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

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

Impact Report

INSIDE

Realizing the promise of personalized medicine

TURNING BACK THE CELL'S CLOCK

Molecular Imaging

Bridging science and drug discovery

About the cover

The University of Texas Medical School at Houston's Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases was established in 1995 to cure the diseases of our time in our time. The Fayez S. Sarofim Research Building is shown on the banks of Brays Bayou with the University Center Tower in the background.

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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 cardiovascular, diabetes, cancer, schizophrenia, and Alzheimer's diseases are unsolved, common, and not caused by a single gene. Discoveries lead to new solutions.

2. New diagnostics and therapies are derivative of discovery and to the benefit of patients. The IMM focuses on these medical solutions. The IMM has organized talent in the Texas Therapeutics Institute to achieve this goal of patient benefit from discovery.

Welcome to the first Brown Foundation Institute for Molecular Medicine (IMM) highlight report, which provides a snapshot of the institute and a vision for our future.

Although the institute was established in 1995, the foresight of our founders and generosity of our donors to open the Sarofim Research Building in 2006 is what has allowed this institute to flourish. In the last few years, our faculty and their research programs have made significant strides. The Center for Stem Cell and Regenerative Medicine is pioneering new cellular therapies that are making a difference in patients with stroke and other debilitating diseases, the Texas Therapeutics Institute is fostering new relations with the pharmaceutical industry, the Center for Molecular Imaging has developed novel technologies to visualize the lymphatic system, the Center for Proteomics and Systems biology have built an innovative infrastructure for personalized medicine.

These are just a few highlights from our outstanding translational research programs. I invite you to read about all of these and other accomplishments in more detail inside. These outstanding medical and research achievements could not be made without the support of our friends at so many levels. We are grateful to those who are as passionate about scientific advances as we are.

We welcome your feedback and your involvement. Please feel free to contact the institute at 713-500-7356.

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

Mission

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

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

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

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

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

Facts about the Fayez S. Sarofim Research Building

- Encompasses 255,748 gross square feet.
- $120 million to build
- Moves 50 percent or more of the air from the offices into the atrium, saving air conditioning costs by providing "semi-conditioned" air to the atrium space
- Can withstand hurricane-force winds on its exterior terracotta rain screen system
- Includes a vivarium sufficient for a rodent population of about 23,000
- Allows natural light to penetrate further into the laboratory spaces by using ceiling "clouds" suspended from laboratory ceilings—convex-shaped and colored white, the panels diffuse light off their surfaces
- Uses glass on 90 percent of the north façade—filling the building interior with natural light and showcasing the building's function
- Divides its area into roughly 50 percent lab space, 50 percent office space, with the intention that the vertex of the wings will foster human interaction
- Employs open laboratory areas with 20 linear feet of island bench in each workspace module—all of the island benches are all easily movable to accommodate the changing dynamics of research
- Has 22 open lab modules along the north side of each of the four lab floors
- Offers an area called "The Institute Gateway" as the public "face" of the IMM—includes the entry vestibule, atrium, auditorium and conference center

Other IMM locations

South Campus Research Building – 3 (SCRB3)

The Department of NanoMedicine and Biomedical Engineering is housed on the sixth floor of the SCRB3 building. SCRB3 is a collaboration between The University of Texas MD Anderson Cancer Center and UTHealth, in cooperation with GE Healthcare and the Texas Enterprise Fund. The

building is a six-story, 315,000 square-foot concrete structure located on the South Campus at the corner of Bertner Street and East Road, with a curtain wall and masonry exterior façade. This facility houses Positron Emission Tomography, Magnetic Resonance Imaging, Optical Imaging Tracers, a Cyclotron, multiple wet labs, and administrative support offices. The building was completed in 2009.

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 that provides space for the school's basic sciences research growth needs.

The laboratory spaces were designed to be extremely flexible, which posed an interesting opportunity for providing fixed utilities to the space.

Overhead raceways were installed to accommodate the electrical power and lab gasses. Mobil laboratory grade casework was utilized so the lab could be reconfigured

to accommodate specific equipment and research grants. The demountable partitions separating the lab spaces also can be moved to increase or decrease the size of the lab.

Affiliates - sponsors of research

Federal

- The National Institutes of Health, a part of the U.S. Department of Health and Human Services, is the nation's premier medical research agency.

State Agencies

- The Cancer Prevention & Research Institute of Texas is a state agency created to fund groundbreaking cancer research and prevention programs and services in Texas.

Foundations and Universities

- The American Cancer Society is a nationwide, community-based voluntary health organization dedicated to eliminating cancer as a major health problem.
- The American Heart Association is the oldest and largest voluntary organization dedicated to fighting cardiovascular diseases.
- The University of Texas MD Anderson Cancer Center is the premier cancer center world renowned for its outstanding programs that integrate patient care, research, prevention, and education.

Proteomics and systems biology: Realizing the promise of personalized medicine

A woman pregnant in her third-trimester rushes to the hospital in what she thinks is early labor. Her physician does a simple blood test, determines it is a false alarm, and sends her home.

A physician targets a cancer patient's tumor with therapies designed specifically to his tumor – down to the proteins on the tumor's cellular surface.

A man's routine PSA test, and new improved ones, determines his risk for prostate cancer.

Each of these examples is in reach today and is the result of the study of proteins, or proteomics.

"We study proteins to be able to understand their variations, which can lead to disease," explains David Gorenstein, Ph.D., who leads the IMM's Center for Proteomics and Systems Biology. "Proteins are the workhorse of the cell. They vary and change throughout life and throughout the disease state, unlike genes, which you have from birth."

By using instruments called mass spectrometers, researchers search for protein signatures, known as biomarkers, which can signal potential disease.

"The goal is to develop protein biomarkers for the early indication of disease and provide personalized medicine," says Dr. Gorenstein, holder of the James T. Willerson Distinguished Chair. "For example, the PSA test is a protein biomarker."

Center investigators are working to discover biomarkers for cancers, Parkinson's disease, and preterm labor.

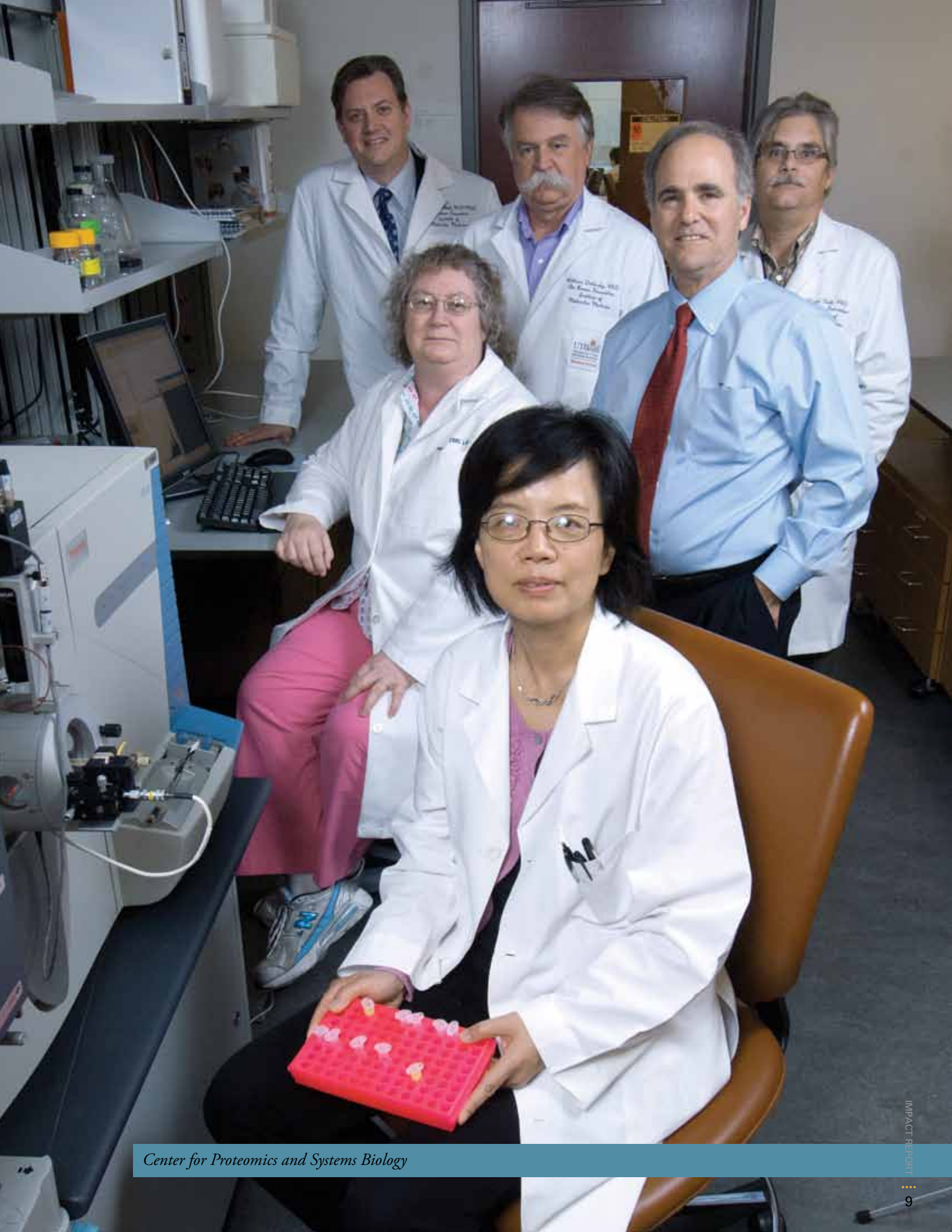
"We use instruments to discover them, and then mathematics – that's the systems biology – to model how they interact and validate their significance," Dr. Gorenstein explains.

The center is involved in several high-profile studies, including the National Children's Study, which is a 20-year research study of 100,000 children and mothers – reviewing the impact of proteins, genetics, environment, and nutrition on health. The center is carrying out the proteomic studies.

The center also is part of one of seven National Institutes of Health Heart Lung and Blood Institute's Proteomics Centers, seeking biomarkers for asthma and lung disease.

In collaboration with The University of Texas MD Anderson Cancer Center, the Center is one of eight National Cancer Institute's Centers for Cancer Nanomedicine to find which proteins to target in the fight against ovarian and pancreatic cancers.

"These are very complex problems, but new solutions that are changing medicine are being developed every day," Dr. Gorenstein says.



Center for Proteomics and Systems Biology

Turning back the cell's clock and advancing health

When Vicki Townsend, of Houston, suffered a stroke, the last thing she expected to do was to return to work the following week. But thanks to an innovative stem cell therapy pioneered at the Medical School, she did just that. Townsend is a patient in the world's first study using a patient's own bone marrow cells for acute ischemic stroke, which is led by Sean Savitz, M.D., associate professor of neurology and a cross-appointed faculty member of the Center for Stem Cell and Regenerative Medicine.

Converting stem cell research from the lab into patient success stories is the ultimate goal of the IMM's Center for Stem Cell and Regenerative Medicine.

"Our center has a strong emphasis in translating our work into medical applications," says Brian Davis, Ph.D., the Annie and Bob Graham Distinguished Chair in Stem Cell Biology and director of the center. "We work with colleagues from the clinical departments of the Medical School to integrate our research into the clinical pipeline, resulting in therapies to help patients. Our center's research encompasses both adult stem cells – for example the bone marrow stem cells used in the Savitz trial, as well as pluripotent stem cells. "

For many years, stem cell research has been embroiled in controversy.

"Embryonic stem cells, being pluripotent, can give rise to any kind of cell or organ tissue, such as lung, skin, or heart. There are ethical issues around how these cells are harvested since they come from a human embryo, and there can be rejection issues as these are taken from one body and then used in another," Dr. Davis explains.

Recently, Japanese scientist Dr. Shinya Yamanaka discovered how to turn adult cells, such as those in the skin or blood, back into this embryonic state. Once harvested from the patient, these adult cells are then induced to revert to an earlier embryonic state, and then can be used therapeutically as whatever cell needed in the same patient without the risk of rejection.

"We imagine the day when healthy patients could have these cells harvested from their bodies and then put in reserve, awaiting the day when they might need them – much like a patient who before a surgery banks their own blood in case they may need a transfusion," Dr. Davis says.

To be able to reprogram and turn back the clock of cells is a game-changer in the therapeutic offerings of stem cells.

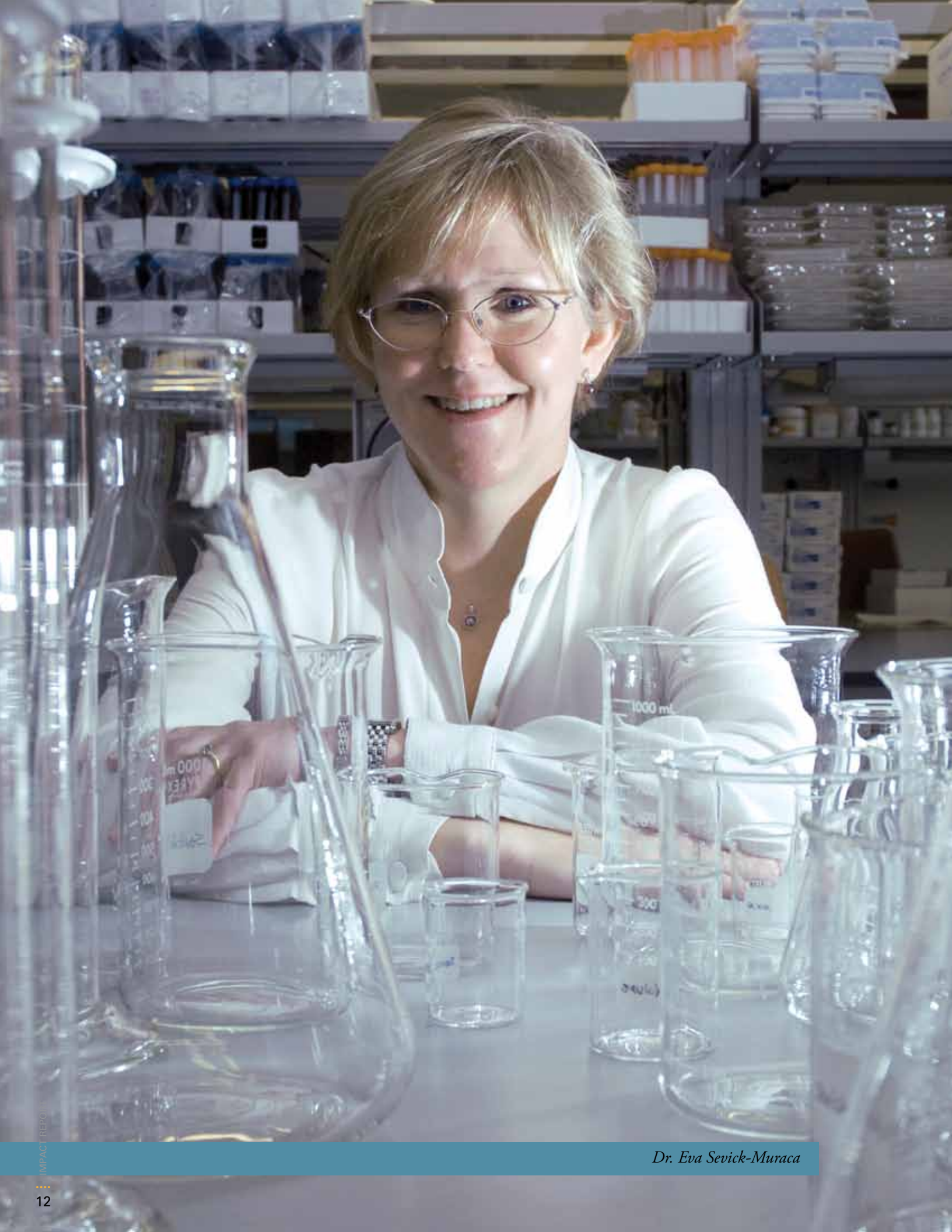
"Five years ago, this kind of induced pluripotent stem cell therapy would have been unimaginable," Dr. Davis says. "The common belief has been that cells once committed to a specific fate – for example to skin cells, could never become anything else.

"Over the next 20 years, we will be working to turn these induced pluripotent stem cells into cells or organs therapeutically appropriate for transplantation – that is the hard part," Dr. Davis says. "We can't magically recreate organs in the lab – but that is one of our long-term goals."

The center is home to 15 primary or cross-appointed faculty members, with plans to grow, as well as serving as the home of the Sen. Lloyd and B.A. Bentsen Center for Stroke Research.

"We are grateful to have such community support and recognition for the work that we do," Dr. Davis says.

Vicki Townsend



Dr. Eva Sevick-Muraca

Molecular imaging: Detecting, treating disease

Lymphedema, or lymphatic obstruction, is an incurable disease resulting from a compromised lymphatic system. Primarily a genetic disease, lymphedema also may be acquired following damage to, or the removal of, the lymph nodes during cancer treatment.

In the United States, it is estimated that 7-9 million cancer survivors are at risk of secondary lymphedema as a result of their treatments.

Until recently, there has not been an accurate way to image the near colorless fluid of the lymphatic system, which has made treating and understanding this disease very difficult.

Eva Sevick-Muraca, Ph.D., director of the Medical School's Institute for Molecular Medicine Center for Molecular Medicine and the Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research, is leading a new method to image the lymphatic system using near-infrared fluorescence-enhanced optical tomography, which offers improved sensitivity, higher resolution with compact instrumentation, and does not require the introduction of a radioactive agent into the patient.

"Our overarching goal is to develop instrumentation, imaging agents, and algorithms for a brand new molecular imaging modality based upon near-infrared light and near-infrared excitable dyes," Dr. Sevick-Muraca says. "The first application we have focused upon is lymphatic imaging since there is a significant unmet clinical need to diagnose lymphatic disorders."

Using dye and light, patients and clinicians can see the lymphatic system directly – before, during, and after treatment. The technique has been able to elucidate the mechanics of the lymphatic system that before were just hypotheses.

"In our studies of cancer patients, we have observed striking lymphatic abnormalities before clinical symptoms of lymphedema occur," Dr. Sevick-Muraca says. "This is important because it is thought that early treatment can ameliorate, or even prevent the disease. If we can detect the onset of the condition prior to the occurrence of symptoms, perhaps we can prevent an otherwise incurable disease.

"We believe that the technique could provide the best means for detecting lyphedema and could personalize treatments for those who are diagnosed with the disease."

The Food and Drug Administration has approved the system for investigational studies, and Dr. Sevick-Muraca is working with the National Cancer Institute to develop a network of centers around the country to facilitate the translation and commercialization of the technology. She and the center recently received funding from the Cancer Prevention and Research Institute of Texas to further these studies.

— Reporting from International Innovations

Texas Therapeutics Institute - The bridge between science and drug discovery

As the drug discovery and development process becomes increasingly complex, a new model of industry/academic partnership is emerging to translate basic biomedical discoveries to life-saving medicines more efficiently and cost effectively.

Across the country, the pharmaceutical industry has been looking outside corporate walls for tomorrow's new drug therapies by reaching out to academic centers. At the same time, universities are taking on these responsibilities, partnering with companies to bring technology out from behind the laboratory environment and into the clinic.

The University of Texas Health Science Center at Houston has had a long history of patenting and commercializing technology, and in 2010, it established the Texas Therapeutics Institute (TTI) as part of the Brown Foundation Institute of Molecular Medicine to specifically bridge the worlds of biomedical research and the pharmaceutical companies.

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

"We are in the process of establishing other sites at UT and beyond to best leverage our academic strengths and foster joint discoveries," says Zhiqiang An, Ph.D., director of the TTI and holder of the Robert A. Welch Distinguished University Chair in Chemistry.

More than 25 faculty and staff members make up the TTI, and their research has resulted in patent filings and disclosures and published research in major journals in a relative short period of time.

"Many of the faculty in this center were recruited from the pharmaceutical industry, and they know how the drug discovery process works," explains Qingyun (Jim) Liu, Ph.D., co-director of the center and holder of the Janice D. Gordon Distinguished Professorship in Bowel Cancer Research. "Our faculty are expected to build academically funded laboratories, create meaningful therapeutics, and serve as a resource for other UT faculty.

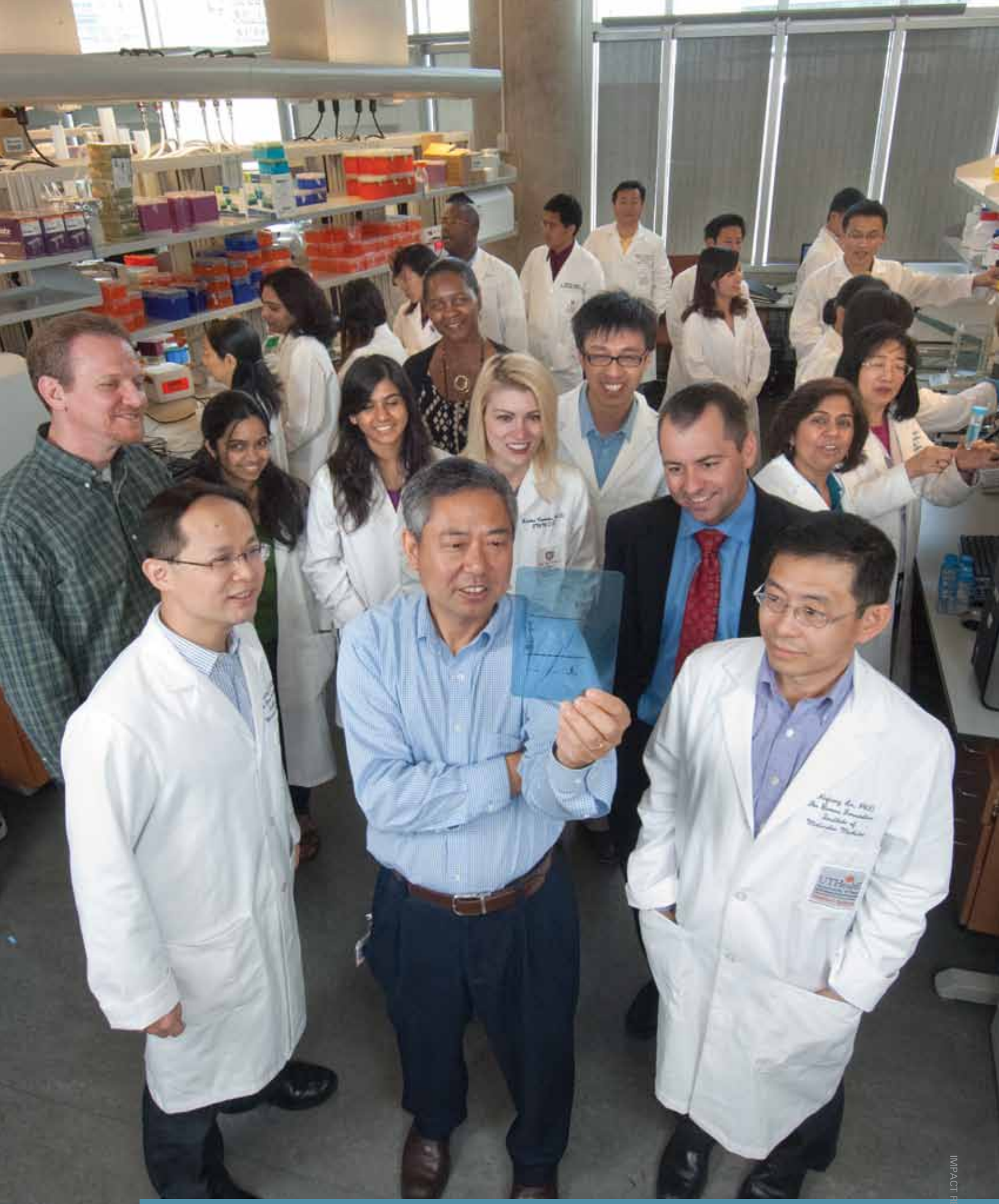
"Our aim is to put discoveries into a later stage, closer to commercialization," Dr. Liu says, adding that a spin-off company deal is expected to be inked before the end of this year.

