementia involves memory lapses, as the disease progresses, persons suffering from dementia will require daily assistance for the remainder of their life. The most common form of dementia is Alzheimer’s dementia (AD), followed by vascular dementia, Lewy-body/Parkinson’s dementia, and fronto-temporal dementia (FTD).

Recent epidemiological and clinical studies are beginning to show that traumatic brain injury (TBI) is a major risk factor for the development of neurodegenerative diseases, such as Parkinson’s Disease, amyotrophic lateral sclerosis (ALS), and chronic traumatic encephalopathy (CTE). PET imaging studies to measure glucose uptake in the brain have shown that decreased glucose uptake may contribute to the development of neurodegenerative diseases. Moreover, these studies have also shown that decreased glucose uptake precedes the presentation of clinical symptoms. For example, in the familial form of AD, decreased glucose metabolism is one of the first pathological changes that transpires (prior to amyloid and TAU deposition), occurring up to 30 years before the onset of symptomology.

Clinical studies have shown that TBI causes a prolonged suppression in glucose metabolism. This suppression has been proposed to be an underlying mechanism for cognitive impairments. Consistent with this, disturbed brain glucose metabolism after TBI has been shown to occur in regions critical to cognition, such as the cortex and the hippocampus. We and others have demonstrated that outcome can be improved by augmenting energy sources (e.g. glucose, lactate) or providing alternative energy-containing compounds (e.g. glyceryl triacetate, acetyl-L-carnitine). However, the link between TBI-triggered decreased energy production and the development of neurodegenerative diseases has not been investigated.

Glucose is the primary energy source for

brain cell survival and function. Glucose is metabolized by glycolysis and oxidative phosphorylation (occurring in mitochondria) to generate ATP, which is used by the cell to carry out various functions. As a part of oxidative phosphorylation, oxygen is consumed which can be monitored as an indicator of mitochondrial function. We have developed and optimized an assay to measure mitochondria respiration in brain tissue that does not require the isolation of mitochondria. Using this assay, we are examining the spatial and temporal changes in glucose metabolism following TBI (and repeat concussion) and its link to the subsequent development of amyloid β aggregation (a key pathology of AD) and Tau aggregation (a key pathology of AD and CTE).

RESEARCH PROJECTS

- To define the temporal and anatomical location of changes in mitochondrial function after TBI and repeat concussion
- To investigate the consequences of mitochondrial plasticity and altered brain energy metabolism after TBI.

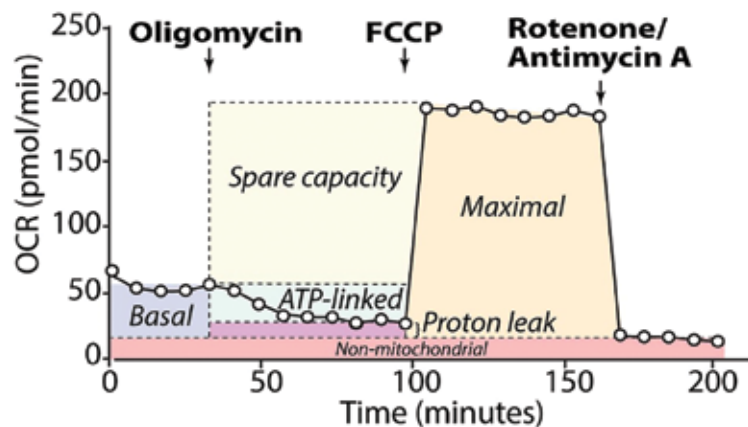
Fischer TD, Dash PK, Liu J, Waxham MN. Morphology of mitochondria in spatially restricted axons revealed by cryo-electron tomography. *PLoS Biol.* 16:e2006169, 2018.

Hood KN, Zhao J, Redell JB, Hylin MJ, Harris B, Perez A, Moore AN, Dash PK. Endoplasmic Reticulum Stress Contributes to the Loss of Newborn Hippocampal Neurons after Traumatic Brain Injury. *J Neurosci.* 38(9):2372-2384, 2018.

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Dash PK, Zhao J, Kobori N, Redell JB, Hylin MJ, Hood KN, Moore AN. Activation of Alpha 7 Cholinergic Nicotinic Receptors Reduce Blood-Brain Barrier Permeability following Experimental Traumatic Brain Injury. *J Neurosci.* 36(9):2809-18, 2016.

KEY PUBLICATIONS



Example of mitochondrial respiration from hippocampal tissue punches. The various aspects of mitochondrial respiration are assessed by the addition of specific inhibitors. Basal respiration is determined by the oxygen consumption rate (OCR) in the absence of any inhibitors. The ATP synthase inhibitor oligomycin decreases proton flux through ATP synthase, thereby reducing electron transport and oxygen consumption. This reduction in OCR represents the respiration related to ATP synthesis. The residual proton flux and related oxygen consumption after oligomycin addition is considered to be a measure of proton leak. The uncoupler FCCP, which abolishes the proton gradient, results in maximal oxygen consumption and is used to measure maximal respiration. Finally, the addition of rotenone (a complex 1 inhibitor) and antimycin A (an inhibitor of cytochrome C reductase) abolishes mitochondrial respiration. The residual oxygen consumption observed after addition of these two compounds is carried out by non-mitochondrial oxygen-consuming enzymes such as oxidases.



Sarah Xuelian Huang, M.B.B.S., Ph.D.

Assistant Professor

Human pluripotent stem cells for lung regeneration and disease modeling

My laboratory is interested in applying human pluripotent stem cells to study the molecular mechanisms of lung cell fate specification in the context of both normal and pathological conditions. The long-term goal is translation of the acquired knowledge into prevention and treatment of currently incurable lung diseases. Lung diseases are among the leading causes of death globally. Lower respiratory infections, chronic obstructive pulmonary disease, and lung cancer together account for approximately 9 million deaths annually worldwide. Despite the huge lung disease burden, we still have very limited understanding of the pathogenic mechanisms responsible for these diseases, and consequently there is a lack of successful therapeutic approaches. The only definitive treatment for end stage lung disease is lung transplantation, which is hampered by the extremely limited number of available donor organs.

Recently, a human pluripotent stem cell-based model has emerged as a novel system for studies of human diseases. The need for such a system stems from the limitations of the existing animal experimental models, which fall short in demonstrating concordance with human studies. In addition, experimental approaches utilizing primary human adult lung cells are inadequate in large part due to the limited availability of lung tissue from healthy subjects (including the additional difficulty of obtaining airway and alveolar cells from the same donor).

Realization of stem cell therapy in lung diseases relies on the successful generation of clinically applicable cell types. As a first, critical step in this direction, we have previously developed a step-wise differentiation strategy that directs human pluripotent stem cells to become different types of upper (airway) and lower (alveoli) respiratory lung epithelial cells at large quantities (Huang *et al.* Nat Biotechnol 2014, Nat Protoc 2015). As a proof of principle, the generated cells have been applied for lung development or disease studies by us

and other research groups. Currently, we are working on culture conditions that can direct the human pluripotent stem cell-derived early lung progenitors toward an enriched population of either airway epithelial cells or distal alveolar cells. The availability of each of these enriched airway- and alveolar- fated cells provides a valid platform for studying lung diseases that originate in both the airway and alveolar. Examples include cystic fibrosis – a genetic disease affects the airway; influenza virus infection induced severe infection and acute respiratory distress syndrome that affects the lower respiratory of the lung; and lung cancers that can arise in both the airway and alveoli cells depending on the subtype.

RESEARCH PROJECTS

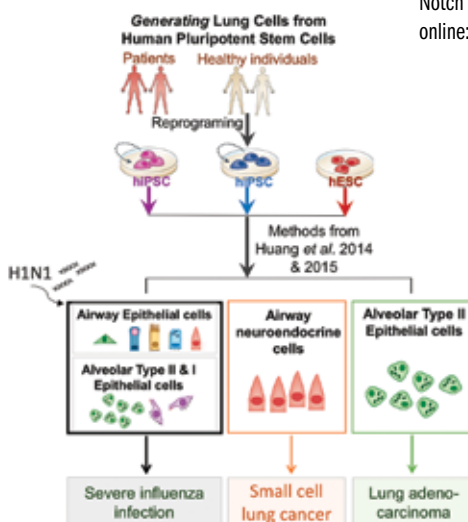
- Use patient hiPSC differentiated lung epithelial cells to examine TLR3 mediated intrinsic immunity and test rare mutations in TLR3 as causative for severe influenza infection induced by H1N1 virus
- Mapping the pathogen recognition patterns and the cellular and molecular responses of the lung epithelial cells to pathogen infection
- Understanding the basic mechanisms of lung lineage specification from NKX2.1⁺SOX2⁺SOX9⁺ NKX2.1⁺SOX2⁺P63⁺ human lung and airway progenitors using molecular, genetic and epigenetic approaches

KEY PUBLICATIONS

Ya-Wen Chen, Sarah X.L. Huang, Ana L.R.T. Carvalho, Siu-Hong Ho, Mohammad N. Islam, Stefano Volpi, Luigi D. Notarangelo, Michael Ciancanelli, Jean-Laurent Casanova, Jahar Bhattacharya, Alice F. Liang, Laura M. Palermo, Matteo Porotto, Anne Moscona & Hans-Willem Snoeck. A three-dimensional model of human lung development and disease from pluripotent stem cells. *Nat Cell Biol.* 2017 May;19(5):542-549. doi: 10.1038/ncb3510. Epub 2017 Apr 24.

Edwin J. Ostrin, Danielle R. Little, Kamryn N. Gerner-Mauro, Elizabeth A. Sumner, Ricardo Ríos-Corzo, Elizabeth Ambrosio, Samantha E. Holt, Nicolas R. Forcioli-Conti, Haruhiko Akiyama, Sam M. Hanash, Shioko Kimura, Sarah X.L. Huang, Jichao Chen. Beta-Catenin maintains lung epithelial progenitors after lung specification. *Development* (2018) 145, dev160788.

Yongchun Zhang, Ying Yang, Ming Jiang, Sarah Xuelian Huang, Wanwei Zhang, Denise Al Alam, Soula Danopoulos, Munemasa Mori, Ya-Wen Chen, Revathi Balasubramanian, Susana M. Chuva de Sousa Lopes, Carlos Serra, Monika Bialecka, Eugene Kim, Sijie Lin, Ana Luisa Rodrigues Toste de Carvalho, Paul N. Riccio, Wellington V. Cardoso, Xin Zhang, Hans-Willem Snoeck, Jianwen Que. 3D Modeling of Esophageal Development using Human PSC-Derived Basal Progenitors Reveals a Critical Role for Notch Signaling. *Cell Stem Cell*, Published online: September 20, 2018.



Schematic illustration of human pluripotent stem cells-derived airway and lung epithelial cells for modeling airway and lung diseases.



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

Advancing the field of neuroscience

and Subarachnoid Hemorrhage. *Stroke*. 2016 Dec;47(12):3005-3013. Epub 2016 Nov 15.

Ying Liu, Yiyang Zheng, Shenglan Li, Haipeng Xue, Georgene W. Hergenroeder, Jiaqian Wu, Yuyuan Zhang, Dong H. Kim, Qilin Cao: Human neural progenitors derived from integration-free iPSCs for SCI therapy. 2016; *Stem Cell Res.* 2017 Jan 5;19:55-64.

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Duran RC, Yan H, Zheng Y, Huang X, Grill R, Kim DH, Cao Q, Wu JQ. The systematic analysis of coding and long non-coding RNAs in the sub-chronic and chronic stages of spinal cord injury. *Scientific Reports*. 2017 Jan 20;7:41008.

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

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

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

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

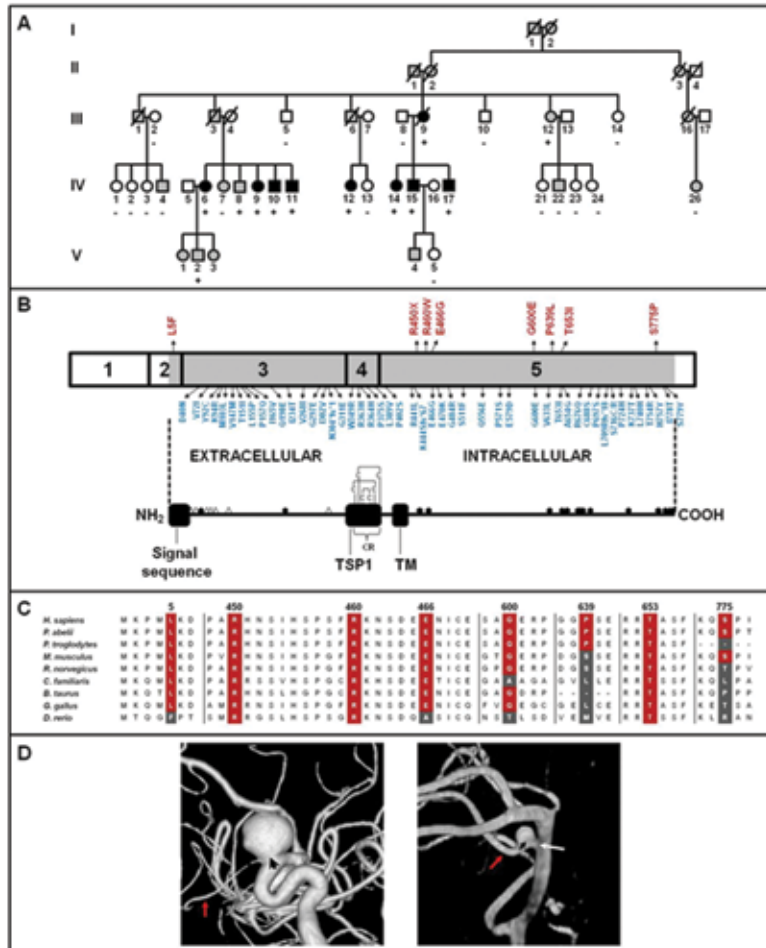
RESEARCH PROJECTS

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

KEY PUBLICATIONS

Santiago-Sim T, Fang X, Hennessy M, Nalbach S, DePalma S, et al. THSD1 (Thrombospondin Type 1 Domain Containing Protein 1) Mutation in the Pathogenesis of Intracranial Aneurysm and Subarachnoid Hemorrhage. *Stroke*. 2016 Dec;47(12):3005-3013. Epub 2016 Nov 15.

Santiago-Sim T, Fang X, Hennessy M, Nalbach S, DePalma S, et al. THSD1 (Thrombospondin Type 1 Domain Containing Protein 1) Mutation in the Pathogenesis of Intracranial Aneurysm



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



Momoko Yoshimoto, M.D., Ph.D.
Associate Professor

Elucidating the mechanisms of multiple waves of hematopoiesis in the mouse embryo

My lab is trying to understand how multiple waves of various hematopoietic cells are produced from “hemogenic endothelial cells” before the first hematopoietic stem cells (HSCs) are produced in the mouse embryo. Specifically, we are asking questions 1) innate B-1 cell progenitors are produced independent from HSCs and persist into postnatal life, and 2) how pre-HSCs mature into adult-repopulating HSCs in a limited time window of embryonic development.

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

At the same time when B-1 progenitors are produced in the embryo, pre-HSC and adult repopulating HSCs are produced from the para-aortic region of the embryo. Pre-HSCs acquire adult-repopulating ability within one day, and its mechanism has yet to be elucidated. We are trying to clarify the molecular mechanisms through which pre-HSCs gain efficient repopulating ability by single-cell RNA sequencing.

Knowledge obtained from above projects will help us to understand the mechanism of HSC

and B-1 cell production *in vivo* and to produce those human counterparts from human iPS cells *in vitro*, which might open a path of cell therapy for hematological disorders and immune deficient patients.

RESEARCH PROJECTS

- Lineage tracing for HSC-independent and/or HSC-dependent B-1 cell development from embryos to adults.
- Elucidating cell intrinsic and cell extrinsic mechanisms for maintaining B-1a cell self-renewal ability.
- Understanding the multiple waves of hematopoiesis and HSC production in the mouse embryo.
- Identify important molecules for HSC maturation in the mouse embryo utilizing single-cell RNA-sequencing.

