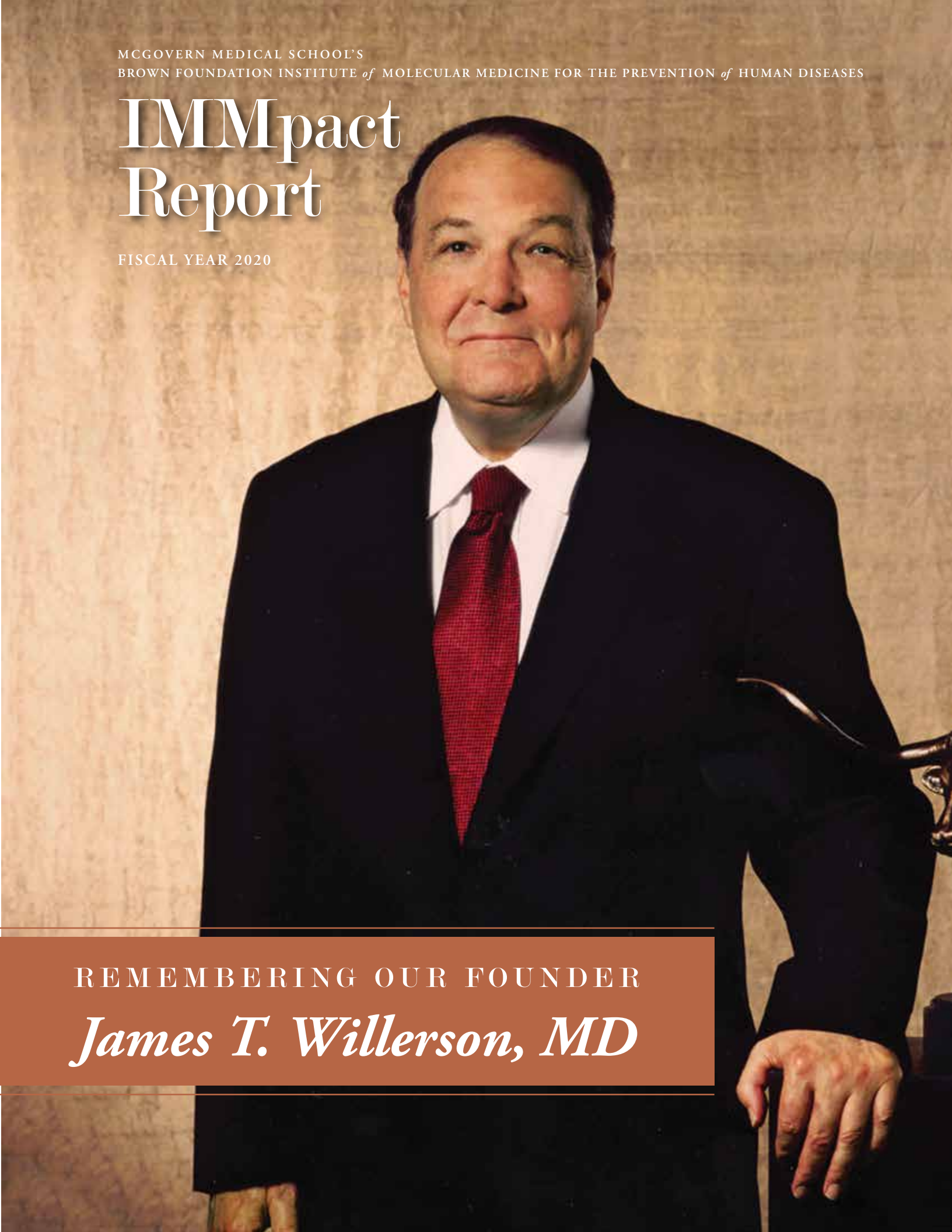
MCGOVERN MEDICAL SCHOOL'S
BROWN FOUNDATION INSTITUTE *of* MOLECULAR MEDICINE FOR THE PREVENTION *of* HUMAN DISEASES

IMMpact Report

FISCAL YEAR 2020



REMEMBERING OUR FOUNDER

James T. Willerson, MD

About the cover

The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases was the brainchild of founder James T. Willerson, MD, who died Sept. 16, 2020. This issue of the IMMPact Report is a tribute to him.

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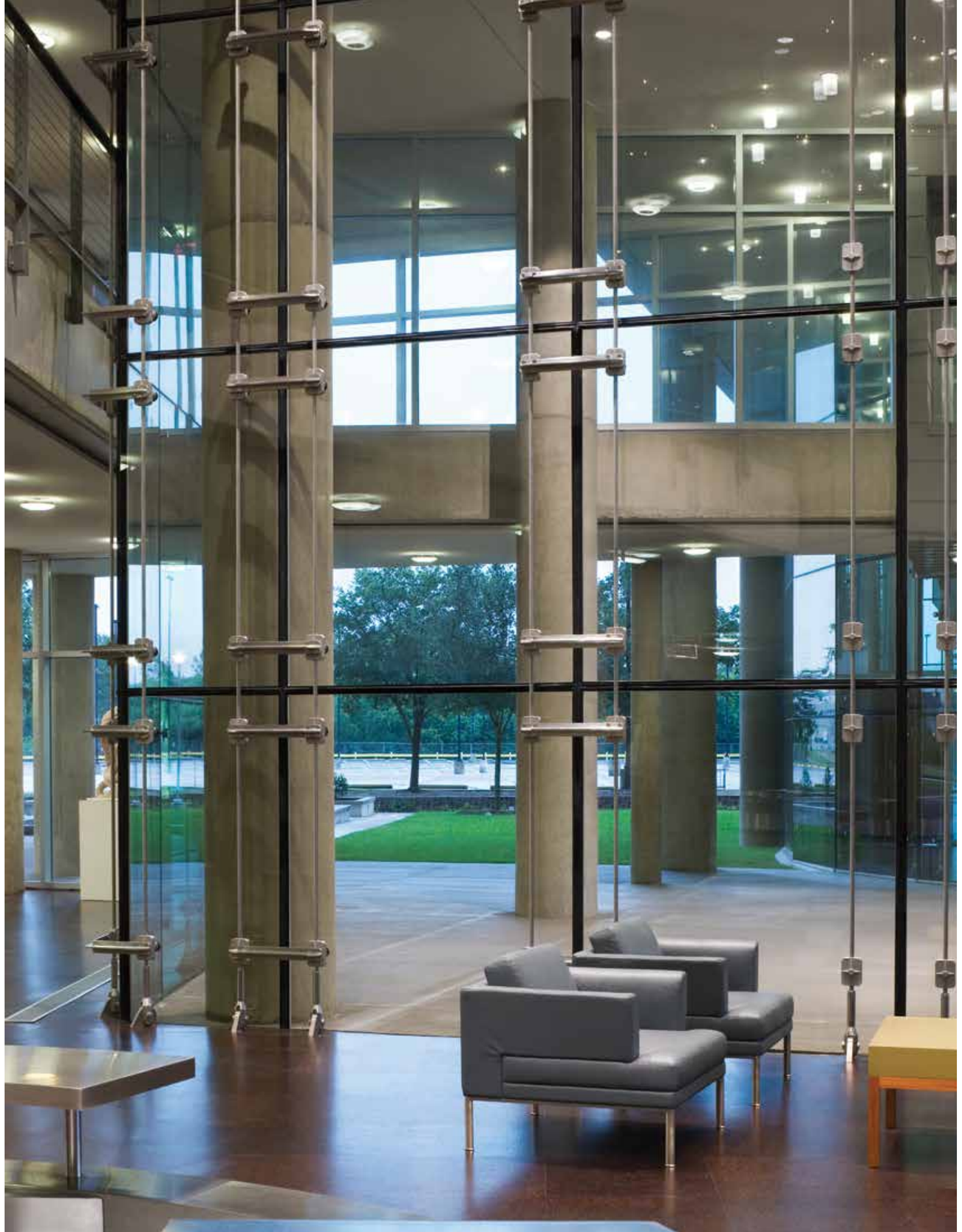

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Director's Message

I am pleased to introduce the latest annual IMMpact report for The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM). The IMM is a stand-alone research institute that is embedded within McGovern Medical School. The IMM mission is to deliver translational outcomes from research in molecular medicine that benefits patients. This mission was proposed by Dr. James Willerson, whose passion for molecular medicine was the driving force for the founding of the IMM. It was his single mindedness coupled with phenomenal fundraising and philanthropy that saw phase one of the project completed in 2006 with the opening of the Fayez Sarofim Research Building that houses the IMM. I say phase one because the opening of this remarkable research building was just the beginning of the outstanding and innovative translational science that continues to define the IMM. Sadly, Dr. Willerson passed on the 16th of September this past year and so to honor him, we are dedicating this year's report to him by going back to the beginning of IMM, to explore how his vision was received by some of the original IMM faculty and the fundraising committee that was tasked with bringing that vision to fruition. Inside the report you will also find his vision in action with in-depth accounts of the innovative research programs pursued by each of our current IMM faculty. There are many metrics that can be used to define research and institutional success, including grant funding, scientific publications, spin out companies, and the capacity to recruit and retain stellar scientists from around the world. By all these metrics the IMM excels; an impressive and enduring legacy of our founder, Dr. Willerson.

As with everywhere, COVID-19 impacted our operations this year although not as extensively as some of our sister institutions within the TMC. Research operations were scaled back in April whilst new operating procedures were put in pace to ensure safe working in the laboratories and then ramped up again in May, such that by summer all labs were operating pretty much as normal, albeit with all workers masked and social distancing observed. Unfortunately, we had to cancel the IMMpact symposium, which was scheduled in April because of COVID concerns. We have not yet decided on a new date, but if the COVID vaccination program, currently underway in Houston, continues to drive down new cases, then a fall symposium may be possible. In the interim we are in the process of developing an IMM Webinar series as an alternative to the symposium, which will showcase short research presentations from our faculty together with question-and-answer sessions and special video coverage of their respective laboratories. We hope to start releasing these webinars next month.

Despite these challenges I am pleased to report, that once again IMM faculty have nevertheless excelled in NIH, DOD, CPRIT and other extramural grant funding. Over the financial year just ended, our new grants and contracts matched last year, which has a best ever for new funding, capping increases in our extramural grant funding for each of the last seven years. It is a testament to the remarkable quality and creativity of our scientists that the IMM remains so successful in attracting research funds. That said, full implementation of our mission remains heavily dependent on attracting support from alternative sources, including research charities and foundations, industry collaborations, and, most importantly, the continuing generosity of our friends and donors. In this context, we are as always deeply appreciative of the strong work and dedication of the IMM advisory council, which plays a key role in the continued growth and development of the IMM.

In conclusion I want to return to Dr. Willerson, his contributions to teaching, education, medical service and research at UTHealth and the broader TMC are too lengthy to list, but for all of us here paramount is his gift of the IMM, and the enduring legacy of scientific and medical discoveries that have only been possible because of it. In Dr. Willerson's own words…. *"Our genes and proteins are the game officials of our lives. They already know if you have a cancer in your future. Or dementia. Or some other devastating disease. We must identify these genes and proteins in our bodies and discover ways in which they might be altered to prevent those diseases from occurring in the first place . . . That research is the role of the IMM"*

We at the IMM are indeed privileged to be realizing Dr. Willerson's vision for molecular medicine at UTHealth. If you would like to investigate how you also can help us further in this regard, I would be very pleased to talk with you personally.

John Hancock, MA, MB, BChir, PhD, ScD
Executive Director, Institute of Molecular Medicine
John S. Dunn Distinguished University Chair in Physiology and Medicine

Mission

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

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

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

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

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

Dr. James Willerson: A vision for excellence

A beautiful burnt orange building in the heart of the Texas Medical Center filled with the world's best scientists working to cure the greatest diseases of our time in our time.

This was the vision of James T. Willerson, MD, which was realized as the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases rose from a plan to reality with the backing of UTHealth and UT System leadership, colleagues, community members, elected officials, and supporters who believed in the future of science.

Dr. Willerson was a pioneer who embraced a vision of excellence in a quest to create a scientific institute unlike any other. Today that institute is known as the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM).

Born Nov. 16, 1939, Dr. Willerson grew up in San Antonio with both parents as doctors in private practice. Dr. Willerson had stated his firm intention to follow in their footsteps early in life. His introduction to Dr. Denton Cooley, founder of the Texas Heart Institute, as a teenager, had a profound impact on the direction of his career.

A proud graduate of The University of Texas at Austin, Dr. Willerson earned his medical degree from Baylor College of Medicine and completed postgraduate training at Harvard Medical School and Massachusetts General Hospital. Prior to his work in Houston, he was on the faculty of The University of Texas Southwestern Medical School in Dallas.

As president of UTHealth, a position he held from 2001-2008, Dr. Willerson aimed to build a university foundation poised for greatness. The IMM was integral to that success.

"I am very proud of the fact that we were able, with Beth Robertson and Rodney Margolis and many friends in Houston, Legislature, UT Regents, to build the IMM and continue to recruit some of the world's best scientists," he said back in 2008. "As I've said about each of our schools, poised for greatness depends on our constant recruitment and retention of the best scientists with a commitment to basic medical science discovery to translate to patients for cure and prevention of their diseases so wonderfully placed in the world's largest medical center with colleagues. I expect great discoveries that benefit mankind to come from our IMM to uplift scientific discovery and translate to our schools, to develop strong research efforts with collaborative grants and educational programs."

Throughout his career, Dr. Willerson always stressed the importance of all three areas of the mission – education, patient care, and research, noting no one area was more important than the other. "We need to be outstanding in each area," he said.

Dr. Willerson always led by example. Not only was he a mentor and teacher, a world-renowned expert pursuing gene therapy and stem cell research, he also was the caring physician for more than 2,000 patients.

The IMM was born in 1989. That was the year Dr. Willerson came to Houston – recruited as chair of the Department of Internal Medicine at The University of Texas Medical School at Houston (now known as McGovern Medical School).

Dr. Willerson imagined the institute as a collaborative environment of scientists not only elucidating the roles of genes in disease but also developing genomic-tailored therapies to combat the most challenging diseases.

"Molecular medicine is a very exciting field, and we must be at the cutting-edge," he once said. "Genes are the

With David Grimes and Dr. Giuseppe N. Colasurdo.

"The vision behind this new building is a vision for understanding the intricate pathways and molecular processes that determine for each individual how human diseases occur."

-James T. Willerson, MD, on the dedication of the Fayez S. Research Building, 2006

> Our success is dependent on scientific talent, and, most importantly, our will to discover and apply new knowledge in technology to better the human condition.
>
> *-James T. Willerson, MD*

drugs of the future. Better yet, if you can predict disease or prevent it altogether, then we can reduce human suffering and the difficulties – including cost – that go with it."

In 1993, Dr. David Low, then-president of the UT Health Science Center, formally announced the university's support of the institute with the kick-off of a $40 million fundraising initiative headed up by Rodney Margolis.

In 1995, Dr. Willerson recruited the first scientific director of the IMM, Hans Muller-Eberhard, MD, PhD. His wife, Irma Gigli, MD, was recruited to lead the IMM's Center for Immunology and Autoimmune Diseases. The next decade was spent growing and focusing the IMM as it moved into temporary space in the Texas A&M Institute of Biosciences and Technology, on the outskirts of the Texas Medical Center.

As the human genome sequencing race transfixed the world, Dr. Willerson capitalized on the scientific fervor, winning over the support of generous community members and elected officials with his vision of the IMM. A campaign was initiated – this one chaired by Beth Robertson and Ben Love, with a fund-raising goal of $200 million.

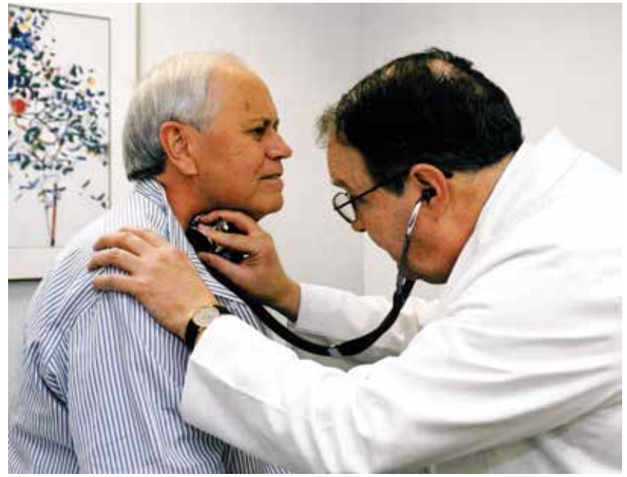
More than $236 million later, the seven-story Fayez S. Sarofim Research Building opened as the Institute's home in 2006, ushering in a new era of research for UTHealth. With scientists in modern labs, pursuing the latest research in a modern environment created to further molecular medicine, the vision became reality.