Another goal of the center is to bring in funding from industry.

"We have received significant funding from outside companies such as Merck and Johnson & Johnson," Dr. An reports.

The center has worked to facilitate relationships between the pharma industry and faculty through workshops and sponsored meetings.

"Our ultimate goal is to benefit patients' health with the creation of new drug therapies that can be brought to market by partnering with the pharmaceutical and biotechnology industries," Dr. An says.



Dr. Jim Liu (center) and Dr. Zhiqiang An (right) direct the Texas Therapeutics Institute.

Center for Cardiovascular Genetics

The IMM Center for Cardiovascular Genetics, established in 2006, focuses on elucidation of molecular genetics and pathogenesis of cardiovascular diseases in humans. Located on the ninth floor of the Denton A. Cooley Building at the Texas Heart Institute at St. Luke's, the center provides specialized clinical services to patients with genetic cardiovascular disorders through the Cardiovascular Genetic Clinic at the Texas Heart Institute Clinic. The research activities at the center entail human molecular genetic studies as well studies in genetic animal models of human heart disease.

The mission of the Center for Cardiovascular Genetic Research is to diagnose and prevent cardiovascular diseases in humans prior to development of the clinical manifestations and to reverse or attenuate the evolving phenotype in those who already have developed the disease.

The ongoing basic research in our laboratory encompasses three groups:

(1) Human Molecular Genetic Studies

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

(2) Functional Studies

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

(3) Clinical studies

- To investigate potential utility of experimental therapies in human patients with hereditary cardiomyopathies through randomized phase I and II clinical studies.

(4) The ongoing clinical research in our laboratory is:

- Recruitment and phenotyping of individuals and families with various genetic cardiovascular diseases, with a primary focus on hereditary cardiomyopathies.
- Pharmacological intervention to prevent, attenuate and reverse the evolving phenotype in hereditary cardiomyopathies.

AJ Marian, M.D.
Center Director & Professor

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

Molecular Genetics and Pathogenesis of Hereditary Cardiomyopathies

Our long-standing research objectives have been to delineate the molecular genetics and pathogenesis of hereditary cardiomyopathies in humans and apply the discoveries to prevent the evolving and reverse the established phenotypes of heart failure and sudden cardiac death in cardiomyopathies. We are currently pursuing active research programs in the three most common forms of hereditary cardiomyopathies, namely hypertrophic, dilated and arrhythmogenic right ventricular cardiomyopathies. Our research programs entail identification of the causative and modifier genetic variants, complemented by *in vitro* and *in vivo* functional studies in isolated cardiac cells and animal models. The mechanistic discoveries are complemented with genetic and pharmacological intervention targeting the pathways that link the causal mutations to the phenotype, in order to prevent and reverse the phenotype initially in the animal models and subsequently in humans.

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

RESEARCH PROJECTS

- Canonical Wnt signaling in the pathogenesis and rescue of cardiac phenotype in arrhythmogenic right ventricular cardiomyopathy
- Molecular genetics and pathogenesis of cardiomyopathies caused by mutations in *TRIM63*, encoding MuRF1 – an E3 ubiquitin ligase.
- Cardiolaminopathy as a disease of premature aging of cardiac cells

KEY PUBLICATIONS

Lombardi, R. Cabreira-Hansen, M. Bell A, Fromm R, Willerson, JT, and Marian AJ. Nuclear Plakoglobin Is Essential For Differentiation of Cardiac Progenitor Cells to Adipocytes in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circulation Research*. 2011; 109; 1342-1353

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

Marian AJ and Belmont J. Strategic approaches to unraveling genetic causes of cardiovascular diseases. *Circ Res*. 2011; 108(10):1252-69

Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ. Resolution of Established Cardiac Hypertrophy and Fibrosis and Prevention of Heart Failure in the β-Myosin Heavy Chain-Q403 Transgenic Rabbits with N-Acetylcysteine. *Circulation* 2009; 119: 1398 - 1407

Lombardi R, Dong J, Rodriguez G, Bell A, Leung TK, Schwartz RJ, Willerson JT, Brugada R. Marian AJ. Genetic Fate Mapping Identifies Second Heart Field Progenitor Cells as a Source of Adipocytes in Arrhythmogenic Right Ventricular Cardiomyopathy. *Circulation Research*. 2009; 104: 1076-8

LAB MEMBERS

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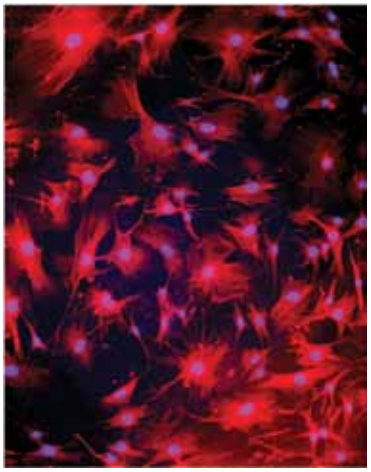
Ruggiero, Alessandra, Post-doctoral fellow
Li, Junjie, Post-doctoral fellow
Suet Nee Chen, PhD Graduate
Grazyna Czernuszewicz, Research Associate
Xu, Jin, Visiting Scientist



Expression of sarcomere protein α-tropomyosin in C-Kit+ cardiac progenitor cells isolated from heterozygous plakoglobin- deficient (PG+/-) mouse heart, a model for arrhythmogenic right ventricular cardiomyopathy. Red: α-tropomyosin; Blue:DNA.

Center for Human Genetics

The investigators of the Research Center for Human Genetics focus their work on common cardiovascular diseases, such as heart disease and stroke. These diseases have a significant impact on the health of our population, and our research combines modern genetic and genomic methods with large-scale human population studies. Progress in the laboratories of our investigators has provided important new understanding of susceptibility to atherosclerosis, coronary artery disease, stroke, and high blood pressure. The ultimate goal of our center is to use genetic approaches to unravel the critical cellular pathways that increase the likelihood that an individual will experience disease and to allow the development and application of new and existing therapeutic and preventive approaches in a way best tailored to individual risk.

This work places us at the forefront of personalized genomic medicine. Genomic medicine is in a remarkable state of rapid progress. This has grown out of the application of next-generation, whole-genome DNA sequencing and genome-wide genetic association studies that have been made possible by innovations in DNA analysis technology. In turn, this has amplified the power of genetic studies to uncover the precise regions of the genome containing genetic variation causing disease risk and then to seek the specific DNA changes that generate this risk. For those types of investigation that cannot be performed in large-scale human population studies, we develop and use a variety of laboratory models of these diseases. This allows us to address aspects of the disease process otherwise inaccessible to investigation. Using these models, we are uncovering how high blood pressure leads to kidney disease and stroke, and how we might modify lipid metabolism to provide new approaches to reduce atherosclerosis and coronary artery disease.

Eric Boerwinkle, Ph.D.
Center Director & Professor

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

Genomic sciences to promote human health

This is a spectacular time to be a human geneticist. Advances in laboratory technologies open the possibility that each and every one of us may have and be able to read our own DNA sequence. At the same time, computers to store and analyze those data have grown in size and speed. The advent of "cloud computing" pushes this envelop even further. The limits to modern genomic research lies within our own imagination and our political will to be the very best. Concurrent with these scientific advances, the population of Texas and the United States continues to grow and age. Therefore, the burden of common chronic diseases, such as coronary heart disease, kidney disease and stroke, is increasing. Our research is discovering the genes and mutations that increase the risk of developing common chronic disease and understanding how these genes interact with the environment to determine health and disease. This work is leading to novel approaches to both treat these conditions in the elderly and prevent their onset in our children. This research combines three powerful biomedical forces: large-scale DNA sequencing, computational analysis, and large samples of individuals with extensive clinical measurements. In addition, this work is not the product of one person at a laboratory bench, desk or doctor's office. Rather, it is the result of a large team of investigators in Houston and around the country working together to tackle big problems in biomedical research and health care. This team-based research program is also an ideal platform for training young biomedical researchers that will lead tomorrow's scientific endeavors.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, Lin CF, Stevens C, Wang LS, Makarov V, Polak P, Yoon S, Maguire J, Crawford EL, Campbell NG, Geller ET, Valladares O, Schafer C, Liu H, Zhao T, Cai G, Lihm J, Dannenfelser R, Jabado O, Peralta Z, Nagaswamy U, Muzny D, Reid JG, Newsham I, Wu Y, Lewis L, Han Y, Voight BF, Lim E, Rossin E, Kirby A, Flannick J, Fromer M, Shakir K, Fennell T, Garimella K, Banks E, Poplin R, Gabriel S, Depristo M, Wimbish JR, Boone BE, Levy SE, Betancur C, Sunyaev S, Boerwinkle E, Buxbaum JD, Cook EH, Devlin B, Gibbs RA, Roeder K, Schellenberg GD, Sutcliffe JS, Daly MJ. (2012) Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature.* Apr 4. doi: 10.1038, PMID: 22495311.

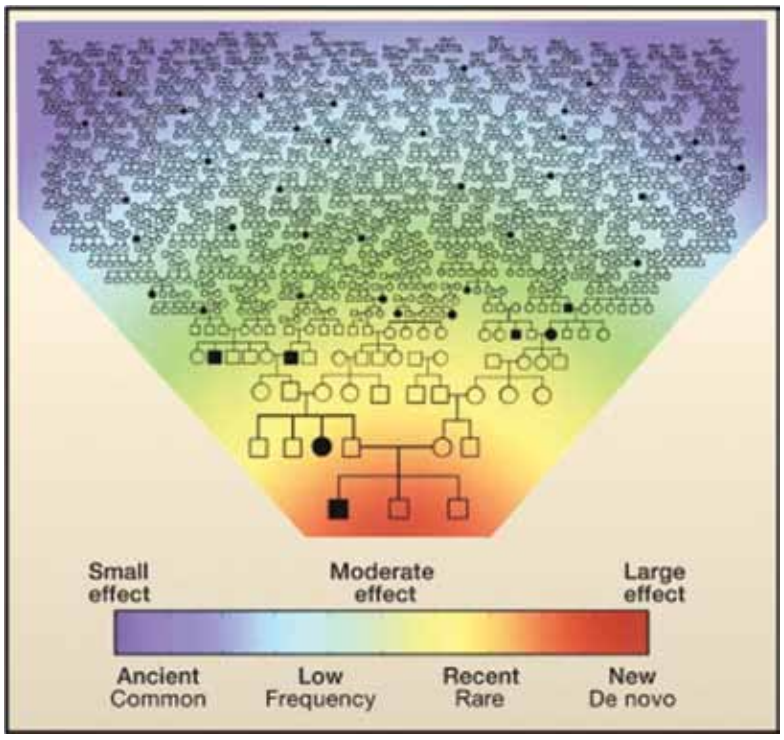
Boerwinkle E (2012) Translational genomics is not a spectator sport. *Genet Epi* 36: 85-87. Doi: 10.1002/gepi21607.

International Consortium for Blood Pressure Genome-Wide Association Studies (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature.* 478:103-9. doi: 10.1038

Lupski JR, Belmont JW, Boerwinkle E, Gibbs RA. (2011) Clan genomics and the complex architecture of human disease. *Cell.* 2011 147: 32-43. PMID: 21962505

LAB MEMBERS

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



Clan GenomicsHeat map and extended pedigree showing the conceptual relationship among de novo mutations leading to disease (red), recent mutations with moderate effects arising within a clan (yellow and green), and older common variants with small effects segregating in the population (blue). An individual's genetic disease risk emerges from the collection of variants he or she has inherited from both parental lineages of distant ancestors (typically common and of individually small effect), more recent ancestors (rare, but potentially larger effect), and de novo mutations.

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

High blood pressure: causes and consequences

As we age, our kidney function declines. The best predictor of whether an individual will lose enough kidney function to require dialysis is whether they have a first- or second-degree relative who has reached end-stage renal disease. This indicates that inherited factors influence risk of renal disease. What is not clear is whether these heritable factors influence risk of diseases that accompany declining renal function, like high blood pressure and diabetes, or whether these diseases are necessary, but insufficient, for renal disease. At present, there are no therapies that provide kidney protection. This is because the mechanism of renal functional decline is not known. Kidneys are difficult to study in humans because they lie deep within the body and their functional units, the glomeruli and nephrons, are microscopic structures. We have developed and study a rat model of renal injury in the presence of high blood pressure. We have two very closely related rat lines that share similar genetic elevation of blood pressure, but one line gets renal disease while the other does not. The renal disease is similar in every way to that present in humans with high blood pressure. By combining functional studies with genetic studies, this model is yielding fascinating insight into the mechanism of disease. Our work indicates that the susceptibility to renal disease is inherited separately from susceptibility to high blood pressure. We have found evidence that genetic variation in important elements of the immune system and the signaling pathways activated by the immune system play a key role in susceptibility to injury. Remarkably, immunosuppressant drugs prevent disease and death in these animals. These observations are leading toward the conclusion that, while high blood pressure or diabetes may injure the kidney, it is the response of the immune system to this injury that determines whether normal renal function is sustained or lost. Our current work is seeking to identify the explicit changes in genes that produce renal disease susceptibility so as to offer the possibility of therapies that target renal disease without producing overall suppressant effect on the immune system. This disease is important: more people die in the US each year from loss of renal function than from breast and prostate cancer combined. Furthermore, even mild loss of kidney function greatly amplifies the risk of death from other cardiovascular diseases.

RESEARCH PROJECTS

- Genetic mechanisms of elevated blood pressure
- Inherited susceptibility to renal disease
- Non-genomic mechanisms of trans-generational trait sharing

KEY PUBLICATIONS

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

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

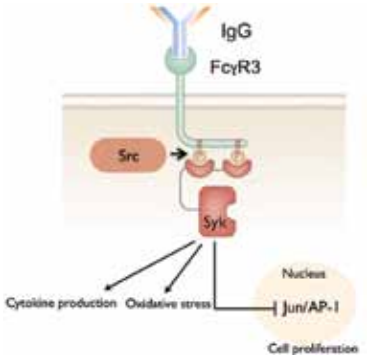
Bell, R., S.M.Herring, N. Gokul, M. Monita, M.L. Grove, E. Boerwinkle, P.A. Doris. High resolution identity by descent mapping uncovers the genetic basis for blood pressure differences between SHR lines. *Circulation (Cardiovascular Genetics)*. 4:223-31, 2011

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

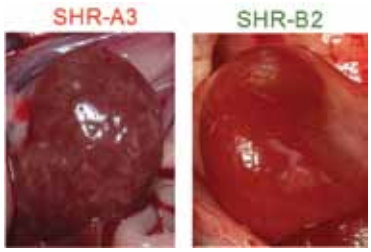
Braun, M.C. and P.A. Doris. Mendelian and trans-generational inheritance in hypertensive renal disease. *Annals Medicine*, 2012. In Press.

LAB MEMBERS

Stacy Herring



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



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

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

Genetic Basis of Brain Vascular Disease

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Fornage M*, Debette S*, Bis JC*, Schmidt H*, Ikram MA*, Dufouil C*, Sigurdsson S*, Lumley T, DeStefano AL, Fazekas F, Vrooman H, Shibata DK, Maillard P, Zijdenbos A, Smith AV, De Boer R, Cushman M , Mazoyer B, Heiss G, Vernoij MW, Enzinger C, Glazer NL, Beiser A, Knopman DS, Cavalieri M, Niessen W, Harris TB, Petrovic K, Lopez OL, Au R, Lambert J-C, Hofman A, Gottesman RF, Garcia M, Heckbert SR, Atwood L, Catellier DJ, Uitterlinden AG, Yiang Q, Smith NL, Aspelund T, Romero JR, Rice K, Taylor KD, Nalls M, Rotter JI, Sharret R, van Duijn CM, Amouyel P, Wolf PA, Gudnason V, van der Lugt A, Boerwinkle E, Psaty BM, Seshadri S, Tzourio C, Breteler MMB, Mosley TH, Schmidt R, Longstreth WT, DeCarli C, Launer LJ. A locus on chromosome 17q25 influences burden of MRI-defined cerebral white matter hyperintensities in individuals of European descent. *Ann. Neurol.* 2011, 69: 928-939 (* denotes equal authors contribution)

Lemaitre RN*, Tanaka T*, Tang W*, Manichaikul A*, Foy M*#, Kabagambe E, Nettleton JA, King IB, Weng L-C, Bhattacharya S#, Bandinelli S, Bis JC, Rich SS, Jacobs Jr DR, Cherubini A, McKnight B, Liang S, Gu X, Rice K, Laurie CC, Lumley T, Browning BL, Psaty BM, Friedlander Y, Djousse L, Hu J, Siscovick DS, Uitterlinden AG, Arnett DK, Ferrucci L*, Fornage M*, Tsai MY*, Mozaffarian D*, Steffen LM*. Genetic Loci Associated with Plasma Phospholipid n-3 Fatty Acids: A Meta-Analysis of Genome-wide Association Studies from the CHARGE Consortium. *PLoS Genetics* 2011, 7: e1002193 (* denotes equal authors contribution; # denotes trainee under my mentorship)

Carty CL, Buzková P, Fornage M, Franceschini N, Cole S, Heiss G, Hindorff LA, Howard BV, Mann S, Martin LW, Zhang Y, Matise TC, Prentice R, Reiner AP, Kooperberg C. Associations Between Incident Ischemic Stroke Events and Stroke and Cardiovascular Disease-Related GWAS SNPs in the Population Architecture Using Genomics and Epidemiology (PAGE) Study. *Circ Cardiovasc Genet.* 2012 Mar 8. (Epub ahead of print)

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

Bis JC*, DeCarli C*, Smith AV*, van der Lijn F*, Crivello F*, Fornage M*, Debette S*, Shulman JM, Schmidt H, Srikanth V, Schuur M, Yu L, Sigurdsson S, Verhaaren BFJ, DeStefano AL, Lambert J-C, Clifford JR, Struchalin M, Stankovich J, Ibrahim-Verbaas CA, Fleischman D, Zijdenbos A, den Heijer T, Choi S-H, Mazoyer B, Coker LH, Enzinger C, Brown M, Amin N, Arfanakis K, van Buchem M, de Bruijn R, Beiser A, Dufouil C, Huang J, Cavalieri M, Thomson R, Nissen WJ, Chibnik LB, Gislason GK, Hofman A, Pikula A, Amouyel P, Freeman KB, Phan T, Oostra BA, Stein JL, Medland SE, Arias Vasquez A, Wright MJ, Frankel B, Martin NG, Thompson PM, the ENIGMA Consortium, Nalls MA, PhD42, Uitterlinden AG, Au R, Elbaz A, Beare R, van Swieten JC, Lopez O, Harris TB, Breteler MMB, De Jager PL, Becker J, Vernooij MW, Knopman D, Fazekas F, Wolf PA, van der Lugt A, Gudnason V, Longstreth Jr. WT, Demoy P, Bennett DA, van Duijn CM*, Mosley TH*, Schmidt R*, Tzourio C*, Launer LJ*, Ikram MA*, Seshadri S*, for the CHARGE consortium. Genome-wide association studies implicate loci on Chromosome 2 in hippocampal volume. *Nature Genetics* 2012 (accepted)

LAB MEMBERS

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

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

Molecular Genetics of Atherogenesis and the Development of Genetic and Cell Therapies for the Treatment of Atherosclerotic Vascular Diseases

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Hua Sun, Amin Samarghandi, Ningyan Zhang, Zemin Yao, Momiao Xiong, and Ba-Bie Teng: PCSK9 Interacts with Apolipoprotein B and Prevents its Intracellular Degradation, Irrespective of the LDL Receptor (2012) *Arteriosclerosis, Thromb, and Vasc Biol,* In press

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

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

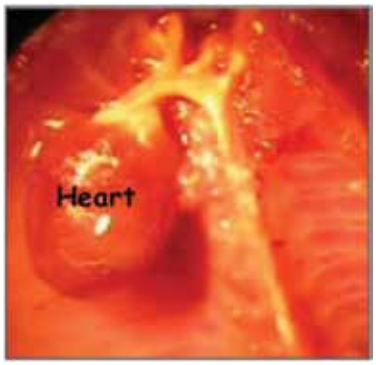
Shumei Zhong, Shihua Sun, and Teng BB: The recombinant adeno-associated virus vector (rAAV2)-mediated apolipoprotein B mRNA-specific hammerhead ribozyme: a self-complementary rAAV2 vector improves the gene expression. (2004) *Genetic Vaccines and Therapy* 2: 5.