KEY PUBLICATIONS

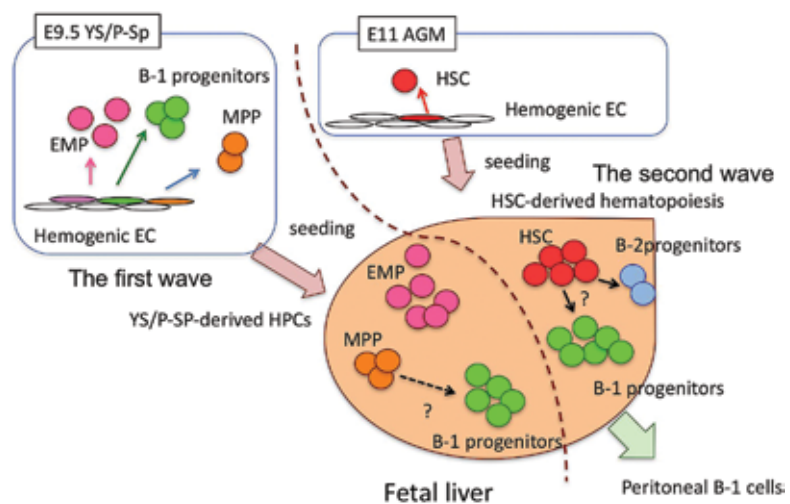
Hadland B, Yoshimoto M. Many layers of embryonic hematopoiesis: new insights into B-cell ontogeny and the origin of hematopoietic stem cells. *Exp. Hematol.* 2018;60:1-9.

Tarnawsky SP, Yoshimoto M, Deng L, Chan RJ, Yoder MC. Yolk sac erythromyeloid progenitors expressing gain of function PTPN11 have functional features of JMML but are not sufficient to cause disease in mice. *Dev. Dyn.* 2017;246(12):1001-1014.

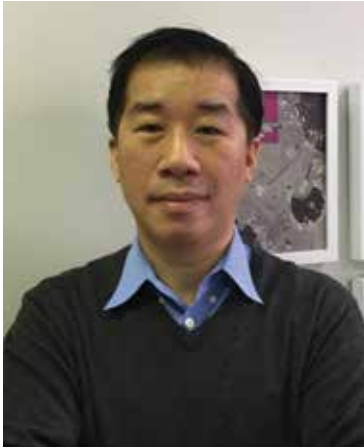
Hadland BK, Varnum-Finney B, Mandal PK, Rossi DJ, Poulos MG, Butler JM, Rafii S, Yoder MC, Yoshimoto M, Bernstein ID. A Common Origin for B-1a and B-2 Lymphocytes in Clonal Pre-Hematopoietic Stem Cells. *Stem Cell Reports.* 2017;8(6):1563-1572.

LAB MEMBERS

Post-doctoral fellow: Nathalia Azevedo Portilho, Ph.D
Assistant professor: Michihiro Kobayashi M.D., Ph.D.



Hypothesis: The first wave of B-1 cell develops from yolk sac hemogenic endothelial cells in the mouse embryo and contributes to B-1 progenitors in the fetal liver and postnatal B-1a cell pool in the adult peritoneal cavity. Hemogenic endothelial cells (ECs) in the yolk sac (YS) and para-aortic splanchnopleura (P-Sp) produce B-1 progenitor cells prior to HSC emergence. These progenitor cells seed fetal liver. Around at E11, the first HSCs emerge from hemogenic ECs at AGM region and also seed fetal liver and expand. Fetal liver B-1 progenitor cells derived from YS hemogenic EC and HSC mature into the peritoneal B-1 cells after birth. (Ann NY Acad Sci 2015)



Dung-Fang Lee, Ph.D.
Assistant Professor

Familial cancer syndromes in a dish

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

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

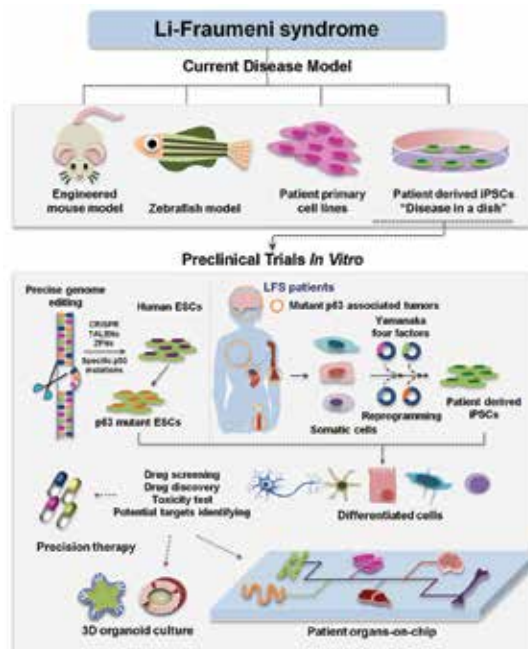
Yu-Hsuan Lin, Brittany E Jewell, Julian Gingold, Linchao Lu, Ruiying Zhao, Lisa L Wang, Dung-Fang Lee. Osteosarcoma: Molecular pathogenesis and iPSC modeling. *Trends Mol Med.* 23(8):737-755, 2017.

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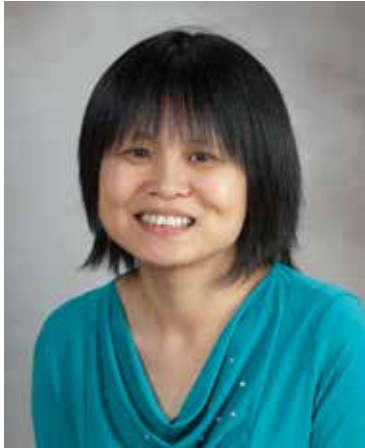
Huensuk Kim, Seungyeul Yoo, Ruoji Zhou, An Xu, Jeffrey M. Bernitz, Ye Yuan, Andreia M Gomes, Michael G Daniel, Jie Su, Elizabeth G. Demicco, Jun Zhu, Kateri A. Moore, Dung-Fang Lee†, Ihor R Lemischka, Christoph Schaniel†. *Proc Natl Acad Sci U S A.* 2018 (In press) (†Corresponding author)

LAB MEMBERS

Post-doctoral fellows: An Xu, Mo Liu, Dandan Zhu
Students: Ruoji Zhou, Brittany E. Jewell
Technicians: Ying Liu
Visiting scholars: Jian Tu, Yu Lin



The application of LFS iPSC model to drug development for LFS and *p53* mutation-associated tumors. LFS iPSC model overcomes the limitations of current LFS disease models like mouse model, zebrafish model and primary cell lines, and holds potentials in modeling LFS associated cancers and facilitating clinical trials in a dish. Precise genome editing techniques make it possible to expand the bank of PSCs with different *p53* mutations, which provides valuable resource for precision cancer medicine. Integration of 3D organoid and organs-on-chip with LFS iPSC disease model offers exciting opportunities for testing existing both WT and mutant *p53*-associated pathway related drugs and discovering new therapeutic compounds.



Ying Liu, Ph.D.
Assistant Professor

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Liu, Y.*, Zheng, Y., Li, S., Xue, H., Schmitt, K., Hergenroeder, G.W., Wu, J., Zhang, Y., Kim, D.H., Cao, Q*. (2017) Human neural progenitors derived from integration-free iPSCs for SCI therapy. *Stem Cell Res.* 2017 Jan 5;19:55-64. doi: 10.1016/j.scr.2017.01.004. [Epub ahead of print] (*corresponding authors) PMID:28073086

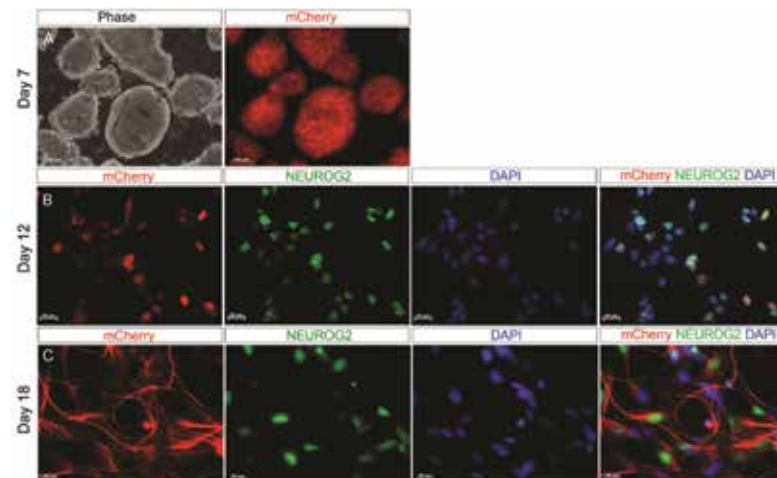
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Long, B., Li, S., Xue, H., Sun, L., Kim, D. H., Liu, Y. (2017). Effects of propofol treatment in neural progenitors derived from human-induced pluripotent stem cells. *Neural Plasticity.* Oct 8, 2017. Article ID 9182748, <https://doi.org/10.1155/2017/9182748> [Epub ahead of print]

Jia D, Li S, Li D, Xue H, Yang D, and Liu Y (2018). Mining TCGA database for genes of prognostic value in glioblastoma microenvironment. *Aging* (Albany NY). 2018 Apr 16. doi: 10.18632/aging/101415. [Epub ahead of print] PubMed PMID: 29676997

LAB MEMBERS

Visiting graduate student: Di Jia
Research associate: Haipeng Xue



A Neurogenin 2 knock-in human iPSC reporter cell line made using the CRISPR/Cas9 system. NEUROG2-mCherry human iPSC clones are induced as embryoid bodies (EBs), which glow red under the fluorescence microscope (A). NEUROG2 antibody staining (green) confirms that mCherry (red, native signal) expression faithfully reflects the endogenous NEUROG2 expression along the differentiation pathway (B, C).



Nami McCarty, Ph.D.

Associate Professor

Jerold B. Katz Distinguished Professorship in Stem Cell Research

Deciphering mechanisms of human cancer cell survival within the bone microenvironment

The behavior of cancer cells is not only dependent on their genomic abnormalities but also requires complex relationships between malignant cells and their local bone marrow niche, which provides an environment for multiple myeloma cell growth as well as protection from chemotherapy-induced apoptosis. The bone marrow niches provide a “hiding place” for dormant clones, which are often resistant to chemotherapeutic agents.

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

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

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

RESEARCH PROJECTS

- Survival mechanisms of dormant multiple myeloma cells and their microenvironment in the bone marrow: We conducted microarray analyses to identify genes expressed in quiescent multiple myeloma cells from the different niches of the bone marrow. We will continue to characterize functions of these genes in the multiple myeloma interaction with bone marrow microenvironment to delineate how dormant multiple myeloma cells evade chemotherapies.
- Development of small molecule inhibitors to target drug-resistant lymphomas: We have conducted high throughput chemical screening to identify the compounds that selectively target mantle cell lymphoma cells that develop drug resistance. We will further develop and test these compounds in animal models for pre-clinical studies and plan to test their efficacies in the patients.
- Delineating transcription factor networks on drug resistant lymphomas: We will continue to address roles for PAX5-BACH2 signaling networks in mantle cell lymphomas. We also will closely work with collaborators at clinicians to determine whether BACH2 sub-cellular localization in the cell determine drug resistance outcome and patient survival.

KEY PUBLICATIONS

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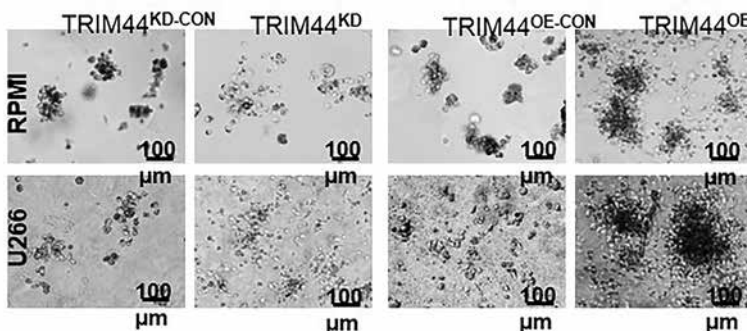
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McCarty, N. Battling quiescence for tumor eradication, is it too good to be true? *Oncotarget* (Invited editorial), 2018

LAB MEMBERS

Post-doctoral fellow: Lyn Liu, Ph.D.

Research assistants: Judy Chen, M.S.



TRIM44 over-expressed cells (TRIM44OE) increased colony formation in methylcellulose medium. Cells were seeded and after 21 days, cell groupings of >40 cells were counted as a colony. The pictures were taken by an inverted microscope (x10).



Naoki Nakayama, Ph.D.

Associate Professor

Annie & Bob Graham Distinguished Chair in Stem Cell Biology

Pluripotent stem cell biology and Synovial joint morphogenesis

Synovial joint is composed of articular cartilage (meniscal cartilage), ligament, and synovial membrane and is formed during embryogenesis from a (multilineage) joint progenitor. Injured joint cartilage is not spontaneously repaired in humans, leading to osteoarthritis. There has been considerable interest in the clinical application of stem cells to the repair of damaged cartilage; however, current cell therapies using expanded articular chondrocytes and adult stem cells, such as mesenchymal stromal cells, face the problems of low yield of cells and their tendency to yield unsuitable, transient cartilage. A transient cartilage, destined to form bone, is typically found in the growth plate. In contrast, joint cartilage is a permanent cartilage. Therefore, we hypothesize that the embryonic joint progenitor would be the best cell-type for the regeneration of joint cartilage, which may also be suitable for ligament regeneration. Pluripotent stem cells (PSCs), whether derived from an embryo, or induced from adult cells, are expected to differentiate into any cell-types of the human body through processes that mimic embryogenesis, making human (h) PSCs a promising source of embryonic cells for regenerative medicine. Therefore, we have been investigating the developmental process from human and mouse (m) PSCs to joint progenitor cells.

What is the molecular basis of human stem/progenitor cells to form permanent cartilage? We have previously developed and purified from hPSCs, progeny representing the three embryonic origins of chondrocytes, and demonstrated that they are able to expand and differentiate into corresponding chondrocyte precursors (chondroprogenitors). All such chondroprogenitors are capable of giving rise to hyaline cartilage in culture. However, most of them are mineralized and turned into bone when transplanted into immunocompromised mice, resembling growth-plate cartilage and adult stem cell-derived cartilage. In order to establish methods to generate permanent cartilage that stays as unmineralized cartilage for a long time after transplantation, we aim to achieve the fol-

lowing two goals; 1) generating the embryonic joint progenitor from hPSCs, and 2) demonstrating that they allow the culture-made cartilage to be stably maintained even after transplantation, and that they show superior capacity of articular cartilage repair to currently available cells. We previously discovered a way to selectively generate, and to a limited extent, expand joint progenitor-like cells from hPSCs. To purify and further characterize the joint progenitor-like cells, we have generated hiPSC lines that carry fluorescence marker genes in the joint progenitor marker gene loci. Furthermore, we have recently discovered that cartilage made with the joint progenitor-like cells is in fact maintained as unmineralized cartilage in mice. In addition, we have determined that controlling cAMP signaling leads to cartilage displaying very limited bone forming capacity after transplantation (i.e. pseudo-permanent cartilage) even from the standard chondroprogenitors. Therefore, we are currently focusing both on the characterization of the joint progenitor-like cells to elucidate molecular basis of their permanent cartilage-forming capacity, and on the elucidation of additional signaling mechanisms required for the formation of permanent cartilage. We expect that such mechanistic studies may lead to a proper method to make clinically relevant adult stem cells more suitable for joint cartilage regeneration.

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

RESEARCH PROJECTS

- Further defining the process of chondrogenesis from the hPSC-derived chondroprogenitors and joint progenitors to give rise to articular chondrocytes.
- Comparative omics analyses to elucidate the molecular basis of permanent, hyaline

cartilage formation from the hPSC-derived joint progenitor-like cells.

- Joint articular cartilage repair with hPSC-derived chondroprogenitors and joint progenitor-like cells in immunocompromised animal models.

KEY PUBLICATIONS

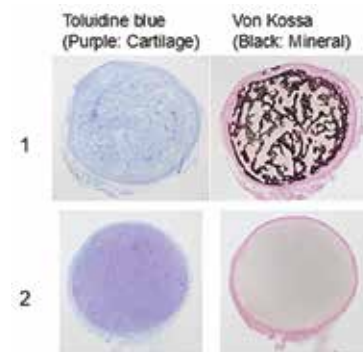
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LAB MEMBERS

Research assistant: Berke E. Sahbazoglu



Transient cartilage from standard chondroprogenitors (1), and permanent cartilage from joint progenitor-like cells (2) recovered from NSG mice after 8 weeks.



Pamela Wenzel, Ph.D.