By 2006, seven research centers had been established at the IMM – each staffed with outstanding faculty pursuing novel work: Cardiovascular Diseases, Cell Signaling, Human Genetics, Immunology and Autoimmune Diseases, Protein Chemistry, Stem Cell Studies, and Nanotechnology. Today's eight centers are targeted to innovative areas to produce discoveries and translational outcomes.

Dr. Willerson never considered his job work. "It's not work, it's opportunity," he once said. "My goal is to make The University of Texas Health Science Center at Houston what it is supposed to be – a health university with excellence at each of our schools."

Following the 9/11 attacks on our nation, he sent a university-wide message, reminding "each one of us has an uncertain number of days on this earth in which to do meaningful things. Let us recommit ourselves to using them wisely and work together to create an environment here in which all of us have the opportunity to be the best we can be."

Dr. Willerson died Sept. 16, 2020, leaving a legacy of excellence – the crown jewel of which was the IMM. At the time of his death, he was the president emeritus, director of cardiology research, and co-director of the Cullen Cardiovascular Research Laboratories at Texas Heart Institute at CHI St. Luke's Health-Baylor St. Luke's. On Nov. 19, 2020, the UT System Board of Regents unanimously named him president emeritus of UTHealth.



Caring for one of his many patients.

Remembering Dr. Willerson

Several of our current Institute of Molecular Medicine faculty were founding faculty members, on staff when Dr. James T. Willerson was leading the Institute. We asked them for their remembrances of our founder.

Ali J. Marian, MD

Professor and Director, Center for Cardiovascular Genetic Research, George and Mary Josephine Hamman Foundation Distinguished Professor in Cardiovascular Research

Dr. Marian published a full paper on the memory of Dr. Willerson in Circulation Research, which may be found at go.uth.edu/willerson An excerpt is included below.

Leadership was natural to him. It was in his genes. It was coupled with his huge vision, the vision of unifying all forces against cardiovascular diseases. He led at The University of Texas at Southwestern and at The University of Texas Health Science Center at Houston. As the president of the university, he built institutions, established programs, and recruited world-class scientists. He was the founding father of the Institute of Molecular Medicine. He had the strong conviction that from the basic science discoveries will come the knowledge the predict, prevent, and cure cardiovascular diseases.

And he was truly a gracious man. When he was editor of *Circulation*, I was one of his associate editors, and we were at the annual meeting of the editorial board. He acknowledged several of the associate editors and forgot to mention my name and likely a few others. This is typically not a big deal as no one expects all of the editors to be acknowledged. A day later, he realized he had missed a few names. I received an apology in person, a personal handwritten note, and a beautiful bouquet of flowers. Of course, none of this was expected, and likely the others he forgot received the same treatment.

Ba-Bie Teng, PhD, FAHA

Professor of Molecular Medicine
The Jerry and Maurry Rubenstein Distinguished Professorship in Heart Research
Center for Human Genetics

I joined UTHealth, Institute of Molecular Medicine, in May of 1998 as a young faculty. I remember meeting Dr. Willerson to discuss a proposed collaborative research project. He greeted me and our other collaborators with his famous, fatherly, warm smile and gave us constructive criticism on how to proceed with our project. Dr. Willerson's enthusiasm for science and medicine was infectious, and it was a motivating force in my career. It was his vision and perseverance that built the Institute of Molecular Medicine and helped it become an icon of research excellence in the Texas Medical Center. I am grateful to have known Dr. Willerson. He will be remembered.

Peter Doris, PhD

Professor and Director, Center for Human Genetics
Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research

In 1979, I enrolled in the PhD program in physiology at the University of California, Riverside. One of the professors who served on my advisory and examination committees was recently arrived in California from her training at UT Southwestern. She was interested in cardiac glycoside drugs and heart function. As I spent time around her in the lab and in classes, I began to hear of a person who she viewed as a legend in cardiology … a person who bridged excellence in clinical medicine with outstanding innovation in heart research. That person was James T. Willerson, MD. I took note of her impressions and held on to them.

In 1997, I visited Houston in connection with a possible faculty opportunity in the newly created Institute of Molecular Medicine. As I learned about how this new institute had come into creation, I was told that, to attract the legendary James T. Willerson, MD from Dallas to Houston, he was offered the opportunity to bring to reality his vision that contemporary biomedical research needed to move into a new era wherein the tools of molecular biology were harmonized and integrated with the problems of clinical medicine. As I understood whose vision this was, my past recollections about Dr. Willerson as a person committed to advocating for medical research that bridged bench and bedside, created a surge of excitement about the new opportunity I was exploring.

During that visit to Houston, I spent 30 minutes with the legend. Not all legends are larger than life. In my mind, such a person was supposed to be huge both in persona and in stature. But Jim Willerson was a compact person with a quiet, deliberate, and modest demeanor. I was impressed that he wasted no energy: his manner was concise and direct, and he was completely lacking in self-doubt. He knew what he believed, and he believed it because it was obvious to him from his own experience in medicine. This was no follower of the ideas of others. I was certain this was a leader who was completely harmonized with my own aspirations in medical science.

During the early years at IMM, Dr. Willerson was heavily engaged in advancing the nascent institute. He was patient, never hurried, but persevered. I saw his persuasiveness. It surprised me. I learned that leadership and innovation was not about loud or noisy claims regarding his own importance or that of his mission. He reached out to the audience of potential donors who might help build and support his vision in his typical earnest, but restrained manner. Quiet, calm, clear, moderate, and confident. The combination was utterly persuasive. He generously moved the spotlight from his own aspirations to the actual investigators who were beginning to bring the vision of IMM to reality.

As we moved forward to actually raising and occupying the splendid building that is now our home and filling it with science, I was sometimes surprised to find that Jim Willerson had little interest in taking credit for the new institute that was forming. For him, I think, the accomplishment was not in being recognized for the achievement that was his vision, but in the knowledge that he had helped create something unique and good and valuable. His effort was genuinely for the benefit of the world he lived in and loved.

Irma Gigli, MD

Professor Emeritus, The Walter & Mary Mischer Distinguished Professor in Molecular Medicine
The Hans J. Müller-Eberhard Chair in Immunology Director Emeritus, IMM Center for Immunology & Autoimmune Diseases

Dr. Willerson's legacy in the IMM is a vision of how you can integrate human beings and different components. The IMM is his major contribution, and the opening of the new building of the institute was a very special day for both of us. I think Dr. Willerson loved the IMM, and his devotion to the institute cannot be denied.

I don't think many people know of how the idea of the institute came about. I was chair of the Department of Dermatology at the University of California San Diego when I was asked to interview a candidate to be the head of cardiology. The candidate was Dr. Willerson. He walked into my office and saw a large poster of a lecture that my husband (Hans Eberhard) had given the year before for an important organization. He was sort of fixed looking at it, and said, "I have a great deal of admiration for that man." And I burst out laughing and said perhaps it's good to be interested in your own husband. We used different names and had our own careers. Dr. Willerson told me he was not interested in the job for which I was to interview him, but said he was looking for good faculty for when he took a position in Houston. He later met with my husband in Germany and said he had an idea of an institute that studied diseases from many different aspects. He continued to develop this in his mind with Hans as the director and me as the codirector. Finally, he got us to agree, and we then looked for architects and made all of the plans. Before the building was built, we had the top two floors of the A&M building on Holcombe and worked in the labs and recruited people. My husband developed advanced prostate cancer and died after a few years. I worked very hard with the architects to develop the concept that eventually became the building of the IMM. It's a place that I love.

Rick Wetsel, PhD

Professor and Director, Hans J. Müller-Eberhard & Irma Gigli Research Center for Immunology and Autoimmune Diseases
Hans J. Müller-Eberhard, MD, PhD, and Irma Gigli, MD Distinguished Chair in Immunology

I will always be grateful to Dr. Willerson for bringing me back to Texas in 1996 as one of the first faculty members of the newly established Institute of Molecular Medicine for the Prevention of Human Diseases. His enthusiasm for the Institute was contagious, and it was a privilege to assist him in the development and fund-raising efforts in those early years. Jim was a visionary, and his ability to raise funds to support his vision was unsurpassed by anyone that I have ever met. He was tenacious in his efforts and in his humble but persistent manner simply would not take no for an answer. His ability to work tirelessly with little sleep was always something I admired but could never hope to emulate. Jim was a thoughtful and supportive mentor for numerous young physicians and biomedical scientists. Through them, his legacy will live on.

History of Leadership

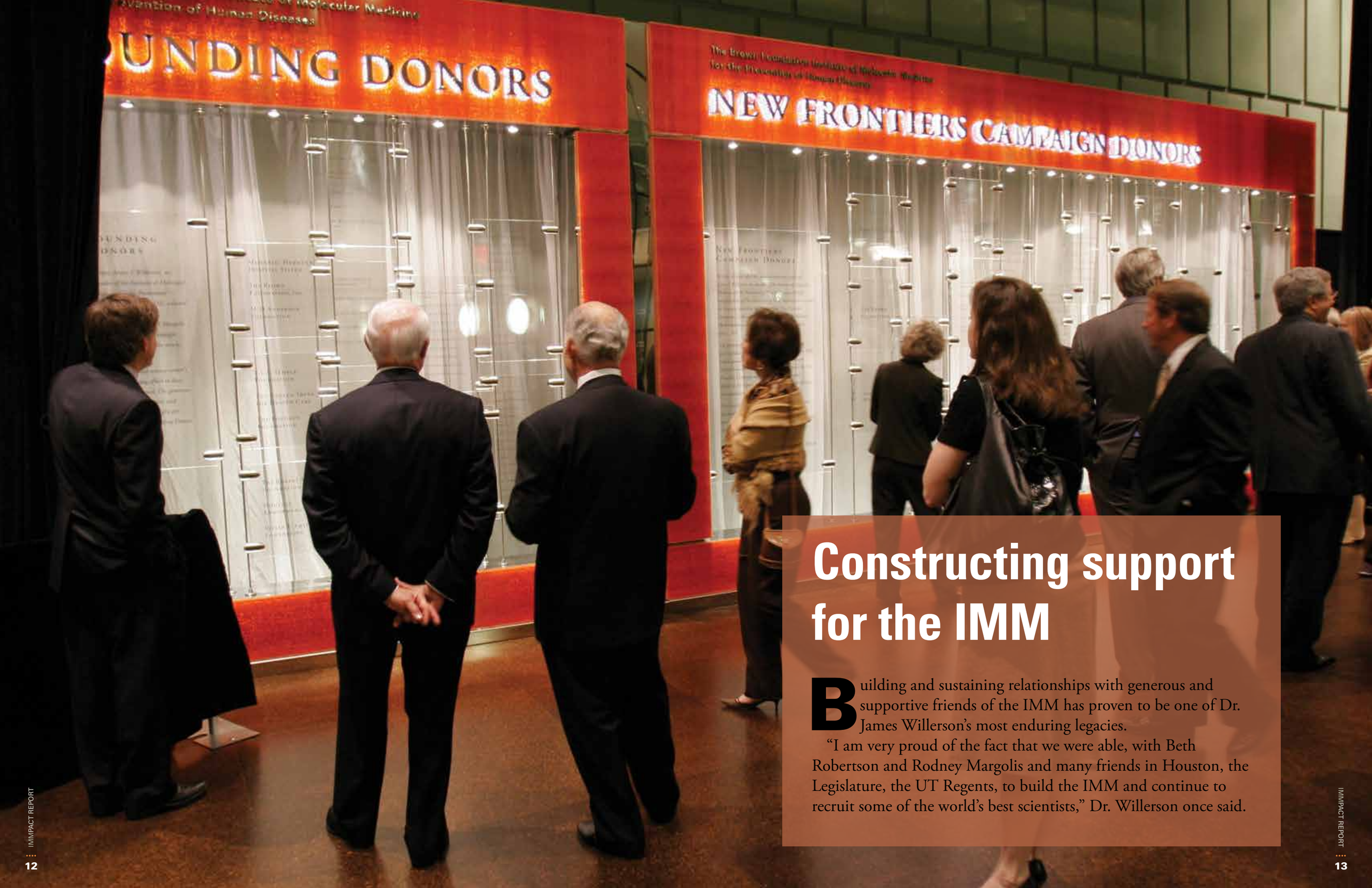
1995 Hans J. Müller-Eberhard, MD, PhD - *Founding Director*

1999 Ferid Murad, MD, PhD - *Director*

Irma Gigli, MD - *Deputy Director*

2006 C. Thomas Caskey, MD - *Director and COO*

2012 John Hancock, MA, MB, BChir, PhD, ScD - *Executive Director*



Constructing support for the IMM

Building and sustaining relationships with generous and supportive friends of the IMM has proven to be one of Dr. James Willerson's most enduring legacies.

"I am very proud of the fact that we were able, with Beth Robertson and Rodney Margolis and many friends in Houston, the Legislature, the UT Regents, to build the IMM and continue to recruit some of the world's best scientists," Dr. Willerson once said.

Houston is a very generous city, and Dr. Willerson was an excellent and caring physician whose scientific knowledge and drive encouraged hundreds to understand, and finance, the power of molecular medicine.

"He told me that when he arrived at UTHealth, nothing was singularly more important to him than accelerating and cementing the institution's research capacity," recalled Randa Safady, PhD, UT System vice chancellor for external relations, communications, and advancement services. "He knew it was the best way to recruit the best and brightest scientists to UTHealth and the TMC, to draw increased sponsored research support, and to be more competitive on a national and international level. He said he would always be 'relentless and in a hurry' with this pursuit. He successfully pushed for more research space to achieve those aspirations."

Former UT System Chancellor Mark Yudof with Dr. Willerson

Focused on growing research, Dr. Willerson recruited Hans J. Muller-Eberhard, MD, as director and his wife, Irma Gigli, MD, as the co-director of the IMM. A team of scientists soon began working in two floors of the Texas Medical Center's Texas A&M research building. Planning quickly started for an independent IMM building, on which Dr. Gigli worked hand-in-hand with the architects. "I put all of myself in what came to be, and the community was happy in seeing what the money could help to develop," she said.

"I still remember vividly his vision for the IMM, which he told me about in 1990," said Ralph Thomas, former chair of the UTHealth development board and senior vice president of Fayez Sarofim & Co. "He impressed me with his vision and dedication. It was inspiring."

In 1995, Dr. Willerson set his sights on realizing a new home for the IMM – a 223,000-square-foot state-of-the-art building, selecting Rodney H. Margolis, Houston community leader, philanthropist, and long-standing friend, to help lead a fundraising initiative to help construct it.

"Jim asked me to head up the campaign and thought Dr. Eberhard was brilliant. We would solicit for philanthropy, and people were so receptive whey they would tell their story.

"We would go to a couple of philanthropic groups who had never given money for molecular medicine, and after he spoke they would say, 'how much money do you want?'" recalled Margolis, who had known Dr. Willerson since their student days at The University of Texas at Austin, where they both were members of the Texas Cowboys.

"A brilliant diagnostician and even better caregiver, Dr. Willerson was everybody's doctor. He had a list of probably 2,500 or 3,000 patients. All 'grateful patients,' but he knew all of them and found time for all of them," said Beth Robertson, who in 2001 stepped up as co-chair, with the late Ben Love, of the New Frontiers Campaign, whose goal was to raise $200 million in support of the IMM.

Dr. Safady recalled the New Frontiers Campaign's early days. "Dr. Willerson was thrilled when Beth said she would lead the campaign. I remember she said she couldn't head up another campaign, and Dr. Willerson said she was the only one he could go to, so she said yes. You can't say no to Dr. Willerson, and she was so committed to him and the IMM, and the results showed," Dr. Safady said.

"We traveled around Houston and the state where

he pitched the idea of the IMM –a world-class institute that would attract world-class researchers/MDs to UT and TMC to make big discoveries that would translate into curing human disease here," Robertson recalled. "And we had amazing results from these grateful patients. They wanted to support Dr. Willerson and his crusade against human disease. We all believed in Dr. Willerson."

"We were able to raise a great deal of money," agreed Dr. Gigli, the Walter and Mary Mischer Distinguished Professor in Molecular Medicine and the Hans J. Muller-Eberhard Chair in Immunology director emeritus. "But the community won't give money unless it is going to something worthwhile."