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

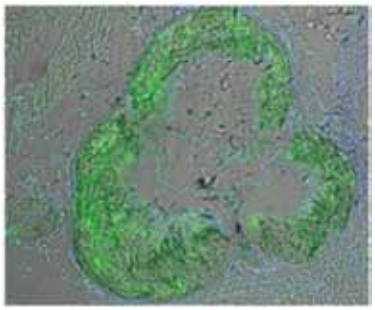
Wang J-P, Enjoji M, Tiebel M, Ochsner S, Chan L, and Teng BB: Hammerhead Ribozyme Cleavage of Apolipoprotein B mRNA Generates a Truncated Protein. (1999) *J. Biol. Chem.* 274: 24161-24170

LAB MEMBERS

Post Docs: Yuchun Wang, MD
PhD Student: Hua Sun
Research Assistants: Hersharan Nischal, Guottua Ji



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



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

Center for Immunology and Autoimmune Diseases

The Hans J. Müller-Eberhard and Irma Gigli Center for Immunology and Autoimmune Diseases is examining the molecular and genetic bases of several different allergic, autoimmune, and infectious diseases involving distinct organs. 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 being used as models for human disease. These animal studies have facilitated the identification of key gene products that play significant roles in regulating the immune system as well as contributing to the pathogenesis of human disease. Results from these research efforts have identified several therapeutic targets for the treatment of asthma, septic shock, and lupus erythematosus. As part of its major interest in pulmonary immunity, the center has recently established a robust research program focused on the development of stem cell therapeutics for the treatment of acute and chronic lung diseases and for genetic deficiencies that affect normal lung function. The center's scientists also are actively pursuing the generation of genetically engineered stem cell lines that will avoid immune mediated graft rejection during transplantation procedures.

Rick Wetsel, Ph.D.
Center Director & Professor

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

Innate Immunology and Inflammation, Lung Disease, and Pulmonary Regenerative Medicine

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Wetsel RA, Wang D, Calame DG. Therapeutic potential of lung epithelial progenitor cells derived from embryonic and induced pluripotent stem cells. *Annu. Rev. Med.* 2011, 62:95-105.

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

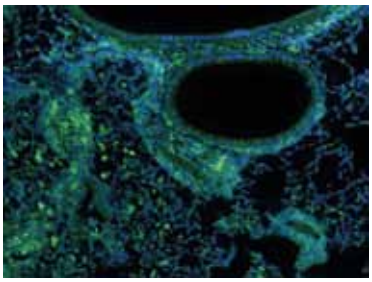
Mueller-Ortiz SL, Wang D, Morales JE, Li L, Chang JY, Wetsel RA. Targeted disruption of the gene encoding the murine small subunit of carboxypeptidase N (CPN1) causes susceptibility to C5a anaphylatoxin-mediated shock. *J. Immunol.* 2009, 182:6533-6539.

Hollmann TJ, Mueller-Ortiz SL, Braun MC, Wetsel RA. Disruption of the C5a receptor gene increases resistance to acute gram-negative bacteremia and endotoxic shock: opposing roles of C3a and C5a. *Mol. Immunol.* 2008, 45:1907-1915.

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

LAB MEMBERS

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



Yeonseok Chung, Ph.D.
Assistant Professor

T cell regulation and function in diseases

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

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

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

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

RESEARCH PROJECTS

- Understanding helper T cell responses in mucosal area upon exogenous stimuli
- Molecular regulation of follicular regulatory T cells and its application
- Role of metabolic factors in shaping adaptive immunity
- Developing novel vaccine approaches for cancer and infectious agents
- Understanding the biology of IL-10 family cytokines

KEY PUBLICATIONS

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

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

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

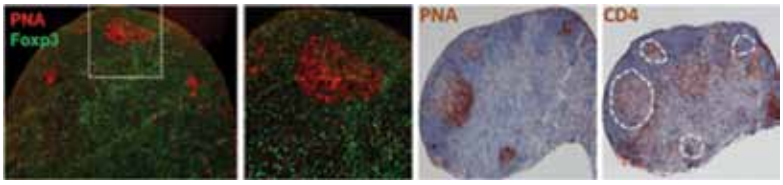
Nurieva RI, Chung Y. Understanding the development and function of T follicular helper cells. *Cell. Mol. Immunol.* 2010: 7: 190

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

LAB MEMBERS

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

Subsets of helper and regulatory T cells



Germinal center reaction

Scott Drouin, Ph.D.
Assistant Professor

Molecular Mechanisms Underlying Airway Obstruction & Inflammatory Diseases of the Lung

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Kiss A, Montes M, Jaensen E, Drouin SM, Wetsel RA, Yao Z, Martin R, Kheradmand F, Corry DB: A Pathogen-Activated Cellular Homing Pathway that Instructs Allergic Inflammation. *J Allergy Clin Immunol.* 2007: 120: 334-342.

Dillard P, Wetsel R, Drouin SM: The Complement Anaphylatoxin C3a Regulates Muc5ac Expression by Airway Epithelial Clara Cells Independently of TH2 Responses. *Am J Resp Crit Care Med.* 2007: 175: 1250-1258.

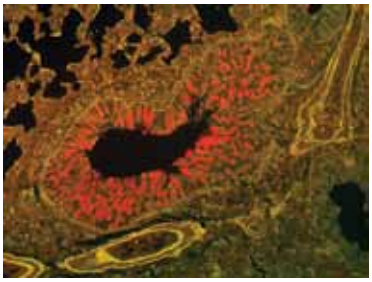
Huber-Lang M, Vidya Sarma J, Zetoune FS, Rittirsch D, Neff TA, McGuire SR, Lambris JD, Warner RL, Flierl MA, Hoesel LM, Gebhard F, Younger JG, Drouin SM, Wetsel RA, Ward PA: Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* 2006: 12: 682-687.

Drouin SM, Corry DB, Kildsgaard J, Hollman TJ, Wetsel RA: Absence of the Complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy. *J. Immunol.* 2002: 169: 5926-5933.

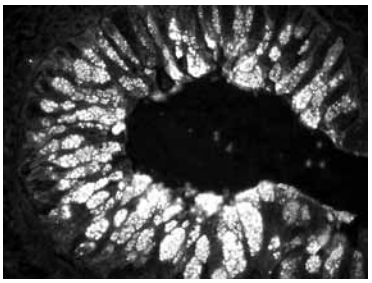
Drouin SM, Corry DB, Kildsgaard J, Wetsel RA: Cutting Edge: The Absence of C3 Demonstrates a Role for Complement in Th2 Effector Functions in a Murine Model of Pulmonary Allergy. *J. Immunol.* 2001: 167: 4141-4145.

LAB MEMBERS

Research Scientist: Dr. Eva Morschl



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



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

Amber Luong, M.D.
Assistant Professor

Environmental triggers regulating immunological mechanisms of chronic airway inflammation

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

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

CRS is clinically classified into 2 groups defined by the absence or presence of nasal polyps (see image 1). This clinical classification has been supported by immunologic profiles of the inflamed sinus tissue in which CRS without nasal polyps are characterized by predominance of neutrophils and elevated T helper cell type 1 (Th1) cytokines while CRS with nasal polyps (CRSwNP) have high presence of eosinophils, mast cells, and basophils and expression of T helper cell type 2 (Th2) cytokines such as IL-4, IL-5, and IL-13.

AFRS is associated with an accumulation of thick entrapped mucus laden with fungal hyphae and eosinophils between the nasal polyps and within sinus cavities. This trapped mucus can cause expansion of sinus cavities and ultimately erosion of bone separating the sinuses from the intracranial and orbital cavities which can result in intracranial complications and blindness, respectively (see image 2).

Dr. Luong marries her clinical and research interests though studies on AFRS. She is currently interested in understanding how fungi stimulate the adaptive immune response in AFRS as well as other CRS subtypes. In addition, she is interested in molecular pathways stimulated by fungi and other environmental triggers such as bacteria and viruses within respiratory epithelial cells that lead to the exaggerated Th2 immune response.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

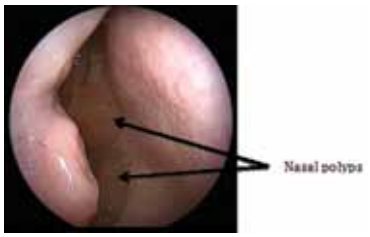
Clark DW, Wenaas AE, Luong A, Citardi MJ, and Fakhri S. Chronic Rhinosinusitis with Nasal Polyps: Elevated serum IgE is associated with Staphylococcus aureus on culture. *Int Forum of Allergy Rhinol.* 2011 Nov;1(6):445-50. PMID:22144053

Isaacs, S, Fakhri S, Luong A, and Citardi MJ. A meta-analysis of topical amphotericin B for the treatment of chronic rhinosinusitis. *Int Forum of Aller and Rhin.* 2011 Jul-Aug;1(4):250-4.

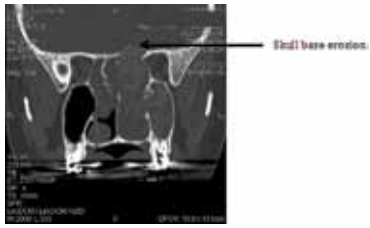
Porter PC, Yan, T, Luong A, Delclos GL, Abramson SL, Kheradmand F, and Corry DB. Proteinases as Molecular Adjuvants in Allergic Airway Disease. *Biochim Biophys Acta.* 2011 Nov;1810(11):1059-65. PMID: 21712069

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

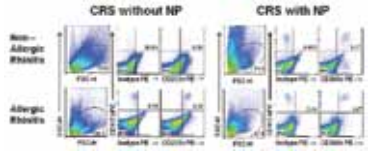
Shaw JL, Ashoori F, Fakhri S, Citardi MJ, and Luong AL. Increased Percentage of Mast Cells within Sinonasal Mucosa of Chronic Rhinosinusitis with Nasal Polyp Patients Independent of Atopy. *Int Forum of Allergy Rhinol*., 2012 Feb 16. [Epub ahead of print]



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



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



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

Dachun Wang, M.D.
Assistant Professor

Lung Stem/Progenitor Cells and Tissue Regeneration

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Wang D., Morales J.E., Calame D.G., Alcorn J.L. and Wetsel R.A. Transplantation of Human Embryonic Stem Cell-Derived Alveolar Epithelial Type II Cells Abrogates Acute Lung Injury in Mice. *Molecular Therapy.* 2010; 10: 3, 526-634 mar.

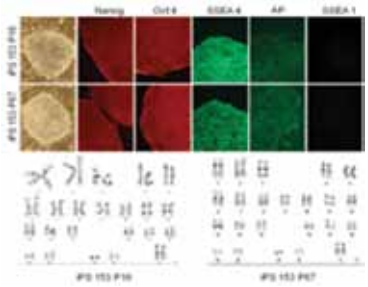
Mueller-Ortiz SL, Wang D., Morales JE, Li L. Change JF. Wetsel RA. Targeted disruption of the gene encoding the murine small subunit of carboxypeptidase N (CPN1) causes susceptibility to C5a anaphylatoxin-mediated shock. J *Immunol.* 2009; 182: 6533-6539.

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

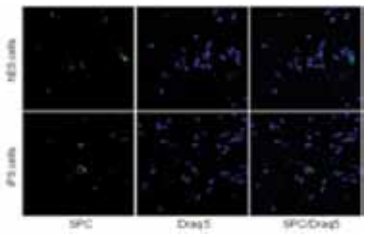
Zhang S., Wang D., Estov Z., Raj S., Willerson JT, and Yeh ET. Both Cell Fusion and Transdifferentiation Account for the Transformation of Human Peripheral Blood CD34-Positivie Cells Into Cardiac myocytes *in Vivo*. *Circulation* Dec. 110 (25):3803-7 (2004).

LAB MEMBERS

Postdoctoral fellow: Dr. Huanhuan Sun
Research Assistant: Yuan Quan



Lung disease-specific iPS ells



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

Eva Zsigmond, Ph.D.
Assistant Professor

Transgenic and Stem Cells Core Facility

The Transgenic and Stem Cells Core Facility was established in 1998 and since that time, it has generated over 700 new transgenic and knock-out mouse animal models for investigators from UTHealth, as well as for scientists from numerous other academic institutions. The laboratory has derived more than 20 new cell lines that have been highly effective for the generation of knock-out/ knock-in mice, as well as for cell differentiation studies. The Core Facility has had a 100% success rate of germline transmission in the production of knock-out mice when using mouse embryonic stem cells that have been derived in the laboratory. In addition to the production, cryopreservation and re-derivation of genetically-engineered mice and rats, the services of the facility also include gene targeting, derivation of new cell lines and technical support in different aspects of animal microsurgery, cell culture and stem cells research.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

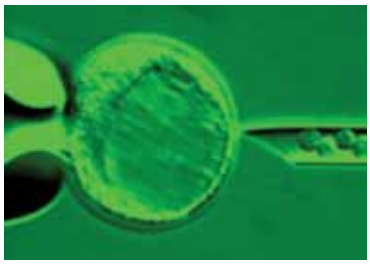
Wang. D., Haviland, D. L., Burns, A.L., Zsigmond, E. and Wetsel, R.A.: A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *PNAS.* 104:4449-4454, 2007.

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

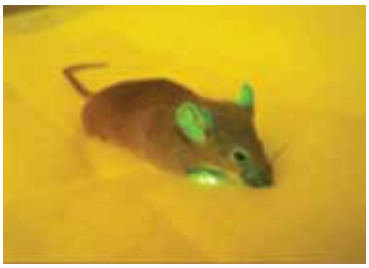
Kildsgaard, J., Zsigmond, E., Chan, L. and Wetsel, R. A.: A critical evaluation of the putative role of C3adesArg, ASP in lipid metabolism and hyperapobetalipoproteinemia. *Molec. Immunol.* 36: 869-876,1999.

LAB MEMBERS

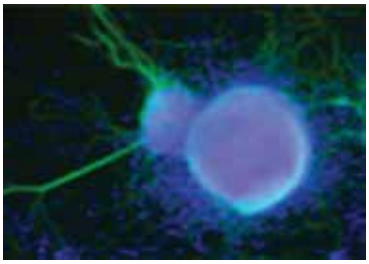
Manager: Aleksey Domozhirov
Research Assistant: Jing Yang



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



GFP-expressing transgenic mouse



Mouse ES cells undergoing neural differentiation

Center for Metabolic and Degenerative Diseases

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

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

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

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

Perry Bickel, M.D.
Associate Professor & Center Director

Perry Bickel, M.D.
Associate Professor and Director of the Center for Metabolic and Degenerative Diseases
Becker Family Foundation Professorship in Diabetes Research

Control of Fat Storage and Fat Burning

An important consequence of "overnutrition" is the accumulation of excess fat in the form of triglycerides in fat tissues and in other tissues such as muscle, liver, and heart. This lipid overload leads to cellular dysfunction that ultimately may progress to type 2 diabetes, nonalcoholic fatty liver disease, and heart failure. My lab seeks to discover the molecular mechanisms of lipid storage and utilization within cells, tissues, and organisms. The overall goal of my research program is to find new molecular targets for the development of nutritional or pharmacological strategies to prevent and treat diabetes and obesity in humans.

My lab focuses on the perilipin family of lipid droplet coat proteins, which are present to different degrees in the various tissues of the body. Our working hypothesis is that the level of these proteins determines the capacity of a tissue to store and/or burn fat in a healthy manner. For example, the protein S3-12 (also know as perilipin-4) is present almost exclusively in fat tissue, and we have shown that driving up the levels of S3-12 results in increased fat accumulation in cells. We have shown that S3-12 coats newly formed fat droplets and likely helps accommodate the acute boluses of fat that occur after a meal. OXPAT, also known as perilipin-5, is present in tissues that burn fat, including muscle, heart, liver, and brown fat tissue (a special form of fat tissue that burns fat to produce heat rather stores fat). In humans, the level of OXPAT in subcutaneous fat tissue decreases with increased obesity.

We have discovered that in cells stimulated to breakdown triglycerides into fatty acids and glycerol (a process known as lipolysis) OXPAT binds to a complex of proteins that regulates the activation of genes important for metabolizing the released fatty acids. Importantly, the presence of OXPAT in this protein complex increases the activation of the genes targeted by the complex. We hypothesize that OXPAT plays a key role in ratcheting up a cell's capacity to fully metabolize the fatty acids released from lipid droplets during lipolysis. Increasing the amount of OXPAT in muscle tissue, as occurs in the muscle of endurance-trained athletes, may protect against obesity-associated metabolic dysfunction, such as insulin resistance and diabetes mellitus.

RESEARCH PROJECTS

- The formation of lipid droplets in cells
- Lipid droplet proteins and gene regulation
- Lipid droplet proteins and mitochondrial function

KEY PUBLICATIONS

Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A, Bickel PE. S3-12, adipophilin, and TIP47 package lipid in adipocytes. *J. Biol. Chem.* 2005. 280(19):19146-19155.

Wolins NE, Quaynor BK, Skinner JR, Tzekov A, Park C, Choi K, and Bickel PE. OP9 mouse stromal cells rapidly differentiate into adipocytes: characterization of a useful new model of adipogenesis. *J. Lipid Res.* 2006. 47: 450-460.

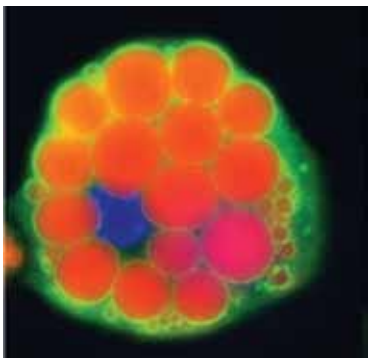
Wolins NE, Quaynor BK, Skinner JR, Tzekov A, Croce MA, Gropler MC, Varma V, Yao-Borengasser A, Rasouli N, Kern PA, Finck BN, and Bickel PE. OXPAT/PAT-1 is a PPAR-induced lipid droplet protein that promotes fatty acid utilization. *Diabetes.* 2006. 55:3418-3428.

Ducharme N and Bickel PE. Lipid droplets in lipogenesis and lipolysis. *Endocrinology.* 2008. 149:942-949.

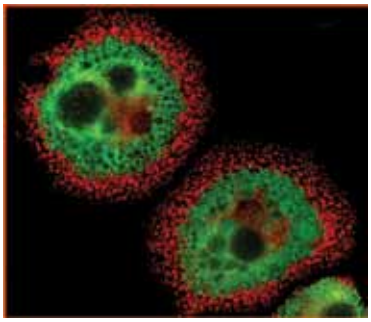
Amati F, Dubé JJ, Alvarez Carnero E, Edreira ME, Chomentowski1 P, Coen PM, Switzer GE, Bickel PE, Stefanovic-Racic M, Toledo FGS, and Goodpaster BH. Skeletal muscle triglycerides, diacylglycerols and ceramides in insulin resistance: Another paradox in endurance-trained athletes? *Diabetes.* 2011. 60(10):2588-2597.

LAB MEMBERS

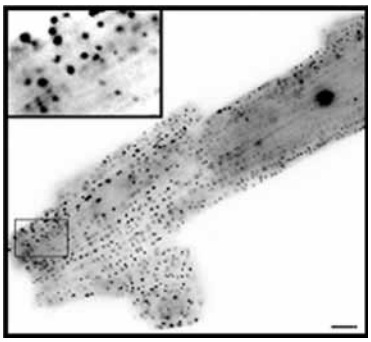
Postdoctoral Fellows: Dr. Violeta Gallardo-Montejano, Dr. Geetu Saxena



An OP9 mouse stromal cell treated with high dose insulin and long-chain fatty acids differentiates into a fat cell loaded with lipid droplets (red) that are coated with perilipin (green). The cell nucleus is stained blue. The Bickel lab characterized OP9 cells as a useful and robust model for studying fat cell biology [Wolins et al. *Journal of Lipid Research*. 2006.]



Staining of lipid droplets for S3-12 (red) and for perilipin (green) during lipid loading of a fat cells reveals spatially organized droplet formation. [Based on image in Wolins et al. *Journal of Biological Chemistry.* 2003.]



OXPAT coats lipid droplets in an adult mouse heart cell. [Wolins et al. *Diabetes.* 2006.]

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

Plasma Membrane Nanostructure and Signal Transduction

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

Specifically, we are investigating how the Ras membrane anchors cooperate with the G-domain and peptide sequences flanking the anchor to drive lateral segregation. Our work suggests new models are needed to explain how lipidated proteins interact with, and use, the plasma membrane to generate signaling platforms. We are interested in how confinement of signaling complexes onto a 2D surface in general and in plasma membrane nanodomains in particular regulates the kinetics and sensitivity of Ras signal signal output. Similarly, as we develop our spatial and proteomic maps of the plasma membrane, we can address how the composition and organization of the membrane alters in response to specific growth factors. We also have a major interest in characterizing the K-Ras endoplasmic reticulum to plasma membrane trafficking pathway. A recent focus of our work is to search for inhibitors of K-Ras plasma membrane association that may have utility as novel anticancer agents.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Zhou Y, Cho KJ, Plowman SJ, Hancock JF. (2012) Nonsteroidal anti-inflammatory drugs alter the spatiotemporal organization of ras proteins on the plasma membrane. *J Biol Chem.* Mar 19. [Epub ahead of print]

Cho KJ, Hill MM, Chigurupati S, Du G, Parton RG, Hancock JF (2011) Therapeutic levels of the hydroxmethylglutaryl-coenzyme A reductase inhibitor lovastatin activate Ras signaling via phospholipase D2. *Mol Cell Biol.* 31: 1110-20.

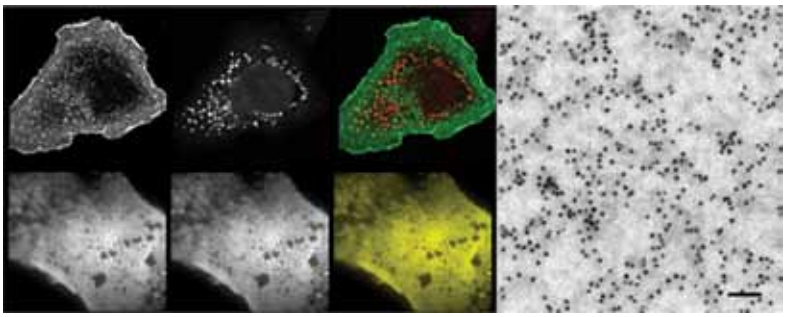
Abankwa D, Gorfe AA, Inder K, and Hancock JF (2010) Membrane orientation and nanodomain localization generate Ras isoform diversity. *Proc Natl Acad Sci USA*. 107:1130-1135.

Kholodenko BN, Hancock JF, Kolch W (2010) Signalling ballet in space and time. *Nat Rev Mol Cell Biol.* 11:414-26.

Hill MM, Bastiani M, Luetterforst R, Kirkham M, Kirkham A, Nixon SJ, Walser P, Abankwa D, Oorschot VM, Martin S, Hancock JF, Parton RG (2008) PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. *Cell.* 132: 113-24.