Assistant Professor

Biomechanical force regulates cell potential and cancer metastasis

RESEARCH PROJECTS

- Mechanobiology of hematopoietic and mesenchymal stem cells
- Fluid flow in initiation of metastasis

KEY PUBLICATIONS

Lee, H.J., Diaz, M.F., Price, K.M., Ozuna, J.A., Zhang, S., Sevic-Muraca, E.M., Hagan, J.P., & Wenzel, P.L. Fluid shear stress activates YAP1 to promote cancer cell motility. *Nature Communications*. 8:14122, 2017.

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Lee, H.J., Ewere, A., Diaz, M.F., & Wenzel, P.L. TAZ responds to fluid shear stress to regulate the cell cycle. *Cell Cycle*. 17(2): 147-153, 2018.

LAB MEMBERS

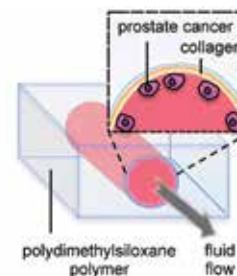
Graduate students: Megan Livingston, Paulina Horton

Research associate: Miguel Diaz

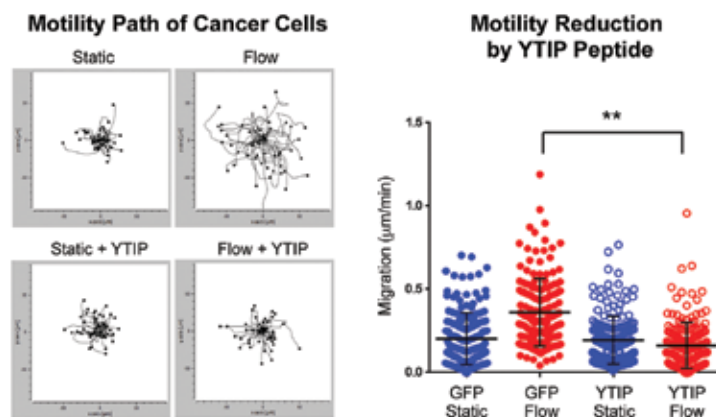
Our lab studies how biomechanical force generated by blood flow in the vasculature and lymph flow in the lymphatics impacts cell potential and behavior.

One arm of our research is designed to address how frictional force promotes stem cell function during embryogenesis, in the adult, and in the context of disease and injury. We are especially interested in how we might use this information in the laboratory to expand improved sources of hematopoietic stem cells and other cellular therapies for treatment of hematologic disorders and neurological trauma. A number of genetic and biochemical pathways are currently under investigation as key players mediating the signaling cascade downstream of fluid force that potentiate stem cell potential and immunomodulatory function of stem cells. We employ various tools and approaches to evaluate stem cell function, including microfluidics, pharmacology, mouse genetics, and transplantation assays.

Fluid flow and hydrostatic pressure also have been implicated in tumor biology, but it remains unclear what role lymphatic or vascular shear stresses may play in regulating metastatic potential of cancer cells. Using biomimetic microchips designed to model the lymphatic vasculature, we modulate the shear stress experienced by cancer cells and evaluate the impact of fluid force on invasive potential and activation of oncogenic pathways that contribute to the systemic spread of cancer from the primary tumor. By application of bioengineering approaches to study the tumor microenvironment, we hope to identify new treatment options for patients affected by cancer.



We engineer biomimetic microchips that permit real-time visualization of cancer cell migration and monitoring of gene activity under conditions that a cancer cell may experience during metastasis through the lymphatic vasculature. A microchannel embedded in the center of the microfluidics device is treated with collagen matrix, followed by introduction of cancer cells. Culture medium is pushed through the microchannel to mimic lymphatic flow.



Flow elevates cancer cell motility, a process important for movement of cells from the primary tumor site to secondary metastatic sites in the body. The increased migration can be blocked by treatment with a small peptide YTIP that interrupts YAP1 function, a proto-oncogene, in the cancer cells. Asterisks represent a significant reduction in cancer cell motility by treatment with YTIP under fluid flow.



Jiaqian Wu, Ph.D.

Associate Professor

Gene transcription and regulation of stem cell differentiation and neural injuries

I am an associate professor in the Vivian L. Smith Department of Neurosurgery and Center for Stem Cell and Regenerative Medicine at McGovern Medical School. During my graduate study at Baylor College of Medicine, I led the NIH Mammalian Gene Collection effort and cloned thousands of mammalian genes, which are publicly available through GE Dharmacon now. I published extensive work in transcriptome complexity, which revealed large amount of non-coding sequence transcription in the mammalian genomes. During my post-doctoral training at Yale University and Stanford University, I was closely involved in the ENCODE project and employed interdisciplinary approaches to study gene expression, transcription factor regulation, and regulatory networks of stem cell self-renewal and differentiation. I was one of the first using RNA-Seq to characterize stem cell neural differentiation process. In my independent laboratory at McGovern Medical School, my lab has carried out unprecedented transcriptome profiling of eight highly purified neuron, glia and vascular cells from brain by RNA-Seq. Our lab identified a large number of novel long non-coding RNAs, and functional and genetic experiments substantiated the role of lncRNA in oligodendrocyte precursor cell (OPC) formation. One of the neurological diseases that we are focusing on is spinal cord injury (SCI). Our lab has already published RNA-Seq studies for acute and chronic SCI phases in mouse and rat contusive injury models. We provided unprecedented data source and a powerful analysis framework for functional investigations of coding and long non-coding RNAs in CNS cell types and SCI. My work has been recognized with prestigious honors and awards, including the National Institutes of Health Ruth L. Kirschstein National Research Service Award for Individual Post-doctoral Fellows, and the International Society for Stem Cell Research (ISSCR) Annual Meeting Travel Award, the National Institute of Health Pathway to Independence (PI) Award (K99/R00), R01 and the Senator Lloyd and B.A. Bentsen Investigator Award. A reviewer for NIH,

New York State Department of Health-Spinal Cord Injury Research Board, MRC, ANR, and many journals, I have presented invited talks and lectures on stem cell biology, neuroscience, and functional genomics at international conferences, the Multiple Sclerosis Research Center of New York, Lawrence Livermore National Laboratory, and the University of Florida, etc. I have developed a patent, authored two books, and wrote many articles that have appeared in *Nature*, *PNAS*, the *Journal of Neuroscience*, *Plos Genetics*, *Genome Research*, and *Scientific Reports* among others.

The laboratory combines stem cell biology and systems-based approaches involving genomics, 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, ChIP-Seq, and ATAC-Seq etc. Our goal is to identify and modulate key regulators as therapeutic targets to direct the differentiation of stem cell into desired neural cell types more efficiently, and to increase transplantation safety.

RESEARCH PROJECTS

- Investigate gene expression and regulatory mechanisms during stem cell differentiation; pinpoint key transcription factors and regulatory RNAs, and modulate key regulators to steer the direction of stem cell differentiation and improve efficiency

- Characterize molecular signatures and identify therapeutic targets for spinal cord injury and neurological diseases
- Network analysis of stem cell differentiation and global network integration of multiple types of omic data

KEY PUBLICATIONS

You, Y., Cuevas-Diaz Duran, R., Jiang L., Dong X., Zong S., Snyder M., Wu J. Q., An integrated global regulatory network of hematopoietic precursor cell self-renewal and differentiation, *Integr. Biol.*, (2018), doi:10.1039/c8ib00059j. PMID: 29892750. Cover Story

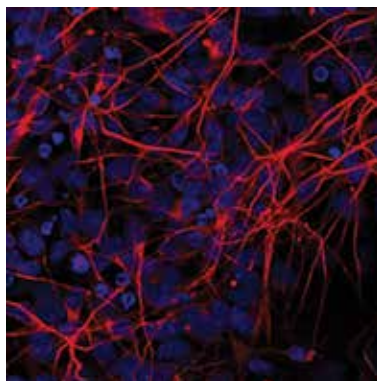
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Cuevas-Diaz Duran, R., Yan, H., Zheng, Y., Huang, X., Grill, R., Kim, D.H., Cao, Q., and Wu, J.Q. The systematic analysis of coding and long non-coding RNAs in the sub-chronic and chronic stages of spinal cord injury, *Scientific Reports*. 2017, Jan. 20; 7:41008. doi: 10.1038/srep41008.

LAB MEMBERS

Post-doctoral fellows: Shiva Nemati, Ph.D.; Haichao Wei., Ph.D.; Xizi Wu, M.D., M.S.; Yanan You, Ph.D.

Undergraduate student: Vy Hong



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



Wa Xian, Ph.D.

Assistant Professor
CPRIT Scholar

Stem cells of regenerative and malignant epithelia

My laboratory develops new technology for capturing the stem cells of highly regenerative tissues, such as the gastrointestinal tract, the lung, and organs such as liver, pancreas, and kidney. These stem cells are quite remarkable as we can grow them for years in a test tube as immature cells and yet on command trigger them to form the organ from which they were derived. Importantly, we are finding that many of the major chronic inflammatory diseases of these organs, such as inflammatory bowel disease, lung diseases such as asthma and COPD, are driven by permanent changes in the stem cell population in these patients. As such, patient-derived stem cells will drive future development of relevant disease models and drug discovery to mitigate these conditions.

A second but related direction of the laboratory is to use our stem cell cloning technology to identify and capture stem cells of highly lethal cancers such as those of the ovaries, pancreas, and esophagus. Remarkably, about 1:2000 tumor cells of a given cancer is a true "cancer stem cell," whereas the rest cannot determine the future of the tumor. Our goal is to understand this minor population of cells in the tumor and how we can target them with drugs that exploit their unique properties.

RESEARCH PROJECTS

• Intestinal stem cell variation underlying inflammatory bowel disease

Inflammatory bowel disease, including Crohn's and ulcerative colitis, remain serious and difficult to manage conditions affecting 1.6 million Americans. The etiology has a genetic component but seems dominated by environmental factors consistent with an interplay between the immune system, the microbiome of the gut, and the intervening intestinal epithelial barrier. We have applied the stem cell technology we developed to identify unusual stem cells in the colons of these patients, which may key for understanding the pathology and chronic features of IBD and identifying new therapeutic targets for resolving this disease.

• Cloning and targeting stem cells of precancerous lesions: Barrett's esophagus

Barrett's esophagus is a precancerous lesion that increases the risk for the development of esophageal adenocarcinoma by 30-100-fold. Given the poor 5-year survival rates for this cancer, we are focused on identifying drugs that eliminate the stem cells of Barrett's esophagus before they have a chance to evolve into dysplasia and malignant cancer.

• Intratumor heterogeneity among stem cells of high-grade ovarian cancer

High-grade ovarian cancer (HGOC) is remarkably sensitive to chemotherapy with many patients achieving a complete response only to suffer relapses 6-24 months later typically

with resistant properties. To understand the cellular and molecular basis of this resistance phenomenon, we are generating large libraries of cancer stem cells from each patient to examine intratumor heterogeneity and how these differences relate to resistant clones.

KEY PUBLICATIONS

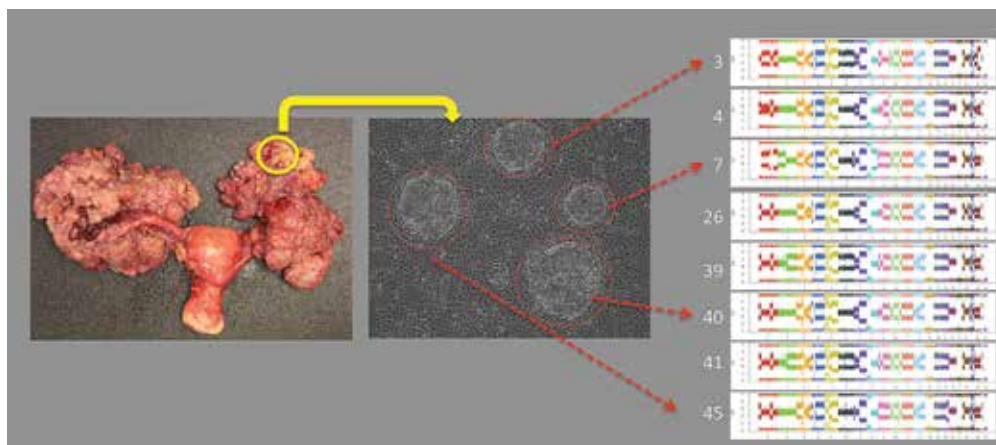
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LAB MEMBERS

Post-doctoral fellow: Weiyi Xu, Ph.D.
Senior research scientist: Brian Wang, M.D.
Research assistant: Jiacui Chen



Derivation of patient-specific cancer stem cell (CSC) library of clones from high-grade ovarian cancer case (left). Four CSC clones sampled for expansion and CNV analysis (right).



The demographics of the American population are shifting toward an increasing elderly population, placing extraordinary demands on our health care system. Aging results in the progressive attrition of homeostasis and functional reserve of all organ systems. As a consequence, the incidence of numerous debilitating diseases, including neurodegeneration, osteoporosis, sarcopenia, sensorineural defects, cardiovascular disease, diabetes, and cancer increases with age. The major focus of the center is to understand the molecular basis of aging and to develop strategies for preventing or delaying age-associated diseases, which represent fundamental and pressing challenges that the medical research community faces today. The precise nature of the damage that is responsible for aging-related degenerative changes remains ill-defined but may include mitochondrial damage, telomere attrition, nuclear dysmorphology, accumulation of genetic mutations, and cumulative DNA, protein, or membrane damage. A universal characteristic of aging is the loss of tissue regenerative potential during the progressive depletion of stem cells, which leads to an impaired ability to respond to stress, and as a consequence, dramatically increases the risk of morbidity and mortality. This, and the exponentially increased incidence of

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

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



Johnny Huard, Ph.D.

Professor & Vice Chair for Orthopedic Research
 Department of Orthopedic Surgery
 Director, IMM Center for Tissue Engineering and Aging Research

Muscle-derived stem/progenitor cell applications

The focus of my research is in the areas of gene therapy, tissue engineering, and regenerative medicine applications based on the use of muscle-derived stem/progenitor cells (MDSPCs). My research team's primary areas of interest include basic stem cell biology and molecular techniques for gene editing and tissue engineering, and application of these techniques for translation to the clinic to aid in repair and regeneration of a variety of tissues. Our research efforts involve investigating applications of MDSPCs for treatment of a variety of diseases, conditions, and injuries that affect the musculoskeletal system, including those resulting from natural and accelerated aging processes. My team has received national and international recognition, and technologies that we have developed have been licensed to industry. Muscle-derived cells that have been isolated by my team are currently being utilized in clinical trials for the treatment of stress urinary incontinence and myocardial infarction. More than 400 women suffering with SU1 in Canada and the United States have volunteered

for this stem cell therapy (Phase III Clinical Trial).

RESEARCH PROJECTS

- Muscle stem cells reprogrammed through genome engineering for autonomously regulated anti-fibrotic therapy and cell autonomous and non-autonomous mechanisms of stem defects with aging
- Development of biological approaches to improve functional recovery after compartment syndrome injury
- Bone abnormalities and healing defects in muscular dystrophy and biomimetic coacervate delivery of muscle stem cells to improve cardiac repair

KEY PUBLICATIONS

Murray IR, Gonzalez González Galofre ZN, Baily J, Dobie R, Wallace RJ, Mackinnon AC, Smith JR, Greenhalgh SN, Thompson AI, Conroy KP, Griggs DW, Ruminski PG, Gray GA, Singh M, Campbell MA, Kendall TJ, Dai XJ, Li Y, Iredale JP, Simpson H, Huard J, Peault B, Henderson NC. α v integrins on perivascular mesenchymal 1 cells critically regulate skeletal and cardiac muscle fibrosis. *Nature Communications*. *Nat Commun*. 2017 Oct 24;8(1):1118. PMID: 29061963.