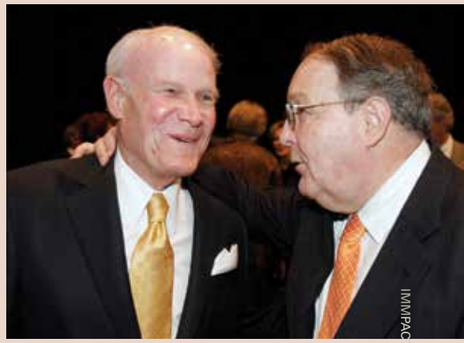
Dr. Gigli also remembered Robertson's involvement. "Beth Robertson was unbelievable," she recalled. "She was very involved and expanded our enthusiasm for the institute."

Throughout the life of both campaigns, more than $240 million was raised through 350 gifts. The largest gifts were $25 million from building namesake Fayez S. Sarofim, founder and owner of the investment firm Fayez Sarofim & Co., and $20 million from The Brown Foundation, Inc., for which the IMM is named.

"The Brown Foundation is proud to join UTHealth in celebrating Dr. Willerson's legacy of excellence and community impact. We are honored to have been an early supporter of his pioneering leadership," said Will Mathis, on behalf of Foundation Trustees.

The generous support from all donors resulted in the recruitment of world-renowned scientists – through the creation of 27 faculty endowments – and ultimately the 2006 grand opening of the Fayez S. Sarofim Research Building, home of the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases. IMM for short.

"We recently had a good laugh about a meeting we had some 14 years earlier about the long name as it would appear in signage and on buildings. He initially insisted on signage in brightly illuminated orange – in fact, he wanted the whole UTHealth



Rodney Margolis lent his support to the IMM.

Beth Robertson co-chaired the New Frontiers campaign.

campus to be illuminated that way," Dr. Safady remembered of Dr. Willerson. "When the Sarofim Building was nearing completion, he wanted large font, orange-lit letters affixed on top of the building to read: The Fayez S. Sarofim Building: The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases. We talked then about how that size and quantity of lettering would be physically impossible without it wrapping it completely around the building. He acquiesced on the exterior orange-lit lettering and agreed to orange up-lighting of the building instead, but his profound dedication to the IMM obviously has made it shine brightly in many additional ways over the years."

Speaking of names, the Beth Robertson Auditorium is the IMM's largest auditorium, a beautifully designed space that hosts invited lectures and speakers, as well as IMM faculty. The Margolis Faculty Lounge, a cozy and inviting space, is located on the third floor of the IMM, overlooking the building's expansive atrium, known as James T. Willerson, MD Discovery Hall.

"I was so intent on naming something for Jim – for the magnitude to his involvement to UT, the magnitude of how he would deprive himself of so much just to practice medicine. He was so dedicated to his work," Margolis remembered.

At the ribbon-cutting of the Sarofim building in November 2006, then-Chairman of the Board of Regents James Huffines and then-UT System Chancellor Mark Yudof were especially proud to be on the platform together and effusive in their praise for Dr. Willerson and the collaboration that achieved the IMM.

"The research done here – every discovery and every advance – will be a testament of generosity that helped to make it happen."

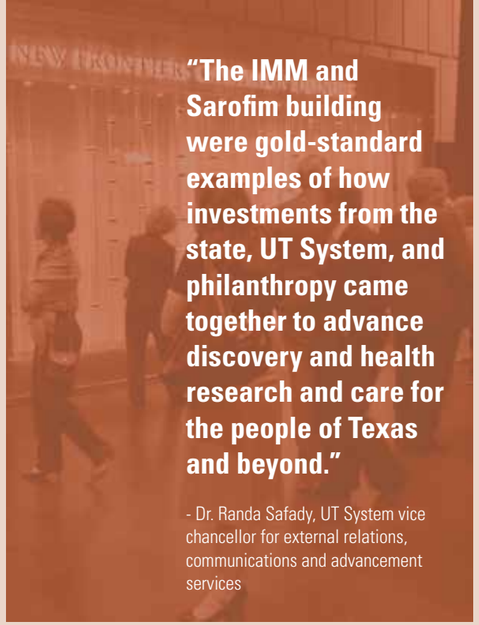
- James Huffines, former chair of the UT Sysem Board of Regents

"The world-class facility, which we've added to the UT System today, was designed to encourage the work of some of the world's best minds as they advance the cause of better health and health care for Texas and the nation," Huffines said at the building's dedication. "The research done here – every discovery and every advance – will be a testament of generosity that helped to make it happen."

"Huffines and Yudof were embarking on a comprehensive UT System Competitiveness Initiative, which resulted in more than 6.5 million square feet of lab and research space across the system's institutions and more than $2.6 billion in new and renovated research and clinical space," Dr. Safady recalled. "The IMM and Sarofim building were gold-standard examples of how investments from the state, UT System, and philanthropy came together to advance discovery and health research and care for the people of Texas and beyond."

Support for the IMM and its mission from the community and generous friends continues today. Dr. Gigli, a longtime member of the Development Board and the IMM's Advisory Council, is unwavering in her passionate promotion of the IMM through gifts of scholarship, the Muller-Eberhard Memorial Lecture Series, and estate gifts.

"I love the IMM, and I think Dr. Willerson loved the IMM. It was put together by three people who loved the idea and loved the place," Dr. Gigli said. "You don't get a place like that in one or two years – it takes a commitment to get the right people."



"The IMM and Sarofim building were gold-standard examples of how investments from the state, UT System, and philanthropy came together to advance discovery and health research and care for the people of Texas and beyond."

- Dr. Randa Safady, UT System vice chancellor for external relations, communications and advancement services

"My impression of Jim, over time, was that he was the most dedicated, multi-talented person I've ever known," Thomas added. "He led innovative research, was a wonderful physician, and an inspiring leader of the UT Health Science Center, and it was a real privilege for me to be a part of this for over 30 years, seeing the execution of his vision."

Dr. Irma Gigli, a founding leader of the IMM.

Dr. Willerson created in the IMM what he envisioned as the standard for medical research, Robertson observed. "He came to UT because he loved the institution. He was a brilliant diagnostician, an incredible leader and had so much energy, doing everything at once.

"He was prepared to demonstrate that this type of quality and world-class research was what he was thinking for the whole institution, that it would give us a vision of how we would take it from the lab to the bed," she said.

His supporters and friends still marvel, remembering his unique abilities and talents.

"There won't be another person like Jim Willerson to cross your path or my path again, for dedication or loyalty again – can't find anyone stronger again," Margolis added. "Jim was so dedicated to the concept of molecular medicine there are not words in the dictionary to celebrate his dedication to medicine."

"How on earth does Jim do it?" Thomas wondered. "There are not that many hours in the day – he had at least three different roles going at the same time and not one was left beside by his emphasis on something else – he was dedicated and well-balanced."

Community supporters included Mayor Bill White.

The IMM, most agree, is Dr. Willerson's crowning achievement.

"The IMM has done what he hoped, I think," Robertson said. "A high-quality pinnacle is what he wanted. Jim Willerson was the high-quality pinnacle person. There was never a dull moment raising money with him. He was not going to be denied. A big UT fan and former UT swimming star, he was very competitive. I admired him – not just for his energy/drive and his thoughtful and encyclopedic knowledge, but also but for his empathic kindness to others. I was blessed to have him as my doctor, but more blessed to have him for my friend."

TIMELINE

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

1989

James T. Willerson, MD joins The University of Texas Health Science Center at Houston and announces his vision to develop an Institute of Molecular Medicine for the Prevention of Human Diseases (IMM) in Houston's Texas Medical Center. Fundraising begins.

1993

APRIL

M. David Low, MD,PhD, president of the UT Health Science Center at Houston, formally announces a plan to establish an institute that specifically will target the prediction and prevention of human diseases – The Institute of Molecular Medicine for the Prevention of Human Diseases. He announces the first receipt of gifts totaling $7.2 million to enhance molecular research. A $40 million fundraising initiative also is announced that will later be expanded to the $200 million New Frontiers Campaign to house and support the new institute.

1995

Hans Müller-Eberhard, MD, PhD, well-known for his pioneering work elucidating the complement system, is recruited by Dr. Willerson as the first scientific director of the new IMM. His wife, Irma Gigli, MD, is simultaneously recruited to lead the IMM's Center for Immunology and Autoimmune Diseases.

1995

MARCH 1

Dr. Gigli becomes the IMM's first faculty member. Plans to house the IMM in the renovated UT Speech and Hearing Building are revised as space is leased and readied in the Albert Alkek Building of the Texas A&M Institute of Biosciences and Technology.

1995

JULY

Müller-Eberhard, MD, PhD, arrives in Houston. Prior to his appointment he was director of the Bernhard Hocht Institute for Tropical Medicine in Hamburg, Germany. He begins develop research programs in immunology, infectious diseases, cardiovascular diseases, neurobiology, and cancer research at the genetic level.Recruitment of scientists in these specialties begins.

1996

IMM occupies first space in the Albert Alkek Building of the Texas A&M Institute of Biosciences and Technology.

1998

MARCH 3

Dr. Müller -Eberhard dies at MD Anderson Cancer Center.

1999

JANUARY

1998 Nobel Laureate Ferid Murad, MD, PhD, is named director of the IMM by Low, then-president of the UT Health Science Center. Dr. Murad continues to direct the institute's Research Center for Cellular Signalling. Dr. Gigli is named associate director of the IMM in addition to her directorship of the Research Center for Immunology and Autoimmune Diseases.

2003

FEBRUARY

The University of Texas System Board of Regents approves plans to move forward with architectural plans and design for a new IMM building. Designed by the Missouri firm of Berkebile Immenschuh Nelson McDowell Architects and Burt Hill Kosar Rittelmann Associates from Pennsylvania, the seven-story building is planned to be adjacent to University Center Tower (UCT).

2003

SEPTEMBER

Groundbreaking event for the IMM's new building.

2004

JANUARY

The Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases becomes the official name of the institute, in recognition of a $20 million gift by The Brown Foundation, Inc.

2005

FEBRUARY

UT System Regents approve renaming the new building The Fayez S. Sarofim Research Building in recognition of the largest gift every received by The University of Texas Health Science Center at Houston—$25 million to advance stem cell research.

2006

FEBRUARY

Thomas Caskey, MD, is named director—and CEO-elect of the IMM, joining the leadership team of Drs. Murad and Gigli as chief operating officer and executive vice president of molecular medicine and genetics.

2006

JANUARY 11

Dr. Willerson announces to the UT Health Science Center Development Board that the New Frontiers development campaign is successfully completed – reaching and surpassing its $200 million goal. Campaign co-chairs, Beth Robertson and the late Ben Love are recognized for their leadership in fundraising.

2006

MAY

First faculty and staff occupy new Sarofim Research Building.

2006

NOVEMBER

Sarofim Research Building is formally dedicated.

2012

John Hancock, MA, MB, BChir, PhD, ScD, is appointed executive director of the IMM.

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

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

Faculty: Priyatansh Gurha, PhD, assistant professor; AJ Marian, MD, professor

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

Research Programs:

The research programs are as follows:

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

We are actively recruiting additional probands and family members.

II. Genomics and epigenetic studies of human heart failure and mouse models of cardiomyopathies: The studies predominantly relate to DCM and ACM and included whole transcriptome analysis by RNA-Seq, DNA methylation analysis, and analyzing chromatin remodeling by ChIP-Sequencing. Specific epigenetic regulators of gene expression are identified and targeted in order to delineate their functions in the heart.

III. DNA damage response in human hereditary cardiomyopathies: We have detected increased double stranded DNA breaks (DSBs) in human hearts from patients with hereditary cardiomyopathies and in mouse models. Studies are ongoing to define genomic characteristics of the DSBs and to define the pathogenic role of DNA damage response pathways in heart failure.

IV. Therapeutic targeting of dysregulated pathways in cardiomyopathies: Dysregulated pathways identified through integrated genomics are targeted through genetic and pharmacological interventions in model organisms and their effects on survival, cardiac function, and clinical outcomes are analyzed. A major focus currently is on the canonical WNT and the Hippo signaling pathway.

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

AJ Marian, M.D.
Center Director & Professor

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

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

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

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

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

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

The overall approach entails an integrated approach that includes human molecular genetic studies through high throughput whole exome and genome sequencing to identify the causal genes and mutations, followed by genomic studies including transcriptomics and epigenetics to define molecular remodeling of chromatin in the presence of causal mutations. The aim is to link the causal mutations to genomic remodeling and to the pathogenic pathways. The responsible molecular mechanisms are identified through molecular mechanistic studies in genetically modified animal models and cultured cells. The mechanistic discoveries are then utilized to intervene in model organisms, utilizing genetic and pharmacological approaches that target the pathogenic pathways, in order to prevent the evolving phenotype and reverse or attenuate the established phenotype. These findings in the model organisms are extended to human studies through pilot randomized placebo-controlled double-blind clinical trials. The findings, if favorable, are pursued through collaborative large-scale clinical trials.

RESEARCH PROJECTS

- Identification of causal genes for heart failure and sudden cardiac death
- Identification and characterization of epigenetic and transcriptomic changes in hereditary cardiomyopathies
- Identification and characterization of the pathogenic molecular pathways in patients with hereditary cardiomyopathies
- Delineation of the role of the mechano-sensing signaling pathways in the pathogenesis of hereditary cardiomyopathies.
- Defining and characterizing the role of DNA damage in hereditary cardiomyopathies and the utilities of the DNA damage pathway in therapeutic targeting.
- Maverick & Explorer studies: Industry – sponsored clinical trials to test efficacy of an ATPase modulator on improve symptoms and exercise tolerance in patients with obstructive (Maverick) and non-obstructive (Explorer) hypertrophic cardiomyopathy.

KEY PUBLICATIONS

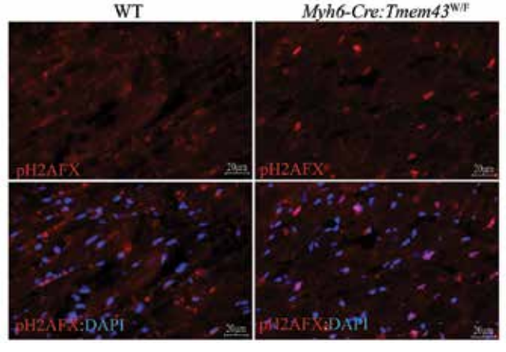
Haplo-insufficiency of Tmem43 in cardiac myocytes activates the DNA damage response pathway leading to a Late-Onset Senescence-Associated pro-fibrotic cardiomyopathy. Rouhi L, Cheedipudi SM, Chen SN, Fan S, Lombardi R, Chen X, Coarfa C, Robertson MJ, Gurha P, Marian AJ. *Cardiovasc Res.* 2020 Oct 18:cvaa300. PMID: 33070193

BET bromodomain inhibition attenuates cardiac phenotype in myocyte-specific Lamin A/C-deficient mice. Auguste G, Rouhi L, Matkovich SJ, Coarfa C, Robertson MJ, Czernuszewicz G, Gurha P, Marian AJ. *J Clin Invest.* 2020. Sept 1, 130 (90 4740-4758, PMID: 32484798

Exercise Restores Dysregulated Gene Expression in a Mouse Model of Arrhythmogenic Cardiomyopathy. Cheedipudi SM, Hu J, Fan S, Yuan P, Karmouch J, Czernuszewicz G, Robertson MJ, Coarfa C, Hong K, Yao Y, Moore HC, Wehrens X, Gurha P, Marian AJ. *Cardiovasc Res.* 2020; 116(6):1199-1213. PMID: 31350552

LAB MEMBERS

Post-doctoral fellows: Sirisha C Marreddy; Leila Rouhigharabaei, PhD
Research assistant: Siyang Fan
Research and clinical nurse: Yanli Tan, RN



Activation of DNA Damage Response (DDR) Pathway, indicated by increased expression of pH2AFX, in the heart in a mouse model of arrhythmogenic cardiomyopathy

Priyatansh Gurha, PhD
Assistant Professor

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

The main objective of my research is to understand the molecular mechanisms that coordinately regulate gene expression and contribute to the pathogenesis of heart failure. Within this theme, we are studying the function of epigenetics and non-coding RNAs in proliferation, differentiation, and maturation of myocytes and how alteration of these interlinked processes eventually leads to cardiac dysfunction and failure. My previous studies have identified epigenetic dysregulation of miR-184 and its role in the pathogenesis of ACM. We have now begun to investigate how reprogramming of epigenetic code governs gene transcription and ensuing cardiac phenotype in human DCM/heart failure (HF). Recently, we uncovered the role of DNA methylation and Lamin Associated Domain in Human HF and identified an epigenetic regulator KDM5, and a novel cardiac myocyte enriched long intergenic non-coding RNA (lincRNA) in the phenotypic manifestation of HF. The role of KDM5 and CM enriched lincRNAs in heart is unknown. We are using induced pluripotent stem cells (iPSCs) and several mouse models to investigate the tissue and cell type-specific contribution of these regulators in cardiac physiology and their contribution toward human HF.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

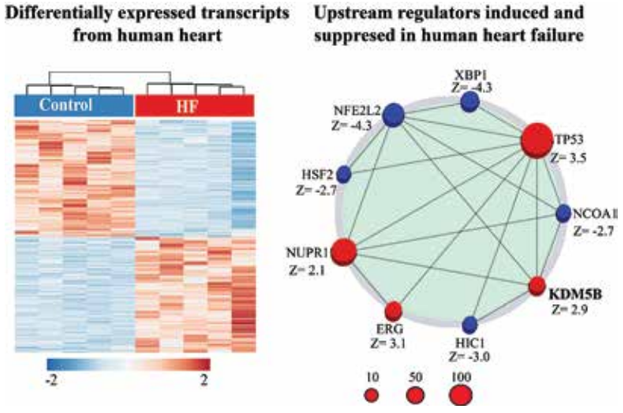
Coste Pradas J, Auguste G, Matkovich SJ, Lombardi R, Chen SN, Garnett Chamberlain K, Riyad JM, Weber T, Singh SK, Robertson MJ, Coarfa C, Marian AJ, Gurha P. Identification of Genes and Pathways Regulated by Lamin A in Heart. *Journal of American Heart Association.* 2020 Aug 18; 9(16): e015690. PMID: 32805188.