LAB MEMBERS

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



Ras localization imaged by confocal, TIRF and electron microscopy

Vihang Narkar, Ph.D.
Assistant Professor

Transcriptional Aerobics in Skeletal Muscle Diseases

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

In our laboratory, we are particularly interested in understanding how nuclear receptors – that are hormone or drug-activated transcriptional factors – regulate metabolic and contractile properties of the skeletal muscle. Recently, we identified a molecular interaction between serine/threonine kinase AMPK and nuclear receptor PPARδ that can be pharmacologically targeted to activate genes linked to mitochondrial biogenesis, fatty acid oxidation, and slow-twitch contractile myofibers in skeletal muscles and improve exercise endurance. These finding reveal that exercise-activated kinases and nuclear receptors are key components of myocellular transcriptional machinery controlling metabolism and fatigue. We are currently investigating the role of estrogen receptor-related receptors (ERR) – a class of orphan nuclear receptors – in skeletal muscle. ERR's and particularly ERRγ is highly expressed in oxidative slow-twitch muscle fibers suggesting a role for these receptors in the regulation of aerobic metabolism. We have genetically targeted ERRγ in mice to investigate the effect of skeletal muscle-specific receptor modification on myocellular gene expression, metabolism and fatigue. Furthermore, we are exploring the potential role of ERRγ in ameliorating diabetes and related muscle microangiopathy as well as muscular dystrophy.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Matsakas A, Yadav V, Lorca S, Evans RM, Narkar VA (2012) Revascularization of Ischemic Skeletal Muscle by Estrogen-Related Receptor-γ. *Circ Res.* In press

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

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

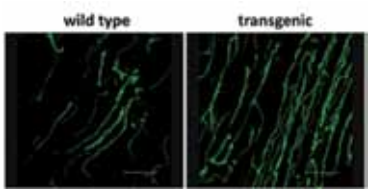
Matsakas A, Narkar VA. (2010) Endurance exercise mimetics in skeletal muscle. *Curr Sports Med Rep.* 9(4): 227-32.

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

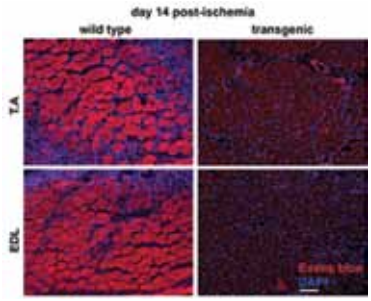
Barish GD, Narkar VA, Evans RM. (2006) PPAR delta: a dagger in the heart of the metabolic syndrome. *J Clin Invest*. 116(3): 590-7.

LAB MEMBERS

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



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



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

Qingchun Tong, Ph.D.
Assistant Professor

Mechanisms Underlying Brain Control of Body Weight and Glucose Homeostasis

Obesity and diabetes are imposing a huge burden on our society, while effective treatment is still lacking. A better understanding of the mechanisms regulating body weight and glucose homeostasis is required to develop new therapeutic strategies. Specific groups of neurons, especially those in the hypothalamus, receive and integrate nutritional status signals, and then adjust food intake and energy expenditure accordingly to maintain energy balance. Previous research has identified important functions of a few groups of hypothalamic neurons (e.g. POMC neurons, AgRP neurons, etc.) and a few hypothalamic genes (POMC, AgRP and MC4R, etc.) in feeding, energy expenditure and glucose homeostasis. However, the mechanisms and the neural pathways with which the brain and hypothalamus regulates energy balance are not well understood.

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

Ultimately we try to delineate specific neural pathways underlying specific physiologic functions, and provide a scientific rationale for effective therapeutic strategies against the current obesity and diabetes epidemic.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

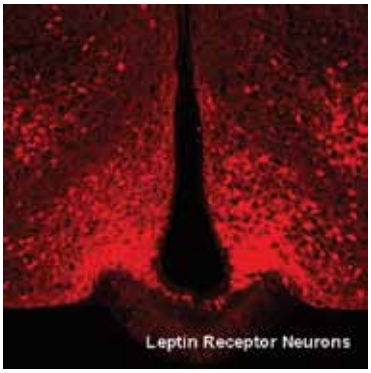
Kong D, Vong L, Parton LE, Ye C, Tong Q Hu X, Choi B, Bruning JC and Lowell BB. Glucose Stimulation of Hypothalamic MCH neurons involves KATP channels, is modulated by UCP2, and regulates peripheral glucose homeostasis. *Cell Metabolism*, 2010, 12(5): 545-552, PMID: 21035764.

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

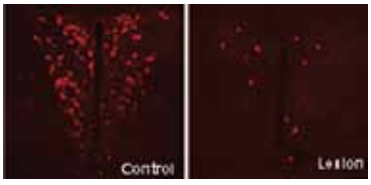
Tong Q, Ye C, McCrimmon RJ, Dhillon H, Choi B, Kramer MD, Yu J, Yang Z, Christiansen LM, Lee CE, Choi CS, Zigman JM, Shulman GI, Sherwin RS, Elmquist JK and Lowell BB. Synaptic Glutamate Release by Ventromedial Hypothalamic Neurons is Part of the Neurocircuitry that Prevents Hypoglycemia. *Cell Metabolism* 5(5):383-393, 2007. PMID: 17488640.

LAB MEMBERS

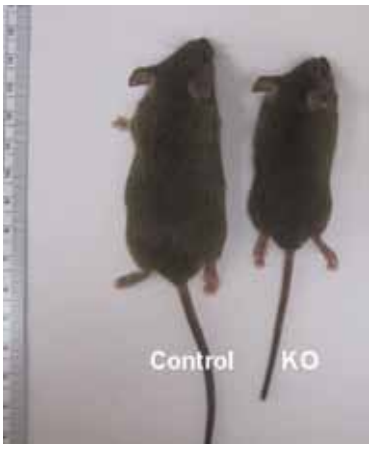
Post Docs: Yuanzhong Xu, Zhaofei Wu, Eun Ran Kim
Research Staff/Associate: Yaming Zhu
Visiting scientist: Rongjie Zhao



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



Specific lesion of oxytocin neurons using mouse genetics.



Disruption of GABA release from a subset of neurons (mainly in hypothalamus) leads to a an anorexia phenotype.

Rodrigo Fernandez-Valdivia, Ph.D.
Instructor

O-glucose regulation of Notch signaling in mammary tumorigenesis

Over 200,000 women will be diagnosed with breast cancer in the US this year, and over 40,000 lives will succumb to this disease. Moreover, survivors will constantly face the possibility of relapse from occult cancer cells (cancer stem cells?) capable of re-growing and metastasize. Notch signaling in mammary morphogenesis and tumorigenesis was first recognized when a frequent insertion site for the mouse mammary tumor virus was mapped to the Notch4 gene. This insertional event triggers expression of a constitutively active form of Notch4 causing mammary tumor formation and neoplastic progression. Moreover, transgenic overexpression of the active version of Notch1, 3, and 4 individually is sufficient to cause mammary tumorigenesis. Elevated expression of Notch pathway components in mammary tumors correlates with poor prognosis in breast cancer patients whereas downregulation of negative regulators of Notch signaling is consistently observed in these tumors.

Both Notch and Wnt pathways regulate mammary gland stem cell (MaSC) homeostasis. Indeed, Notch signaling is required for Wnt1 induced transformation of primary human mammary epithelial cells both in vitro and in vivo, indicating that a cross talk between these pathways is required for breast cancer progression. Remarkably, the Notch pathway is upregulated in both human and MMTV Wnt1 murine breast cancer stem cells. These observations indicate that Notch signaling exerts important roles in the etiopathogenesis of breast cancer, and that therapeutic targeting of the Notch pathway might provide an excellent opportunity to treat this disease.

By using innovative mouse and Drosophila genetics we recently discovered Rumi, the sole "protein O glucosyltransferase" present in mammals. Rumi regulates Notch signaling by adding glucose to specific serine residues on Notch receptors. Importantly, the number of O linked glucose residues on Notch determines the strength of signaling, and even removing one copy of Rumi affects Jagged1-induced signaling. I am testing the hypothesis that genetic ablation of Rumi can suppress breast cancer stem cell expansion and tumor progression in mouse models of breast cancer. In addition, I'm using my recently developed "GAP-repair mutagenesis" technology to engineer a new genetic mouse model that will allow specific genetic ablation in MaSCs. If my proposed work proves that a genetic decrease in Rumi can halt the progression of breast cancer in mice, pharmacological targeting of this new enzyme in human patients could serve as a novel therapeutic approach to prevent breast cancer relapse by inhibiting Notch signaling in breast cancer stem cells.

RESEARCH PROJECTS

- Rumi's control of Notch signaling in Mammary gland morphogenesis and tumorigenesis.
- Regulation of mammary gland stem cell (MaSC) homeostasis by O-linked glucose.
- Protein O-glucosylation on Notch-Wnt signaling cross-talk in mammary morphogenesis and tumorigenesis

KEY PUBLICATIONS

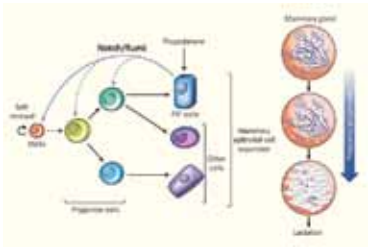
Fernandez-Valdivia R, Takeuchi H, Samarghandi A, Lopez M, Leonardi J, Haltiwanger RS, Jafar-Nejad H (2011) Regulation of the mammalian Notch signaling and embryonic development by the protein O-glucosyltransferase Rumi. *Development* 138(10):1925-1934.

Leonardi J*, Fernandez-Valdivia R*, Li Y, Simcox A, Jafar-Nejad H (2011) Multiple O-glucosylation sites on Notch function as a buffer against temperature-dependent loss of signaling. *Development* 138(16):3569-78. *Co-first authors.

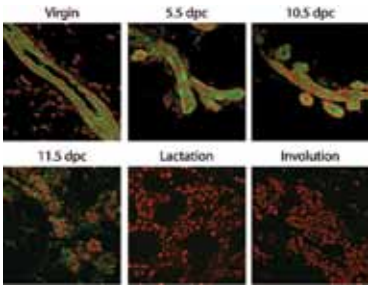
Takeuchi H, Fernández-Valdivia R, Caswell D, Nita-Lazar A, Rana N, Garner T, Weldeghiorghis T, Macnaughtan M, Jafar-Nejad H, Haltiwanger R (2011) Rumi functions as both a protein O glucosyltransferase and a protein O xylosyltransferase. *PNAS* 108(40):16600-16605.

Fernandez-Valdivia, R., Mukherjee A., Ying, Y., Li, J., Paquet, M., DeMayo, F., Lydon, J. P. (2009) The RANKL signaling axis is sufficient to elicit ductal side-branching and alveologenesis in the mammary gland of the virgin mouse. *Developmental Biology* 328(1):127-139.

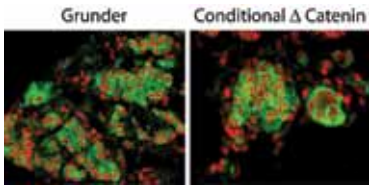
Fernandez-Valdivia, R., Mukherjee, A., Creighton, C. J., Buser, A. C., DeMayo F. J., Edwards D. P., Lydon J. P. (2008) Transcriptional Response of the Murine Mammary Gland to Acute Progesterone Exposure. Endocrinology 149(12):6236-50.



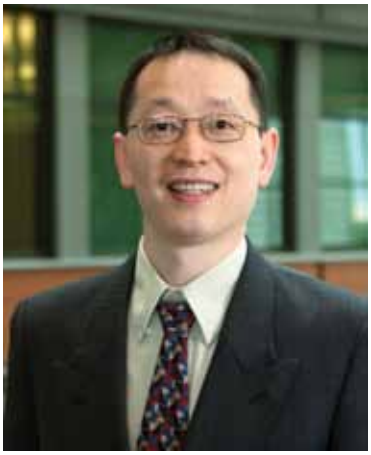
Rumi's control of Notch signaling in steroid hormone-dependent mammary gland morphogenesis and tumorigenesis



Dynamic expression of Rumi in mammary gland development.



Rumi expression in progesterone-dependent (Grunder) and Wnt signaling conditional activation mammary gland tumor models.



Sheng Zhang, Ph.D.
Assistant Professor

Studying Human Brain Degenerative Diseases Using the Fruit Fly

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

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

Our laboratory is focusing on the following projects:

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

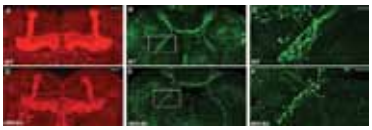
Zhang S, Xu L, Lee J, Xu T. (2002). *Drosophila* Atrophin homolog functions as a transcriptional co-repressor in multiple developmental processes. *Cell*, 108 (1): 45-56.

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

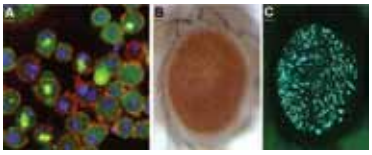
Hu G, Zhang S, Vidal M, Baer JL, Xu T, Fearon ER. (1997). Mammalian homologs of *seven in absentia* (sina) regulate DCC via the ubiquitin-proteasome pathway. *Genes & Development.* 11(20):2701-14.

LAB MEMBERS

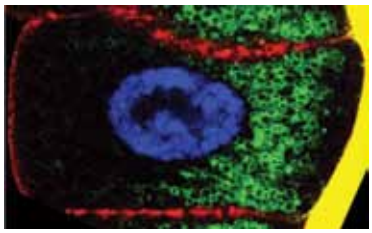
Post Docs: Dr. Zhen Xu, Dr. Yanning Rui, Dr. Dongsheng Chen
Students: Antonio Tito, Michael McCarthy
Technicians: Yamin Sun, B.S., Research Assistant I, Zhihua Chen, Ph.D., Research Associate



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



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



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

Center for Molecular Imaging

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

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

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

Currently, the team effectively translates new NIR molecular imaging technologies literally from "bench-to-bedside and back again," in efforts that embrace its division and clinical partners in the Texas Medical Center. The CMI is one of four centers in the United States comprising the National Cancer Institute's Network for Translational Research.

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

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

Eva Sevick-Muraca, Ph.D.
Professor, Cullen Chair of Molecular Medicine, & Center Director

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

Molecular Imaging and Diagnostics

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Maus EA, Tan IC, Rasmussen JC, Marshall MV, Fife CE, Smith LA, Guilliod R, Sevick-Muraca EM. :Near-infrared fluorescence imaging of lymphaticsin head and neck lymphedema. Head Neck. 2012 Mar; 34(3):448-53.doi: 10.1002.hed.21538. Epub 2010 Nov 12. PMID: 22311465[PubMed – in process]

Lapinski PE, Kwon S, Lubeck BA, Wilkinson JE, Srinivasan RS, Sevick-Muraca E, King PD: RASA1 maintains the lymphatic vasculature in a quiescent functional state in mice. J Clin Invest. 2012 Feb 1;122(2):733-47. Doi: 10.1172/JCI46116. Epib 2012 Jan 9. PMID:22232212[PubMed – indexed for MEDLINE] Free PMC article

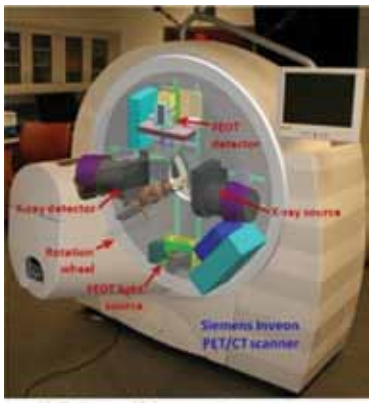
Lu Y, Zhu B, Darne C, Tan IC, Rasmussen JC, Sevick-Muraca EM: Improvement of fluorescence-enhanced optical tomography with improved optical filtering and accurate model-based reconstruction algorithms. J Biomed Opt. 2011 Dec; 16(12):126002. PMID:22191919[PubMed – in process]

Sevick-Muraca EM: Translation of near-infrared fluorescence imaging technologies: emerging clinical applications. Annu Rev Med. 2012;63:217-31. Epub 2011 Oct27. PMID: 22034868[PubMed – in process]

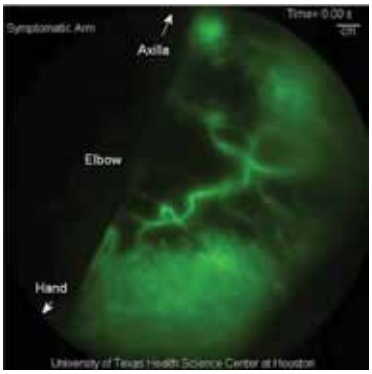
Hall MA, Kwon S, Robinson H, Lachance PA, Azhdarinia A, Randanathan R, Price RE, Chan W, Sevick-Muraca EM: Imaging prostate cancer lymph node metastases with a multimodality contrast agent. Prostate. 2012 Feb 1:72(2):129-46. Doi 10.1002/pros.21413. Epub 2011 May 2. PMID: 21538422[PubMed – indexed for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

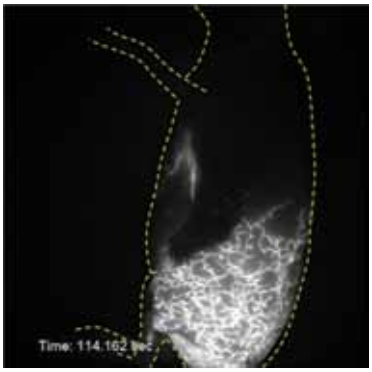
LAB MEMBERS

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



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





Ali Azhdarinia, Ph.D.
Assistant Professor

Molecular imaging probe development

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

RESEARCH PROJECTS

- Development of molecular imaging probes with radioactive and near-infrared labels
- Synthesis of novel chelation platforms for radiolabeling and drug design
- Design of new near-infrared dyes
- Pharmacological evaluation of imaging probes targeting tumors and other molecular processes

KEY PUBLICATIONS

Hall, M.A., Kwon, S., Robinson, H., Lachance, P.A., Azhdarinia, A., Ranganathan, R., Price, R.E., Chan, W., Sevick-Muraca, E.M., Imaging prostate cancer lymph node metastases with a multimodality contrast agent. *Prostate*, 2012, 72, (2), 129-146.

Rodenberg, E., Azhdarinia, A., Lazard, Z.W., Hall, M.A., Kwon, S.K., Wilganowski, N., Salisbury, E., Merched-Sauvage, M., Davis, E.A., Sevick, E.M., Davis, A.R. MMP-9 is a diagnostic marker of heterotopic ossification in a murine model. *Tissue Eng Part A.* 2011 May 20. [Epub ahead of print], PMID:21599541.

Azhdarinia, A., Wilganowski, N., Robinson, H., Ghosh, P., Kwon, S., Lazard, Z.W., Davis, A.R., Olmsted-Davis, E., Sevick-Muraca, E.M. Characterization of chemical, radiochemical and optical properties of a dual-labeled MMP-9 targeting peptide. *Bioorg Med Chem.* 19(12):3769-76, 2011. PMID:21612930.

Azhdarinia, A., Ghosh, P., Ghosh, S., Wilganowski, N., Sevick-Muraca, E.M. Dual-labeling Strategies for Nuclear and Fluorescence Molecular Imaging: a Review and Analysis. *Mol Imaging Biol.* 2011 Dec 13. [Epub ahead of print], PMID:22160875.

Moss, J.A., Vãvere, A.L., Azhdarinia, A. Design of Peptide Imaging Agents for Whole-body and Intraoperative Molecular Imaging. Curr Med Chem, 2012 (accepted).

LAB MEMBERS

Research scientists: Sukhen Ghosh, Pradip Ghosh

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

Multimodality imaging of new bAU8 bone formation with ^{64}Cu-M_2. NIR fluorescence (left) and μPET/CT images (middle) acquired on day 4 post-implantation show early detection of matrix metalloproteinase activity by molecular imaging prior to anatomical changes that were visualized on the follow-up μCT scan (right) performed on day 11. (adapted from Rodenberg, E. et al., *Tissue Eng Part A*, 2011, 17, (19-20), 2487-2496).

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

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

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

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

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

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

In addition, we are working with several other clinicians to identify genetic markers associated with genetic disorders like schizophrenia, Tuberous Sclerosis Complex, Dercum's disease, Adiposis dolorosa and Madelung's disease.

RESEARCH PROJECTS

- Genome and Bioinformatics Analysis of patients with Lymphedema.
- Genetic diagnostics using next generation sequencing: The CEO Genome Project.
- Identification of markers for Schizophrenia in patients from Houston .
- Identification of new alleles for Tuberous Sclerosis Complex (TSC) and Spina Bifida Cystica. Collaborator of Hope Nurthrup.
- Dercum's disease, Adiposis dolorosa, Madelung's disease. Collaborator of Karen L Herbst, M.D. UC San Diego.

KEY PUBLICATIONS

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

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

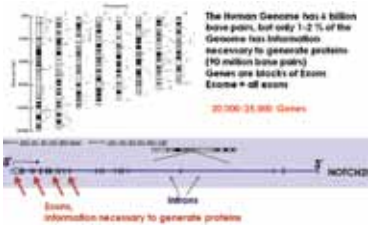
Sea Urchin Genome Sequencing Consortium. 2006. The genome of the sea urchin Strongylocentrotus purpuratus. *Science* 314:941-52. PMID: 17095691

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

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

LAB MEMBERS

Sr. Research Coordinator: Otis Hall, Jr.



Exon Capture and Exomes.

Basic steps in our variant analysis pipeline.