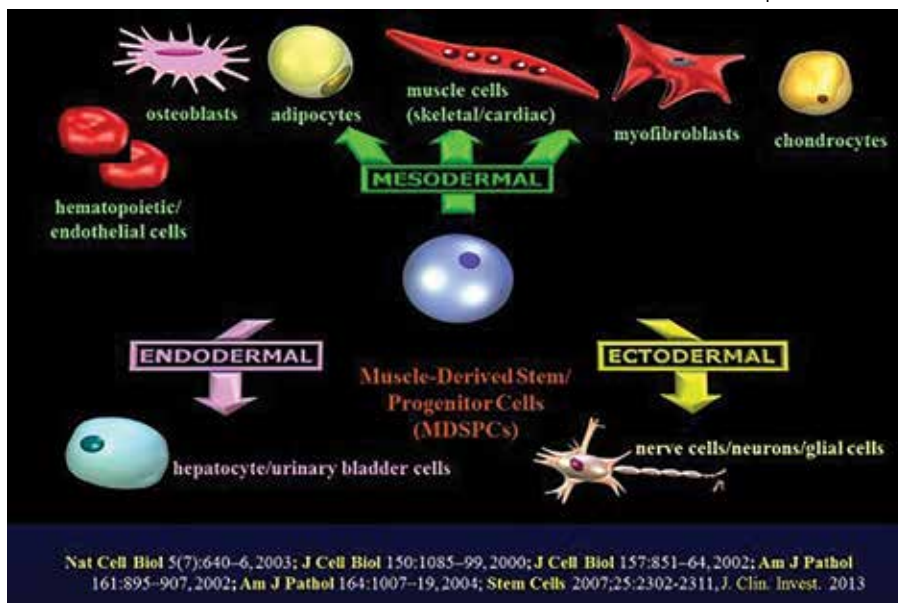
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B, Weiss K, Huard J. RhoA/ROCK inhibition improves the beneficial effects of glucocorticoid treatment in dystrophic muscle: implications for stem cell depletion. *Hum Mol Genet*. 2017 Aug 1;26(15):2813-2824. PMID: 28549178.

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LAB MEMBERS

Assistant professors: Ping Guo, Ph.D.; Aiping Lu, M.D.; Xiaodong Mu, Ph.D.; Xueqin, M.D., Ph.D.; Krishna Sinha, Ph.D.
 Post-doctoral research fellows: Shanshan (Ellen), Ph.D.; Chieh (Judy) Tseng, Ph.D.; Xuying Sun, Ph.D.; William Sealy Hambricht, Ph.D.
 Senior research scientists: Polina Matre, M.B.A., Ph.D.; Yan Cui, M.D.
 Visiting scientist: Fan Yang, Ph.D.
 Visiting students: Zhenhan (Stephen) Deng, Chih-Yi (Amy) Lin
 Research team: Haiying Pan; Haizi Cheng, M.D.; Ling (Jeannie) Zhong; Martha Pena; Sarah Amra; Michelle Ramirez; Mary Hall, M.B.A., Ph.D.; Barbara Lipari



Muscle-Derived Stem/Progenitor Cells (MDSPCs) from Murine and Human Skeletal Muscle



Xueqin Gao, M.D., Ph.D.
Assistant Professor

Muscle-derived stem cells for bone and cartilage regeneration and repair

I am a member of Dr. Johnny Huard's research team. My research focuses on using muscle-derived stem cells (MDSCs) and gene therapy for bone and cartilage repair. I conduct translational studies to use MDSCs for the treatment of bone defects, non-union fractures, and age-related bone and cartilage conditions, such as osteoporosis and osteoarthritis. I am also investigating bone biology in a disease model of muscular dystrophy.

Human muscle-derived stem cells for bone regeneration

Large segmental bone defects and non-union fractures caused by traumatic injury or cancer resection represent major issues in clinical orthopaedics. We are investigating new vectors and growth factors to mediate *ex vivo* gene therapy and using biomaterials to deliver growth factors to enhance human muscle-derived stem cell (hMDSC)-mediated bone regeneration. We also are investigating the effects of the age of both donor hMDSCs and hosts on hMDSC-mediated bone repair.

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

The application of stem cells, including murine muscle-derived stem cells (mMDSCs) in murine models, for treating osteochondral defects or for osteoarthritis repair are clinically translational. In this project, we are using viral vectors and biomaterials to deliver growth factors for hMDSC-mediated cartilage repair, particularly for the treatment of monoiodoacetate (MIA)-induced osteoarthritis in a murine model.

Investigation of interactions between muscle and bone in a muscular dystrophy model

Duchenne muscular dystrophy (DMD) is a deadly muscle disease that inflicts about 1 in 3,000 boys. Patients often become wheelchair-bound in their second decade of life. We have found bone abnormalities in a dystrophin/utrophin double knock out (dko) model that closely mimics the clinical manifestations of human DMD. For this project, we are investigating how muscular dystrophy affects the bone quality

and defect healing and how muscle and bone interact in this mouse model in order to unveil mechanisms, which may be translated into new strategies to improve the life quality of DMD patients.

RESEARCH PROJECTS

- Utilizing human muscle-derived stem cells and gene therapy for bone tissue repair
- Using human muscle-derived stem cells for cartilage and osteoarthritis repair using *ex vivo* gene therapy and biomaterial scaffolding.
- Bone abnormalities in muscular dystrophy (NIH R01 awarded to Dr. Huard)

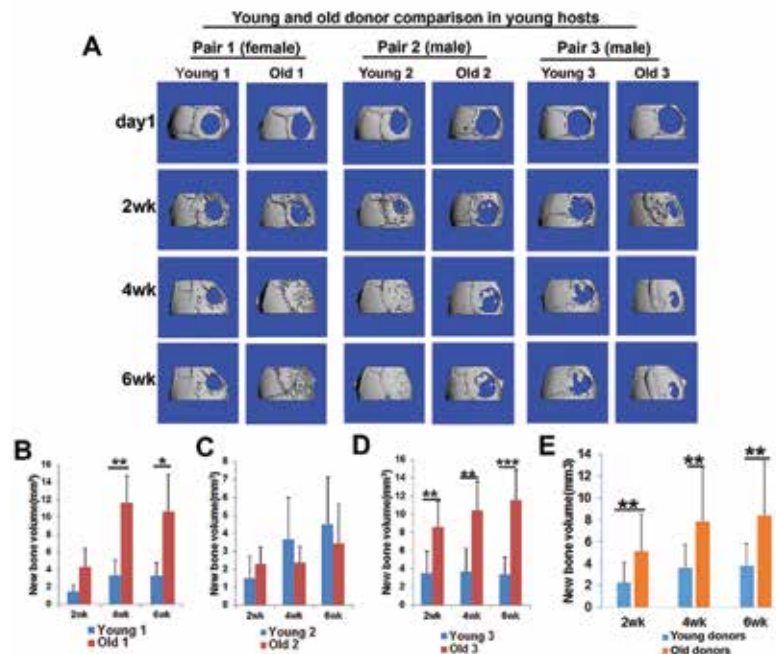
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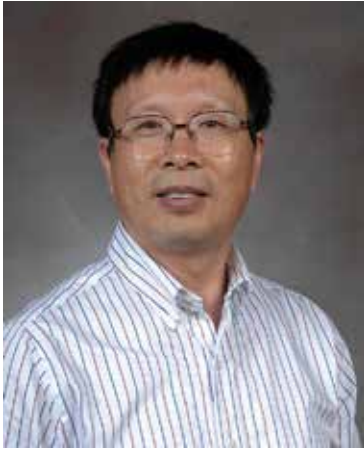
muscle-derived stem cell-mediated bone regeneration. *Stem Cell Res Ther.* 2018 Nov 21;9(1):316. doi: 10.1186/s13287-018-1066-z. PMID:30463597

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Old human MDSCs are as efficient as young hMDSCs for bone regeneration in critical size parietal bone defect when transduced with Lenti-BMP2/GFP (X, Huard J *et al.*, *Stem Cell Research & Therapy*, 2018).



Ping Guo, Ph.D.
Assistant Professor

Stem cell/gene therapy and tissue engineering for treating sports-related diseases

We have three major areas of research.

RESEARCH PROJECTS

Human Muscle-derived Stem Cells Prevent Cardiac Muscle Dysfunction in Aged Dystrophic Mice through a Paracrine Effect---*In Vivo* Tracking of Donor Cells

Duchenne muscular dystrophy (DMD), an X-linked progressive muscle wasting disease, is caused by a deficiency in dystrophin and affects approximately 1 in 3,600 boys in the world. The lack of sarcolemma dystrophin in DMD patients not only affects skeletal muscle but also myocardium, which causes cardiomyopathy that eventually leads to heart failure and premature death. Previously our group has demonstrated the robust regenerative capacity of human muscle-derived stem cells (hMDSCs) in the ischemic hearts. Recently, we demonstrated that hMDSCs were capable of preventing cardiac dysfunction in the aging dystrophic mice. However, how the hMDSCs participated in the cardiac remodeling remains unclear. To elucidate whether the hMDSCs directly contributed to cardiac remodeling by differentiating into cardiac muscle cells (cardiomyocytes) or via a paracrine mechanism, we utilized a viral mediated luminescent tracking system to transduce hMDSCs with luciferase and injected the cells into aged dystrophic mice. Our results showed that luciferase activity significantly decreased 2 weeks after hMDSC transplantation; moreover, there were no luciferase or GFP positive cells detected in the heart. These observations suggest that the hMDSCs prevent cardiac dysfunction in aged dystrophic heart via the secretion of stimulatory factors, which may improve cardiac muscle regeneration. Identification of these secreted factors will be critical for developing future therapeutic options for treating cardiomyopathy in DMD patients.

Microsphere-Depleted VEGF Improves the Beneficial Effect of Platelet Rich Plasma for Cartilage Healing after Osteoarthritis

Adult stem cell therapy and platelet-rich plasma (PRP) are emerging therapies in regen-

erative medicine for repair of musculoskeletal defects due to injury or aging. Stem cells after transplantation readily participate in tissue regeneration, while PRP provides growth factors and cytokines that accelerate and enhance endogenous cells, inflammatory cells, etc. to participate in the repair process. PRP contains several growth factors, including platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor- β 1 (IGF- β 1), and transforming growth factor- β 1 (TGF- β 1). Some of these growth factors can enhance healing in certain tissue lineages but may be harmful in others. For example, VEGF promotes angiogenesis, which is beneficial for skeletal muscle healing, but promotes fibrocartilage formation. Studying the tissue-specific effects of PRP while blocking certain growth factors can improve our understanding of its biological nature and potential therapeutic utility in musculoskeletal injuries.

Stem cells and regeneration in digestive tract organs.

The pancreas is a vital part of the digestive system and a critical controller of blood sugar levels. The homeodomain transcription factor Pdx1 is crucial for pancreas formation. Pdx1-expressing cells are first observed at embryonic day 8.5 (E8.5), prior even to the earliest indication of morphogenesis, in endodermal cells designated to give rise to the pancreas. In adult mouse, Pdx1 protein is transiently expressed in pancreatic ducts when pancreas is injured, such as Langerhans islets damage in alloxan induced

diabetic mice, implying Pdx1 may be necessary for the neogenetic formation of β -cells from mature ducts. In order to study the role of pdx1 in the pancreatic duct for pancreatic regeneration, most investigators use the Cre-lox system to generate duct-specific Pdx1 deletions in mice. This method is able to delete Pdx1 specifically from tissue or cells with or without a tamoxifen-inducible (cre-ERT2) system; however, several caveats and limitations exist with tamoxifen-induced depletion. In addition, the cre-ERT2 system may be leaky, resulting in constitutive rather than inducible activation in some cells. To overcome these flaws from the Cre-ERT2/lox system, we decided to use a novel CRISPR-Cas9 gene editing technique to delete pdx1 in pancreatic ducts.

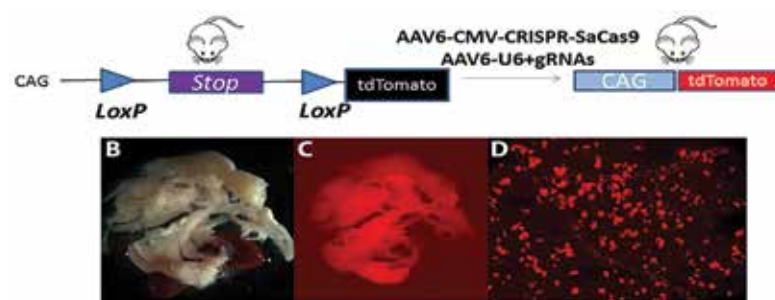
KEY PUBLICATIONS

Xiangwei Xiao, Ping Guo et. al. and George Gittes, *Cell Stem Cell*. 2018 Jan 4;22(1):78-90. e4.

Johnny Huard, Aiping Lu, Xiaodong Mu, Ping Guo, Yong Li, *Cells Tissues Organs*, 2016, 202:227-236.

LAB MEMBERS

Post-doctoral fellows: Shanshan
Graduate students: MacGregor Hall, Isaac Castillo
Research assistants: Elizabeth Morris, Sabrina Gonzalez.



Verification of SaCas9 and gRNA *in vivo* deletion of loxP sites in ROSA26-LSL tomato mice pancreases.



Aiping Lu, M.D.
Assistant Professor

Improving stem cell-based therapeutic outcomes by enhancing the muscle stem cell microenvironment for treatment of muscular disease and accelerate aging

Our group focuses on identification of circulating factors and progenitors for treatment of muscle injury, muscular disease and accelerate aging.

Pregnancy improves wound healing in the murine model. Pregnancy represents a unique biological model of a shared circulatory system between the mother and fetus. We have demonstrated that pregnant mice exhibit accelerated wound healing following laceration injury when compared to non-pregnant control mice. We also observed less fibrosis in muscle of pregnant mice than non-pregnant mice at 2 weeks post injury. Our results suggested that circulating factors present during pregnancy may play an important role in wound healing by enhancing the function of progenitor cells in the skin and muscle. Identifying rejuvenating factors within blood circulation during pregnancy could have potential for the development of novel therapies for wound healing.

Enhance stem cell therapeutic outcome by improving microenvironment for treatment of muscular dystrophy. Duchenne muscular dystrophy (DMD) is a lethal genetic disease and there is still no effective cure for DMD. Transplantation of muscle progenitor cells from healthy donors for treatment in DMD has been widely investigated but with unsatisfied outcome due to the harmful macroenvironment in the muscle of DMD patients. We hypothesized blood exchange or parabiosis between young WT and dystrophic mice will improve the environment of stem cells. This will not only enhance the outcome of stem cell transplantation from donor but also have a beneficial effect for the function of muscle stem cells in the host. The results obtained from this study will develop novel and clinical relevant therapies to alleviate stem cell dysfunction and improve the histopathologies in muscle dystrophy.

RESEARCH PROJECTS

- To analyze the cell non-autonomous effects induced by aging and stress on MDSPCs using heterochronic parabiosis and tissue-specific

inactivation of ERCC1. (NIH, P01)

- To investigate the cell-cell interaction between myogenic cells and non-myogenic cells.
- The role of pregnancy in muscle injury and wound healing (NIH R21)
- Parabiotic pairing between dystrophic mice and WT mice for improving muscle and heart histopathology.

KEY PUBLICATIONS

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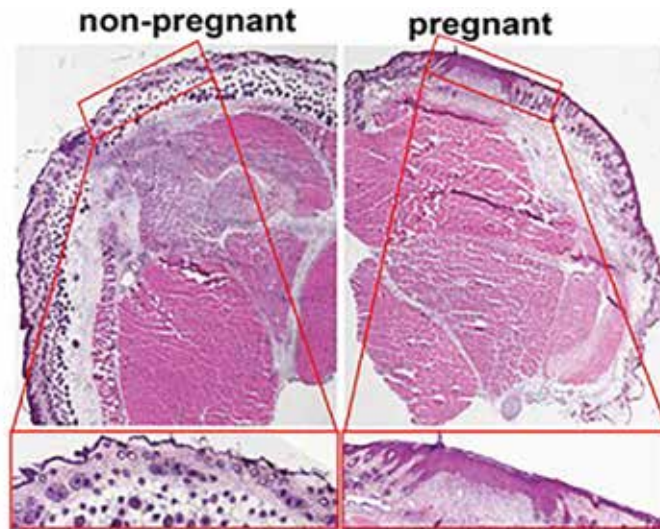
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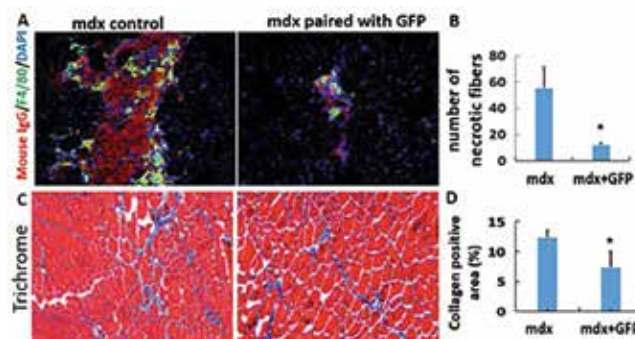
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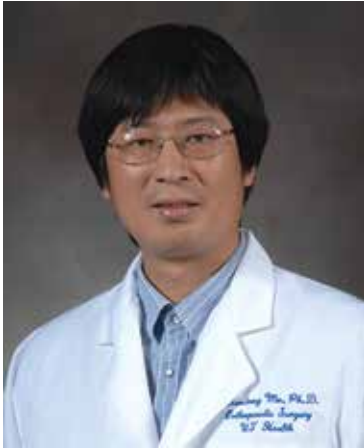
Huard J, Mu X, Lu A. *Clin Pharmacol Ther*. 2016 Aug; 100(2):142-6.