Marreddy Cheedipudi S, Matkovich SJ, Coarfa C, Hu X, Robertson MJ, Sweet ME, Taylor M, Mestroni L, Cleveland JC, Willerson JT, Gurha P#, Marian AJ#. Genomic Reorganization of Lamin-Associated Domains in Cardiac Myocytes is Associated with Differential Gene Expression and DNA Methylation in Human Dilated Cardiomyopathy. *Circ Res.* 2019 Feb 11. PMID: 30739589 (# Co-corresponding authors).

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

LAB MEMBER

Post-doctoral fellow: Manisha Deogharia



Heat plot of differentially expressed transcript and dysregulated upstream transcriptional regulators in human heart failure (HF).

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

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

Progress in the laboratories of our investigators continues to yield exciting and important insights. Our human population geneticists, working under the direction of Dr. Myriam Fornage, are global leaders in their field, and are making notable progress in the study of susceptibility to stroke and age-related decline in cognitive function. A significant fraction of sudden cardiac death results from rhythm disruptions that arise in genetic variation in the proteins processing the electrical activity within the heart. Our newest faculty member, Dr. Ashish Kapoor, is an emerging leader in this field. We have shown that kidney injury associated with increased blood pressure results from the emergence of auto-antibodies that damage tissues. This unexpected finding from Dr. Doris' lab points to a role of immune system genetic variation in creating disease risk. Dr. Ba-bie Teng continues to advance understanding of susceptibility to atherosclerosis and the interplay between new drug targets, such as PSCK9, and lipoprotein uptake by cells. As our understanding of the complexity of information storage and retrieval in the genome expands, our colleague Dr. Sidney Wang is addressing approaches to assess, extract, and exploit new levels of genomic complexity that will inform work in this field.

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

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

Peter A. Doris, PhD
Professor/Center Director
Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research

Genetics of cardiovascular end organ injury

High blood pressure is a frequent cause of renal injury, but the risk of renal disease in patients with high blood pressure is best predicted by family history, indicating a genetic predisposition. At present we have almost no knowledge of why high blood pressure creates kidney disease in some people, but not others. To try to fill this knowledge gap, we study a genetic model comprising inbred laboratory rats that have high blood pressure. The divergence of hypertensive renal disease risk seen in humans is also present in these rats. Some lines get progressive renal injury, other lines don't. Therefore, this model provides a means to investigate what genetic differences can drive kidney disease. We can take what we have learned and conceive of treatment approaches to prevent disease and test them in the model.

What we have learned so far:

Genes influencing antibody formation affect the emergence of hypertensive renal disease.

We have identified important genetic variation in the immunoglobulin heavy chain gene, which encodes antibodies. We also have identified genetic deletion in the gene, Stim1. This is a key gene in lymphocyte function. T and B lymphocytes comprise the adaptive immune system. The mutation in Stim1 blocks normal T and B cell function and leads to antibody-mediated autoimmune disease.

Genetic and pharmacological suppression of antibodies eliminates hypertensive renal disease.

To prove that antibodies causes hypertensive renal injury in our model organism, we deleted the immunoglobulin gene. These animals cannot generate antibodies. They have high levels of blood pressure but have no renal injury. An immunosuppressive drug that inhibits B cell function has a similar effect.

Gut bacteria activate the hypertensive immune system and create antibodies that cause disease

When hypertensive rats unable to produce antibodies are raised without antibody replacement, they experience blood infection (sepsis). Blood culture indicates that the infecting bacteria are non-pathogenic bacteria that live in the gut. When antibiotics are given to hypertensive rats prone to injury, renal injury was markedly reduced. The bacteria induce antibodies to a common bacterial protein. This protein is highly conserved in mammals as well as bacteria. These antibodies may prevent this protein from functioning to protect the kidney from pressure-induced injury.

Key questions that are the focus of our current interest:

Do the pathogenic mechanisms active in rats give insight into renal disease in humans? Common genetic variants occur in humans that alter the control of antibody formation and may contribute to disease risk.

KEY PUBLICATIONS

Dhande, I.S., S.C. Kneedler, A.S. Joshi, Y. Zhu, M.J. Hicks S.E. Wenderfer, M.C Braun, P. A. Doris. Germ-line genetic variation in the immunoglobulin heavy chain creates stroke susceptibility in the spontaneously hypertensive rat. *Physiological Genomics.* 51:578-585, 2019 PMID 31608789

Dhande, I.S., S.C. Kneedler, Y. Zhu, A.S. Joshi, M.J. Hicks, S.E. Wenderfer, M.C. Braun, P.A. Doris. Natural genetic variation in Stim1 creates stroke in the spontaneously hypertensive rat. *Genes and Immunity.* 2020. PMID 32300198

Dhande, I.S., Y. Zhu, S.C. Kneedler, A.S. Joshi, M.J. Hicks, S.E. Wenderfer, M.C. Braun, P.A. Doris. Stim1 polymorphism disrupts immune signaling and creates renal injury in hypertension. *J. Amer. Heart Association.* 9(5):e0141422019, 2020. PMID 32075490

Dhande, I.S., and P.A. Doris. Pulling the hood off genetic susceptibility to hypertensive renal disease. *J. Amer. Soc. Nephrol.,* 31(4):667-668, 2020. PMID 32123053

LAB MEMBERS

Post-doctoral fellow: Isha S. Dhande, PhD
Research assistants: Yaming Zhu, MD; Aniket Joshi, BS

We have used Pac-Bio long read genomic sequencing to investigate genetic changes in the gene encoding antibodies (the immunoglobulin heavy chain gene, IGH). This is a new sequencing method that allows genetic differences that occur over larger scales to be revealed. The large IGH gene is comprised of different segments that play different functions during the course of antibody development. In SHR-A3 we discovered that two of these segments have been accidentally duplicated more than once and the duplicated segments have been retained and are still functional. This may disturb normal antibody function and provide one input that creates disease susceptibility.

Myriam Fornage, PhD
Professor
The Laurence and Johanna Favrot Distinguished Professorship in Cardiology

Molecular epidemiology of the aging brain

Throughout our lifetime our brain changes more than any other part of our body. Beginning in midlife, aging brings about subtle changes in brain structure, chemistry, and function. These changes are detectable by neuroimaging techniques such as magnetic resonance imaging (MRI) and are associated with a greater risk of future stroke, cognitive and functional impairment, dementia, and death. Novel 'omics' technologies allow us to characterize and quantify the sets of biological molecules that make up cells, tissues, and organisms on a population scale. These powerful technologies have opened new avenues toward biomarker discovery for risk prediction and risk stratification, enabling informed preventive and therapeutic interventions to slow or reverse brain aging.

A decline in cognitive function, such as reasoning, attention, memory, and language, is strongly correlated with brain aging. Our research program investigates the risk factors that influence cognitive aging using genetic data. In collaboration with researchers in the United States and Europe, we apply genome sequencing technologies to identify genes and gene variants that influence risk for cognitive impairment and associated Alzheimer's disease and stroke. We focus on diverse populations, especially those of Hispanic and African ancestry, who disproportionately suffer from these diseases of aging.

We also utilize knowledge about the common genetic variations that control modifiable exposures, such as high blood pressure, to investigate the causal relations between these modifiable risk factors and health outcomes in large cohorts. This is a technique known as Mendelian randomization. Using this approach, we studied the effect of high blood pressure on cognitive health during middle age, a pivotal period in the life course when cognitive function begins to decline among healthy adults. We showed that high blood pressure, especially high systolic pressure, is causally associated with poorer processing speed, verbal memory, and executive function during midlife. By providing support for a causal relationship between blood pressure and cognitive health in middle age, our study underscores the need for further investigations of the mechanisms of blood pressure dysfunction on cognitive health across the lifespan, which may inform on early intervention and timely treatment of hypertension to maintain brain health.

Besides genetic factors, we also study the link between other molecules, such as DNA methylation, proteins, and metabolites with disease of the aging brain. For example, methylation levels measured at defined sites across the genome are correlated with an individual's chronological age. We showed that accelerated epigenetic aging, which is operationalized as a DNA methylation-based measure of age that is higher than an individual's chronological age is associated with lower verbal fluency in middle-aged adults.

RESEARCH PROJECTS

- Discovering DNA sequence variants influencing Alzheimer's Disease, stroke and neuroimaging markers of brain aging.
- Discovering novel epigenetic (DNA methylation) variants that influence risk for brain small vessel disease and its related neurocognitive outcomes.
- Discovering novel genetic variants for high blood pressure using gene-lifestyle interactions and pathway analysis. In particular, discovering how depression and anxiety affects genetic risk of hypertension.
- Investigating the genetic determinants of cognitive function in diverse Hispanics/Latinos

KEY PUBLICATIONS

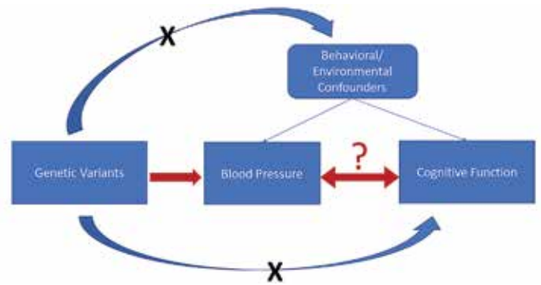
Sun D, Thomas EA, Launer LJ, Sidney S, Yaffe K, Fornage M. Association of blood pressure with cognitive function at midlife: a Mendelian randomization study. *BMC Med Genomics.* 2020;13(1):121.

Jian X, Sofer T, Tarraf W, Bressler J, Faul JD, Zhao W, Ratliff SM, Lamar M, Launer LJ, Laurie CC, Schneiderman N, Weir DR, Wright CB, Yaffe K, Zeng D, DeCarli C, Mosley TH, Smith JA, González HM, Fornage M. Genome-wide association study of cognitive function in diverse Hispanics/Latinos: results from the Hispanic Community Health Study/Study of Latinos. *Transl Psychiatry*. 2020;10(1):245.

Bressler J, Marioni RE, Walker RM, Xia R, Gottesman RF, Windham BG, Grove ML, Guan W, Pankow JS, Evans KL, Mcintosh AM, Deary IJ, Mosley TH, Boerwinkle E, Fornage M. Epigenetic Age Acceleration and Cognitive Function in African American Adults in Midlife: The Atherosclerosis Risk in Communities Study. *J Gerontol A Biol Sci Med Sci.* 2020;75(3):473-480.

LAB MEMBERS

Post-doctoral fellow: Yunju Yang, PhD
Graduate Students: Songmi Lee (PhD program); Nitesh Enduru (MPH program)
Biostatisticians: Bin Shi, PhD; Emy Thomas, MS; Rui Xia, PhD
Research Associate: Ping Wang, PhD



Mendelian randomization uses gene variants to interrogate cause and effect in human cohort data. For example, we have examined whether persons genetically predisposed to having high blood pressure are more likely to have cognitive impairment.

Ashish Kapoor, PhD
Assistant Professor

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

Despite the progress in the prevention and treatment of cardiovascular diseases in general, sudden cardiac death (SCD) remains a major public health problem. SCD, defined as a sudden and an unexpected pulseless condition due to a cardiac arrhythmia (when heart beats out of rhythm) without evidence of a non-cardiac cause, is the leading cause of deaths in US (~500,000 each year) and accounts for ~15% of all-cause deaths and ~50% of deaths from cardiovascular diseases. Moreover, in almost half the cases, SCD is the first sign of an underlying cardiovascular condition. Although many forms of heart disease can lead to SCD, the most common process underlying SCD is ventricular fibrillation (VF), an irregular and uncoordinated contraction of cardiac muscles of ventricles (lower chambers of heart) due to disorganized electrical signals. VF is usually fatal if not reversed by defibrillation immediately. Most of the existing cardiovascular risk factors are poor at predicting SCD, even in those individuals with a history of heart disease, clearly showing that other environmental and/or genetic factors are likely to play a role in developing VF and SCD. Indeed, from population- and family-level studies there is evidence for genetic susceptibility to SCD. However, studies to identify genetic factors underlying susceptibility to SCD directly have had limited success due to pooling of the very diverse forms of heart diseases leading to SCD into one group. Instead, we focus on the electrocardiographic QT interval, an intermediate observable characteristic/trait (phenotype) that predisposes to SCD. Electrocardiography, also known as ECG, measures the electrical activity of heart chambers and the QT interval in an electrocardiogram corresponds to the time taken by ventricles to depolarize (activated state) and repolarize (resting state) in every heart beat. In the general population, QT interval varies across individuals and is a useful clinical marker as both prolongations and shortenings of the QT interval have been known to be associated with increased risk of cardiac arrhythmias and SCD. We are interested in identifying the genes that underlie this variation with the aim that understanding the genetic factors for QT interval variation will potentially impact our understanding of SCD risk and its management. Our studies have the prospect to identify the genetic causes for QT interval variation, some of which in turn could serve as potential therapeutic (drug) targets or potential biomarkers (genes and gene products) to identify individuals at high risk for SCD. What we as a community have learned so far is that many genes together contribute to QT interval variation and that majority of DNA changes leading to QT interval variation do so not by altering the form of the gene product rather by altering the amount of the gene product made by our heart cells. Starting with known genetic associations between DNA sequence variants and the QT interval in the general population, our work involves pinpointing the causes behind these associations to identify the underlying gene defects and how they impact QT interval.

RESEARCH PROJECTS

- Molecular characterization of QT interval genome wide association study (GWAS) signals to identify the underlying causal variants, genes and their mechanisms.
- Evaluation of heart-restricted *Nos1ap* null mice to understand its role in cardiac electrophysiology.
- Functional genomic approaches to understand cardiac gene expression regulation.

KEY PUBLICATIONS

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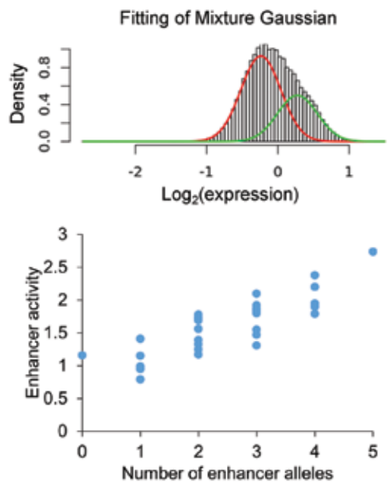
Lee D, Kapoor A, Safi A, Song L, Halushka MK, Crawford GE, Chakravarti A. Human cardiac cis-regulatory elements, their cognate transcription factors, and regulatory DNA sequence variants. *Genome Research* 28:1577-88, 2018.