Barrett Rowland Harvey, Ph.D.
Assistant Professor

Therapeutic and Diagnostic Antibody Development

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Davies-Venn CA, Angermiller B, Wilganowski N, Kwon S, Aldrich MB, Wu G, Harvey BR, and E.M. Sevick-Muraca, "Albumin-binding domain conjugate for near-infrared fluorescence lymphatic imaging," *Molecular Imaging and Biology,* 2011. PMID: 21688052

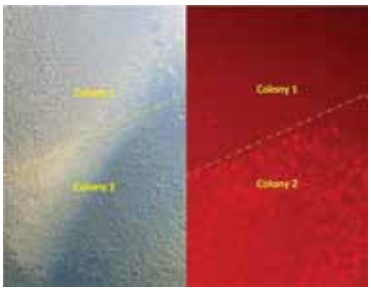
Gao P, Pinkston KL, Nallapareddy SR, van Hoof A, Murray BE, Harvey BR. "The *Enterococcus faecalis rnjB* is required for pilin gene expression and biofilm formation." *Journal of Bacteriology,* 192(20): 5489-98, 2010. PMID: 20729365

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

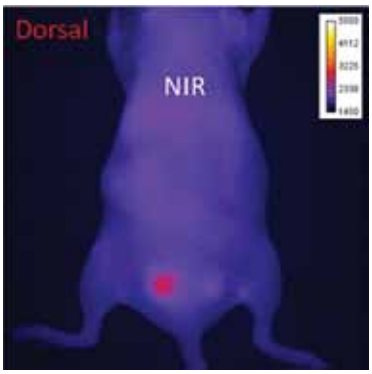
Harvey BR, Georgiou G, Hayhurst A, Iverson BL, and GK Rogers, "Anchored Periplasmic Expression (APEx), a Versatile Technology for the Isolation of High Affinity Antibodies from *E.coli* Expressed Libraries," *Proc Natl Acad Sci U S A,* 101(25): 9193-8, 2004. PMID: 15197275

LAB MEMBERS

Kenneth L. Pinkston – Research Coordinator II
Dr. Peng Gao – Research Instructor
Karina Vazquez-Arreguin – Research Assistant
Sarah Ho – Rice Undergraduate Student (Bios310)

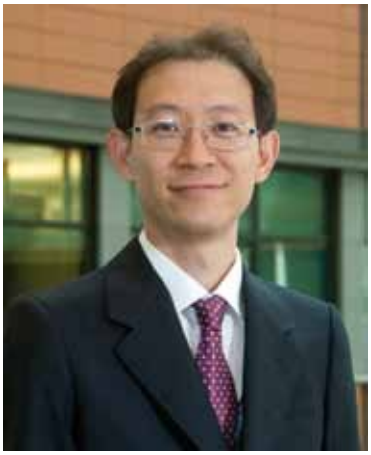


Evaluation of colonies from tetracarcinoma cell line for ligand induced DsRed signaling.



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

High throughput antibody selection.



Sunkuk Kwon, Ph.D.
Assistant Professor

Functional Imaging in Health and Disease

The main research interest of my program focuses on investigating the microcirculatory movement of fluid and macromolecules, particularly in the lymphatic system using fluorescence optical imaging techniques. The lymphatic system plays an important role in edema prevention, immune surveillance, cancer metastasis, as well as fluid/protein homeostasis. The alteration of lymphatic function can cause obesity, edema, diabetes, as well as other diseases. Although the importance of the lymphatic system in physiological and pathophysiological conditions has been well recognized, non-invasive imaging of lymphatic function has significant difficulties, due to the lack of diagnostic imaging approaches. Recently, we have developed non-invasive, dynamic near-infrared fluorescence (NIRF) imaging methods for imaging and quantifying lymphatic function in health and disease. Therefore, non-invasive NIRF imaging can be used to image changes of lymphatic function and architecture in disease and potentially to provide diagnostics and information in response to therapy.

Another area of interest is to non-invasively and quantitatively image intestinal motility. We recently demonstrated non-invasive NIRF imaging with sufficient temporal resolution and sensitivity to quantitatively assess different patterns of dynamic contractile function of the murine intestine resulting from the secretion of fluorescent bile after injection of ICG. Moreover, we non-invasively imaged for the first time intestinal motions using autofluorescence induced by standard murine diet containing chlorophyll without an exogenous imaging agent. Based upon preliminary data, my research focuses upon imaging altered intestinal contractile function in genetically engineered models of GI motility disorders/dysfunction and in animal models of post-infectious and post-inflammatory irritable bowel syndrome.

Other direction of my scientific interests is multi-modality molecular imaging. The Center for Molecular Imaging is developing and translating imaging agents, which are dual-labeled with a PET/SPECT radiotracer and a NIR fluorescent dye. I lead the preclinical imaging core for the Network for Translational Research (NTR) that is focused upon demonstrating agent feasibility. For example, I will conduct molecular imaging of (i) neurogenesis, (ii) myocardial infarction and heterotopic bone formation using matrix metalloproteinase (MMP)-2 and -9 inhibitors, (ii) inflammation using COX-2 inhibitors, and (iii) cancer and LN metastasis using antibodies and etc.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

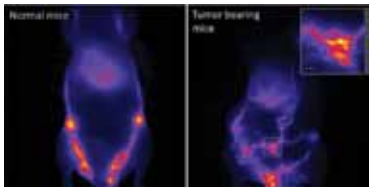
Lakshmi S., Kwon S., Ke S., Schiff R., and Sevick-Muraca E. M., Dual-labeled Trastumab-based imaging agent for the detection of HER-2 receptor overexpression in metastatic breast cancer. *Journal of Nuclear Medicine*, 48; 1501-1510, 2007 (Cover of issue).

Kwon S. and Sevick-Muraca E. M., Functional lymphatic imaging in tumor-bearing mice. *Journal of Immunological Methods*, 360; 167-172, 2010.

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

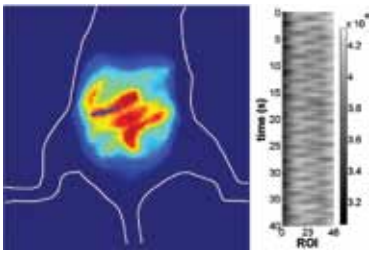
Kwon S. and Sevick-Muraca E. M., Non-invasive, dynamic imaging of murine intestinal motility. *Neurogastroenterology and Motility*, 2011. 23: 881-e344.

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



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

Extensive lymphatic vessel hyperplasia in induced RASA1 deficient mice.



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

John Rasmussen, Ph.D.
Assistant Professor

NIRF Imaging of Lymphovascular Disorders

Our research focuses on the use of optical imaging to further our understanding of biological systems and to develop diagnostic and therapeutic approaches for a variety of diseases, including cancer and lymphovascular disorders. The lymphatic system is a secondary circulation system that plays a significant role in fluid homeostasis, protein transport, immune response, and cancer metastasis. The extreme case of lymphatic failure or lymphedema is a poorly understood, incurable disease marked by chronic swelling, tissue fibrosis, impaired immune response, and significant reduction in quality of cancer survivorship. Traditional clinical imaging modalities such as scintigraphy, X-ray, MRI, and ultrasound lack the spatial and/or temporal resolutions needed to resolve fine lymphatic architecture and contractile function and/or require quantities of contrast agent not easily introduced into the lymphatics.

Over the past few years, we have developed and translated near-infrared fluorescence (NIRF) optical imaging as a way to noninvasively image and characterize human lymphatics and quantify their contractile function in health and disease using microdose amounts of fluorescent contrast agent. My work currently focuses on the continued development of NIRF imaging and its application to new biological and clinical questions. Specifically we are using NIRF imaging to study the growth and reorganization of the lymphatics, termed lymphangiogenesis, to elucidate its role in the development of lymphovascular diseases such as lymphedema and cancer metastasis, as well as the role of the lymphatics in other diseases, such as rare adipose disorders, that may have a lymphovascular component. I am also working on further developing NIRF imaging instrumentation for clinical applications to evaluate emerging therapeutics against lymphangiogenesis and developing new analytical tools to facilitate lymphatic image processing.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

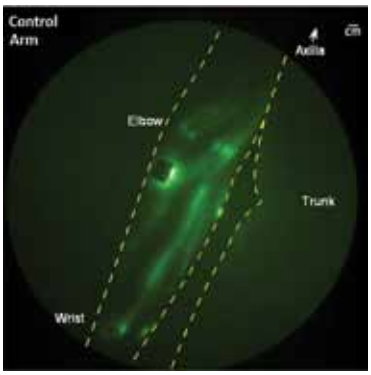
Rasmussen, J.C., Tan, I., Marshall, M.V., Adams, K.A., Kwon, S., Fife, C.E., Maus, E.A., Smith, L., Covington, K.R., and Sevick-Muraca, E.M., "Human lymphatic architecture and (dys)function imaged using NIR fluorescence," *Translational Oncology,* 3(6):362-372, 2010.

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

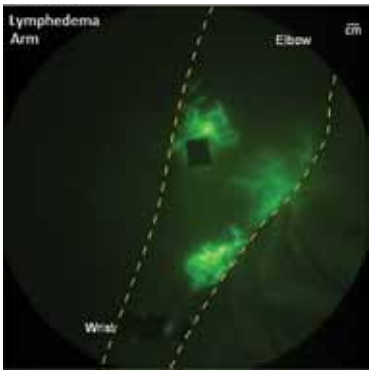
Rasmussen, J.C., Tan, I.C., Marshall, M.V., Fife, C.E., and E.M. Sevick-Muraca, "Lymphatic Imaging in humans with near-infrared fluorescence," *Current Opinion in Biotechnology,* 20: 74-82, 2009.

Sevick-Muraca, E.M. and Rasmussen, J.C., "Molecular Imaging with Optics: A Primer and a Case for Near-Infrared Fluorescence Techniques in Personalized Medicine," *Journal of Biomedical Optics,* 13(4): 041303, 2008.

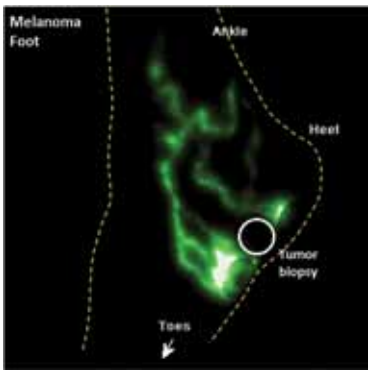
NOTE: all images are adapted from Rasmussen *et al.*, ABME, 40(2):408, 2012.



Lymphatics in a control arm



Lymphatics in an arm with lymphedema



Lymphatics draining a melanoma tumor

Center for Proteomics and Systems Biology

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

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

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

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

Hubs of Research Collaboration within the Center

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

David Gorenstein, Ph.D.
Professor, Center Director, & Deputy Director

David Gorenstein, Ph.D.
Professor and Director of the Center for Proteomics and Systems Biology
James T. Willerson Distinguished Chair in Cardiovascular Research

NanoMedicine and Proteomics in Cancer and Cardiovasculature Disease

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Gavin C. Bowick, Kizakhe V. Soman, He Wang, Judith F. Aronson, Bruce A. Luxon, Lee O. Lomas, David G. Gorenstein & Norbert K. Herzog, "Proteomic Analysis of Arenavirus Infection Identifies Differential Expression of Prothymosin-α," *J. Biomedicine and Biotechnology,* 2010;2010. pii: 956823. Epub 2010. PMID: 20706531

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

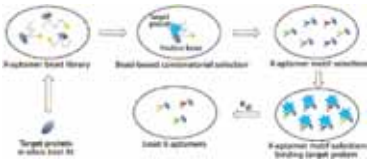
Aman Mann, Rohan Bhavane, Anoma Somasunderam, Brenda Liz Montalvo-Ortiz, Ketan B. Ghaghada, David Volk, René Nieves-Alicea, K. Stephen Suh, Mauro Ferrari, Ananth Annapragada, David Gorenstein, Takemi Tanaka, "Thioaptamer Conjugated Liposomes for Tumor Vasculature Targeting", *Oncotarget,* April, Vol.2,pp.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

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

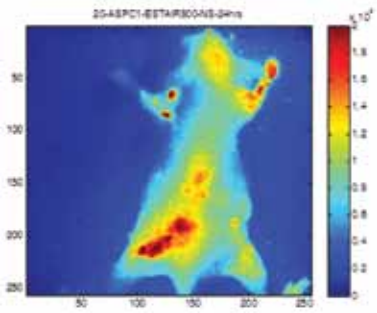
Aman P. Mann, Takemi Tanaka, Anoma Somasunderam Xuewu Liu, David G. Gorenstein, Mauro Ferrari, "Bone marrow targeted delivery of multistage vector via E-selectin", *Advanced Healthcare Materials,* 23, H278-H282 (2011) (Front page cover).

LAB MEMBERS

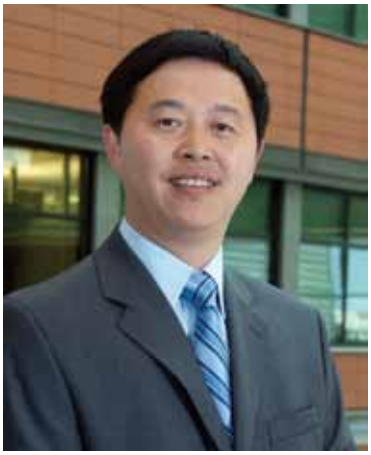
Research Scientists: Lokesh Rao, PhD, Hongyu Wang, PhD
Research Assoc.: Xin Li, MS, Li Li, PhD
Post Docs: Miguel-Angel Elizondo-Riojas, Weiguo He
Medical Students: Angela Sung, Max Polansky
Graduate Students: Sai Gandham, Kurtis Anderson



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



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



Chuantao Jiang, M.D., Ph.D.
Assistant Professor

Alpha-Synuclein for Parkinson's Disease

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

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

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

RESEARCH PROJECTS

- Biomarkers for early diagnosis of Parkinson's disease (PD)
- The functional roles of α-synuclein in PD pathogenesis
- α-synuclein knockdown in adult PD mouse model

KEY PUBLICATIONS

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

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

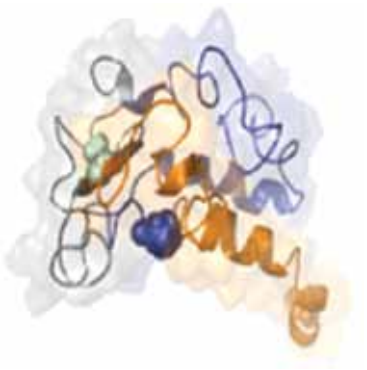
Jiang CT, Xiong W, Lu BY, Gonda MA, Chang JY. (2010) Synthesis and Immune Response of Non-native Isomers of Vascular Endothelial Growth Factor. *Biochemistry.* 49:6550-6556.

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

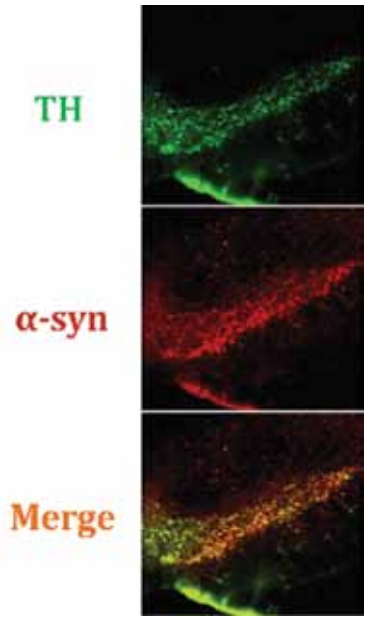
Xiong W, Hong DP, Chang JY, Mandredsson FP, Jiang CT*. (2012) Increased alpha-synuclein antibodies reduce brain alpha-synuclein levels without improving behaviors and pathology (In submission)

LAB MEMBERS

Research Associates: Wei Xiong (Currently appointed in MD Anderson Cancer Center)
Dong-Pyo Hong (Currently appointed as Assistant Professor at University of South Florida)



Alpha-synuclein Protein



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

Vimentin is a novel AKT1 target mediating motility and invasion

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Denner, L., Rosenblatt, K.P., Bodenburg, Y., Jiang, J., Nolan, R., Chilvers, R., Schnell, V.L., Ahmed, M., and Urban, R.J. (2012) IRE1α Regulates Steroidogenesis Through a Noncanonical Pathway. Molecular Endocrinology, in press.

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

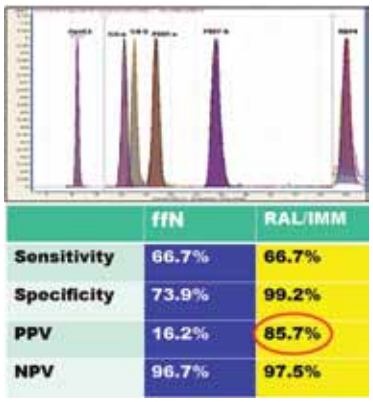
Rosenblatt, K.P., Huebschman, M.L., and Garner, H.R. (2012) Construction and Hyperspectral Imaging of Quantum Dot Lysate Arrays. In Methods of Molecular Biology: Individualized Molecular Medicine. Espina, V. and Liotta, L.A., eds. (New York: Humana Press, Inc.), pp 311-324.

Zhao, Y., Banerjee, S., Dey, N., Lejeune, W.S., Sarkar, P.S., Brobey, R., Rosenblatt, K.P., Tilton, R.G., and Choudhary, S. (2011) Klotho Depletion Contributes to Increased Inflammation in Kidney of the db/db Mouse Model of Diabetes Via RelA (Serine)536 Phosphorylation. Diabetes 60: 1907-1916. PMID: 21593200

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

LAB MEMBERS

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



	ffN	RAL/IMM
Sensitivity	66.7%	66.7%
Specificity	73.9%	99.2%
PPV	16.2%	85.7%
NPV	96.7%	97.5%

Multi-Marker Standards for BirthStat Blood Test for Pre-Term Labor. Using Selective Reaction Monitoring, a quantitative mass spectrometry platform for measuring multiple protein analytes without affinity reagents such as antibodies, we created standardized assays for measuring indicators and predictors of pre-term birth in maternal blood samples. The test has high Positive and Negative Predictive Value for diagnosing birth within 2 days after pregnant mothers experience symptoms (i.e. versus false labor).

David Volk, Ph.D.
Assistant Professor

Metabolomics, Proteomics and Nanomedicine

By combining powerful statistical analysis and bioinformatics methods with NMR- or MS-based measurements of metabolite or protein levels in living systems, mechanisms of disease pathways, or onset of disease can be studied. Most recently we have used such techniques to investigate the effects of ethanol and fatty liver disease, and the ingestion of chemicals used to refine uranium and plutonium and the resulting metabolite profile. We are using similar statistical methods to investigate protein markers of biobank sample stability and degradation in connection with the National Children's Study and with the onset of diabetes, to name a few areas.

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

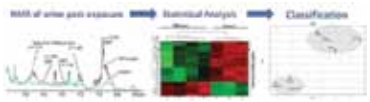
1H and 31P NMR-Based Lipidomics of Ethanol-Induced Fatty Liver. H. Fernando, S. Kondraganti, K. K. Bhopale, D. E. volk, M. Neerathilingam, B. S. Kaphalia, B. A. Luxon, P. J. Poor, G. A. S. Ansari, *Alcoholism: Clinical and Experimental Research* 34,: 1937-1947, 2010.

1H NMR Metabonomic Study of Rat Responce to Tri-Phenyl Phosphate and Tri-Butyl Phosphate Exposure. T.M. Alam, K. M. Alam, M. Neerathilingam, D. E. Volk, S. Sarkar, G. A. Shakeel Ansari, B. A. Luxon. *Metabonomics* 6: 386-394, 2010

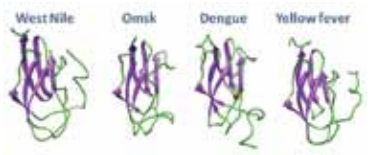
Yellow Fever Virus Envelope Protein Domain III. D. E. Volk, F. J. May, S. H. Gandham, A. Anderson, J. von Lindern, D. Beasley, A. D.T. Barrett and D. G. Gorenstein. *Virology* 394:12-18, 2009.

LAB MEMBERS

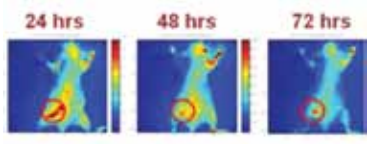
Research Scientists: Lokesh Rao, PhD, Hongyu Wang, PhD
Research Assoc.: Xin Li, MS, Li Li, PhD
Post Docs: Miguel-Angel Elizondo-Riojas, Weiguo He
Medical Students: Angela Sung, Max Polansky



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



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



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

Center for Stem Cell and Regenerative Medicine

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

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

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

Brian Davis, Ph.D.
Associate Professor and Center Director

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

Genetically Corrected Stem Cells for Treatment of Inherited Blood and Lung Diseases

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

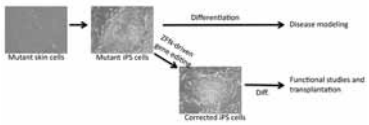
B.R. Davis and F. Candotti: Revertant somatic mosaicism in the Wiskott-Aldrich Syndrome. *Immunologic Research* 44:127-131, 2009.

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

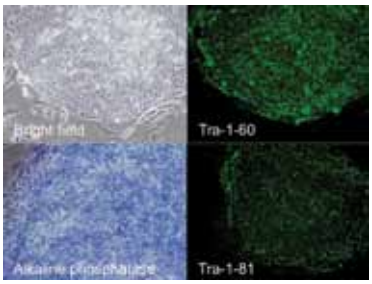
Davis BR, Yan Q, Bui JH, Felix K, Moratto D, Muul LM, Prokopishyn NL, Blaese RM and Candotti F. Somatic Mosaicism in the Wiskott-Aldrich Syndrome: Molecular and Functional Characterization of Genotypic Revertants. *Clinical Immunology* 135:72-83, 2010.

LAB MEMBERS

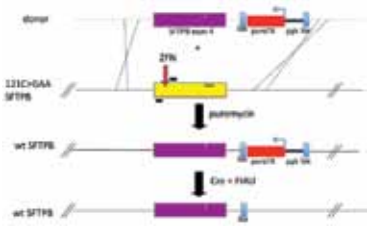
Research Scientist: Dr. Ana M. Crane
Postdoctoral Fellows: Dr. Philipp Kramer, Dr. Xuan Shirley Li, Dr. Rasoul Pourebrahamin
Ph.D. Students: Jacquelin Bui, Zita Garate (visiting student from Spain), Tamara Laskowski
Technician: Wei Liao



Application of Mutant and Corrected Induced Pluripotent Stem Cells



Characterization of Induced Pluripotent Stem Cells



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

Nathalie Brouard, Ph.D.
Instructor

Development of the Hematopoietic Stem Cell Niche

Stem cells reside in a specific microenvironment that is commonly referred to as the stem cell niche. Hematopoietic Stem Cells (HSC) are located in the adult in the bone marrow that is the site of production for all blood cells from birth. The HSC niche in the bone marrow is composed by a combination of cells, extracellular matrix, and growth factors. The nature and localization of the niche change during development. The first emergence of definitive HSC is observed in the Aorta Gonad Mesonephros (AGM) region, then the fetal liver becomes the site of a major expansion of the number of HSC that colonize the bone marrow that will be the definitive site of hematopoiesis. At each of these stages the function of the niche is different: emergence of HSC in the AGM, expansion of the HSC pool in the fetal liver, and homeostatic maintenance in the bone marrow.