Improvement of wound healing after injury in pregnant mice.



Heterogenetic parabiotic pairing improved dystrophic skeletal muscle histopathology



Xiaodong Mu, Ph.D.
Assistant Professor

Calcification paradox in bone and soft tissues is mediated by aberrant RhoA activation

Our “Stem Cells in Aging and Cancer” research team is part of Dr. Johnny Huard’s research group in the Center for Tissue Engineering and Aging Research and the Department of Orthopaedic Surgery. Our studies involve stem cell biology, wound regeneration, fibrosis prevention, muscular dystrophy, premature aging, and cancer biology (osteosarcoma). Currently, we are especially interested in studying the mechanisms of stem cell senescence and premature aging, and the mechanisms of various musculoskeletal disorders and conditions (i.e., Duchenne Muscular Dystrophy, cancer cachexia-related muscle atrophy, heterotopic ossification, and osteosarcoma).

Currently, I am focusing my research in the following areas:

1) Understanding the cellular and molecular mechanisms of muscle stem cell senescence in diseased and aged muscles, and the potential effect of senolytics in delaying aging.

Accelerated exhaustion, senescence, and loss of regenerative potential of stem cells have been observed in diseased skeletal muscle, such as resulting from progeria and muscular dystrophic disease. Senolytic drugs are starting to be accepted as novel strategy to remove senescent cells and delay aging, and I am interested in studying how it may rescue muscle aging and diseases.

2) Investigating the mechanisms of muscle atrophy in osteosarcoma-induced cancer cachexia and potential effects of muscle stem cells on reducing muscle atrophy.

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

3) Improvement of wound healing by reducing

fibrosis or application of stem cells and nanofibers.

We previously reported the effects of the hormone relaxin and MMPs in the prevention of fibrosis during the healing process of injured skeletal muscle or amputated digits. Since healing of diseased skeletal muscle (i.e., dystrophic muscle) is usually accompanied by excessive fibrosis, we are investigating how to enhance regeneration of diseased soft tissues with the application of multipotent stem cells and novel nanofibers carrying muscle rejuvenating factors.

RESEARCH PROJECTS

- Understanding the cellular and molecular mechanisms of muscle stem cell senescence in diseased and aged muscles, and the potential effect of senolytics in delaying aging.
- Investigating the mechanisms of muscle atrophy in osteosarcoma-induced cancer cachexia and potential effects of muscle stem cells on reducing muscle atrophy.
- Improvement of wound healing by reducing fibrosis or application of stem cells and

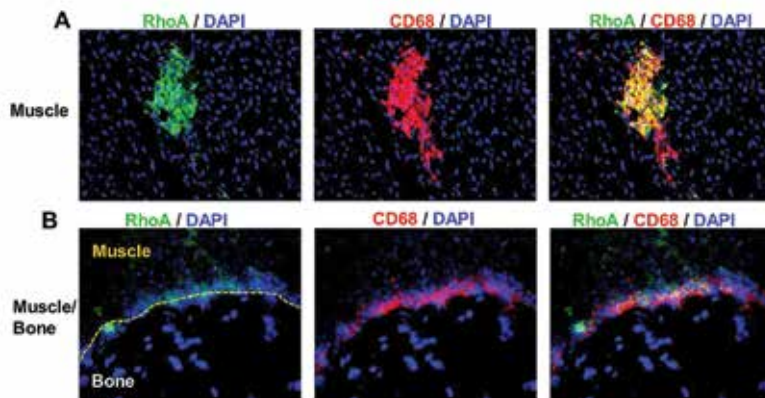
nanofibers.

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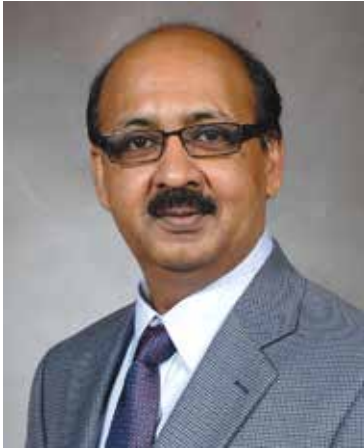
Mu X, Tang Y, Takayama K, Chen W, Lu A, Wang B, Weiss K, Huard J. RhoA/ROCK inhibition improves the beneficial effects of glucocorticoid treatment in dystrophic muscle: implications for stem cell depletion. *Hum Mol Genet.* 2017 Aug 1;26(15):2813-2824. PMID: 28549178.

Mu X, Agarwal R, March D, Rothenberg A, Voigt C, Tebbets J, Huard J, Weiss K. Notch Signaling Mediates Skeletal Muscle Atrophy in Cancer Cachexia Caused by Osteosarcoma. *Sarcoma.* 2016.



Extensive accumulation of CD68⁺/RhoA⁺ cells in degenerative/calcification tissue in the dystrophic muscle of mice model can be involved in calcification paradox.

A. The CD68⁺ macrophages accumulating at the degenerative/calcification sites in the dystrophic muscle are activated for RhoA expression. B. The CD68⁺ macrophages accumulating at the muscle and bone interface are also activated for RhoA expression.



Krishna Sinha, Ph.D.
Assistant Professor

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

My long-term research interests focus on epigenetic control of progenitor/stem in repair of musculoskeletal tissues after injuries as well as prostate cancer-induced skeletal metastases. Our research plan is to identify and study gene function in age-related bone disorders in order to provide pivotal information that will inform the development of preventive measures and treatments for bone disorders. Since joining Dr. Huard's research group, I have broadened my research interests to include gene therapy approaches that use multipotent skeletal muscle-derived stem cells in the regeneration of musculoskeletal tissues during aging, diseases, and bone malignancies.

Epigenetic control in multipotent potentials of skeletal muscle-derived stem cells.

Skeletal muscle derived progenitor / stem cells (MPCs) are highly regenerative and multipotent and have ability to differentiate into various cell types, including skeletal muscle, bone, and cartilage. Epigenetic mechanisms play important role in self-renewal capacity and function of adult stem cells. My current research focuses on the epigenetic regulation by histone lysine methylation in control of those genes that are involved in the multipotent abilities of MPCs and their regenerative capacity (Figure). Small molecule compounds and CRISPR/Cas9 gene editing tools are currently used to regulate the function of epigenetic and transcriptional

regulator to improve the tissue regenerative capacity.

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

RESEARCH PROJECTS

- To study epigenetic mechanisms of self-renewal and depletion of skeletal muscle-derived stem cells, and in the repair of musculoskeletal tissues using next-gen sequencing approaches.
- To test the therapeutic potential of MPC's secretory factors in inhibiting prostate cancer cell proliferation.
- To investigate the oncogenic function of NO66 in PCa progression and bone metastasis, and develop stem cell therapy to inhibit the development of PCa in bone.
- Understanding the molecular mechanisms of

HIF1a and the role of MPCs in high healing capacity of *MRL/MpJ* (super-healer) mouse strain.

KEY PUBLICATIONS

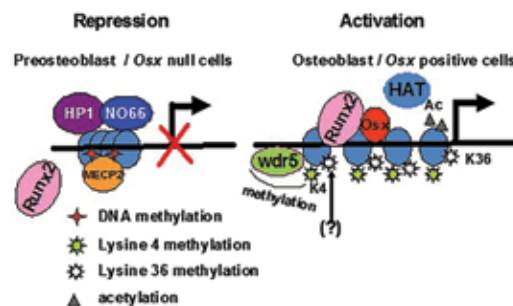
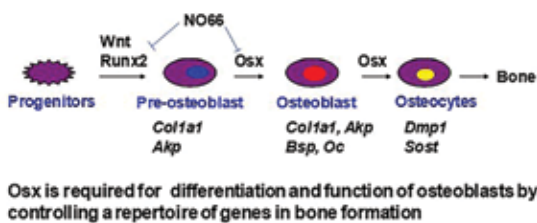
Sinha KM, Yasuda H, Coombes MM, Dent SR and de Crombrughe B. Regulation of the osteoblast-specific transcription factor Osterix by NO66, a Jumonji family histone demethylase. *EMBO J*, e-Pub 11/2009.

Brien et al. (2012). The Polycomb PHF19 binds H3K36me3 and recruits PRC2 and the NO66 demethylase to hitherto active genes during embryonic stem cell differentiation. *Nature Structure and Molecular Biology*, 2012 Dec;19(12):1273-81.

Yue Tao, Minhao Wu, Xing Zhou, Dandan Zhang, Benoit deCrombrughe, Krishna M Sinha, Jianye Zang (2013). Structural Insights into the Interactions of Histone Demethylase NO66 with Osterix to Repress Gene Transcription. *Journal of Biological Chemistry* 2013, 288, 16430-16437.

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



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



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

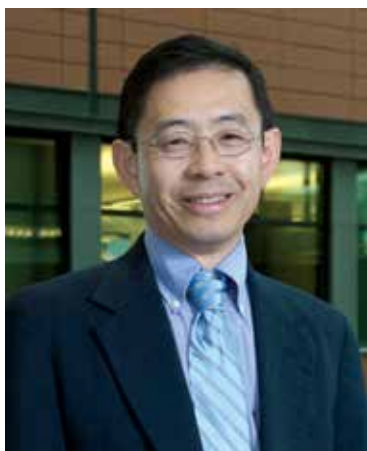
TTI-IMM investigators have brought in significant funding from the pharmaceutical and the biotechnology industry, such as Merck, and the National Institutes of Health, the Cancer Prevention and Research Institute of Texas, and the Department of Defense, and have made significant scientific discoveries in the areas of cancer biology, fungal natural products, and cancer antibody drug development.

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

2) discovery of biologics, natural products, and synthetic small molecules that modulate the activity of these targets as potential lead molecules for drug discovery; and 3) characterization of antibodies from animals and humans in response to viral infections and experimental vaccines. On the translational side, research from TTI has granted licenses for multiple biologic therapeutics.

In addition to the basic and translational research programs, TTI is building two major drug discovery platforms: 1) the Therapeutic Monoclonal Antibody Lead Optimization and Development Platform and 2) the Natural Products and Small Molecular Drug Discovery Platform. The drug discovery platforms not only support TTI internal projects, but they also support collaborative projects with scientists from the IMM, the Texas Medical Center, Texas based Institutions, and national and international institutions.

Zhiqiang An, Ph.D.
Professor & Center Director
Robert A. Welch Distinguished University Chair in Chemistry



Zhiqiang An, Ph.D.

Professor and Co-Director of the Texas Therapeutics Institute
Robert A. Welch Distinguished University Chair in Chemistry

Discovery and development of therapeutic antibodies and antibiotics

Our group focuses on the discovery and development of therapeutic antibodies and antibiotics against human diseases, including cancer and infectious diseases. Currently, we have four major research areas.

RESEARCH PROJECTS

- Cancer antibody drug resistance mechanisms. Immune suppression is recognized as a hallmark of cancer and this notion is largely based on studies on cellular immunity. Our recent studies have demonstrated a new mechanism of cancer suppression of immunity by impairment of antibody effector function mediated by proteolytic enzymes in the tumor microenvironment.
- HER3 mediated cell signaling and HER3 targeting antibodies for cancer therapy. Ablated regulation in the HER/ErbB family receptor signaling has been implicated in various cancer types. Agents targeting EGFR and HER2 exhibited clinical benefits for the treatment of some cancer types, but drug resistance is widespread. Current understanding of the drug resistance mechanisms is limited and HER3 has been implicated in the resistance to current EGFR and HER2 therapies. Our group is working on: 1) HER3mediated cell signaling; 2) the role HER3 plays in resistance to current anti-HER2 and EGFR antibody therapies; and 3) generation of HER3 targeting antibodies and their mode of actions.
- Antibodies response to viral infections and vaccination. Design of highly immunogenic vaccines that induce neutralizing antibodies against a broad range of clinical isolates is one of the approaches in developing effective viral vaccine. We have an ongoing project to aid the design of HCMV and dengue vaccines by profiling antibody response to the experimental vaccines in rhesus and humans.
- Cancer therapeutic monoclonal antibody drug discovery. Our group has built a comprehensive antibody drug discovery platform with a focus on antibody lead optimization technologies, such as antibody phage display, deep

sequencing of antibody encoding genes from individual antibody expressing B cells, affinity maturation, and humanization. Currently, we have multiple collaborative antibody drug discovery projects targeting various cancer types.

KEY PUBLICATIONS

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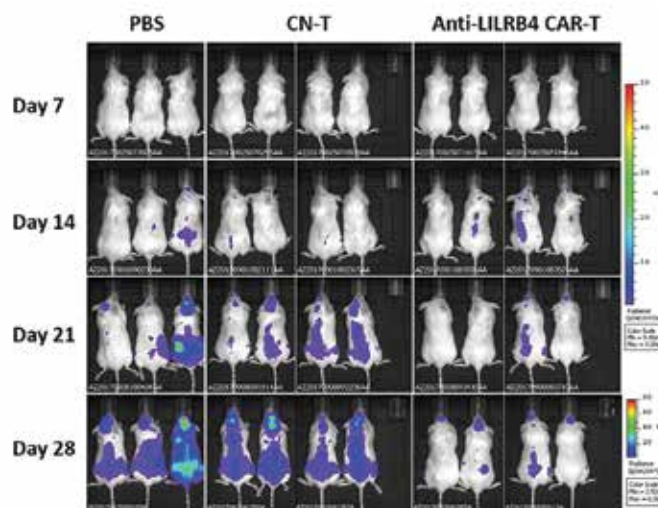
Anami et al. 2018. Glutamic acid-valine-citrulline cleavable linkers ensure high stability and efficacy of antibody-drug conjugates in mouse

models. *Nature Communications* 9:2512 | DOI: 10.1038/s41467-018-04982-3.

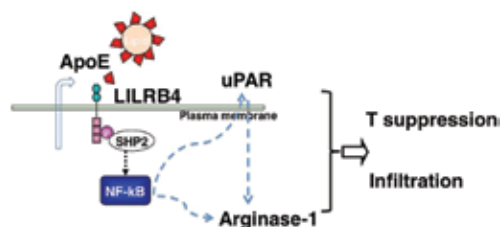
Noh et al., 2017. Differential effects of EGFL6 on tumor versus wound angiogenesis. *Cell Reports*. 21(10): 2785-2795

LAB MEMBERS

Post-doctoral fellows: Xun (Mark) Gui, Zhiqiang Ku, Leike (Simon) Li, Qihui Wang, Xiaohua Ye, Wei (Waker) Xiong
Graduate students: Hang Su
Research coordinator: Georgina T. Salazar
Research scientist: Ahmad S. Salameh
Instructor: Yi Du



Anti-LILRB4 CAR-T cells significantly reduce leukemia burden in MV4-11 AML xenograft mouse model. NSG mice were irradiated on day -1, injected with MV4-11 AML cells on day 0, and treated with PBS, control (untransduced)-T cells or anti-LILRB4 CAR-T cells on day 5. Weekly BLI of mice treated with PBS, control-T cells, and anti-LILRB4 CAR-T cells (John et al., 2018. A novel anti-LILRB4 CAR-T cell for the treatment of monocytic AML. *Mol Ther.* 2018 Aug 7. pii: S1525-0016(18)30372-1. doi: 10.1016/j.ymthe.2018.08.001).



Schematic for the mechanisms by which LILRB4 suppresses T cells and promotes leukaemia infiltration. LILRB4-SHP2-NFκB-uPAR-ARG1 is a signalling pathway in monocytic AML cells that suppresses immune activity and supports leukaemia migration (Deng et al. 2018. Inhibitory receptor signaling in leukemia cells mediates tumor infiltration and T cell suppression. *Nature* 562:605-609).



Gerald F. Bills, Ph.D.