Kapoor A, Auer DR, Lee D, Chatterjee S, Chakravarti A. Testing the Ret and Sema3d genetic interaction in mouse enteric nervous system development. *Human Molecular Genetics* 26:1811-20, 2017.

LAB MEMBERS

Research assistant: Alexa Smith, BS
Post-doctoral fellow: Parul Singh, PhD



Enhancer activities (reporter expression) of ~500 QT interval associated variants-centered test elements using a massively parallel reporter assay performed in mouse cardiomyocyte HL1 cells and fitting of Mixture Gaussian to the observed distribution (top); Combined additive effect of five SCN5A causal cis-regulatory elements and their enhancer variants on luciferase reporter activity in HL1 cells (bottom).

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

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

Atherosclerosis is an inflammatory disease in the aorta that increases its severity as we age. The disease includes imbalance lipid metabolism that leads to hyperlipidemia and maladaptive immune responses that affect the arterial vasculature. Our research focuses on understanding the development of atherosclerosis and to elucidate the cross-regulation between atherosclerosis and immunity. We generated a mouse model that mimics humans with hyperlipidemia by deleting both LDL receptor (LDLR) and RNA editing enzyme (Apobec1) genes *(LDb=Ldlr-/-Apobec1-/-)*. These mice develop atherosclerosis as they age. Feeding on a Western high-fat diet accelerates their atherosclerosis development. Moreover, male mice develop atherosclerosis faster and more severe than females.

PCSK9 (proprotein convertase subtilisin/kexin type 9) is a causative gene for hyperlipidemia. Patients with elevated PCSK9 levels have increased plasma cholesterol and premature coronary artery disease. We delete PCSK9 gene from *LDb* mice, the *LTp (Ldlr-/-Apobec1-/-Pcsk9-/-)* mice showing decreased atherosclerosis with improved function of endothelial cell. PCSK9 modulates autophagy signaling pathway. PCSK9 modulates SREBP-1c lipogenesis, using lipidomics technology, we demonstrated that PCSK9 induced the production of cholesteryl ester, phosphatidic acid class and phosphatidyl serine class in the liver (see Figure). Oxidized phospholipids have been shown to be associated with increased atherosclerosis development. We analyzed the effect of PCSK9 on the differential gene expressions in the aorta. Our results revealed that PCSK9 upregulated the expression of Cytokine-cytokine receptor interaction pathway, genes such as CCL2, CCL7, IL23A, IL6 and TGFB3 were elevated (see Figure). Collectively, PCSK9 contributes to atherosclerosis through its multiple effects on autophagy, hepatic lipid metabolism and cellular immune function in endothelial cells.

Immune cells are either recruited, or locally activated, resulting in their proliferation in the atherosclerotic lesions, and in turn, contributing to the development of vascular atherosclerosis. *LDb* atherogenic mice exhibit increased plasma interleukin-17 (IL-17), which is associated with increased numbers of T helper 17 cells (Th17). By deleting PCSK9 from *LDb* mice, these triple knockout *LTp* mice have opposite effects with decreased IL-17 and reduced Th17 cells. Thus, PCSK9 is associated with changes in T cell programming that contributes to the development of atherosclerosis.

In the coming years, we plan to use single-cell RNA-sequencing analysis to create a comprehensive single-cell atlas of all cells in the aorta. We will define the differences in sex and age associated with each signature of cell population. We will identify cell subpopulations that influences vascular disease development.

RESEARCH PROJECTS

- The role of PCSK9 in autophagy, inflammation, and atherosclerosis.
- Using CRISPR/Cas9 technique, we generated IL-17 RC knockout mice in the background of LDb. We are currently studying its effect on atherosclerosis.
- Using genetic therapy such as mRNA for the treatment of atherosclerotic vascular diseases.
- Using single-cell RNA sequencing (scRNA-Seq) to sequencing aorta to reveal the atlas of cells in the development of atherosclerotic disease.

KEY PUBLICATIONS

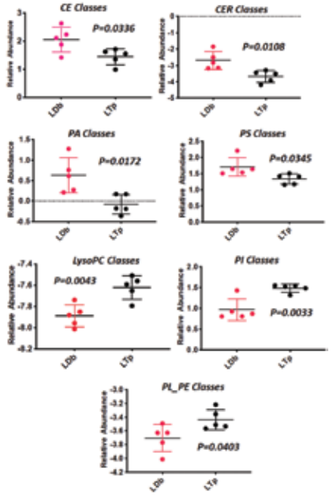
PCSK9 Deficiency Reduces Atherosclerosis, Apolipoprotein B Secretion and Endothelial Dysfunction: Hua Sun, Ronald M. Krauss, Jeffrey T. Chang, and Ba-Bie Teng. *J Lipid Res* 59: 207-223 (2018). PMID: 29180444*.

A critical role of PCSK9 in mediating IL-17-producing T cell responses in hyperlipidemia: Young Uk Kim, Patrick Kee, Delia Danila and Ba-Bie Teng. *Immune Network 19* (6), e41 (2019). PMID: 31921471.

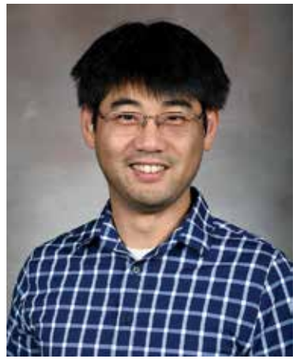
Epac1 (Exchange Protein Directly Activated by cAMP1) upregulates LOX-1 (Oxidized Low-Density Lipoprotein Receptor 1) to promote foam cell formation and atherosclerosis development: William G. Robichaux III, Fang C. Mei, Wenli Yang, Hui Wang, Zhen Zhou, Dianna M. Milewicz, Ba-Bie Teng, and Xiaodong Cheng (2020). *In Press ATVB*.

LAB MEMBERS

Research Associate: Xin Li



PCSK9 modulates lipogenesis via SREBP-1c pathway, affecting autophagy, lipid metabolism and atherosclerosis. Using Lipidomic analyses, we showed that Cholesteryl ester (CE), Ceramide (CER), Phosphatidic acid (PA) and PhosphatidylSerine (PS) were upregulated, whereas LysoPC, PhosphoInositol (PI) and PL_PE classes were downregulated in hyperlipidemic mice containing PCSK9 (LDb).



Sidney Wang, PhD
Assistant Professor

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

Regulation of gene expression is fundamental to a wide range of biological processes. From cell fate determination during development to malignant transformation during tumorigenesis, precise control of gene expression forms the basis of these processes. Our current understanding of gene regulation is, however, far from complete. Most published studies that profile gene expression are transcript-centric (i.e. they focus on measuring mRNA levels and levels of transcription factor binding). While these efforts revealed intricate networks of cooperativity amongst transcription factors in shaping complex biological processes, much of the post-transcriptional regulation are left unexplored. It remains unclear whether the process of protein translation is regulated by a network of factors to an extent of complexity similar to transcription regulation. We ask questions such as "Do sequence specific RNA binding proteins (RBP) cooperate in controlling translation?" "Are there translational regulatory networks that orchestrate critical biological processes?"

Our research program focuses on addressing these questions in biological contexts that are relevant to human health. Our immediate goals are to develop novel tools to systemically study RBP binding; to investigate regulatory functions of upstream Open Reading Frames (uORFs); and to integrate these functional genomics annotations with results from genetic studies, in order to fine map the regulatory variants and to provide mechanistic understanding for disease associated variants.

RESEARCH PROJECTS

- **Regulation of protein translation by uORF in stress response.** Translation regulation by uORF has long been hypothesized based on supports from studies of a handful of uORFs. We have reported a systemic survey of uORF impact on protein translation and identified genetic variants associated with this impact (Figure 1). We are further expanding this line of research in the context of stress response, where global scale changes in translational regulation are expected.
- *Using RNA binding protein footprint sequencing to investigate translational regulation of protein synthesis.* RNA binding proteins are known to regulate protein translation. We aim to develop a general and effective tool to facilitate research in this area.
- **Identification of functional novel coding regions across multiple tissues.** We have previously identified 7,273 novel coding regions from a single cell type using ribosome profiling data. While we provided evidence of active translation at these loci, the biological function and importance of these loci remains unknown. We are following up on this line of research by designing knockout screens to identify loci that are essential for cell survival. We are also expanding our efforts in identifying novel coding regions through performing ribosome profiling experiments in additional cell types and tissues.
- **Gene expression buffering at the post-translational level.** Gene expression at the transcript level are often assumed to propagate to the protein level. In a series of studies, we have demonstrated that, in our cell line model system, the variations observed at the transcript level is often buffered at the protein level through post-translational processes. In order to evaluate how general this observation is, we are now expanding our analysis to other tissue types and species.

KEY PUBLICATIONS

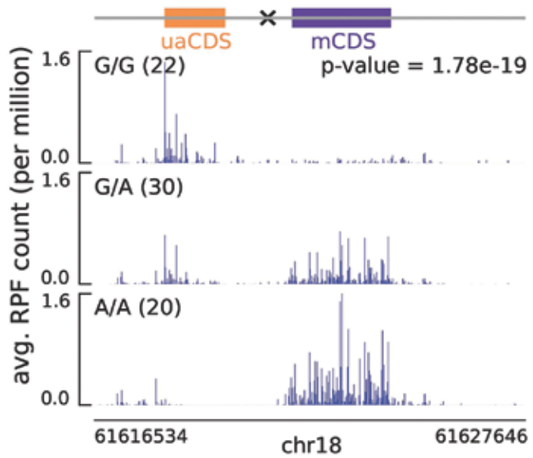
Raj A, Wang SH, Shim H, Harpak A, Li YI, Engelmann B, Stephens M, Gilad Y, Pritchard JK. Thousands of novel translated open reading frames in humans inferred by ribosome footprint profiling. *eLife.* 2016 May 27; 10.7554/eLife.13328

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

Wang SH and Elgin SCR. The impact of genetic background and cell lineage on the level and pattern of gene expression in position effect variegation. *Epigenetics & Chromatin.* 2019; 12(70)

LAB MEMBERS

Post-doctoral fellow: Sandeep Bansal



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

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

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

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

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

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

Research interests include:

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

Rick Wetsel, PhD
Center Director & Professor
Hans J. Müller-Eberhard, MD, PhD and Irma Gigli, MD Distinguished Chair in Immunology

Rick Wetsel, PhD

Professor and Director of the Center for Immunology and Autoimmune Diseases
Hans J. Müller-Eberhard, MD, PhD and Irma Gigli, MD Distinguished Chair in Immunology

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Simmons KT, Mazzilli JL, Mueller-Ortiz SL, Domozhirov AY, Garcia CA, Zsigmond EM, Wetsel RA. Complement Receptor 1 (CR1/CD35)-expressing retinal pigment epithelial cells as a potential therapy for age-related macular degeneration. *Mol Immunol. 2020:118:91-98. (PMID: 31862673).*

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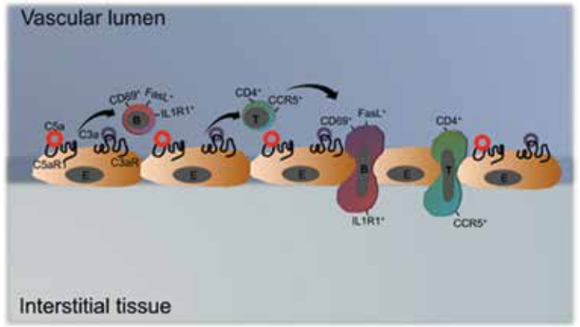
Mazzilli JL, Domoshirov AY, Mueller-Ortiz SL, Garcia CA, Wetsel RA, Zsigmond EM. Derivation and characterization of the human embryonic stem cell line CR-4: differentiation to human retinal pigment epithelial cells. *Stem Cell Res.* 2017: 18: 37-40 (PMID: 28395800).

LAB MEMBERS

Senior research scientist: Stacey Mueller-Ortiz, PhD
Senior research associate: Aleksey Y. Domozhirov, MS



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

Michael R. Blackburn, PhD

Executive Vice President & Chief Academic Officer, UTHealth
Dean and John P. McGovern Distinguished Professor of Biomedical Sciences,
MD Anderson UTHealth Graduate School
Professor and Vice Chairman, Department of Biochemistry and Molecular Biology
William S. Kilroy Sr., Distinguished University Chair in Pulmonary Disease
Dean of Research, ad interim, McGovern Medical School

Adenosine signaling and the regulation of chronic lung disease

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

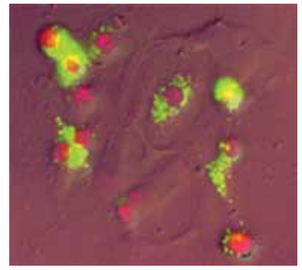
Philip, K.; Mills, T. W.; Davies, J.; Chen, N. Y.; Karmouty-Quintana, H.; Luo, F.; Molina, J. G.; Amione-Guerra, J.; Sinha, N.; Guha, A.; Eltzschig, H. K.; and Blackburn, M. R. (2017) HIF1A up-regulates the ADORA2B receptor on alternatively activated macrophages and contributes to pulmonary fibrosis. *FASEB* J. 31, 4745-4758. PMID: 12871304

Luo, F.; Le, N. B.; Mills, T.; Chen, N. Y; Karmouty-Quintana, H.; Molina, J. G.; Davies, J.; Philip, K.; Volcik, K. A.; Liu, H.; Xia, Y.; Eltzschig, H. K.; and Blackburn, M. R. (2016) Extracellular adenosine levels are associated with the progression and exacerbation of pulmonary fibrosis. *FASEB* J. 30, 874-883. PMID: 26527068

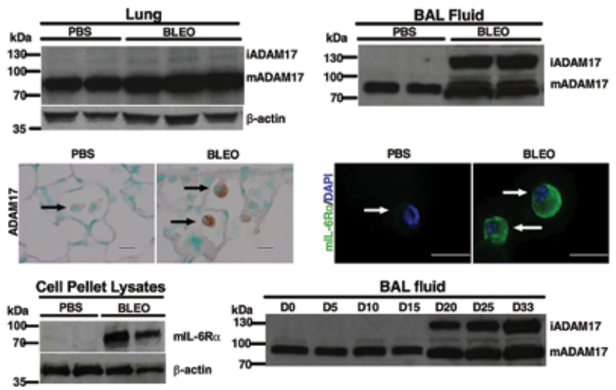
Karmouty-Quintana, H.; Philip, K.; Chen, N. Y.; Weng, T.; Molina, J. G.; Luo, F.; Davies, J.; Acero, L.; Le, Bao; Bunge, I.;Volcik, K.; Le, T.; Johnston, R. A.; Xia, Y.; Eltzschig, H. K.; and Blackburn, M. R. (2015) Deletion of ADORA2B from myeloid cells dampens lung fibrosis and pulmonary hypertension. *FASEB* J. 29, 50-60. PMID: 25318478

LAB MEMBERS

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



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



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

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

Environmental triggers regulating innate immune responses in chronic airway inflammation

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

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

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

Epithelial cells

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

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

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

In addition, my lab is interested in the molecular characterization of fungi-mediated signaling pathway(s) and the fungal component responsible for signaling in the inflammatory response in some CRS subtypes. We currently believe allergic fungal rhinosinusitis may result from a defect in local anti-fungal immune response. This has led us to our recent interest of establishing a mouse model of eosinophilic upper and lower airway inflammation and the protocols to evaluate the sinus inflammation. Current studies are focused on the pathways that regulate antimicrobial peptides with antifungal activity as it relates to CRS.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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Tyler, M.A.; Russell, C.B.; Smith, D.E.; Rottman, J.B.; Dietz, C.J.; Hu, X.; Citardi, M.J.; Fakhri, S.; Assassi, S.; and Luong, A. Large scale gene expression profiling reveals distinct type 2 inflammatory patterns in chronic rhinosinusitis subtypes. *J Allergy Clin Immunol,* 2017 Mar;139(3):1061-1064.