Bone marrow transplantation is the treatment option of choice for hematological malignancies. More recently, umbilical cord blood (UCB) has been successfully used as an alternative source of hematopoietic stem cells based on the ease of procurement and a reduced risk of Graft Versus Host Disease. However a major disadvantage of UCB is the low cell dose available, which first restricted its use to pediatric patients. The low cell dose results in delayed neutrophil and platelet engraftment and a high rate of graft failure in adult patients. To overcome this problem one of the strategy proposed is to subject the UCB sample to an *ex vivo* expansion step prior to transplant. While the expansion of some committed progenitors has been successfully obtained, the amplification of immature HSC remains limited.

We hypothesized that the HSC niche in fetal liver niche represents a model of expansion of HSC. Reproducing these events *in vitro* would be a key to the development of *ex vivo* expansion of UCB derived HSC suitable for transplantation. We have identified cellular components of the fetal HSC niche that support the maintenance of transplantable HSC for at least 2 weeks. The identification of secreted factors or cell surface protein uniquely expressed by these cells will lead to the discovery of new effectors of the early expansion of HSC.

This work will also contribute to the development of methodology to produce HSC from human pluripotent cells. We propose to use the appropriate developmental niches or signals derived from the niche to improve the production of HSC from pluripotent cells.

RESEARCH PROJECTS

- Identification and characterization of the HSC niche during development
- Derivation of huHSC from embryonic stem cells or Induced pluripotent stem cells

KEY PUBLICATIONS

Suire, C., N. Brouard, K. Hirschi & P. J. Simmons. (2012). Isolation of the stromal-vascular fraction of mouse bone marrow markedly enhances the yield of clonogenic stromal progenitors. *Blood.* (In Press)

Robinson, S. N., P. J. Simmons, M. W. Thomas, N. Brouard, J. A. Javni, S. Trilok, J. S. Shim, H. Yang, D. Steiner, W. K. Decker, D. Xing, L. D. Shultz, B. Savoldo, G. Dotti, C. M. Bollard, L. Miller, R. E. Champlin, E. J. Shpall & P. A. Zweidler-McKay. (2012). Ex vivo fucosylation improves human cord blood engraftment in NOD-SCID IL-2Rgammanull (NSG) mice. *Experimental Hematology.* (In Press)

Brouard, N. (2011). Mesenchymal Stem Cell Circulation and Trafficking. *Mesenchymal Stem Cells,* ed. by Y. Xiao. Brisbane, Australia: Nova Science Publishers, Inc.

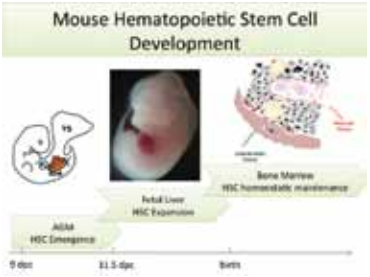
Brouard, N., R. Driessen, B. Short & P. J. Simmons. (2010). G-CSF increases mesenchymal precursor cell numbers in the bone marrow via an indirect mechanism involving osteoclast-mediated bone resorption. *Stem Cell Res* 5.65-75.

Short, B. J., N. Brouard & P. J. Simmons. (2009). Prospective isolation of mesenchymal stem cells from mouse compact bone. *Methods in Molecular Biology* (Clifton, N.J.) 482.259-68.

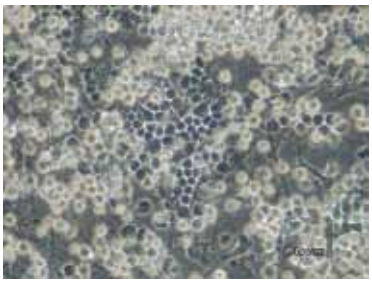
Spooncer, E., N. Brouard, S. K. Nilsson, B. Williams, M. C. Liu, R. D. Unwin, D. Blinco, E. Jaworska, P. J. Simmons & A. D. Whetton. (2008). Developmental fate determination and marker discovery in hematopoietic stem cell biology using proteomic fingerprinting. *Mol Cell Proteomics* 7.573-81.

LAB MEMBERS

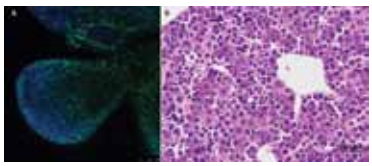
Research Assistant: Pooja Ghandi
Post Doc: Nadine Matthias, DMV.



Changes in the anatomical location and function of the Hematopoietic Stem Cell Niche during development.



Hematopoietic Stem Cell cultured on fetal liver niche



Mouse Fetal Liver. A: In toto immunostaining for endothelial cells of day 11.5 Fetal Liver . B: Resin section of day 13.5 Fetal Liver.

Qi Lin Cao, Ph.D.
Associate Professor

Stem Cells for Neurological Diseases

Despite extensive research, the central nervous system has little ability for repair with no therapeutic approach current available to restore the functions after many neurological diseases. Stem cells have shown great therapeutic potential for neurological disorders and may represent an effective novel therapy for these devastating diseases. Grafted stem cells can replace the lost neural cells, such as neurons, oligodendrocytes, or astrocytes. They also can facilitate host repair by suppressing secondary injury to preserve more neural tissue and enhance plasticity of spared neuronal circuits in the host. Human embryonic stem cells (ESCs) may be an ideal source of neural stem and progenitor cells for transplantation to treat neurological diseases. They can expand readily without depletion and differentiate into neural stem and progenitor cells to derive various neural cell types. Inducible pluripotent stem cells (iPSC), which are newly developed remarkable pluripotent, ESC-like cells reprogrammed from somatic cells, offer significant additional advantages in terms of availability of source material without ethical concerns of embryo use, and have the ability to generate isografts without the need of immunosuppression. However, several critical issues need to be resolved in order to realize the full therapeutic potential of human ESC or iPSC for neurological diseases. It still remains very challenging but is essential to direct human ESC or iPSC to differentiate into desired neural stem or precursor cells *in vitro* and then purify these cells before transplantation without the contamination of undifferentiated ESC or iPSC since undifferentiated ESC or iPSC will form teratoma *in vivo* after transplantation. It also remains to be determined the ideal grafting cell types to restore functions for different neurological diseases. Importantly, the mechanisms by which NSC or NPC grafting might enhance the functional recovery are unknown. My laboratory is studying the molecular mechanisms to regulate the neural differentiation of human ESCs and iPSCs and developing standard methods to differentiate and purify ideal neural cells for different neurological diseases. We are testing the therapeutic potential and long-term safety of human ESCs- and iPSC-derived neural stem or precursor cells in preclinical animal models of spinal cord injury, traumatic brain injury and stroke. These studies will help us to develop novel stem cell-based therapies for these neurological disorders, which can be translated to clinical application in the near future.

RESEARCH PROJECTS

- Identification of molecular mechanisms to regulate neural differentiation of human ESCs and iPSCs.
- The long-term therapeutic efficacy and safety of human ESCs and iPSC-derived neural stem or precursor cells for spinal cord injury and stroke.
- Identification and characterization of key regulators for oligodendrocyte differentiation and remyelination after spinal cord injury.
- The molecular mechanisms to regulate astrogliosis and the functions of astrogliosis after spinal cord injury, traumatic brain injury, or stroke using conditioned knockout mice models.
- Screening and identification of novel neuroprotection agents for spinal cord injury.

KEY PUBLICATIONS

Cao QL, Xu XM, DeVries WH, Enzman GU, Ping P, Tsoulfas P, Wood PM, Bunge MB, and Whittemore SR (2005) Functional recovery after transplantation of multineurotrophin-expressing glial-restricted precursor cells into traumatically injured spinal cord. *J. Neurosci.* 25: 6947-6957.

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

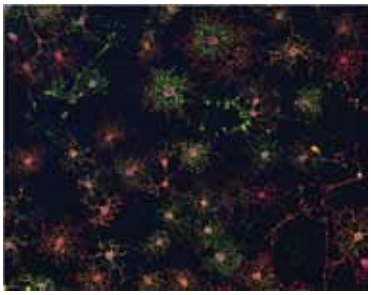
Zhu Y, Park J, Hu XM, Li H, Cao QL, Feng GS and Qiu MS (2010) Control of oligodendrocyte generation and proliferation by Shp2 protein tyrosine phosphatase. *Glia* 58: 1407-1414.

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

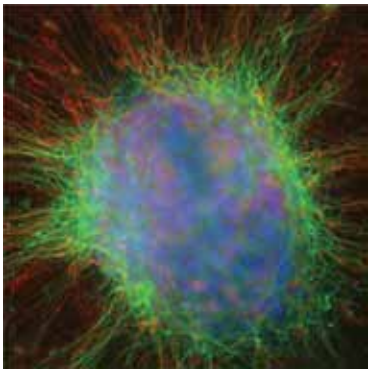
Wang YP, Cheng XX, He Q, Kim DH, Whittemore SR and Cao QL (2011) Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult OPCs by increasing the expression of bone morphogenetic proteins. *J Neurosci* 31(16):6053– 6058.

LAB MEMBERS

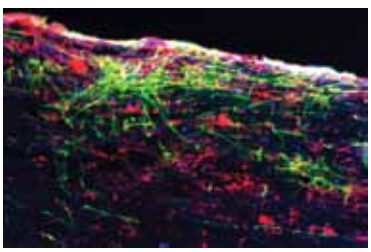
Postdoc Research Associate: Ping Bu, Hezou Lu, Junlin Yang, Yiyan Zheng
Technician: Ling Chen



Oligodendrocyte precursor cells in culture



Neuronal differentiation of human ESCs *in vitro*



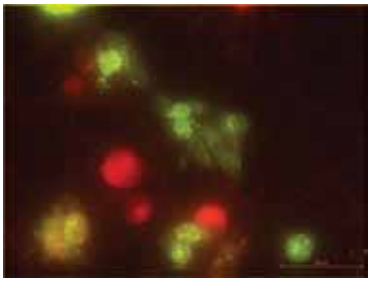
The survival and differentiation of grafted neural stem cells after spinal cord injury

Charles Cox, Jr., M.D.
Professor

Cellular Therapies for Neurological Injury

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

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



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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

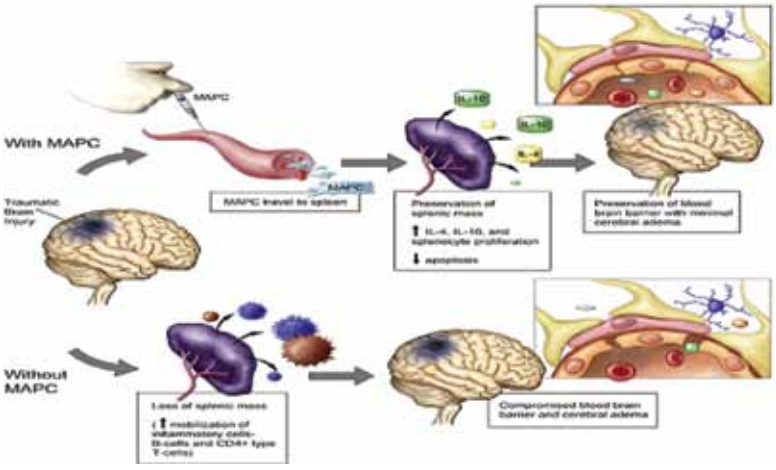
Walker PA, Shah SK, Jimenez F, Gerber MH, Xue H, Cutrone R, Hamilton JA, Mays RW, Deans RA, Pati S, Dash PK, Cox CS. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: Preserving the blood-brain barrier via interaction with splenocytes. *Exp Neurol* 225:341-352, 2010

Walker P, Harting MT, Jimenez F, Pati S, Dash PK, Cox CS. Direct intrathecal implantation of mesenchymal stromal cells leads to enhanced neuroprotection via an NFκβ mediated increase in IL-6 production. Stem Cells Dev.19: 867-876, 2010. PMC Journal – In Process.
Harting MT, Sloan LE, Jimenez F, Baumgartner JE, Cox CS. Subacute neural stem cell therapy for traumatic brain injury. *J Surg Res* 153:188-194, 2009. PMC Journal – In Process.

Harting MT, Jimenez F, Xue H, Fischer UM, Baumgartner JE, Dash PK, Cox CS. Intravenous mesenchymal stem cell therapy for traumatic brain injury? *J Neurosurgery* 110:1189-1199, 2009. PMC Journal – In Process.

LAB MEMBERS

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



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

Dong Kim, M.D.

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

Advancing the field of Neuroscience

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

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

Dr. Kim is noted for his research into the origin, development and treatment of brain aneurysms. He leads basic science efforts, such as identifying the genes that lead to an inherited risk for aneurysms and genetic changes in brain tumors, and translational projects that directly affect clinical practice.

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

Dr. Kim is a graduate of Stanford University and the University of California, San Francisco (UCSF) School of Medicine. After general surgery training at Harvard, he completed his neurosurgery training under Dr. Charles Wilson at UCSF. He went on to complete a fellowship in cerebrovascular surgery and skull base tumors with Dr. Arthur Day.

He has held faculty and hospital appointments at Harvard Medical School, Brigham and Women's Hospital, the Dana-Farber Cancer Institute, Cornell University Medical College, The New York Hospital and Memorial Sloan Kettering Cancer Center.

Dr. Kim specializes in the following diseases:

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

RESEARCH PROJECTS

- Stem Cell Therapy for Spinal Cord Injury
- Genetic Aneurysm Research
- Neuro Trauma Research

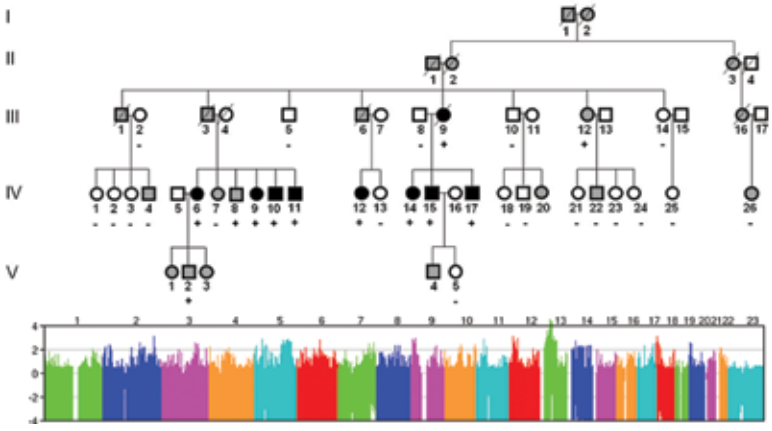
KEY PUBLICATIONS

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

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

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

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



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

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

The role of adipose stem cells in obesity and cancer

Efforts of our Laboratory converge on stem cells, obesity, and cancer. Specifically, the focus is on intercellular interactions in adipose tissue and tumors and the role of adult progenitor cells in pathology. These studies are based on the analysis of clinical specimens and mouse models. We have discovered the phenomenon of adipose cell mobilization in obesity and cancer. By using tissue transplantation models, we demonstrated trafficking of adipose stromal cells to tumors where they engage as components of tumor microenvironment stimulating angiogenesis and cancer progression. These findings have provided new insights on the association between increased adiposity and cancer. The molecular mechanisms of adipose cell migration to tumors and their role in tumor microenvironment are under investigation. The laboratory has extensive experience in phage display library screening, which we perform in vivo to isolate differentially expressed vascular cell surface molecules. These studies are based on our combined expertise in angiogenesis, molecular biology, and bioinformatics. Initially established in the mouse model and then translated to screens in cancer patients, they have led to several tissue-specific cell surface protein interactions that are now pursued as therapy targets. A drug prototype based on a peptide targeting IL-11 receptor in the prostate is being clinically tested in cancer patients. In another project, an approach to obesity reversal through directed ablation of white fat vasculature has been established: a compound targeting prohibitin protein on the surface of adipose endothelium has proven effective as an experimental anti-obesity drug (Adipotide) in rodent and monkey models. Recently, by combining peptide library screening technology with our expertise in cell population separation from organs by flow cytometry, we identified delta-decorin as the first known marker selectively expressed on adipose progenitor cells. An agent designed to target adipose progenitor cells through binding to delta-decorin is being pre-clinically tested for long-term treatment of obesity and a combination cancer therapy. Other projects are focused on identification of molecules targeting brown adipose tissue for whole body imaging applications, adipose tissue engineering in three-dimensional culture, identification of liposarcoma-initiating cells, and exploring the role of adipose tissue in leukemia progression.

RESEARCH PROJECTS

- Investigating the function of adipose stromal cells in cancer and other pathologies
- Experimental targeting adipose stromal cells as an approach to obesity and cancer treatment
- Investigating the function of prohibitin / annexin 2 interaction in adipose tissue
- Assessing the role of chemokine gradients and of SPARC/integrin signaling in adipose stromal cell migration
- Development of compounds targeting brown adipose tissue for whole body imaging applications
- Adipose tissue engineering in three-dimensional culture
- Characterization of cell hierarchy in human liposarcomas
- Identification of new stem markers of mesenchymal stem cells
- Exploring the role of adipose tissue in leukemia progression

KEY PUBLICATIONS

Kolonin M.G., Saha P. K., Chan L., Pasqualini R., Arap W. Reversal of obesity by targeted ablation of adipose tissue. *Nature Medicine*. 10, 625-32, 2004.

Nie J., Chang B., Traktuev D., Sun J., March K.L., Chan L., Sage E.H., Pasqualini R., Arap W. and Kolonin M.G. Integrin $\alpha 5/\beta 1$ is a receptor for the matricellular protein SPARC on adipose stromal cells and a prospective therapeutic target, *Stem Cells*. 26 (10), 2735-2745, 2008.

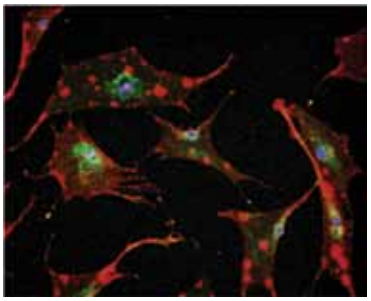
Zhang Y, Daquinag A., Traktuev D., Amaya F., Simmons P. J., March K.L., Pasqualini R., Arap W. and Kolonin M. G. Mouse white adipose tissue is a source of cells that are recruited by tumors and promote cancer progression, *Cancer Research*. 15 (69) 5259-5266, 2009.

Kolonin M.G. and Simmons P.J. Combinatorial stem cell mobilization, *Nature Biotechnology*. 27 (3) 252-253, 2009.

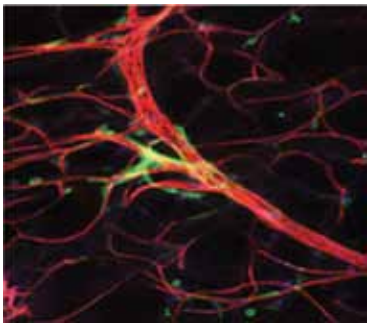
Daquinag A., Zhang Y., Amaya F., Simmons P. and Kolonin M.G. Cleavage Fragment of Decorin Interacts with Adiponectin and Resistin on the Surface of Adipose Stromal Cells, *Cell Stem Cell*. 9 (1):74-86, 2011.

LAB MEMBERS

Alexis Daquinag: postdoctoral fellow
Olga Sirin: postdoctoral fellow
Yan Zhang: research scientist
Felipe Amaya-Manzanares: research associate
Chieh Tseng: graduate student
Fernando Florez: Laboratory Technician



Adipose Stem Cells (ASC) with SPARC Protein on Cell Surface (red).



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

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

Enlarge Stem Cell Pool for Regenerative Medicine

Our team has developed several novel techniques for molecular, cellular and small animal-based research to focus on two major areas of study: 1) exploring the properties of the dedifferentiation/transformation of terminally differentiated cells into various stem cells for regenerative medicine and tissue engineering applications, and 2) studying the processes involved with fibrous scar formation and prevention in the injured and diseased tissues of the neuron and musculoskeletal system. The laboratory is also interested in translational study and clinical application of stem cells and engineered tissue for treating congenital diseases and traumatic injuries.

We have also set up classic amphibian models of tissue regeneration; newts and salamanders can rebuild most missing body parts such as limbs, liver, lens and heart after amputation injury. However, injured mammalian tissue, including that of humans, is usually replaced with fibrotic scar tissue at the end of the healing process. Our aim is to determine the mechanism(s) behind the regenerative process in amphibians and ascertain the relationship(s) to human tissue or organ regeneration. We will investigate the cellular dedifferentiation process for increasing the stem cell population and methods of preventing fibrotic scar tissue formation during wound healing. Our expectation is to transfer our learning from amphibian regenerative models to enlarge stem cell pool for regenerative medicine applications, and to build various functional human tissues/organs for human patients' demand.

RESEARCH PROJECTS

- Children's Regenerative Medicine: The project will use various cell sources combined with bioengineering scaffolds to build functional tissues for repair of pediatric defects, such as children's diaphragmatic hernia.
- Dedifferentiation and Stem Cell Populations: The project aims to enlarge the stem cell pool without genetic modification as a cell source for regenerative medicine.
- Adult Embryonic Potential Stem Cells and Application: Obtain natural embryonic potential stem cells from adult tissue for utilization in tissue engineering and regenerative medicine, such as central neurologic disorder and disease.
- Fibrosis and Prevention Studies: Investigate the mechanism behind the fibrosis process after injuries and diseases, and seek methods for prevention of fibrous scar tissue formation.
- Newt model: Combination of mammalian cells with amphibian cells to investigate the potential of tissue/organ regeneration process in the Newt model and the mechanisms.

KEY PUBLICATIONS

Mu XD, Peng HR, Pan HY, Huard J, Li Y. Study of muscle cell dedifferentiation after skeletal muscle injury of mice with a Cre-Lox system. *Plos One*. 2011 February 3 ;6(2):e16699, doi:10.1371.