Professor

Kay and Ben Fortson Distinguished Chair in Neurodegenerative Disease Research

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

Microorganisms have produced many of our most important drugs. Their hyper-biodiversity and genetic capacity for synthesis of organic molecules continue to yield breakthrough molecules for invention in human disease. Multidisciplinary microbial biomedical research in the Texas Therapeutics Institute and the Institute of Molecular Medicine brings together members of our lab and collaborators from diverse backgrounds, including pharmaceutical sciences, organic chemistry, biochemistry, molecular biology, and microbiology. Our research involves testing microbial natural products for therapeutic applications, making natural products through fermentation to support medicinal chemistry synthesis, and elucidating biosynthetic pathways of bioactive natural products. We seek to test various hypotheses that natural product-producing microorganisms harbor biosynthetic gene clusters and novel biosynthetic mechanisms that can be harnessed to generate new bioactive chemistry useful in intervention in infectious diseases and cancers. In parallel, we use pathway genetics and genomic manipulation in the producing organisms to aid in supplying large quantities of these natural products to support synthesis of new derivatives.

Our lab employs genomics to interpret and predict genetically encoded chemical diversity of microorganisms using filamentous fungi as model organisms, especially biosynthetic families relevant for pharmaceutical intervention in human diseases. For example, we have characterized biosynthetic pathways responsible for the family of echinocandin antifungal drugs, including pneumocandin B0, the starting molecule for the antifungal drug CANGIDAS. We have re-programmed pneumocandin biosynthesis to produce new strains with improved product purity and new analogues with increased potency. In parallel, we use pathway genetics and genomic manipulation in the producing organisms to aid in supplying large quantities of these natural products to support synthesis of new derivatives and overproduce drug-precursor

molecules.

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

RESEARCH PROJECTS

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

- Discovery of new antifungals and other therapeutic agents.

KEY PUBLICATIONS

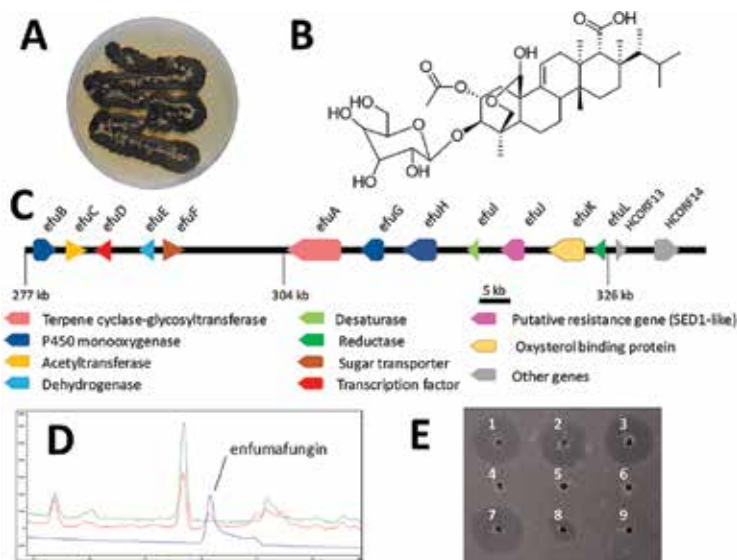
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Li, Y., Q. Yue, J. Dinith, D. Swenson, G. Bartholomeusz, Z. An, J. Gloer & G. Bills. 2017. Anti-*Cryptococcus* phenalenones and cyclic tetrapeptides from *Auxarthron pseudauxarthron*. *Journal of Natural Products* 80:2101-2109.

LAB MEMBERS

Post-doctoral fellows: Dr. Nan Lan, Dr. Bruno Perlati



Identification of the gene cluster encoding antifungal drug precursor enfumafungin. A. enfumafungin-producing fungus, *Hormonema carpetanum*, growing in culture. B. Chemical structure of enfumafungin. C. Genes cluster encoding the biosynthesis of enfumafungin. D. Comparison of HPLC-UV chromatograms derived from wild-type (red line) and $\Delta efuA$ mutant (green line) extracts. The enfumafungin standard is blue. E. Agar-well-diffusion assays of wild-type and mutant extracts against *Candida albicans*. Wells 1-3: wild-type; 4-6: $\Delta efuA$ mutant; 7: enfumafungin; 8: amphotericin B; 9: methanol.



Kendra S. Carmon, M.S., Ph.D.

Assistant Professor

Therapeutic targeting and mechanisms of LGR5 in colon tumors and cancer stem cells

Emerging evidence has shown that within several different malignant tumors types there exists a subpopulation of cancer cells that behave like normal stem cells. These cells are referred to as cancer stem cells (CSCs), or tumor-initiating cells, since they have the capacity to fuel tumor growth. CSCs have been implicated in drug resistance, metastasis, and relapse, making them a major impediment for the effective treatment of cancer. Therefore, it is essential to develop novel therapies that can ultimately target and destroy CSCs.

Recent studies have unequivocally established that the adult stem cell marker LGR5 (Leucine-rich repeat-containing, G protein-coupled Receptor 5) is highly expressed in primary colon tumors and that only LGR5-positive colon CSCs are capable of driving tumor growth and metastasis. In addition, LGR5 expression has been shown to be significantly elevated in several other major tumor types, including liver, gastric, and ovarian carcinomas. However, the actual function and mechanism of LGR5 in CSCs is still relatively unknown. My previous work with Dr. Qingyun's (Jim) Liu's laboratory led to the discovery that LGR5 functions as a receptor of the secreted growth factors R-spondins to promote cancer cell adhesion and regulate Wnt signaling, an important pathway in stem cell survival and tumor growth. Altogether, these findings suggest that LGR5 plays an important role in cancer and could serve as a promising new target for the development of CSC-based therapies.

My research is focused on elucidating the function and signaling mechanisms of LGR5 in colon CSCs using colon cancer cell lines and patient-derived tumors models. This work will lead to identifying the role of LGR5 in the control of tumor growth, metastasis, and drug resistance. Furthermore, we are developing innovative therapeutics called antibody-drug conjugates (ADCs) that target and destroy colon tumors and CSCs, similar to guided missiles. ADCs are comprised of a highly specific monoclonal antibody attached to a cytotoxic

chemical "warhead" that is only released once. The ADC binds and enters target tumor cells. We have successfully generated LGR5-targeted ADCs that incorporate the cytotoxin monomethyl-auristatin E (MMAE) and showed they could destroy colon cancer cells and tumors in mice. Currently, we are taking novel approaches to modify and improve our LGR5-targeting ADCs in order to effectively treat a larger number of tumors. Our lab also is identifying and characterizing new cancer targets for future ADC development. In collaboration with Dr. Ali Azhdarinia's group, we are using PET/CT imaging to select optimal therapeutic antibodies and evaluate them as diagnostics to stratify tumors, which would respond best to LGR5-targeted ADC therapy. Our work will lead to the elucidation of the function and mechanism of LGR5 in CSCs and generate innovative therapeutic leads to target CSCs for the treatment and eradication of colon cancer.

RESEARCH PROJECTS

- Development of antibody-drug conjugates to target colon tumors and cancer stem cells
- Investigation of the LGR5 signaling mechanism and its role in cancer stem cells, metastasis, and drug resistance

- Identification of novel therapeutic targets and associated signaling pathways in colon cancer

KEY PUBLICATIONS

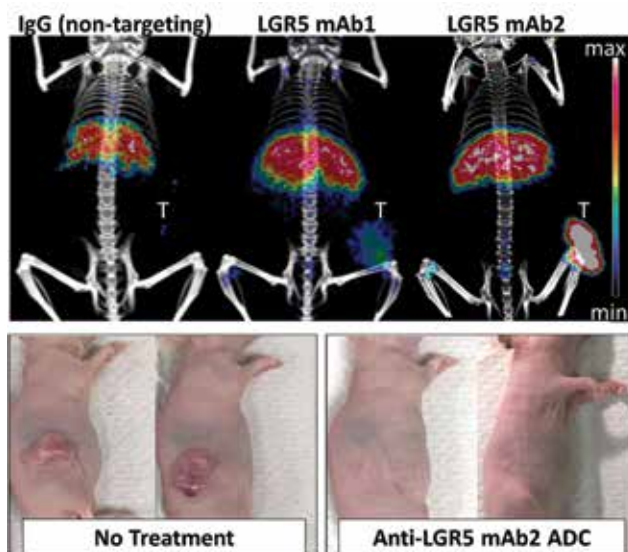
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Carmon K.S., Gong X., Yi J., Wu L., Thomas A., Moore C.M., Masuho I., Timson D.J., Martemyanov K.A., Liu Q.J. LGR5 receptor promotes cell-cell adhesion in stem cells and colon cancer cells via the IQGAP1-Rac1 pathway. *J Biol Chem.* 292(36):14989-15001, 2017.

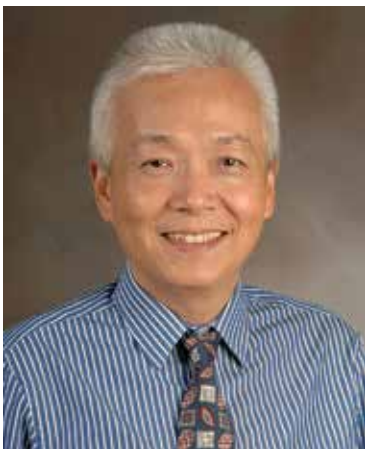
Gong X., Azhdarinia A., Ghosh S.C., Xiong W., An Z., Liu Q., and Carmon K.S.* LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence, *Mol. Cancer Ther.* 15(7):1580-90, 2016.

LAB MEMBERS

Research associate: Sheng Zhang



PET/CT imaging was used to evaluate tumor uptake of non-targeting antibody (IgG) and 2 different LGR5-targeting antibodies (mAb1 and mAb2) in LGR5-expressing colon tumors (upper panel). T, indicates tumor site. mAb2 exhibited significant tumor uptake and was selected for ADC development. Treatment with anti-LGR5 mAb2 ADC eradicates tumors in a xenograft model of colon cancer (bottom panel).



Xiaodong Cheng, Ph.D.

Professor

Walter and Mary Mischer Distinguished Professor in Molecular Medicine

cAMP - mediated cell signaling and drug discovery

KEY PUBLICATIONS

Robichaux, W. G. and Cheng, X. Intracellular cAMP Sensor EPAC: Physiology, Pathophysiology and Therapeutics Development. *Physiological Reviews*. 98:919-1053, 2018.

Yang, W., Mei, F.C. and Cheng, X. EPAC1 regulates endothelial Annexin A2 cell surface translocation and plasminogen activation. *FASEB J*. 32:2212-2222, 2018.

Singhmar, P., Huo, X., Li, Y., Dougherty, P. M., Mei, F. C., Cheng, X., Heijnen, C. J. and Kavelaars, A. An orally active Epac inhibitor reverses

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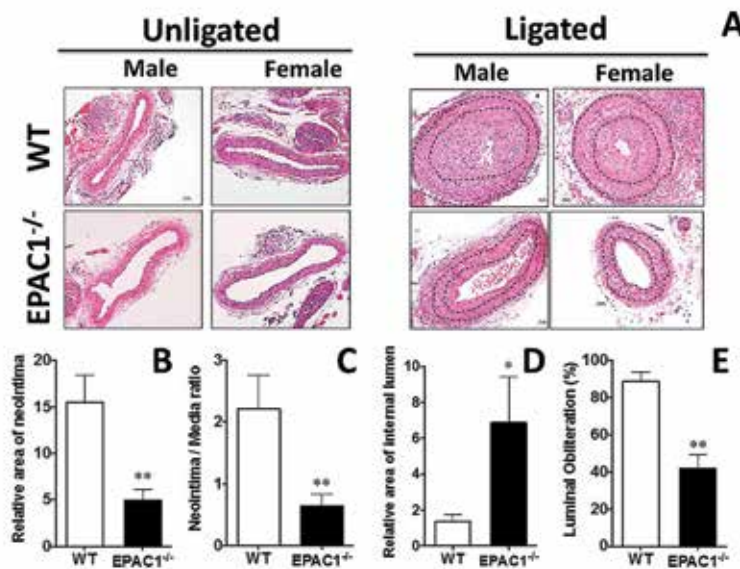
LAB MEMBERS

Research assistant professor: Fang Mei
 Research scientist: Wenli Yang
 Instructor: William Robichaux
 Research associate: Wei Lin

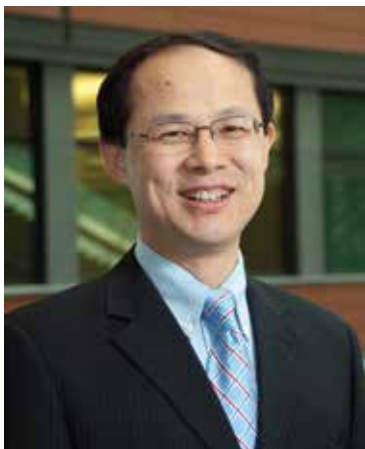
Our laboratory studies intracellular signaling associated with second messenger cAMP, a major stress signal implicated in the development of human diseases. We apply multidisciplinary approaches, coupling biochemistry, biophysics, and cell biology with pharmacology and chemical biology, to understand the structure and function of a family of cAMP sensors: exchange proteins directly activated by cAMP (EPAC). Our goals are to unravel the signaling intricacies of EPAC proteins and to design pathway specific modulators for these important signaling molecules so that their functions can be exploited and controlled pharmaceutically for the treatment of human diseases. We have developed first-in-class EPAC selective inhibitors and EPAC knockout mouse models to study the physiological functions and diseases relevance of this family of important signaling molecules. Recently, we have identified a potential use of EPAC inhibitors in the prevention and treatment of fatal rickettsioses. Currently, we are actively engaged in developing second generation isoform specific EPAC inhibitors and agonists and in exploring their potential uses in various human diseases including chronic pain and cardiovascular diseases.

RESEARCH PROJECTS

- Structural and functional analyses of the exchange proteins directly activated by cAMP (EPAC).
- Examine the roles of EPAC proteins in major human diseases, such chronic pain and heart failure using EPAC knockout mouse models and pharmacological inhibitors.
- Preclinical development of novel drug candidates targeting EPAC for the treatment of microbial infections caused by tick-borne bacteria Rickettsia.



Epac1 knockdown alleviates neointima formation in carotid arteries after ligation *in vivo*. Left side carotid artery from wild type (N = 7) and Epac1 deletion mice (N = 7) were ligated and analyzed at day 28 after ligation. Luminal obliteration was significantly reduced in the carotid artery from Epac1 deletion mice compared to WT mice as shown by H&E staining.



Wenliang Li, Ph.D.

Associate Professor

Studying and targeting cancer metastasis and drug resistance

My research programs are (1) to obtain critical new knowledge of cancer metastasis and drug resistance of human cancer cells, (2) to identify new biomarkers and drug targets for the development of better therapeutics for human cancers.

Cancer metastasis, the spread of tumor to other parts of patient's body, is responsible for over 90% of cancer death. However, cancer metastasis is still poorly understood, and the current approaches to prevent or treat human metastatic cancers are mostly unsuccessful. Therefore, there is a huge unmet medical need to better understand cancer metastasis and to develop new therapies against cancer metastasis. Through genomics, RNAi and cDNA functional screens, my lab has identified several crucial but previously unknown regulators for cancer metastasis. Some of the novel regulators control epithelial-mesenchymal transition (EMT), while some others are essential for survival and proliferation of highly metastatic cancer cells (i.e. essential genes). EMT, a developmental process, is believed to play a key role in cancer metastasis, drug resistance, organ fibrosis, and stem cell phenotypes. Essential genes for metastatic cancer cells may be the key to understand colonization, the rate-limiting step of cancer metastasis. Signaling pathways and molecular mechanisms of these novel regulators are under investigation with molecular, cellular, biochemical, genomic, proteomic approaches, and mouse models. These studies are yielding critical new insights for cancer metastasis and facilitating the development of new therapeutics and biomarkers.

Another research topics in our lab is to investigate the mechanisms of cancer cell plasticity and drug resistance. In particular, we study how prostate cancers become resistance to a new generation of androgen receptor pathway inhibitors (ARPIs), and how non-small cell lung cancers (NSCLC) become resistant to EGFR inhibitors. The common theme in this topic is to better understand and to target a process called neuroendocrine differentiation (NED),

which is increasingly accepted as a critical process in cellular plasticity and drug resistance of these two major solid cancer types. Upon the acquisition of resistance to ARPIs, some AR-positive prostate adenocarcinoma cancers become AR-low/negative aggressive neuroendocrine prostate cancers. Similarly, after becoming resistant to EGFR inhibitors, some NSCLC demonstrates phenotypes of small cell lung cancer, which is neuroendocrine in nature and very aggressive. NED is still poorly understood and currently there is no effective treatments to prevent or overcome drug resistance related to NED. We investigate the underlying mechanisms of NED, cellular plasticity, and drug resistance, especially the roles and mechanisms of action of several novel epigenetic regulators.