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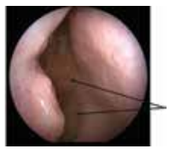
LAB MEMBERS

Hua Sun, PhD, Yi-Dong Li



Nasal polyps

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

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

- How does replicative senescence of adipocyte progenitors underlie diabetes development?
- How do adipocyte-derived fatty acids contribute to diabetes and cancer progression?
- Can cells of adipose tissue be targeted for therapeutic purposes?
- How is angiogenesis, fibrosis, and inflammation implicated in metabolic dysfunction?
- How do stress hormones regulate energy utilization in diabetes?
- What vascular genes can be targeted to treat muscle disease and diabetes?
- How does the brain and circadian clock control the body's energy balance?
- How does the circadian clock protect against liver disease and cancer?
- How does the brain control glucose homeostasis in diabetes?
- What are the functions of the genes mutated in neurodegenerative diseases?
- How does disruption of cellular homeostasis cause neurodegeneration?
- How does stress promote Alzheimer's disease and post-traumatic stress disorder?

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

Mikhail Kolonin, PhD
Center Director & Professor
Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research

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

Adipocyte progenitor cells: Dysfunction in disease and aging

Our group is interested in the mechanisms underlying aging-related diseases and developing new approaches to target them. Specifically, we focus on the role of fat (adipose) tissue in the context of obesity, type-2 diabetes, muscle degeneration, and cancer. While white adipocytes store lipids to release them in times of energy scarcity, brown adipocytes burn lipids off to keep the body warm. In obesity, overgrown white fat becomes inefficient in holding lipids, hence causing diabetes, cardiovascular disease, and cancer. In contrast, active brown fat can prevent the onset of metabolic disease. Both white and brown adipocytes are continuously replaced as they undergo senescence, and their pools in fat tissue are maintained by adipose stem cells (ASCs). In obesity, increased numbers of white fat ASCs are generated. We have discovered that tumors recruit these ASCs that fuel cancer progression. Taking advantage of our expertise in targeted therapeutics, we have developed the first experimental drug (D-CAN) targeting ASCs. Our publications demonstrate that D-CAN prevents obesity and suppresses tumor growth in mice. In more recent work, we used this experimental drug to investigate the mechanism through which ASC promote cancer progression to chemotherapy resistance and metastasis and validated them as a drug target. We are also applying ablation of ASC as a new therapeutic approach to Duchenne muscular dystrophy treatment. In collaboration with bariatric surgeons, we recently showed that D-CAN targets human ASCs. Our reports indicate that D-CAN treatment spares brown fat ASCs, leads to generation of brown adipocytes, and enables a short-term metabolic benefit. However, our recent data indicate the importance of maintaining functional ASCs and preventing their replicative senescence in healthy aging. As we age, fat cell numbers decrease and the deficient fat tissue fails to effectively absorb lipids, which start spilling into other organs. This can cause inflammation and metabolic disorders accounting for cancer and organ failure in the elderly. Our experiments in mice lacking telomerase (TERT) in ASC models suggest that adipocytes run out because ASCs lose replicative potential with age due to telomere shortening and become 'exhausted', which is accelerated by obesity. Understanding the mechanisms and function of fatty acid transport in the context of type-2 diabetes and cancer is our most recent pursuit. Another research direction is focused on the role of inflammatory signaling and fat tissue remodeling in metabolic response to anti-diabetes drugs.

RESEARCH PROJECTS

- Adipose stromal cells: heterogeneity, function in health, and targeting in disease
- Aging-associated exhaustion of adipocyte progenitors and its role in metabolic disease
- Molecules mediating intercellular interactions and signaling in obesity and cancer

KEY PUBLICATIONS

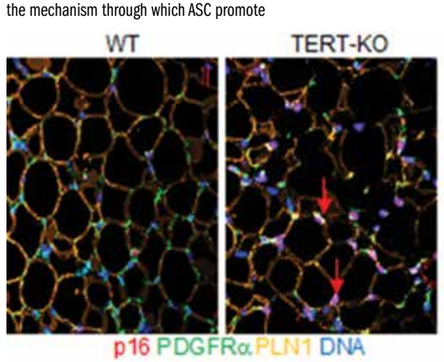
Age-associated telomere attrition in adipocyte progenitors predisposes to metabolic disease, Gao Z, Daquinag A, Fussell C, Zhao Z, Dai Y, Rivera A, Snyder B, Eckel-Mahan KL, Kolonin MG., *Nature Metabolism.* 2020

Ablation of Stromal Cells with a Targeted Proapoptotic Peptide Suppresses Cancer Chemotherapy Resistance and Metastasis. Su F, Wang X, Pearson T, Lee J, Krishnamurthy S, Ueno NT, Kolonin MG. *Molecular Therapy Oncolytics.* 2020

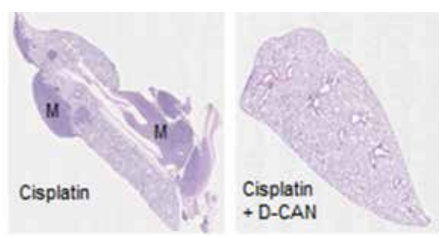
Adipose Stromal Cell Expansion and Exhaustion: Mechanisms and Consequences. Eckel-Mahan K, Ribas Latre A, Kolonin MG. *Cells.* 2020

LAB MEMBERS

Sr. research scientists: Alexis Daquinag, Zhanguo Gao
PhD student: Shraddha Subramanian
Research assistant: Cale Fussell



Immunofluorescence on sections of adipose tissue showing expression of senescence marker p16 in PDGFRa-positive stromal cells accumulating in mice lacking telomerase (TERT) in adipose stroma. PLN1: adipocyte marker yellow. Nuclei (DNA) are blue.



Sections of lungs from mice developing spontaneous breast cancer metastasis and treated with chemotherapy (cisplatin) alone (Left) or a combination of chemotherapy and a peptide D-CAN targeting adipose stromal cells. Note the suppression of metastases (M) by D-CAN.

Rebecca Berdeaux, PhD
Associate Professor
Director, Graduate Program in Biochemistry and Cell Biology

Novel pathways regulating type 2 diabetes and muscle regeneration

Muscle weakness and compromised muscle metabolism reduce quality of life for aging people or those afflicted with type 2 diabetes. Our lab studies how hormones regulate skeletal muscle function and repair after injury. Our long-term goal is to identify new drug targets to help prevent muscle weakness and loss with aging, to improve sugar and fat burning in people with metabolic disease who may not be able to exercise, and to increase muscle stem activity with aims of improving muscle repair after trauma. Our approach is specifically designed to study proteins and protein complexes that are inherently "druggable" because they are usually turned on and off by hormones such as insulin and adrenaline.

RESEARCH PROJECTS

- Determine how a stress activated kinase tunes sugar and fat utilization in skeletal muscle in obesity
- Identify gene networks that drive muscle stem cell replication after injury
- Comprehensively define muscle stem cells and inflammatory cells in human muscle during recovery from traumatic muscle and bone injury

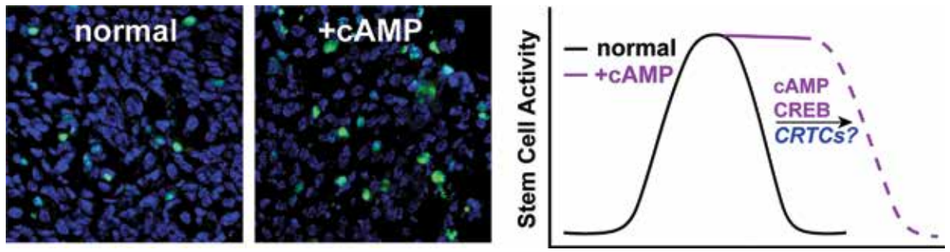
KEY PUBLICATIONS

Hollstein, P.E., Eichner, L.J., Brun, S.N., Kamireddy A., Svensson, R.U., Vera, L., Ross, D.S., Rymoff, T.J., Hutchins, A., Galvez, H., Williams, A., Shokhirev, M.N., Screaton, R.A., Berdeaux, R., and Shaw, R.J. (2019) The AMPK-related kinases SIK1 and SIK3 mediate key tumor suppressive effects of LKB1 in NSCLC. *Cancer Discovery*, pii: CD-19-1261.

Nishimori, S., O'Meara, M.J., Castro, C., Noda, H., Cetinbas, M., da Silva Martins, J., Ayturk, U., Brooks, D.J., Bruce, M., Nagata, M., Ono, W., Janton, C.J., Bouxsein, M.L., Foretz, M., Berdeaux, R., Sadreyev, R.I., Gardella, T.J., Juppner, H., Kronenberg, H.M., Wein, M.N. (2019) Salt inducible kinases dictate PTH1R action in bone development and remodeling. *J Clin. Invest.*, 129(12): 5187-5203.

LAB MEMBERS

Post-doctoral fellows: Laura Bobart, Mariane Martinez
Research assistants: Elena Dyukova, Chase Hutchins, Daniel Hancock
Graduate tutorial student: Daisy Diaz-Rohena
Medical student: Victor Gonzalez
Undergraduate student: Lindsey Hill



Hormones like adrenaline stimulate cAMP. We genetically engineered mice to have high cAMP in muscle stem cells. After injury, high cAMP is sufficient to prolong the activation of these muscle stem cells center photo and graph. We are testing how cAMP activated DNA binding proteins (CREB/ CRTCs) participate in muscle stem cell activity with the goal of identifying new drug targets for muscle healthspan during aging.

Kristin Eckel-Mahan, PhD
Assistant Professor

Circadian rhythms in health and disease

The goals of my lab center on the role of our internal 24-hour biological (i.e. circadian) clock in health and disease. The circadian clock in an exquisite time-keeping system present in all cells of our body that drives daily rhythms in physiology and tissue-specific function. Examples of our daily rhythms include the sleep/wake cycle, food intake, internal body temperature, and hormone secretion. This internal clock adapts to and is aligned with the rotation of the earth on its axis. Recent evidence from large epidemiological studies reveals that chronic circadian disruption increases our risk of several diseases. Examples of circadian disruption include travel across time zones (jet-lag), working a night shift or rotating shifts ("social jet lag"), and light contamination by white and blue light sources. In addition, some clock gene mutations lead to sleep disorders. When the circadian clock is disrupted genetically or environmentally, it increases the risk for several diseases including cancer and several types of metabolic disease. We are trying to understand why circadian disruption produces these effects.

While the central clock of the brain is predominantly controlled by light, circadian oscillations in peripheral organs are heavily influenced by other *zeitgebers* ("time-givers") such as food. Poor quality nutrients as well as food intake at the wrong time can impair circadian communications across the body, increasing the risk of metabolic diseases such as obesity and diabetes. Our current experiments include those designed to reveal which zeitgebers are most important for tissue-specific clock function and the mechanisms by which tissue-specific clocks protect against metabolic disease.

Our lab and others have shown that food intake has a strong influence on peripheral circadian clocks, such as the biological clocks in the liver, muscle, and adipose tissue. Over time, nutrient excess disrupts 24-hour rhythms in several insulin sensitive tissues that become insulin resistant under such feeding conditions. Susceptible tissues include the liver, muscle, and adipose tissue. Our studies point to tight regulation of circadian lipid metabolism in the liver as well as diurnal proliferation of adipocyte precursor cells. These 24-hour activities are disrupted under conditions of nutrient challenge, predisposing an organism to metabolic disease, such as obesity and type II diabetes.

In addition to metabolic disease, circadian disruption is associated with an increased risk of cancer. We have identified that a liver protein ("HNF4a") has circadian functions that are altered in the context of specific liver cancers. Attempts to restore the circadian function in these cells causes tumor cell death and impairs tumor growth. We are using this information to determine whether liver tumors that show circadian dysregulation can be effectively treated with molecules that boost the circadian activity in these cells.

RESEARCH PROJECTS

- Mechanisms by which circadian disruption leads to metabolic disease
- Mechanisms linking circadian disruption to liver cancer
- Mechanisms underlying daily proliferation of adipocyte progenitor cells
- Understanding the role of the circadian clock in human adipose tissue

KEY PUBLICATIONS

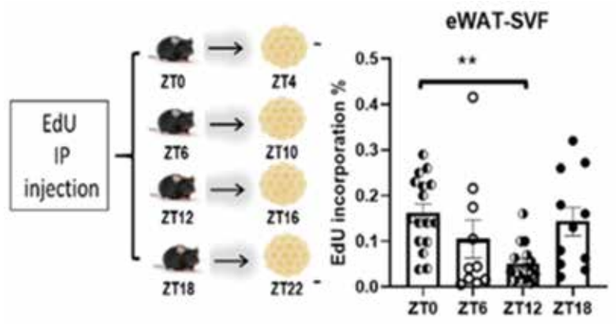
"Incompatibility of the circadian protein BMAL1 and HNF4α in hepatocellular carcinoma" Baharan Fekry, Aleix Ribas-Latre, Corrine Baumgartner, Jonathan R. Deans, Christopher Kwok, Pooja Patel, Loning Fu, Rebecca Berdeaux, Kai Sun, Mikhail G. Kolonin, Sidney H. Wang, Seung-Hee Yoo, Frances M. Sladek, and Kristin Eckel-Mahan *Nature Communications* 2018; 9:4349

"HNF4α-deficient Fatty Liver Provides a Permissive Environment for Sex-independent Hepatocellular Carcinoma" Baharan Fekry, Aleix Ribas Latre, Corrine Baumgartner, Alaa M.T. Mohamed, Mikhail G. Kolonin, Frances M. Sladek, Mamoun Younes, and Kristin L. Eckel-Mahan *Cancer Research* Nov. 2019 Volume 79, Issue 22, pp. 5860-5873

"Adipose Stromal Cell Expansion and Exhaustion: Mechanisms and Consequences" Kristin Eckel-Mahan, Aleix Ribas Latre and Mikhail G. Kolonin *Cells* 2020 Apr 9(4):863

LAB MEMBERS

Instructors: Baharan Fekry, PhD
Post-doctoral fellows: Rafael Bravo Santos, PhD
Graduate student: Rachel Van Drunen, Jamie Tran



Injection of EdU at different times of the day to label proliferating cells reveals rhythmic, daily proliferation of adipocyte progenitor cells (quantified on the right), with highest proliferation of these cells within the fat pad at the end of the feeding period.

Nicholas Justice, PhD
Associate Professor

The impact of stress on psychiatric and neurodegenerative diseases

High levels of stress cause anxiety that if continuously present leads to devastating mental illnesses, most commonly depression, generalized anxiety disorder and PTSD. Stress impacts the progression of other diseases, in part due to elevated levels of the stress hormone, cortisol, which is released in response to stress. Diseases that can be exacerbated by stress and high cortisol levels include metabolic diseases like diabetes, cardiovascular disease, and age-dependent neurodegeneration in diseases such as Alzheimer's Disease, Parkinson's disease, and loss of cognitive ability that occurs naturally with age. Our lab is focused on how our bodies perceive stress, react to stress acutely, and are impacted by chronic stress exposure that change our physiology to cause, drive, and exacerbate mental and neurodegenerative diseases such as Alzheimer's Disease.

RESEARCH PROJECTS

Local signaling that controls endocrine, autonomic, and behavioral stress responses via direct signaling with stress-activated CRF neurons.

At the IMM, we have developed many new genetic tools, allowing the discovery and functional interrogation of specific stress-responsive circuits in the brain. Our first discovery was a new type of neuron in the hypothalamus that controls release of stress hormones by stress. We have now demonstrated in published experiments a new mechanism that functions to control hormone release in response to stress and limit over-production of Cortisol. Our continued study of this circuit has revealed additional roles for this new type of neuron that is responsive to Corticotropin Releasing Factor (CRF) and thereby can coordinate hormone release with autonomic, endocrine, and behavioral changes made in response to stress exposure. This new class of neurons can be selectively manipulated allowing interrogation of their function to an unprecedented degree. We are determined to find the mechanisms used by these neurons to control the stress response to help us avoid diseases that are caused or negatively impacted by stress.

· CRF and other neuroendocrine neurons in the PVN project to key motor circuits to influence movement choice

We identified another unprecedented neural circuit that connects stress hormone release with the central circuits responsible for coordinating movement. This movement circuit, termed "the basal ganglia" is the system that malfunctions in Parkinson's Disease as well in other neurodegenerative disorders and is associated with loss in the ability to move and control movements. We hypothesize that this newly found circuit communicates stress-relevant information to the basal ganglia to influence which movements are made in response to threats in our environment. In this way, stress circuits shape our reaction to a stressful threat, perhaps causing escape behavior and running away from a threat in certain contexts, and hiding from the threat other contexts. Given our identification of this new circuit connecting (stress) hormone release and core neural circuits that guide movement, endless possibilities for future discovery lay ahead which we pursue with passion.