Mu XD, Li Y. Conditional TGF-beta1 treatment increases stem cell-like cell population in myoblasts. *J Cellular Molecular Medicine* 2011;15(3):679-690

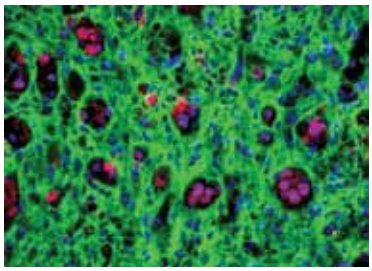
Mu XD, Xiang GH, Rathbone CR, Pan HY, Bellayr IH, Walter TJ, Li Y. Slow-adhering stem cells derived from injured skeletal muscle have improved regenerative capacity. *American J Pathology*. 2011;179(2):931-941

Mu X, Urso ML, Murrary K, Fu F, Huard J, Li Y. Relaxin regulates MMP expression in myogenic cells and promotes satellite cell activation during muscle dealing of both young and aged mice. *American J Pathology* 2010;177(5):2399-410

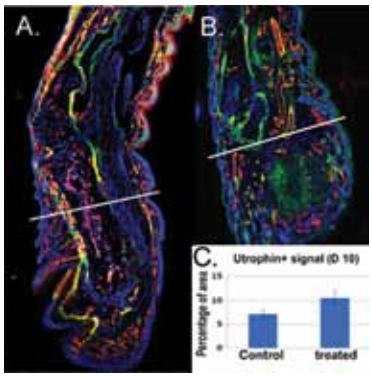
Wang W, Pan HY, Jefferson M, Li Y. MMP1 promotes muscle cells migration and differentiation. *American J Pathology* 2009;174(2):541-9.

LAB MEMBERS

Administrator: Stephanie Baca
Lab senior technician/manager: Haiying Pan
Postdoc research fellow: Dr. Yohan Choi; Shen Yang
Student: Yinda Tang

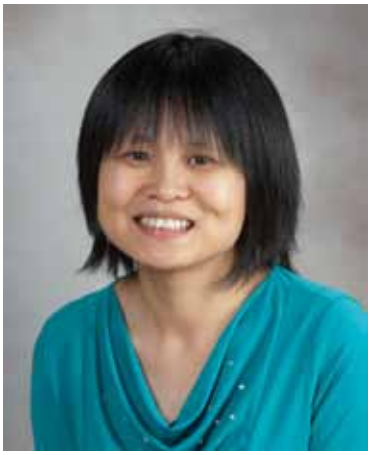


Fibrosis formation and potential mechanisms



Finger regrowth after amputation injury

Newts regeneration model



Ying Liu, Ph.D.
Assistant Professor

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

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

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are promising therapeutic tools for regenerative medicine. They can proliferate indefinitely in culture and have the capacity to differentiate into any cell types of the body. Protocols for directed differentiation of hESCs and hiPSCs into neural stem cells (NSCs) have been established. These NSCs can be maintained in a chemically defined medium and proliferate in culture for at least 20 passages without going into senescence or changing their multipotential properties. When induced, they become functional neurons and glia as directed. The number of NSCs can be amplified to satisfy clinical demands. However, ethical issues, the possibility of immune rejection, and tumorigenicity have precluded hESCs and their derivatives to be applied to the clinical settings. hiPSCs, which are reprogrammed from somatic cells, have the potential to circumvent some of these problems. By transient overexpression of four transcription factors, OCT4, SOX2, KLF4 and C-MYC, somatic cells such as dermal fibroblasts, keratinocytes, and blood cells, can be reprogrammed to pluripotent state and share many hESC characteristics. Most critically, hiPSCs provide autologous materials for patients, which theoretically omit the need for immune suppression. We have set up systems to optimize the more clinically relevant, integration-free hiPSC generation protocol. We perform directed differentiation of patient-specific iPSCs into NSCs, neuronal and glial progenitors, as well as mature cell types for disease modeling, transplantation studies, neural regeneration and repair, and drug screening and testing. We also have developed efficient procedures to genetically label and purify hESC- and hiPSC-derived lineage specific cells for in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

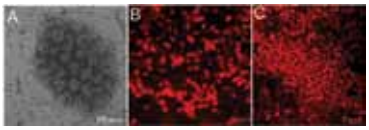
Liu, Y., Rao, M. (2011) Gene targeting in human pluripotent stem cells. Methods Mol Biol. 767:355-367.

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

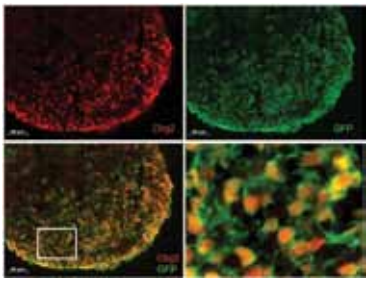
Liu, Y., Thyagarajan, B., Lakshmipathy, U., Xue, H., Lieu, P., Fontes, A., MacArthur, C.C., Scheyhing, K., Rao, M.S., and Chesnut, J.D. (2009). Generation of a platform human embryonic stem cell line that allows efficient targeting at a predetermined genomic location. Stem Cells Dev 18, 1459-1472

LAB MEMBERS

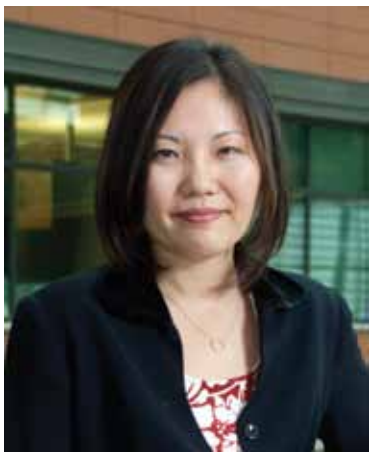
Research Associate: Haipeng Xue



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



Human embryonic stem cell (hESC) knockin GFP reporter recapitulates endogenous expression of targeted neural lineage specific transcription factor



Nami McCarty, Ph.D.
Assistant Professor

Biological and Molecular Heterogeneity of Blood Cancers

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Jung, H-J., Zheng, C., Wang, M., Fayad, L., Romaguera, J., Kwak, L.W., and McCarty, N. (2012) Calcium blockers decrease the bortezomib resistance in mantle cell lymphoma (MCL) via manipulation of tissue transglutaminase activities. *Blood.* Jan 31. Epub ahead of print.

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

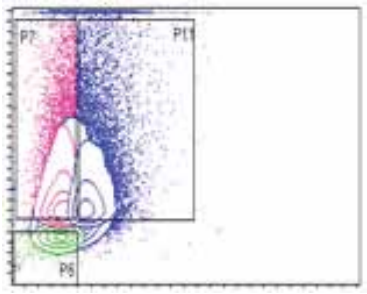
Jung, H-J., Zheng, C., and McCarty, N. (2011) Stem-like tumor cells confer drug resistant properties to mantle cell lymphoma. *Leukemia and Lymphoma.* 52:1066-1079.

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

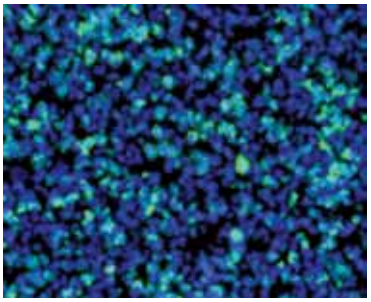
Alvarez Arias, D.A, McCarty, N*., Lu, L., Maldonado, R., Shinohara, M.L., and Cantor, Unexpected role of clathrin adaptor, AP-1 in MHC-dependent positive selection of T cells. *Proc. Natl. Acad. Sci.* 107:2556-2561, 2010 *Co-first author.

LAB MEMBERS

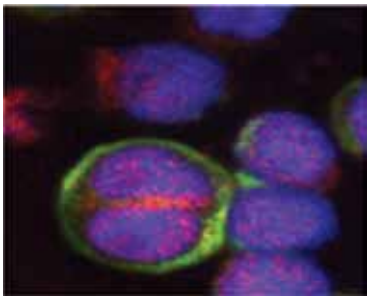
Research Associate: Judy Chen
Postdoctoral Associate: Dr. Junhyoung Park, Dr. Amit Kumar



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



FACS analysis of cell cycle using Pyronin and Hoechst staining.



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

Naoki Nakayama, Ph.D.
Associate Professor

Stem Cell Differentiation and Lineage Specification

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

Development of mesodermal and neural crest-like chondroprogenitor cells: The cartilage of joints is not spontaneously repaired after injury in human. There has been considerable interest in the clinical application of stem cells to the repair of damaged cartilage; however, current adult stem cell therapies face the problems of low yield of cells and their tendency to yield fibrotic cartilage that does not stably integrate into the surrounding joint cartilage. Joint is formed during embryogenesis. Therefore, embryonic chondroprogenitors responsible for limb and vertebral joint formation are likely to be the best source of cells for the regeneration of joint cartilage in the adult. We have developed and purified from human PS cells paraxial mesoderm and neural crest progeny with the capacity to expand, differentiate into chondroprogenitors, and generate hyaline-like cartilage particles that are stably maintained *in vivo*. We are interested in developing lateral plate mesoderm and limb chondroprogenitors, and comparing the cartilage regeneration abilities of such embryonic chondroprogenitors and adult stem cells in animal models.

Development of hematopoietic stem cells (HSCs): Attempts to derive and isolate hematopoietic cells from PS cells began nearly 20 years ago using mouse embryonic stem cells, later moving to human PS cells. However, all early studies, including our own, failed to reproducibly generate hematopoietic cells that fulfill the stringent definition of stem cells: significant levels of multilineage marrow repopulation in serial transplants. HSCs are born from the endothelium of the dorsal aorta in an embryonic region called the aorta-gonad-mesonephros (AGM) and expanded in fetal liver during embryogenesis. We have developed and isolated from human PS cells hemoangiogenic cells that give rise to progeny displaying a weak marrow-repopulating activity. We are interested in examining the effects of AGM and fetal liver stromal cells on the genesis of HSCs with a stronger engraftment potential.

RESEARCH PROJECTS

- Prospective isolation of three embryonic chondroprogenitors (sclerotome, limb mesenchyme and ectomesenchyme), generated from human pluripotent stem cells through their corresponding developmental intermediates (i.e. paraxial mesoderm, lateral plate mesoderm and neural crest, respectively).
- Joint development and joint cartilage regeneration with the pluripotent stem cell-derived chondroprogenitors.
- Specification and prospective isolation of hematopoietic stem cells from human pluripotent stem cells
- Molecular and cellular basis of endothelial hemogenesis.

KEY PUBLICATIONS

Umeda, K., Zhao, J., Simmons, P., Stanley, E., Elefanty, A., and Nakayama, N. (2011) *"Human chondrogenic paraxial mesoderm, directed specification and prospective isolation from pluripotent stem cells".* In revision.

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

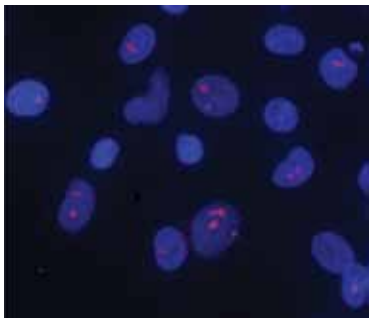
Tanaka, M., Jokubaitis, V., Wood, C., Wang, Y., Brouard, N., Pera, M., Hearn, M., Simmons, P., and Nakayama, N. (2009) "BMP inhibition stimulates WNT-dependent generation of chondrogenic mesoderm from embryonic stem cells". *Stem Cell Res.,* 3:126-141.

Nakayama, N., Han, C.-Y., Cam, L., Lee, J.I., Pretorius, J., Fisher, S., Rosenfeld, R., Scully, S., Nishinakamura, R., Duryea, D., Van, G., Bolon, B., Yokota, Y., and Zhang, K. (2004) "A novel chordin-like BMP inhibitor, CHL2, expressed preferentially in chondrocytes of developing cartilage and osteoarthritic cartilage". *Development*, 131, 229-240.

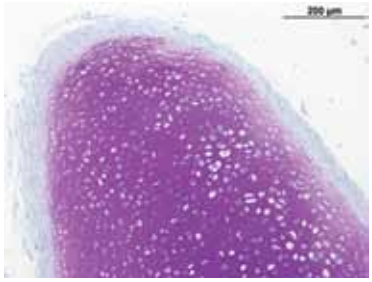
Nakayama, N., Duryea, D., Manoukian, R., Chow, G., and Han, C.-Y.E. (2003) "Macroscopic cartilage formation with embryonic stem cell-derived mesodermal progenitor cells". *J. Cell Sci.* 116, 2015-2028.

LAB MEMBERS

Research Associates: Jiangang Zhao, Qing Yan, PhD
Research Technician: Suprita Trilok
Animal Specialist: Nadine Matthias, DVM



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



Cartilage generated with human PS cell-derived paraxial mesoderm and maintained for 12 weeks in an immunocompromised mouse: The cartilage area is purple.

Pamela Wenzel, Ph.D.
Assistant Professor

Regulation of Stem Cell Potential by Biomechanical Force

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

One arm of our research is designed to address how biomechanical force activates the embryonic hematopoietic program and how we might use this information in the laboratory to expand improved sources of hematopoietic cells that can be used for patients in the clinic. A number of genetic and biochemical pathways are currently under investigation as key players mediating this signaling cascade, and we employ various approaches to evaluate their role in blood development, including biomechanics, microfluidics, pharmacology, embryonic stem cell modeling, mouse genetics, and transplantation assays.

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

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

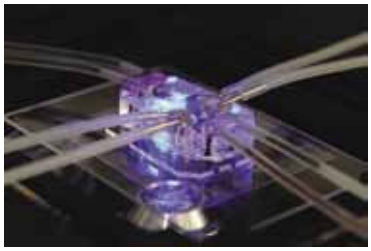
Naveiras, O., Nardi, V.*, Wenzel, P.L.*, Hauschka, P.V., Fahey, F., and Daley, G.Q. (2009). Bone marrow adipocytes as negative regulators of the hematopoietic microenvironment. *Nature* 460: 259-263. *Equal contribution.

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

LAB MEMBERS

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

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



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

Jiaqian Wu, Ph.D.
Assistant Professor

Gene Transcription and Regulation of Stem Cell Differentiation

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

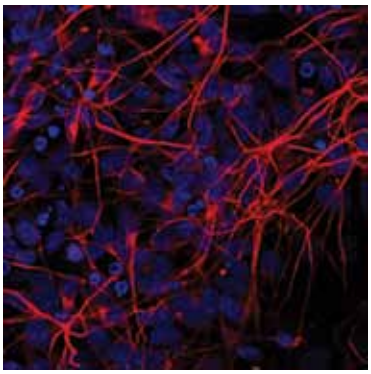
Wu, J. Q., Habegger, L., Noisa, P., Szekely, A., Qiu, C., Hutchison, S., Raha, D., Lin, H., Egholm, M., Weissman, S., Cui, W., Gerstein, M., and Snyder, M. (2010). Dynamic Transcriptomes during Neural Differentiation of Human Embryonic Stem Cells Revealed by Integrating Short, Long, and Paired-end Sequencing.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

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

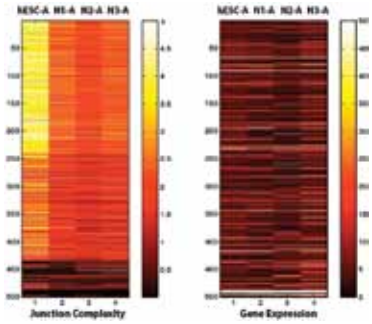
Wu, J. Q., Seay, M., Schulz, V., Hariharan, M., Tuck, D., Lian, J., Du, J., Shi, M., Ye, Z. J., Gerstein, M., Snyder, M., and Weissman, S. (2012). Tcf7 is a key regulator of the self-renewal and differentiation switch in a multipotential hematopoietic cell line. *PLoS Genet 8*(3): e1002565.

LAB MEMBERS

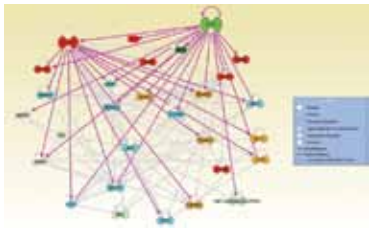
Postdoctoral Fellow: Kenian Chen
Research Assistant: Shuyun Deng



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



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



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

Texas Therapeutics Institute

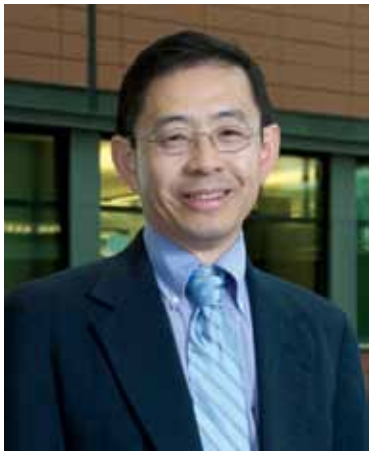
Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) is the headquarters of TTI, which is a consortium of The University of Texas Health Science Center Houston (UTHealth), M D Anderson Cancer Center, and The University of Texas at Austin. TTI was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas Systems, and each of the three universities for the discovery, development, and commercialization of potential therapeutic and diagnostic agents.

Toward this goal, TTI faculty were nearly all recruited from pharmaceutical companies, and they are here to conduct research that is directly related to the identification and validation of drug targets, and establishment of proof-of-principle for therapeutics.

Current research at TTI-IMM is focused on the signaling mechanisms of receptors and enzymes that have critical roles in tumor initiation, progression, or metastasis and on the discovery of biologics that modulate the activity of these targets as potential lead molecules for drug discovery.

TTI-IMM has quickly brought in significant funding from pharmaceutical industry and government grant agencies, as well as made significant scientific discoveries in the area of cancer research.

Zhiqiang An, Ph.D.
Professor and 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

HER3 mediated cell signaling and HER3 targeting antibodies for cancer therapy

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

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

RESEARCH PROJECTS

- HER3 mediated cell signaling and the development of HER3 targeting monoclonal antibodies for cancer therapy
- Evaluation of vaccine-induced antibody responses in preclinical animal models and humans
- Therapeutic antibody discovery and development

KEY PUBLICATIONS

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

H. Glantschnig, R. Hampton, P. Lu, J. Zhao, S. Vitelli, L. Huang, P. Haytko, T. Cusick, C. Ireland, S. Jarantow, R. Ernst, N. Wei, P. Nantermet, K. Scott, J. Fisher, F. Talamo, L. Orsatti, A. Reszka, P. Sandhu, D. Kimmel, O. Flores, W. Strohl, Z. An, and F Wang. 2010. Generation and Selection of Novel Fully Human Monoclonal Antibodies that Neutralize Dickkopf-1 (DKK1) Inhibitory Function in vitro and Increase Bone Mass *in vivo*. *Journal of Biological Chemistry* 17;285(51):40135-47.

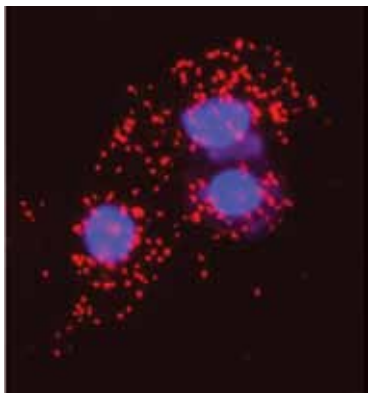
Y. Yu, P. Lee, Y. Ke, Y. Zhang, Q. Yu, J. Lee, M. Li, J. Song, J. Chen, J. Dai, F. J. R. D. Couto, Z. An, W. Zhu, and G. Yu. 2010. A humanized anti-VEGF rabbit monoclonal antibody inhibits angiogenesis and blocks tumor growth in xenograft models. *Plos One* 5(2):e9072 (1-12).

Z. An, G. Forrest, R. Moore, M. Cukan, P. Haytko, L. Huang, S. Vitelli, J. Z. Zhao, P. Lu, J. Hua, C. R. Gibson, B. Harvey, D. Montgomery, D. Zaller, F. Wang, and W. R. Strohl. 2009. IgG2M4, an engineered antibody isotype with reduced Fc function. *mAbs* 1(6):572 - 579.

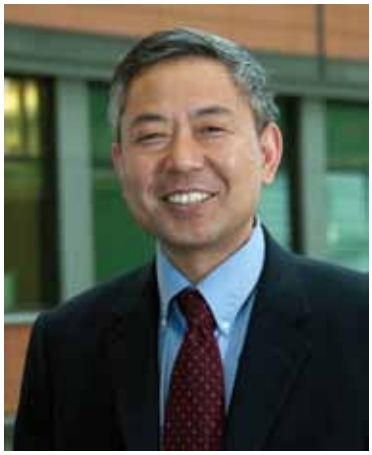
Montgomery, D. L., Y-J. Wang, R. Hrin, M. Luftig, B. Su, M. D. Miller, F. Wang, P. Haytko, L. Huang, S. Vitelli, J. Condra, X. Liu, R. Hampton, A. Carfi, A. Pessi, E. Bianchi, A. Finnefrock, J. Joyce, D. Lowe, R. Geleziunas, D. Bramhill, V. M. King, W. Strohl, Z. An. 2009. Affinity maturation and characterization of a human monoclonal antibody against HIV-1 gp41. *mAbs* 1(5):462-474.

LAB MEMBERS

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



EGFR/HER3 dimerization in breast cancer cells



Qingyun (Jim) Liu, Ph.D.
Professor and Co-Director of the Texas Therapeutics Institute
Janice Davis Gordon Distinguished Professorthsip for Bowel Cancer Research

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

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

Our research is focused on delineating the mechanisms of a group of cell surface receptors that are essential for the survival of normal stem cells and determining their roles in the maintenance and proliferation of cancer cells. We also are engaged in the validation of these receptors as potential drug targets and identification of lead molecular as potential anticancer therapeutics. We modulate the activity of these receptors in normal and cancer cells using a variety of techniques and measure their effect on the survival and growth of the cells. Most recently, we successfully identified the factors that are essential for the activation of the receptors, representing an important step toward the understanding of the mechanisms of these receptors. We are now in the process of further characterizing these receptors and screening for their inhibitors as leads for the discovery of potential therapeutics.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

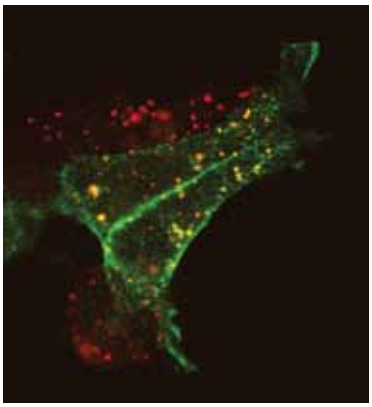
Carmon, K.S., Gong, X, Lin, Q., Thomas, A., and Liu, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. Proc Natl Acad Sci U S A, 108:11452-11457 (2011).

Carmon, K.S., Lin, Q., Xing, G., Thomas, A., and Liu, Q. LGR5 Interacts and Co-Internalizes with Wnt Receptors to Modulate Wnt/ beta-catenin Signaling. Mol. Cell. Biol., in press (2012).