RESEARCH PROJECTS

- Mechanisms of action for novel regulators of cancer metastasis.
- New pathways and mechanisms of epithelial-mesenchymal transition.
- Lineage plasticity and acquired resistance to cancer therapeutics.
- Epigenetic mechanisms of beta adrenergic signaling in tumor progression and angiogenesis.

KEY PUBLICATIONS

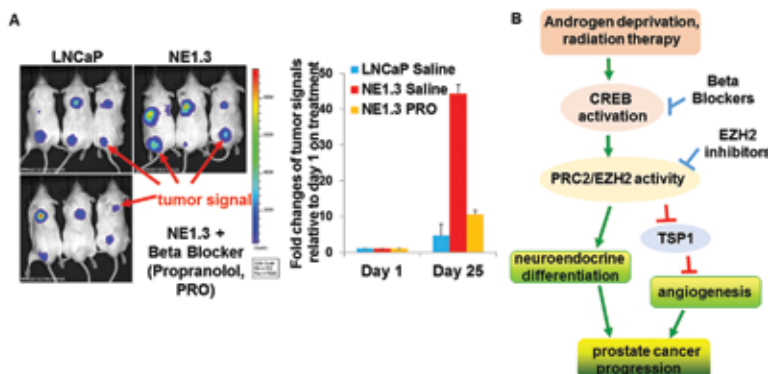
Li, L., Su, N., Zhou, T., Zheng, D., Wang, Z., Chen, H., Yuan, S., Li, W*. Mixed lineage kinase ZAK promotes epithelial-mesenchymal transition in cancer. *Cell Death & Disease* 2018 Feb 2; 9(2):143. *corresponding author

Zhao, Y., Li, W*. Beta-adrenergic signaling on neuroendocrine differentiation, angiogenesis, and metastasis in prostate cancer progression. *Asian Journal of Andrology* 2018 May 29. *corresponding author

Zhang Y, Zheng D, Zhou T, Song H, Hulsurkar M, Su N, Liu Y, Wang Z, Shao L, Ittmann M, Gleave M, Han H, Xu F, Liao W, Wang H, Li, W*. Androgen deprivation promotes neuroendocrine differentiation and angiogenesis through CREB-EZH2-TSP1 pathway in prostate cancers. *Nature Communications*, 2018 Oct 4; 9(1):4080. *corresponding author

LAB MEMBERS

Post-doctoral fellows: Zheng Wang, Junwei Lian, Ying Jing, Jinbang Xu
Research assistant II: Han Yang



We identified a new critical pathway that contributes to the progression of prostate cancer to the aggressive tumors resistant to hormonal therapy, called neuroendocrine prostate cancer (NEPC). (A) Hormonal resistant NEPC cells NE1.3 form tumors much better than its parental prostate cancer cells LNCaP that are sensitive to hormonal therapy, which can be inhibited by propranolol, a beta blocker commonly used for heart diseases and hypertension in patients. (B) A summary model of our main findings in the critical pathway that be blocked by inhibitors such as beta blockers and EZH2 inhibitors.



Qingyun (Jim) Liu, Ph.D.

Professor

Janice Davis Gordon Chair for Bowel Cancer Research

Investigation of normal and cancer stem cells for the discovery of cancer therapeutics

Adult stem cells are specialized cells that can self-renew and give rise to all the other types of differentiated cells in the tissue where the stem cells reside. They are essential for the maintenance of tissues with high turnover rate, such as the gut and skin, and for tissue repair after injury. However, these cells also are believed to be the cells-of-origin for many types of cancer as they are programmed to divide indefinitely. Furthermore, tumor tissues are also heterogeneous in which only a subpopulation of cells can self-renew and provide daughter cells that make up the bulk of the tumor. These self-renewing cancer cells, designated cancer stem cells or tumor-initiating cells, often bear great similarity to normal stem cells in molecular profile and regulatory systems. Understanding of the mechanisms that govern the control of the self-renewal and differentiation of normal and cancer stem cells will provide crucial knowledge to the discovery and development of novel therapeutics for regenerative medicine and cancer treatment.

Our research is focused on delineating the function and mechanisms of a group of cell surface receptors called LGR4, LGR5, and LGR6 (LGR4-6) that play critical roles in the survival of normal stem cells and tumor cells. Previously, we discovered that LGR4-6 function as receptors of a group of stem cell factors called R-spondins (RSPOs) that are essential for the survival and growth of stem cells. We have now elucidated how RSPOs and LGRs work together to regulate cell growth and migration. In particular, we uncovered that RSPO3-LGR4 has a major role in the aggressiveness of lung adenocarcinomas and colorectal cancer. Most recently, we showed that drug conjugates of anti-LGR5 antibodies showed excellent anti-tumor efficacy in preclinical models of colon cancer. We also have identified and characterized a series of anti-LGR4 antibodies and generated their drug conjugates. The modified antibodies displayed robust anti-tumor activity in animal models of colorectal and ovarian cancer. Our current efforts are focused on further optimizing

these drug leads targeting the RSPO-LGR system as potential treatment for colorectal cancer and other types of malignancies.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

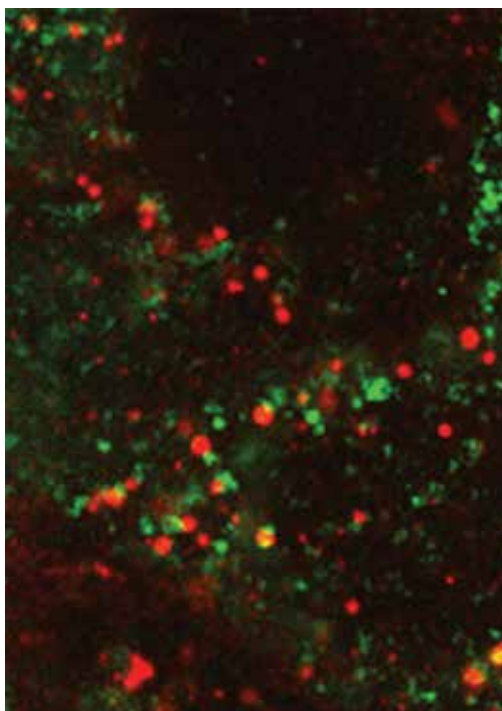
Gong, X., Azhdarinia, A., Ghosh, S., Xiong, W., An, Z., Liu, Q., Carmon, K.S. (2016). LGR5-targeted antibody-drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol Cancer Ther.* 15:1580-1590 (2016).

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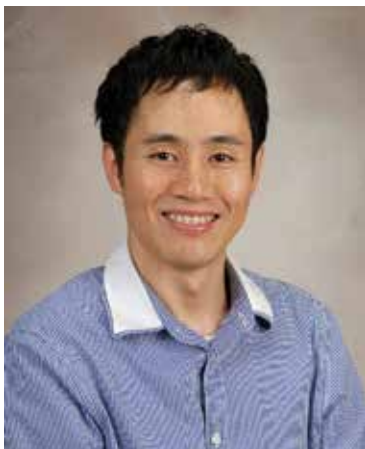
Park, S., Cui, J., Yu, W., Wu, L., Carmon, K.S., Liu, Q.J. Differential activities and mechanisms of the four R-spondins in potentiating Wnt/ β -catenin signaling. *J. Biol. Chem.* 293:9759-9769 (2018).

LAB MEMBERS

Post-doctoral fellows: Soohyun Park, Jianghua Tu
Sr. research associates: Wangsheng Alice Yu, Ling Wu



Internalization and localization of anti-LGR4 antibody into ovarian cancer cells. LGR4 antibody (Red) is internalized into the lysosome (Green) in cancer cells.



Kyoji Tsuchikama, Ph.D.

Assistant Professor

Linker technology for constructing efficacious antibody-drug conjugates (ADCs) toward innovative cancer therapeutics

Antibody-Drug Conjugates (ADCs) represent a rapidly growing and extensively potent class of anticancer therapeutics. As demonstrated with the 4 FDA-approved ADCs (Adcetris[®], Kadcyla[®], Besponsa[®], and Mylotarg[®]) and more than 60 promising ADCs in clinical trials, successful treatment outcomes using ADCs have inspired scientists and clinicians to further advance this new molecular format for developing effective cancer therapeutics. ADCs deliver potent anticancer drugs selectively to tumors while avoiding healthy tissues, enabling the use of highly active drugs that have been too toxic to be used alone for cancer treatment. An appropriate chemical linker between the antibody and the highly active drug provides a specific bridge, enabling selective delivery and precise release of the highly active drug only at the tumor sites. Thus, the use of proper ADC linkers is a key for successful implementation of ADC-based chemotherapy.

Our primary interest is in the development of novel chemical ADC linkers by taking advantage of the power of organic chemistry, medicinal chemistry, and chemical biology. We have developed dual-loading ADC linkers enabling simple and easy installation of two drug molecules onto an antibody. We have revealed that our dual-loading ADCs exert greater potency than those constructed with traditional single-loading linkers. Recently, we also developed a

glutamic acid-valine-citrulline tripeptide linker as a new-generation ADC linker. The conventional valine-citrulline linker has been used in about half of successful ADCs. However, it is known that valine-citrulline linkers can undergo premature degradation in mouse circulation. This issue complicates drug design strategy and preclinical evaluation of ADC candidates. We demonstrated our linker exhibited greater plasma stability and treatment efficacy in mouse tumor models than does a conventional variant. These technologies could add flexibility to antibody-drug conjugate design and help minimize failure rates in preclinical studies caused by linker instability.

With our technology platform in hand, we are currently working on producing next-generation ADCs for combating the cancer drug resistance and heterogeneity issues. The drug resistance and tumor heterogeneity are unsolved issues in cancer chemotherapy leading to discontinuation of medication and recurrence of malignancy. We envisage that our novel ADC linkers will help not only ourselves but also other researchers establish various approaches for overcoming such unsolved issues, which will lead to a number of valuable additions to the current list of antitumor drug candidates in the future clinical studies.

RESEARCH PROJECTS

- Design, synthesis, and evaluation of novel branched chemical linkers for constructing multi-loading ADCs
- Structural optimization of ADC linkers for high plasma stability and rapid drug release

- Modulation of the ADC function by chemical modification for organ-specific delivery
- Quorum sensing-guided drug delivery

KEY PUBLICATIONS

Tsuchikama, K. *, Shimamoto, Y., Anami, Y. (2017) Truncated Autoinducing Peptide Conjugates Selectively Recognize and Kill *Staphylococcus aureus*. *ACS Infectious Diseases*, 3(6), 406–410.

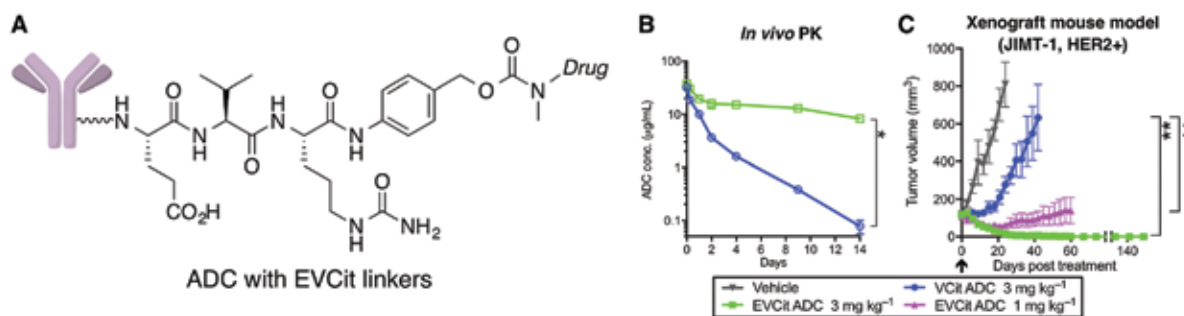
Anami, Y., Xiong, W., Gui, X., Deng, M., Zhang, C. C., Zhang, N., An, Z., Tsuchikama, K. * (2017) Enzymatic Conjugation Using Branched Linkers for Constructing Homogeneous Antibody-Drug Conjugates with High Potency. *Organic & Biomolecular Chemistry*, 15, 5635–5642.

Tsuchikama, K. *, An, Z. (2018) Antibody-drug conjugates: recent advances in conjugation and linker chemistries, *Protein & Cell*, 9, 33–46. DOI:10.1007/s13238-016-0323-0.

Anami, Y., Yamazaki, C. M., Xiong, W., Gui, X., Zhang, N., An, Z., Tsuchikama, K. * (2018) Glutamic acid-valine-citrulline linkers ensure stability and efficacy of antibody-drug conjugates in mice, *Nature Communications*, 9:2512.

LAB MEMBERS

Post-doctoral fellows: Yasuaki Anami, Ph.D.; Chisato Tsuchikama, Ph.D.; Aiko Yamaguchi, Ph.D.



The glutamic acid-valine-citrulline (EVCit) linker maximizes stability and therapeutic potency of ADCs in mouse models. (A) Structure of the EVCit linker. (B) Pharmacokinetics (PK) of anti-HER2 ADCs constructed using EVCit or conventional VCit linker in BALB/c mice (3 mg/kg each, n = 3). (C) *In vivo* comparison of ADCs with VCit and EVCit linkers in the HER2+ JIMT-1 xenograft mouse model (n = 5). All tumor-bearing mice were treated with a single dose of 1 or 3 mg/kg each ADC at Day 0 (indicated with a black arrow).



Ningyan Zhang, Ph.D.

Associate Professor

Cancer resistance mechanisms to therapeutic antibodies and modulation of anticancer immunity

Monoclonal antibodies are becoming a major drug modality for cancer treatment and have shown clinical success for treatment of various types of cancer. Tumor targeting monoclonal antibodies, such as trastuzumab against HER2 and bevacizumab targeting tumor angiogenesis factor VEGF, have been successfully used for treatment of many types of cancer. However, both innate and acquired resistance to these therapeutic antibodies are widely reported. Understanding the mechanism of cancer resistance to therapeutic antibodies is of paramount importance for improvement of these cancer targeted therapies to benefit more cancer patients. Our current research programs are centered on better understanding of tumor evasion of antibody immunity and develop therapeutic strategies to modulate anticancer immunity for improvement of cancer treatment.

Cancer immune evasion is recognized as a hallmark of cancer. Our research has demonstrated the prevalence of proteolytic impairment of antibody IgG in the tumor microenvironment. Trastuzumab and pertuzumab (anti-HER2 antibody) with a single hinge cleavage showed a loss of immune effector function against cancer cells *in vitro* and reduced antitumor efficacy *in vivo*. Based on our recent findings and reports by others, we hypothesize that antibodies recognizing tumor associated antigens (TAA) in the tumor microenvironment are susceptible to proteolytic impairment through a hinge cleavage by matrix metalloproteinases (MMPs). Such proteolytic hinge cleavage of antibodies not only weakens antibody anticancer immunity but also leads to an immune suppressive tumor microenvironment. To test our hypothesis, we employ a wide array of experimental approaches including *in vitro* 2D and 3D cell co-cultures, mouse tumor models, and studies with clinical samples from cancer patients to determine factors influencing proteolytic impairment and to identify mechanisms of cancer immune evasion triggered by proteolytic impairment of antibody hinge. State-of-the-art technologies are used in our studies, such as high content fluorescence

imaging, mass spectrometry, fluorescence activated cell sorting (FACS), and single-cell cloning of antibodies. We have established a monoclonal antibody platform technology to discover and select novel anticancer antibodies for functional evaluation and preclinical development. The long-term goal of our research is to understand mechanisms of cancer evasion of antibody and cellular immunity and to identify key molecular targets for development of effective anticancer immunotherapies.

RESEARCH PROJECTS

- Determine the role of proteolytic hinge cleavage of antibodies in cancer immune suppression.
- Understand mechanisms of cancer evasion of antibody immunotherapeutics.
- Develop platform technology for discovery of therapeutic antibodies.