· Oxytocin neurons become responsive to CRF only in mothers who have had offspring

Using an array of transgenic animals designed to visualize and manipulate CRF receptor neurons, we found that Oxytocin neurons, which are involved in parturition and lactation, but also in social bonding that occurs between a mother and her offspring. Interestingly, we only see CRFR1 positive Oxytocin neurons in mothers; both virgin females and male animals lack CRFR1 expression in Oxytocin neurons. Moreover, CRFR1 is expressed by Oxytocin neurons for the rest of the mother's life. We are just beginning to understand how CRF responsiveness alters how Oxytocin neurons serve their known functions in pup-rearing by mothers. However, this molecular change that occurs only in mothers has the potential to alter how mothers respond to stress long after they have finished raising children. This project has introduced a new field in our study of stress biology: how stress influences maternal behaviors. We are determined to uncover how, and perhaps why, oxytocin neurons receive information about the stress status of an animal surrounding the time of parturition, and whether it is beneficial or detrimental to successful reproduction.

KEY PUBLICATIONS

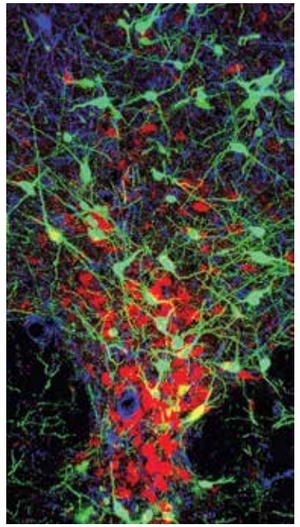
Hunt AJ, Dasgupta R, Rajamanickam S, Jiang Z, Beierlein M, Chan CS, Justice NJ (2018) Paraventricular hypothalamic and amygdalar CRF neurons synapse in the external globus pallidus. *Brain Struct Funct* 223:2685–2698.

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

Justice NJ (2018) The relationship between stress and Alzheimer's disease. *Neurobiol Stress* 8:127–133.

LAB MEMBERS

Post-doctoral fellow: Shivakumar Rajamanickam, PhD
Research assistant: Jonathan Tao



Tracing connections between CRF receptor positive Serotonin neurons (red) and the neurons that make direct synapses onto these neurons to control their activity.

Vihang Narkar, PhD
Associate Professor
George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research

Gene regulation in metabolic-vascular syndromes

Our laboratory broadly studies transcriptional regulation of metabolic and vascular homeostasis using nuclear receptors as model signaling molecules. Currently, we are investigating the cellular and physiological functions of orphan nuclear receptors (e.g. estrogen-related receptors) and their co-regulators (e.g. PGC1's). We use a wide-ranging approach, including genetically engineered mice, murine disease models, high-throughput gene expression analysis (e.g. RNA-sequencing, ChIP-sequencing), pharmacology, cell signal and *in vitro* systems in our studies. These tools are being used to investigate the role of ERR's and PGC1's in (I) cellular processes such as genome-wide gene orchestration, mitochondrial biogenesis, and angiogenesis; (II) physiological phenomenon, such as exercise adaptation and whole-body metabolism; as well as (III) diseases such as obesity/diabetes, peripheral arterial disease and muscular dystrophies. Our ongoing work has uncovered the therapeutic role of estrogen-related receptors (ERR's) via metabolic and angiogenic regulation in peripheral arterial disease (PAD), and in Duchenne muscular dystrophy (DMD). Similarly, our studies on peroxisome proliferator activator receptor delta (PPAR-delta) have yielded insights in to exercise mimicking cellular mechanisms that can be harnessed to boost metabolism, protect against obesity, and prevent diabetes. On the other hand, we also have uncovered the detrimental role of nuclear receptor co-activator PGC1-beta in PAD and muscle degeneration via regulation of anti-angiogenic, apoptotic, and autophagic pathways. Our work spanning the area of metabolic vascular syndromes that include obesity, diabetes and its cardiovascular complications has been published in journals including *Cell, Cell Metabolism, Cell Reports, Circulation Research, eLife* and *Nature Communications.*

RESEARCH PROJECTS

- Transcriptional regulation of muscle metabolism, vascularization, mass, and fitness by nuclear receptors.
- Nuclear receptor target discovery for muscle recovery in peripheral arterial disease, Duchenne muscular dystrophy, obesity, and diabetes.
- Role of nuclear receptors in blood vessel growth and diabetic retinopathy.

KEY PUBLICATIONS

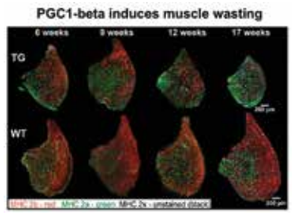
Knudsen, N.H., Stanya, K.J., Hyde, A.L., Chalom, M.M., Alexander, R.K., Liou, Y.H., Gang, M.R., Jacobi, D., Liu, S., Sopariwala, D.H., Fonseca-Pereira, D., Garrett, W.S., Narkar, V., Ortlund, E.A., Kim, J.H., Paton, C.M., Cooper, J.A., Lee, C.H. Interleukin-13 drives metabolic conditioning of muscle to endurance exercise. *Science.* 368(6490): eaat3987, 2020

Araujo, H.N., Lima, T.I., Guimarães, DSPSF, Oliveira, A.G., Favero-Santos, B.C., Branco, R.C.S., Araújo, R.M.D.S., Dantas, A.F.B., Castro A., Chacon-Mikahill, M.P.T., Minatel, E., Geraldo, M.V., Carneiro, E.M., Rodrigues, A.C., Narkar, V.A.*, Silveira, L.R. Regulation of Lin28a-miRNA let-7b-5p pathway in the skeletal muscle cells by PPARδ. *Am J Physiol Cell Physiol.* 2020 (*Co-corresponding Author.)

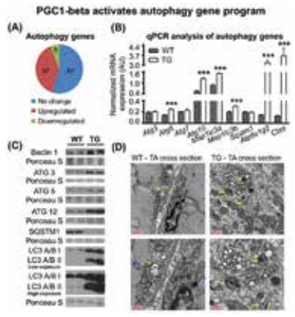
Roy, A, Sharma, A.K., Nellore, K., Narkar, V., Kumar, A. TAK1 preserves skeletal muscle mass and mitochondrial function through redox homeostasis. *FASEB BioAdvances.* 2020; 00: 1–16.

LAB MEMBERS

Post-doctoral fellow: Danesh Sopariwala
Research assistants: Nitya Narayana, Lisa Lin



Progressive loss of muscle mass by PGC1-beta activation in transgenic (TG) compared to control (WT) mice.



PGC1-beta induces muscle wasting by activating a process called autophagy. Panels (A-D) represent activation of autophagy transcriptional program (A), autophagy biomarker genes (B) and proteins (C), and electron microscopy images of PGC1-beta mediated autophagosome formation (arrows) (D) in muscle.

Kai Sun, MD, PhD
Assistant Professor

Adipose tissue remodeling and metabolic health

My laboratory investigates and discovers novel factors that regulate the dynamics of adipose tissue remodeling during obesity development. The long-term goal of our research is to address the clinical significance of these factors in human obesity, diabetes, and cardiovascular diseases.

In the past years, we have revealed that high fat diet-induced obesity shapes a hypoxic microenvironment that initiates the local fibrosis and inflammation in adipose tissue. The unhealthy adipose tissue further leads to systemic insulin resistance and cardiovascular dysregulation. Intriguingly, we found that VEGF-A-induced angiogenesis ameliorates the pathological changes by suppressing the local hypoxia and stimulating sympathetic innervation in both white and brown adipose tissue. Our study further reveals that the hypoxia-induced MT1-MMP facilitates the healthy expansion of adipose tissue by stimulating angiogenesis in combination with VEGF-A and leptin, thus relieving the pathological conditions. Furthermore, we found that MT1-MMP cleaves collagenous proteins to increase the ECM flexibility in adipose tissue.

Most recently, we analyzed the dynamics of lipid droplet-associated proteins during adipose tissue remodeling by mass spectrometry. We have successfully identified several novel proteins that translocalize onto lipid droplets and the interface of endoplasmic reticulum (ER)-lipid droplets in response to different stimuli. Particularly, we found that one of the identified proteins named Carboxyl Esterase 3 (Ces3) targets lipid droplets upon β-adrenergic-stimulation where it exerts the lipolytic function on the lipids. We further discovered that another factor called Dynamin-Related Protein 1 (DRP1) translocates onto ER where it promotes the fission of the nascent lipid droplets from the ER in response to lipid stress. We are applying state-of-the-art tools and techniques to investigate the mechanisms governing the functions of the novel factors on the dynamics of lipid droplets

RESEARCH PROJECTS

- Hypoxia induced pathological changes in adipose tissue.
- Sympathetic innervation in adipose tissue and energy expenditure.
- Reversibility of adipose tissue fibrosis by novel anti-fibrotic therapies.
- Regulation of the dynamics of lipid droplets and metabolic health.

KEY PUBLICATIONS

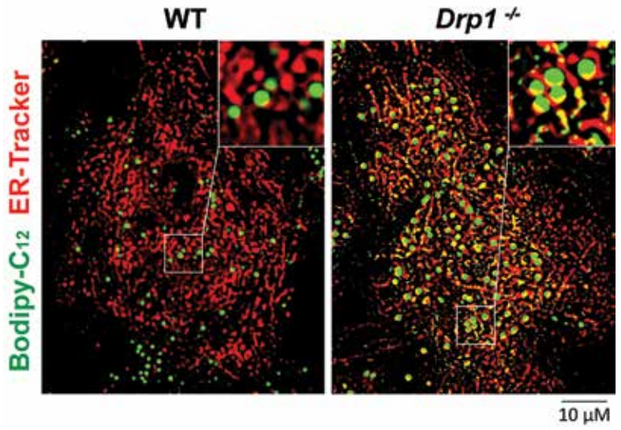
Yang L, Li X, Tang H, Gao Z, Zhang K, Sun K. A Unique Role of Carboxylesterase 3 (Ces3) in β-Adrenergic Signaling-Stimulated Thermogenesis. *Diabetes.* 2019 Jun; 68(6):1178-1196.

Li X, Zhao Y, Chen C, Yang L, Lee HH, Wang Z, Zhang N, Kolonin MG, An Z, Ge X, Scherer PE, Sun K. The critical role of MMP14 in adipose tissue remodeling during obesity. *Mol Cell Biol.* 2020 Mar; 40(8): e00564-19.

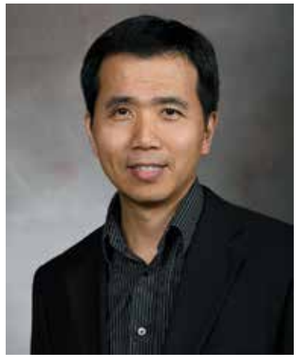
Li X, Yang L, Mao Z, Pan X, Zhao Y, Gu X, Eckel-Mahan K, Zuo Z, Tong Q, Hartig SM, Cheng X, Du G, Moore DD, Bellen HJ, Sesaki H, Sun K. Novel role of dynamin-related-protein 1 in dynamics of ER-lipid droplets in adipose tissue. *FASEB J.* 2020 Jun; 34(6):8265-8282.

LAB MEMBERS

Post-doctoral fellows: Xin Li, MD, PhD; Gang Li PhD



Drp1 ablation leads to retention of lipid droplets in ER. Left: The nascent lipid droplets (green) dissociate from ER (red) and form cytosolic droplets during the biogenesis process in the wild type (WT) HeLa cells. Right: The nascent lipid droplets are retained in the ER lumen or ER surface in the *Drp1-/-* HeLa cells (Yellow area in the right top window) (Live cells imaged with n-SIM super-resolution microscope).



Qingchun Tong, PhD
Associate Professor
Cullen Chair in Molecular Medicine

Brain control of feeding, body weight, and glucose metabolism

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

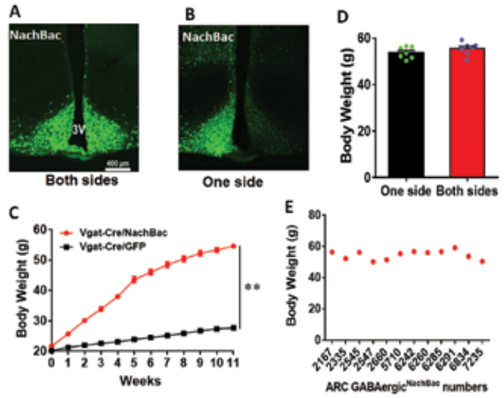
Paraventricular hypothalamus mediates diurnal rhythm of metabolism. Kim ER, Xu Y, Cassidy RM, Lu Y, Yang Y, Tian J, Li DP, Van Drunen R, Ribas-Latre A, Cai ZL, Xue M, Arenkiel BR, Eckel-Mahan K, Xu Y, Tong Q. *Nat Commun.* 2020 Jul 30;11(1):3794. doi: 10.1038/s41467-020-17578-7.PMID: 32732906 Free PMC article.

Profound and redundant functions of arcuate neurons in obesity development. Zhu C, Jiang Z, Xu Y, Cai ZL, Jiang Q, Xu Y, Xue M, Arenkiel BR, Wu Q, Shu G, Tong Q. *Nat Metab.* 2020 Aug;2(8):763-774. doi: 10.1038/s42255-020-0229-2. Epub 2020 Jul 27.PMID: 32719538

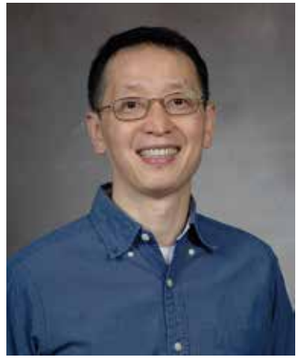
Disrupted hypothalamic CRH neuron responsiveness contributes to diet-induced obesity. Zhu C, Xu Y, Jiang Z, Tian JB, Cassidy RM, Cai ZL, Shu G, Xu Y, Xue M, Arenkiel BR, Jiang Q, Tong Q. *EMBO Rep.* 2020 Jul 3;21(7):e49210. doi: 10.15252/embr.201949210. Epub 2020 May 27.PMID: 32462726

LAB MEMBERS

Instructor: Yuanzhong Xu, MD, PhD
Post-doctoral fellows: Zhiying Jiang, PhD; Santosh Mandal, PhD
Graduate students: Jessie Morrill, Jing Cai, Hongli Li (visiting)
Technician: Claire Young



Our recent studies reveal that Arc GABA neurons exert a profound and redundant role in obesity development. A) Injection of Cre-dependent adenoassociated viral vectors expressing NachBac (sodium channel in bacteria, causing chronic activation of neurons) in bilateral arcuate nucleus (Arc) of the hypothalamus of Vgat-Cre mice (i.e. GABA neurons). 3V = third ventricle. Scale bar- 250μM. B) Injection of Cre-dependent adenoassociated viral vectors expressing NachBac (sodium channel in bacteria) in unilateral arcuate nucleus (Arc) of the hypothalamus of Vgat-Cre mice (i.e. GABA neurons). 3V = third ventricle. Scale bar- 250μM. C) Body weight curve of mice with chronic activation of Arc GABA neurons versus controls. D) Comparison in body weight between mice with NachBac expression in bilateral versus unilateral sides of the Arc. E) Relationship of body weight with the number of Arc GABA neurons with NachBac expression, demonstrating chronic activation of small subset of Arc GABA neurons is sufficient to drive obesity.



Sheng Zhang, PhD
Associate Professor
Becker Family Foundation Professor in Diabetes Research

Molecular mechanisms of neurodegenerative diseases

As we live longer and enjoy unprecedented longer life expectancy, we are also becoming increasingly vulnerable to aging-related neuronal degenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). As these incapacitating brain diseases are inflicting unbearable emotional and financial tolls to patients and their families, they are becoming a pressing threat to our society. However, by now there is little effective prevention and treatments against these maladies.

We are trying to address these challenges by studying how neurons can stay healthy during normal aging. Our senses, reasoning, and responses are realized through neurons and their functional connections inside our body. However, unlike other cells, such as those from skin and blood that are constantly dividing and being replenished, neurons face unique challenges. In particularly, once they are born and mature into interconnected functional units, they mostly lose the ability to reproduce and no longer can be replaced for the rest of life. To maintain longevity, these long-lived neurons harbor robust self-clearance machineries to stay healthy and ward off internal crisis and external insults for decades to come.

The self-maintenance machines inside cells include *chaperones* that help proteins to stay in shape, and proteasomes as well as *autophagy* (meaning "self-eating" in Greek) and lysosomal systems that act as internal clearance machineries to clean up and recycle worn-out or toxic cellular materials. In neurodegenerative diseases, these protective machineries often become inefficient or nonfunctional, leading to excessive buildup of toxic wastes (known as aggregates, tangles, or plaques) inside the brain, causing eventual neuronal loss.