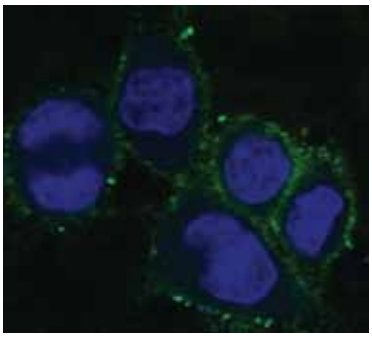
Xing, G., Carmon, K.S., Lin, Q., Thomas, A., Yi, J., and Liu, Q. LGR6 is a high affinity receptor of R-Spondins and potentially functions as a tumor suppressor. PlosOne. In press (2012).

LAB MEMBERS

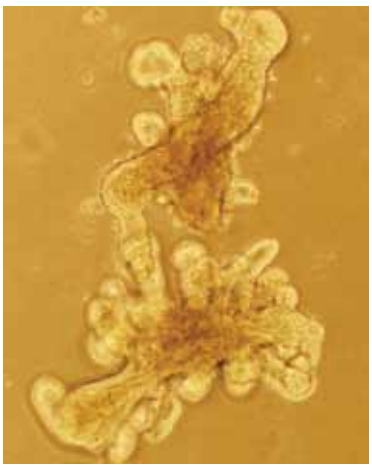
Instructor: Xing Gong
Research Scientist: Qiushi Lin
Postdoctoral fellows: Kendra Carmon and Jing Yi
Technicians: Anthony Thomas



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



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



Organoid cultures of LGR5-positive intestinal stem cells

Nathan S. Bryan, Ph.D.
Assistant Professor

The Role of Nitric Oxide in Health and Disease

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

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

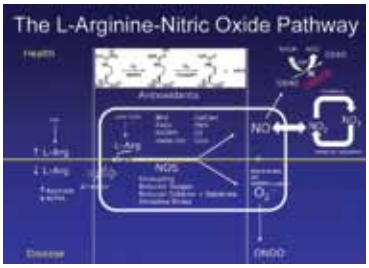
Hord NG, Ghannam J, Garg HK, Berens PD, Bryan NS: Nitrate and nitrite content of human, formula, bovine and soy milks: implications for dietary nitrite and nitrate recommendations *Breastfeeding Medicine* 2010 Oct 19.

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

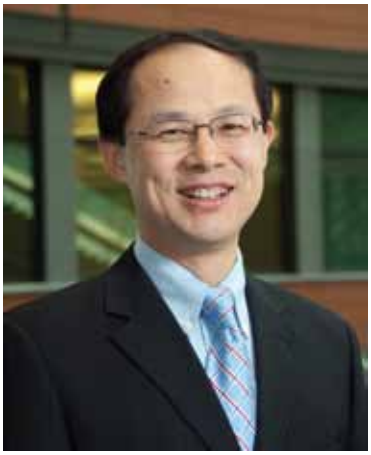
Bryan NS and Loscalzo J (Editors) *Nitrite and Nitrate in Human Health and Disease* – Springer Humana Press New York ISBN: 978-1-60761-615-3, May 2011

LAB MEMBERS

Hong Jiang, Ph.D. – Senior Research Scientist
Ashley C. Torregrossa, B.S. – Research Associate
Deepa K. Parthasarathy, B.D.S., M.P.H.,



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



Wenliang Li, Ph.D.
Assistant Professor

Molecular Mechanisms of Cancer Metastasis

My research is to study novel molecular mechanisms of metastasis with the goal of identifying new biomarkers and drug targets for the development of better therapeutics for human cancers.

Metastasis is still poorly understood, and the current approaches to prevent or treat human metastatic diseases are mostly unsuccessful. Through genomics, RNAi and cDNA functional screens, Our lab is identifying human genes that may play important but previously unknown roles in cancer metastasis. Signaling pathways and molecular mechanisms of these interesting candidates are under investigation with molecular, cellular, biochemical, genomic, proteomic approaches, and mouse models.

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

RESEARCH PROJECTS

- Novel regulators in cancer metastasis
- Epithelial-mesenchymal transition (EMT)
- Cancer stem cell
- Drug resistance

KEY PUBLICATIONS

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

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

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

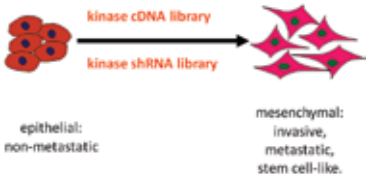
Baldwin A, Li W, Grace M, Harlow E, Münger K and Grueneberg DA. II. Genetic Interaction Screens Identify Alterations in Kinase Requirements Following HPV16 E7 Expression in Cancer Cells. Proceedings of the National Academy of Sciences USA (PNAS). 2008 Oct 28;105(43):16478-83.

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

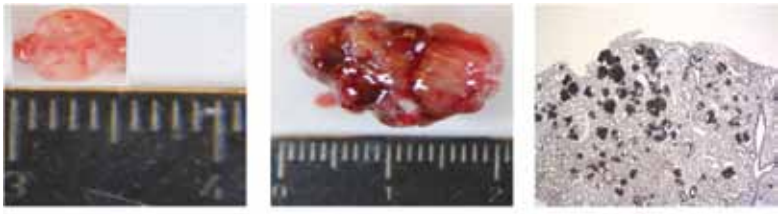
Note: These 4 PNAS papers I-IV were selected as Signaling Breakthroughs of 2008 (the most exciting advances in signaling transduction research in 2008) in the popular annual Editorial Guide of journal Science Signaling (formerly Science STKE), a Science family journal.

LAB MEMBERS

Postdoc Fellows: Nanping Ai, Linna Li



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



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

Kalpana Mujoo, Ph.D.
Assistant Professor

Role of Nitric Oxide-cyclic GMP signaling in Stem cells and Cancer

The nitric oxide-cyclic GMP (NO-cGMP) pathway mediates important physiological functions associated with various integrative body systems, including the cardiovascular and nervous systems. Furthermore, NO regulates cell growth, survival, apoptosis, proliferation, and differentiation at the cellular level. To understand the significance of the NO-cGMP pathway in development and differentiation, studies have been conducted both in developing embryos and stem cells.

We are interested in understanding if manipulation of the NO-cGMP pathway by employing activators and inhibitors as pharmacological probes and/or genetic manipulation of NO signaling components will regulate stem cell differentiation. To that end, we have demonstrated differential expression and function of various NO-cGMP signaling components in stem and differentiated cells. Furthermore, our studies demonstrate that combination of NO donors and allosteric sGC (NO receptor) activators enhance the differentiation of stem cells into cells of myocardial lineage.

Recent discovery of induced pluripotent cells has opened up new avenues for understanding the molecular mechanism(s) of their cell proliferation and differentiation. Our preliminary studies with induced pluripotent cells demonstrate aberration in cyclic GMP signaling downstream of NO receptor soluble guanylyl cyclase. Therefore, we are interested in elucidating the underlying molecular mechanisms involved in such aberration by focusing on events downstream of NO receptor sGC.

Prostate cancer is the second leading cause of cancer-related mortality with more than 32,000 patient deaths reported in recent Cancer Statistics data. Small rare population of "cancer-initiating cells" (CICs) with in tumors are cells capable of self-renewal, differentiation, and reoccurrence of prostate cancer. We are interested in evaluating effective molecular biomarker (s) and therapeutic modalities for early detection and targeting of metastatic prostate cancer. Cyclic GMP pathway exhibits important physiological role in cardiovascular system but its significance is not well understood in cancer. Since PKG and cyclic GMP play an important role in physiological function of normal prostate, loss of their expression and function may lead to aggressive form of cancer. Our results demonstrate loss of sGC and PKG activity and expression in many cancer cells including prostate cancer. We believe that proposed research should reveal novel molecular biomarkers and drugs, which could be evaluated to alleviate suffering and death of patients with prostate cancer.

RESEARCH PROJECTS

- Nitric oxide-cyclic GMP signaling in embryonic and induced pluripotent stem cells
- Role of small molecules in differentiation of stem cells
- NO receptor soluble guanylyl cyclase (sGC) and protein kinase G (PKG) as molecular biomarker (s) for human prostate cancer

KEY PUBLICATIONS

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

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

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

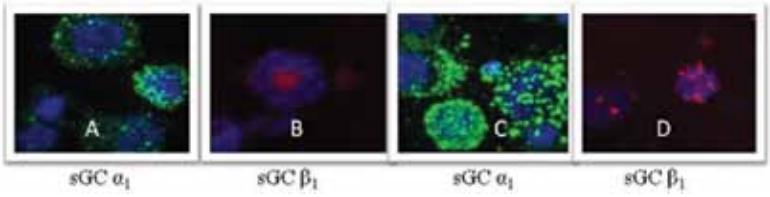
Mujoo, K.*, Sharin, V.G., Bryan, N., Krumenacker, J.S., Sloan, C., Parveen, S., Kots, A., Murad, F*. (2008). Role of nitric oxide signaling components in differentiation of embryonic stem cells into myocardial cells. *Proc Natl Acad Sci. USA,* 105: 18924-18929.

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

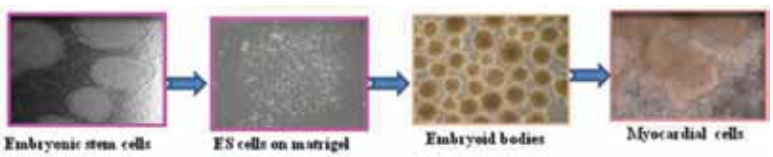
*Corresponding author

LAB MEMBERS

Former Post Docs: Chitralekha Bhattacharya, Ph.D.
Former Technicians: Lubov Nikonoff



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



Differentiation protocol of human ES cells

Ningyan Zhang, Ph.D.
Associate Professor

Trastuzumab resistance mechanisms in cancer

Antibodies are rapidly becoming a major drug modality for cancer treatment, and they are among the most efficacious targeted therapies available today. This trend is continuing as about 50% of the new drugs in various stages of clinical development are antibodies, and cancer therapeutic antibodies constitute a majority. Human epidermal growth factor receptor (EGFR) family consists of four closely related type 1 transmembrane tyrosine kinase receptors (EGFR/HER1, HER2, HER3 and HER4), and both EGFR and HER2 are proven oncogenes and have been successfully targeted using several monoclonal antibodies for treatment of various types of cancers, including non-small cell lung cancer, colon cancer and breast cancer. Trastuzumab is a humanized anti-HER2 IgG1 antibody and has shown clinical success for treatment of breast cancer patients with HER2 over-expression at both adjuvant and neoadjuvant settings. However, mechanisms of action are still not fully understood and multiple mechanisms have been proposed including inhibition of HER2 signaling, HER2 receptor downregulation, prevention of HER2 extracellular domain shedding, and antibody dependent cell cytotoxicity (ADCC) mediated through antibody Fc interaction with immune effector cells. Both innate and acquired resistance to trastuzumab have been widely reported, which presents significant challenges in the clinic.

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

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

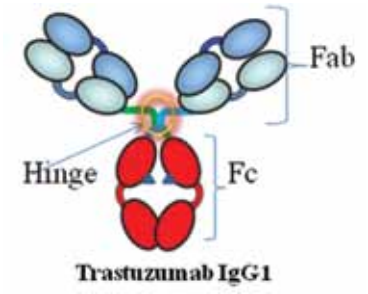
Miguel Aste-Amézaga1, Ningyan Zhang et al. (2010) Characterization of Notch1 Antibodies that Inhibit Signaling of both Normal and Mutated Notch1 Receptors, *Plos One* 5 (2) e9094

Li Y. Burns JA, Cheney C., Zhang NY, Bett AJ, Chastain A., Aste-Amezaga, AM., Huber H., Audoly, LP. and Zhang ZQ. (2010) Distinct expression profiles of Notch-1 protein in human solid tumors: implications for development of targeted therapeutic monoclonal antibodies *Cancer Biology and Therapy*, 4:1-9

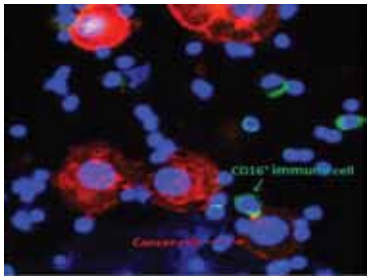
Zhang NY, Williams B, Lu P, An Z, and Chin C-N. (2009) Therapeutic antibodies in clinic use and leading clinical candidates. In: *Therapeutic Antibodies: from Bench to Clinic, Z An* ed., John Wiley & Sons, Inc. Hoboken, NJ. pp 711-762.

LAB MEMBERS

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

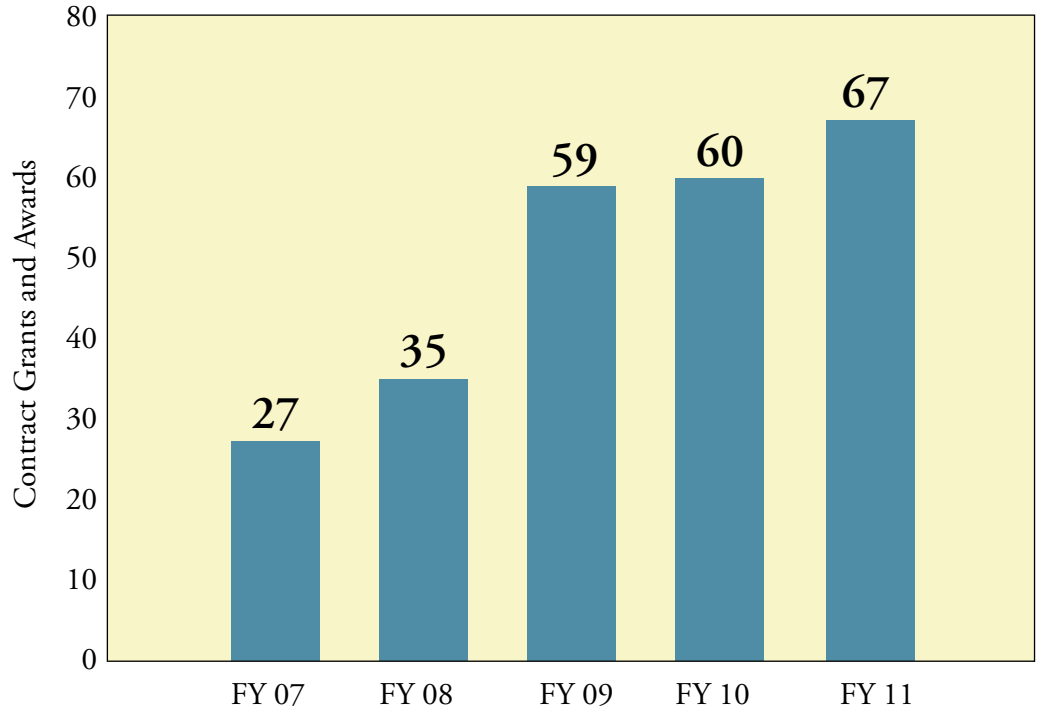


Schematic diagram of antibody

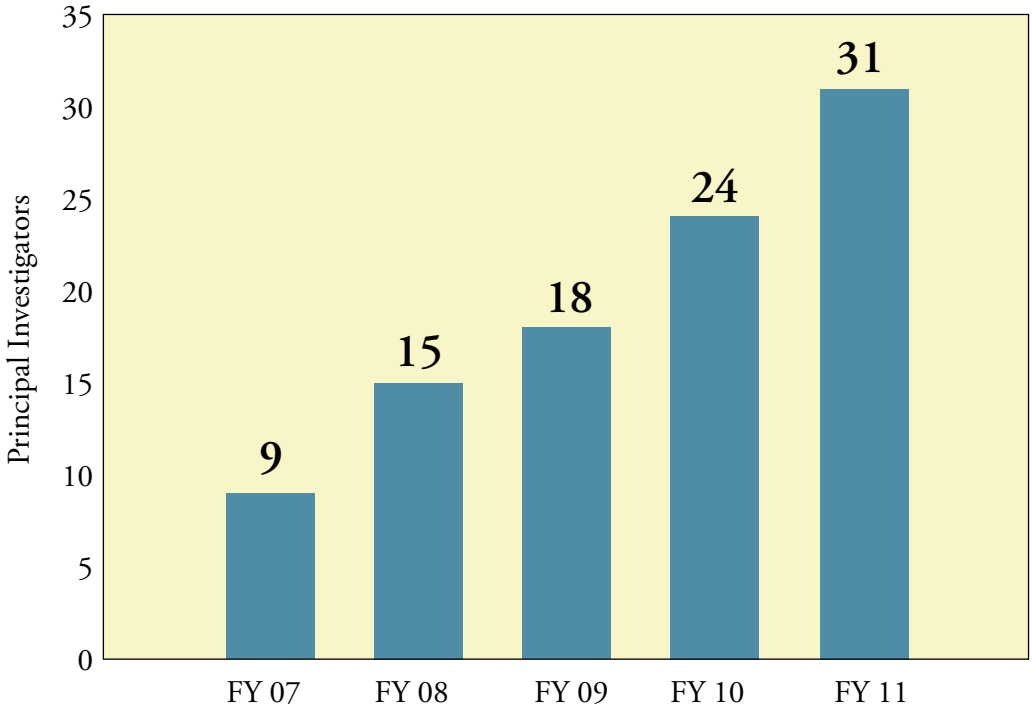


IImmune cells/cancer cells interaction in a co-culture condition

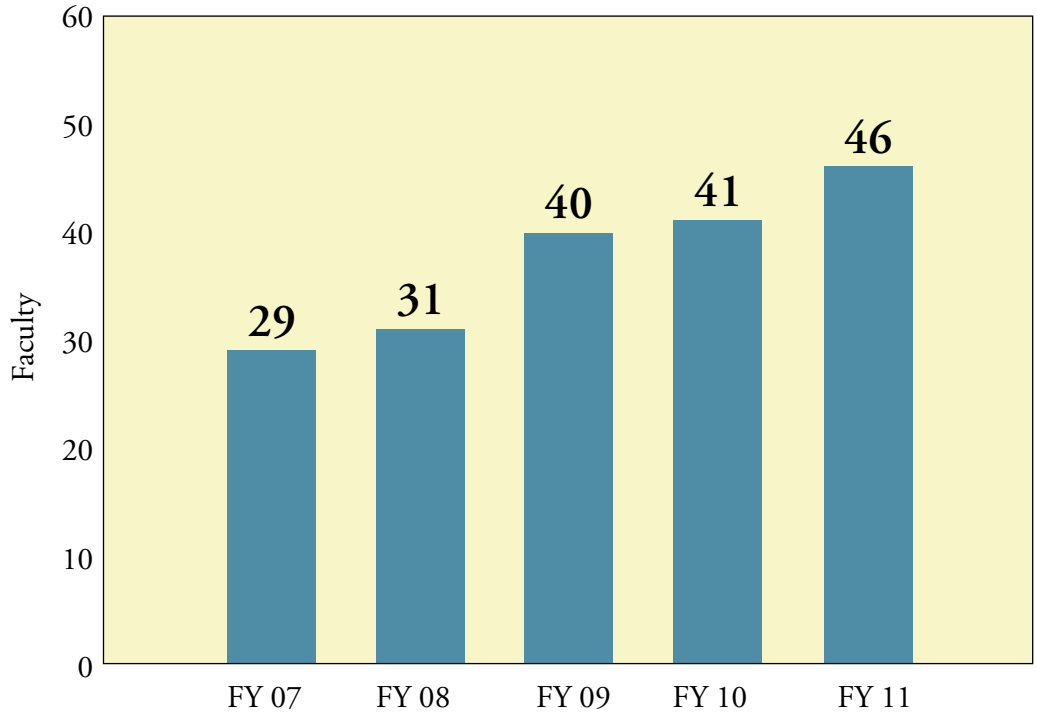
Number of contract grants and awards



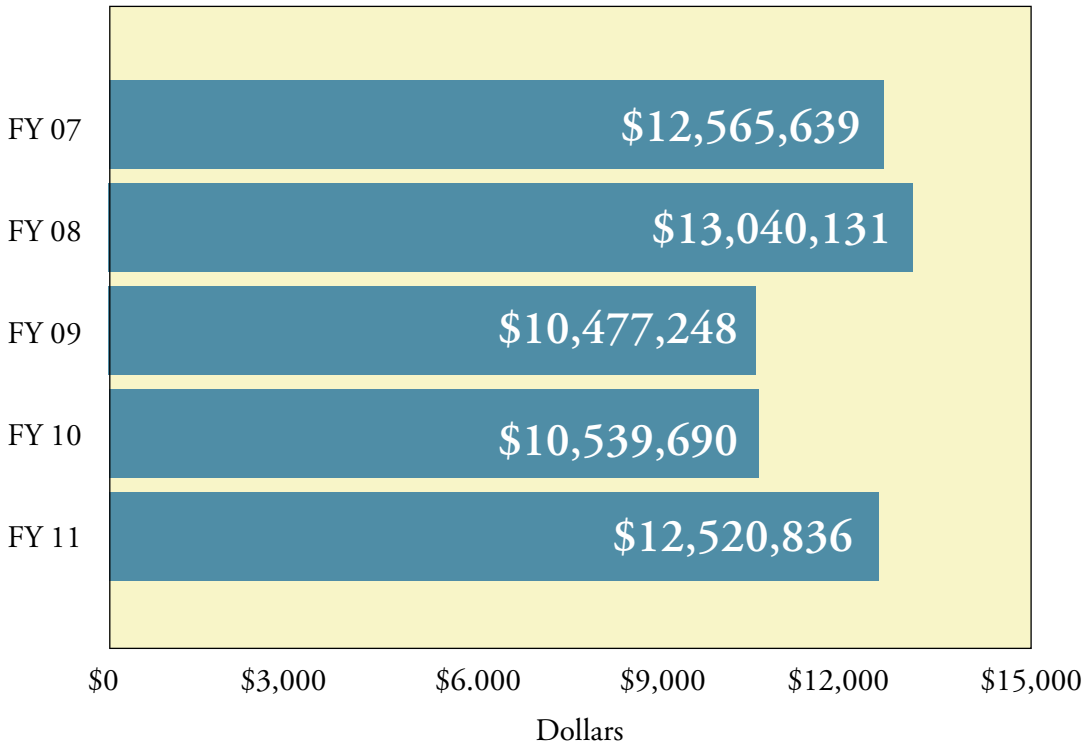
Number of Principal Investigators



Number of Faculty



Research Expenditures



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