KEY PUBLICATIONS

Mi Deng, Xun Gui, Jaehyup Kim, Xie Li, Weina Chen, Zunling Li, Yuanzhi Chen, Heyu Chen, Weiguang Luo, Zhigang Lu, Jingjing Xie, Hywyn Churchill, Yixiang Xu, Zhan Zhou, Guojin Wu, Chenyi Yu, Samuel John, Kouyuki Hirayasu, Nam Nguyen, Hui Deng, Haidong Tang, Yizhou Zou, Benjamin Chen, Hisashi Arase, Ningshao

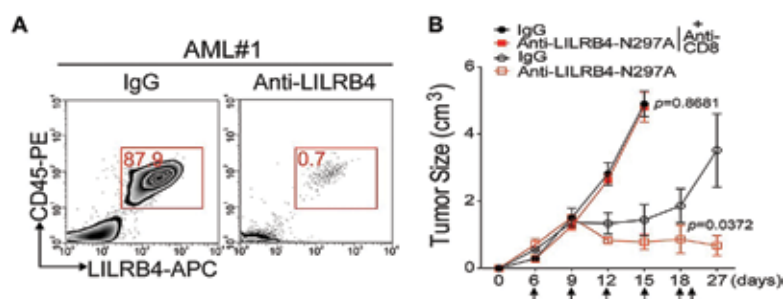
Xia, Youxing Jiang, Robert Collins, Yang-Xin Fu, Zhiqiang An, Junke Zheng, Ningyan Zhang*, and Cheng Cheng Zhang. (2018) Inhibitory receptor signaling in leukemia cells mediates tumor infiltration and T cell suppression *Nature*, doi.org/10.1038/s41586-018-0615-z.

Yi Yasuaki Anami, Chisato Yamazaki, Wei Xiong, Xun Gui, Ningyan Zhang, Zhiqiang An, Kyoji Tsuchikama. (2018) Glutamic acid-valine-citrulline cleavable linkers ensure high stability and efficacy of antibody-drug conjugates in mouse models. *Nature Communications*, DOI: 10.1038/s41467-018-04982-3.

Hao-Ching Hsiao, Xuejun Fan, Robert E. Jordan, Ningyan Zhang *and Zhiqiang An. (2018). Proteolytic single hinge cleavage of pertuzumab impairs its Fc effector function and antitumor activity *in vitro* and *in vivo*. *Breast Cancer Research*, 20:43, DOI: 10.1186/s13058-018-0972-4.

LAB MEMBERS

Research associate/scientists: Hui Deng, M.S.; Xuejun Fan, M.D., Ph.D.; Wei Xiong, Ph.D.
Post-doctoral fellows: See the list under Dr. Zhiqiang An's group.



Blockage of human primary AML cancer cell infiltration into bone marrow by our novel anti-LILRB4 antibody (A); Inhibition of AML tumor growth by the anti-LILRB4 antibody depends on activation of T cell immunity in a mouse syngeneic tumor model (B). From M. Deng et al. (2018) *Nature*.

IMM SERVICE CENTERS

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

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

ANTIBODY ENGINEERING AND EXPRESSION SERVICE CENTER

Antibody therapeutics represents a major breakthrough in combating human diseases, including cancer. Even though the pharmaceutical and biotechnology industries are in the center stage of drug discovery and development, academic researchers are increasingly engaged in discovering new antibody drug candidates. However, advancement of some the promising antibodies in the early stage of discovery from academic research laboratories is often hindered by the lack of access to the expertise and infrastructure required for antibody engineering and other related key technologies. Our antibody engineering and expression service center will fill the gap of the much needed expertise in early discovery of monoclonal antibodies and lead optimization for the research and drug discovery communities. The objective of the service center is to provide technical support and services to antibody identification, molecular cloning, antibody expression, and purification. Results generated from the service center will strengthen the collaborators' ability to attract external funding to continue development of

the optimized therapeutic antibodies with the ultimate goal of translating basic research to novel therapies.

CLINICAL AND TRANSLATIONAL PROTEOMICS SERVICE CENTER

Current trends in biomedical research are increasingly focused on translational studies not only for the understanding of disease processes and therapies but also for disease diagnosis and the evaluation of therapeutic efficacy. These studies often require extensive analyses of research and clinical specimens to identify steady or perturbation-induced proteome alterations associated with a disease status or biological state. Such proteome alterations may include changes in protein composition and expression, post-translational modifications (PTMs), protein functions, as well as protein interaction with proteins and other biomolecules. Our service center provides state-of-the-art proteomics services to UTHealth, the Texas Medical Center research community, and other external organizations.

The basic services provided are designed to identify and quantitate proteins and their PTMs in a broad range of research specimens, from purified protein samples to complex mixtures such as cell and tissue extracts, plasma/serum, and/or other biofluids. We also provide advanced supports, such as biomarker discovery and verification, development of targeted proteomics assays, and metaproteomics for microbiome profiling. The service center contains the cutting-edge instrumentation and trained personnel to provide an integrated proteomics analysis, including sample preparation, mass spectrometric analysis, and bioinformatics data processing.

COLLABORATION IMAGING SERVICE CENTER

The IMM Center for Molecular Imaging is a facility that all researchers at UTHealth who are, or wish to be, involved in small animal/translational imaging studies should be acquainted with. The Center is directed by Dr. Eva Sevick and led by engineering and basic science faculty members whose research focuses on different aspects of molecular imaging, including new instrumentation, design and chemistry of targeted probes, innovative algorithms, and pioneering translation of new imaging technologies into clinical trials. The newly formed Molecular Imaging “collaboration” center utilizes this existing expertise to interact with clinicians, clinician-scientists, as well as academic and industry researchers across the nation on translational projects in cancer, drug discovery, autoimmune disorders, gastrointestinal disorders, nanotechnology, chronic wound care, peripheral vascular disease, and others. Facilities include a Siemens hybrid PET/CT small animal scanner with custom fluorescence tomography capabilities and an array of custom bioluminescence and fluorescence instrumentation that is paired with unique imaging agents/gene reporter systems. Generalized protocols are available to investigators to maximize benefit from the latest developments in molecular imaging.

FLOW CYTOMETRY SERVICE CENTER

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

Thousands of cells can be analyzed per second as they pass through liquid in front of the lasers. 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 and additionally isolate cell populations to be sorted. The service center provides training, instrumentation, and technical expertise for both analysis and cell sorting. These instruments are

available on a fee-for-services charge to all research investigators from UTHealth and external organizations.

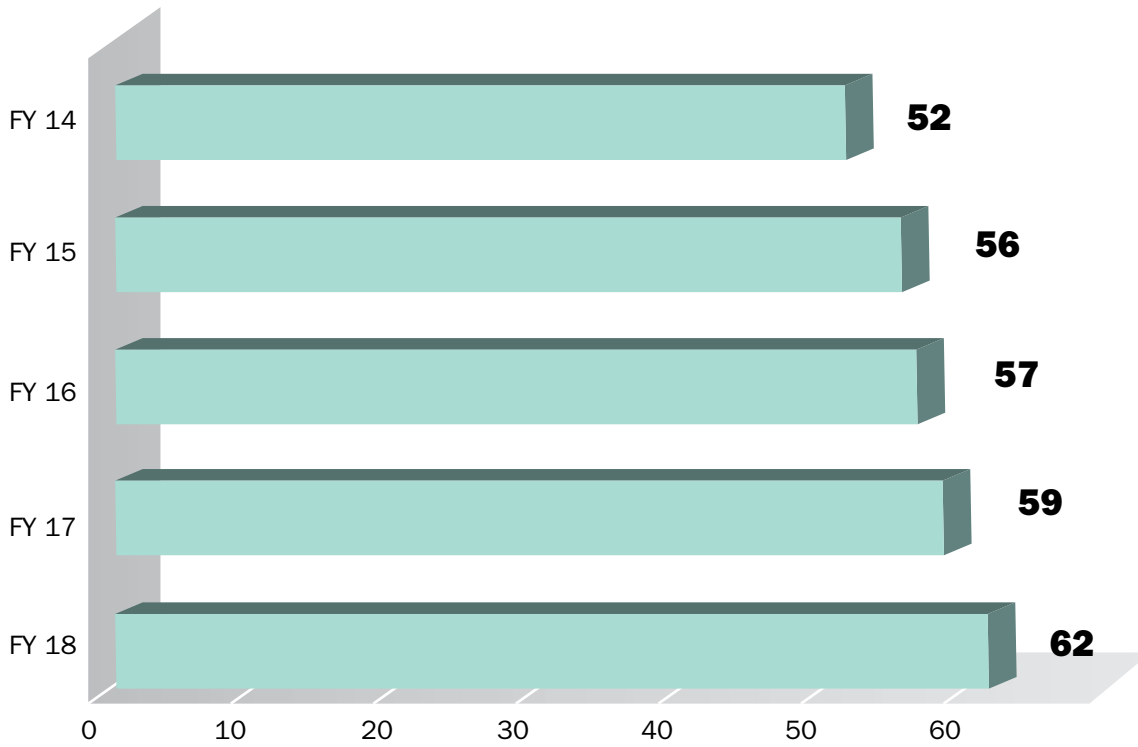
TRANSGENIC AND STEM CELL SERVICE CENTER

Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cells service center, which was established in 1998. It has generated over 800 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, CRISPR/Cas9 genome editing, derivation of new cell lines, and intellectual/technical support in different aspects of microsurgery, cell culture, and stem cells research.

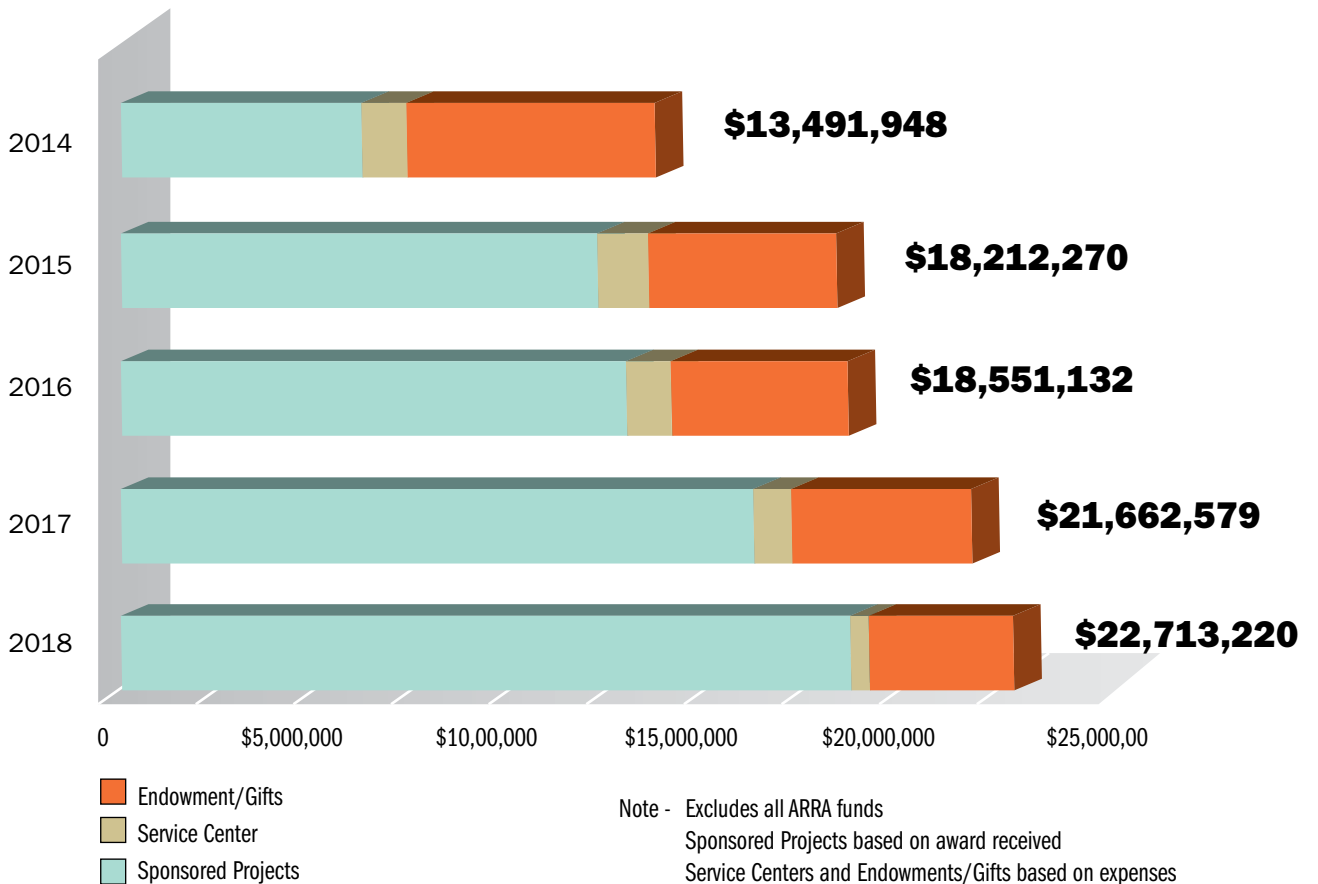
NANOCHEMISTRY SERVICE CENTER

The Nanochemistry Service Center provides state-of-the-art 3D printing and aptamer development services. We provide 3D printed models of human organs and novel surgical tools, in prototype or final production models. We have both traditional FDM thermoplastic and multi-color, resin-based high-resolution (14 micron) 3D printers with large print beds. A wide range of materials with varying Shore A values (hardness) is available. STL files, SolidWorks, or medical imaging files can produce 3D Models. Our other focus is the synthesis and development of novel DNA aptamers, X-aptamers, or RNA for targeting proteins or cells. In particular, most of this work focuses on delivering therapeutic agents to cancers, such as ovarian, breast, and pancreatic cancers, or targeting proteins upregulated in other disease states. Chemical conjugation, nanoparticle production, and other chemistries are available. We are located on the 3rd floor of the Fayed S. Sarofim Research Building.

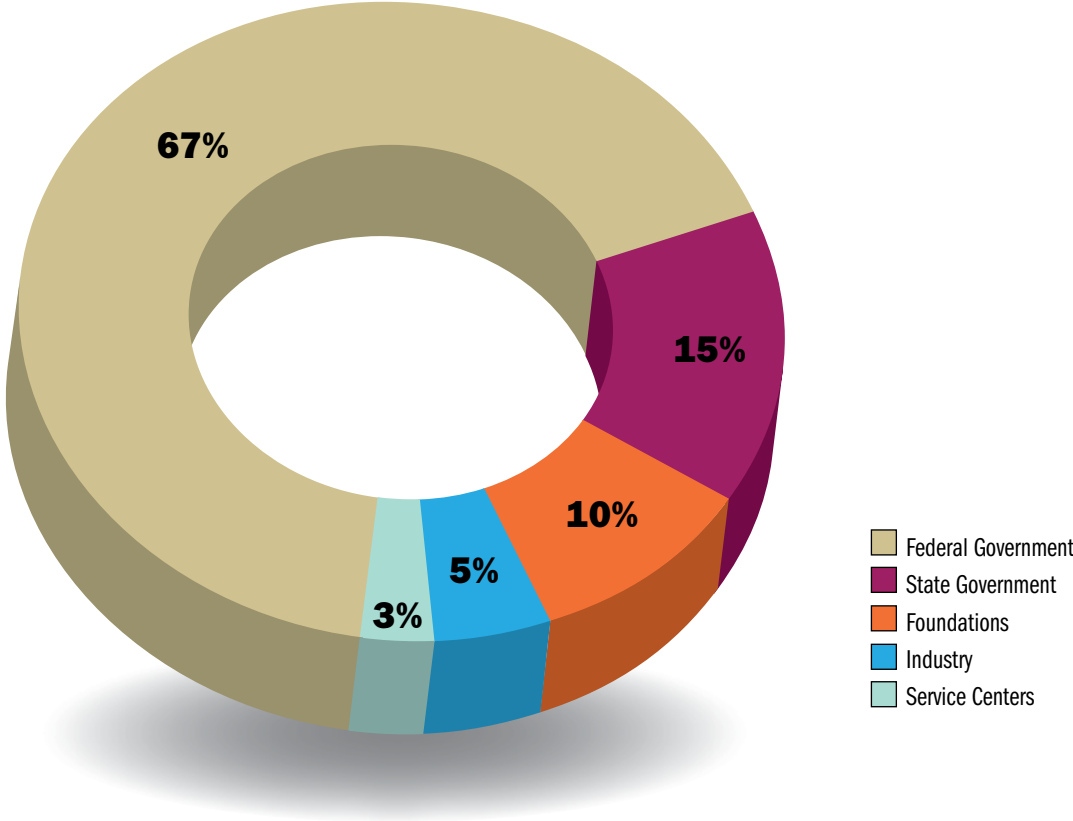
NUMBER OF FACULTY



TOTAL FUNDS SUPPORTING RESEARCH



TOTAL EXPENSES SUPPORTING RESEARCH



GIFT REPORT

New Gifts and Bequests Fiscal Year 2018

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The Carolyn Frost Keenan Professor in Cardiovascular Disease Research

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TO RECRUIT AND RETAIN TOP SCIENTISTS FROM AROUND THE WORLD.**

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