Using genetic, biochemical, and cell biology tools in different model systems from invertebrate *Drosophila* to mammalian mouse and cultured human cells, we are trying to understand how these self-maintenance machines recognize and efficiently clear away internal toxins, while spare and protect normal cellular constituents. Our eventual goal is to be able to command these innate protection machineries to fight against devastating brain degenerative diseases.

Currently we are focusing on the following studies:

(1) Chaperone Hsp110 on neuronal function and survival.

haperone Hsp110 is one of the most abundant proteins in the brain. It helps other proteins to fold into proper shapes to function properly. It is also a major component of the dis-aggregase machinery that dismantles tightly packed protein aggregates.

(2) Biogenesis of autophagosomes and other specialized cellular organelles and their dysfunction in brain diseases

Cells produce many specialized cellular organelles, such as the autophagosome, lysosome-related organelles and synaptic vesicles. Autophagosomes are garbage bags produced by a cell during autophagic process to collect unwanted or harmful cellular components for their eventual disposal and recycling. These specialized organelles control many aspects of neuronal function and survival, while their disruptions are linked to a spectrum of disorders including AD, PD, HD and schizophrenia.

(3) Huntington's disease gene Huntingtin.

Huntingtin is important for neuronal survival and is involved in autophagy, but its exact roles in versatile cellular pathways remain to be fully elucidated.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

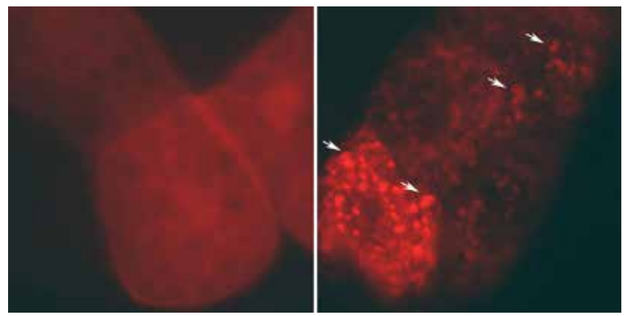
David-Morrison G, Xu Z, Rui YN, Charng WL, Jaiswal M, Yamamoto S, Xiong B, Zhang K, Sandoval H, Duraine L, Zhang S*, Bellen HJ*. (2016) "WAC Regulates mTOR Activity by Acting as an Adaptor for the TTT and Pontin/Reptin Complexes." *Dev Cell.* 36(2):139-51. (*corresponding authors)

Xu Y, Zhang S and Zheng H. (2019): "The cargo receptor SQSTM1 ameliorates Tau pathology and spreading through selective targeting of pathological Tau". *Autophagy* 15(4): 583-598.

Mao DX, Lin G, Senturk M, Zuo ZY, Tepe B, Zhang S, Arenkiel, BR, Sardiello M, and Bellen HJ. (2019): "Vap proteins are required for autophagic and lysosomal degradation". *Autophagy* 15(7): 1214-1233.

LAB MEMBERS

Instructor: Shiyu Xu, PhD
Graduate Students: Yue Yu; Amanda Solbach; Heather Tsong (rotating graduate student)
Research assistants: Xin Ye, PhD; Mrs. Lili Ye



Induction of autophagosomes by human ULK1 gene
(Right panel) ULK1 gene is an autophagy inducer. Cells expressing ULK1 produced numerous autophagosomes (red puncta) inside the cell. (Left panel) Control cells without ULK1 expression.

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.

The CMI houses a diverse, interdisciplinary team of scientists and engineers who develop and use multi-modality molecular diagnostics and imaging techniques, including nuclear imaging, X-ray computed tomography, bioluminescence, fluorescence, and near-infrared fluorescence (NIRF) to enable new understandings of disease and chronic conditions. Sponsored industry, philanthropic, and federal research funding focuses upon autoimmune disorders, neuroinflammation, cancer metastases, hemo- and lymph-vascular diseases, and lymphedema. The team has experts in instrumentation, imaging agent development, antibody engineering, animal models of human disease, and translational science that effectively moves inventions and discoveries, "bench to bedside," and when discoveries are made in the clinic, from "bedside back to bench."

A highlight of the CMI is the basic science/clinical translational team that engages clinicians at UTHealth and at partnering institutions in the Texas Medical Center and in the Houston suburbs. These FDA-approved clinical studies enable visualization of the lymphatic system using photonics technologies for better diagnosis and directed treatments. Conditions such as vascular anomalies, congenital heart disease, peripheral vascular disease, breast cancer, and head and neck cancer are under investigation using our investigational imaging technologies. Earlier, translational activities further explore visualization of brain function in neonates, and in preclinical models of human disease, CSF outflow into the lymphatics, and intraoperative detection of lymph node metastases and tumor margins. Our team focuses upon translating new NIRF molecular imaging agents using validated standards that can be applied across difference photonics device platforms.

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

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

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

Understanding how the lymphatic watersheds mediate immune health and chronic disease

As higher vertebrates evolved from the sea into land dwellers, terrestrial antigen exposure increased and the adaptive immune system evolved from a centralized lymphatic system to one dependent upon regional draining lymph nodes. The decentralized lymphatic system is organized into watersheds that drain into lymph node basins before emptying into the hemovascular circulatory system. In the regional draining lymph nodes, antigens are presented to activate immune cells that then leave the lymph node and disseminate through the body via the blood vasculature.

This organization enables regional processing of immune responses to multiple antigens without overwhelming the immune system and breaking central tolerance, or tolerance to self. Yet despite the watershed organization of lymphatics, drugs that are intended to alter immune responses by targeting key signaling molecules within the lymphatics are administered or dosed systemically. Whether dosed to stimulate the immune system as in cancer checkpoint blockade immunotherapies, or to attenuate immune responses against self as in autoimmune therapies, these pharmaceutical strategies frequently lead to suboptimal results and, perhaps not surprisingly, adverse immune responses that break central tolerance.

In addition, all tissues drain to at least one lymphatic watershed not only to ensure immunosurveillance, but also to collect cellular waste products and excess fluid for return to the hemovascular circulatory system. Lymphatic insufficiency can result in the build-up of waste products and unresolved inflammation. For example, in the lower extremities of aging populations, we have found that lymphatic insufficiencies accompany peripheral vascular disease and precede ulcer formation. In the brain, the cerebrospinal fluid (CSF) and interstitial fluid (ISF) drains into the cervical lymphatic watershed, and in animal models of Alzheimer's disease, is impaired and presumably leads to Aβ aggregation and plaque formation.

In our research program, we employ near-infrared fluorescence imaging of the lymphatic vasculature and its function in order to understand chronic conditions that involve the lymphatics and to more effectively deliver therapeutics that can modulate immunity. Specifically, we conduct translational imaging of infants, children, and adults in the Texas Medical Center with chronic conditions and investigate the corresponding animal models of these conditions. Our studies are designed to develop new biological insights that could lead to better prevention and treatment of these conditions. We also engineer new methods of lymphatic imaging to provide better diagnostics of chronic conditions.

RESEARCH PROJECTS

- Lymphatic delivery of immunotherapies for cancer and autoimmune disorders.
- Evaluating the role of CSF outflow in brain health and Alzheimer's.
- Assessing the role of lymphatics in metabolic disorders.
- Refining measurements of lymphatic anatomy and function.

KEY PUBLICATIONS

Kwon, S., Velasquez, F.C., Rasmussen, J.C., Greives, M.R., Turner, K., Morrow, J.R., Hwu, W.J., Ross, R.F., and E.M. Sevick-Muraca, "Nanotopography-directed lymphatic delivery of checkpoint blockade immunotherapy for improved anti-tumor responses," *Theranostics*, 9(26):8332-8343, 2019.

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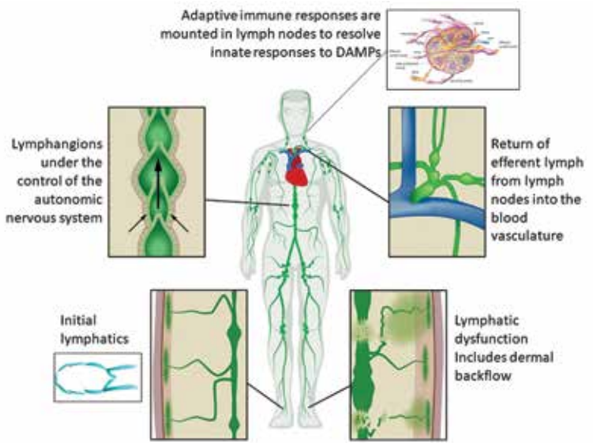
Rasmussen, J.C., Kwon, S., Pinal, A., Bareis, A., Velasquez, F.C., Janssen, C.F., Morrow, J.F., Fife, C.E., Karni, R.J., and E.M. Sevick-Muraca, "Assessing the lymphatic route of CSF outflow and peripheral lymphatic contractile activity during head down tilt using near-infrared fluorescence imaging," *Physiol. Reports*, 8(4):e14375, 2020. PMID: 32097544.

LAB MEMBERS

Post-doctoral fellows: Carolina Mantilla-Rojas, PhD
Research assistants/associates: Janelle Morton, BS, Fred Christian "CJ" Velasquez, BA



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

Melissa B. Aldrich, MBA, PhD
Assistant Professor

Imaging in immunology

Cancer survivors face the possibility of developing a devastating side effect of cancer treatment: lymphedema (LE), which manifests as a permanently swollen arm, leg, neck, or trunk. LE requires constant compression garment wear, meticulous skin care, and specialized massage. LE patients suffer discomfort, depression, cellulitis bouts, and there is no cure—only palliative treatment. Studies have shown that, if caught early in development, LE treatment can reverse the disease. Near-infrared fluorescence lymphatic imaging (NIRF-LI) imaging delivers high-resolution, low-cost images of lymphatic vessel architecture and pumping. In disease states such as LE, NIRF-LI imaging can provide information for early diagnosis and evaluation of treatment efficacy. I lead a five-year prospective and longitudinal study using NIRF-LI surveillance of breast cancer patients to identify early LE development and biomarkers that could suggest pharmacological treatment. My recent research shows that certain plasma cytokines are elevated in breast cancer patients destined to develop LE a year later, providing a prognostic tool to enable early identification of at-risk patients for pre-habilitation treatment referral. I have presented the first lymphatic-visual (NIRF-LI) evidence at an international conference showing that LE is reversible at early stages. I also lead a three-year CPRIT-funded clinical study of reparative lymphatic microsurgeries, which are gaining in popularity for LE patients for whom traditional palliative care fails. This study will objectively assess whether the surgeries actually improve lymphatic drainage in affected limbs, and may suggest ways to improve outcomes, including decreasing cellulitis risk that plagues LE patients.

I have participated as a team member imaging treatment responses to head and neck LE, which affects ~90% of head and neck cancer patients. The treatment used, pneumatic compression therapy, removes stagnant lymph in LE patients, but needs NIRF-LI to visually "prove" to medical insurers that the therapy actually works. I am very active in the LE community, and I was recently appointed to the Scientific and Medical Advisory Council of the Lymphatic Education & Research Network (LE&RN), an international organization of researchers, physicians, therapists, and patients, dedicated to advancing lymphatic health. I also chaired the committee that established standards for LE&RN's Centers of Excellence designation, which now enable patients to locate health institutions with lymphatic expertise.

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

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

Chylothorax occasionally affects neonatal heart surgery patients. I and my colleagues here at CMI and Memorial Hermann Hospital have used NIRF-LI to help visualize the source of pleural effusion in babies with chylothorax. We also have imaged numerous pediatric patients with lymphovenous anomalies to help physicians direct optimal care.

RESEARCH PROJECTS

- Longitudinal study of breast cancer-related LE
- Longitudinal study of reparative microsurgeries for LE
- Imaging of lymphatics in lipedema
- Imaging of neonatal chylothorax and pediatric lymphovenous anomalies

KEY PUBLICATIONS

Aldrich M.B., Rasmussen J.C., Fife C.E., Shaitelman S.F., Sevick-Muraca E.M. The development and treatment of lymphatic dysfunction in cancer patients and survivors. *Cancers (Basel)* 12:2280, 2020.

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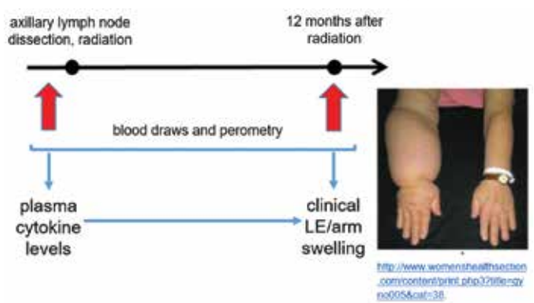
Schaverien M.V., Aldrich M.B. New and emerging treatments for lymphedema. *Semin Plast Surg* 32:48-52, 2018.

LAB MEMBERS

Medical student: Kay Pham



Near-infrared fluorescence lymphatic imaging (NIRF-LI) of arm lymphatics in a patient with breast cancer-related lymphedema. Green fluorescent dye is visualized through the skin, pumping through vessels (in a healthy arm) or pooling as backflow (in lymphedema). Medical insurers need objective evidence that currently prescribed physical therapy for lymphedema actually works. These images are the first visual proof of reversal of lymphedema in response to therapy.

John Rasmussen, PhD
Assistant Professor
Carolyn Frost Keenan Professor in Cardiovascular Disease Research

Device translation for lymphatic imaging

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

One of our primary focuses is the relationship between the lymphatics and the blood circulatory system. It has been known for many years that patients with advanced chronic venous disease, often co-develop lymphedema, a condition of chronic swelling with fibrotic tissue changes and poor immune response. We recently imaged a group of patients with early venous disease and observed a degradation of lymphatic anatomy as evidenced by the appearance of segmented lymphatic vessels and increased incidence of dermal backflow, or abnormal movement of contrast agent into the dermal tissues, as venous disease progressed. In addition, the lymphatic pumping rate initially increased to compensate for the increased venous load (C3 disease) but then decreased by nearly half as the disease continued to progress to C4 disease. A better understanding of the role of the lymphatics in early vascular disease may enable the development of more efficacious therapeutic approaches.

The drainage of cranial lymphatics have been implicated in the development of neurological disorders, including spaceflight-associated neuro-ocular syndrome, where microgravity conditions result in fluid shifts from the body to the head, The resulting chronically high cranial pressures can damage the optical nerves of astronauts. We recently completed a small study assessing the impact of gravity on cranial lymphatic drainage. In this study, subjects were imaged in a head down tilt position to mimic microgravity conditions as well as while laying on their back and sitting up. The images revealed delayed cranial drainage when the subject was in the head down position, indicating that under normal conditions gravity aids cranial lymphatic drainage.

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

RESEARCH PROJECTS

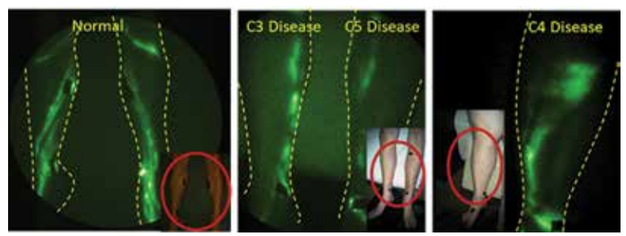
- Understanding the role of lymphatics in the development of peripheral venous disease
- Assessing the development of cancer-related lymphedema and its response to intervention
- Understanding the role of lymphatics in the development of neurological conditions

KEY PUBLICATIONS

Rasmussen, J.C. (corresponding author), Zhu, B., Morrow, J.R., Aldrich, M.B., Sahihi, A., Harlin, S.A., Fife, C.E., O'Donnell, T.F., and E.M. Sevick-Muraca, "Degradation of Lymphatic Anatomy and Function in Early Venous Insufficiency," *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, In Press, Available Online September 2020.

Rasmussen, J.C., Kwon, S., Pinal, A., Bareis, A., Velasquez, F.C., Janssen, C.F., Morrow, J.R., Fife, C.E., Karni, R.J., and E.M. Sevick-Muraca, "Assessing lymphatic route of CSF outflow and peripheral lymphatic contractile activity during head-down tilt using near-infrared fluorescence imaging," *Physiological Reports,* 8(4):e14375, 2020.

Zhu, B., Kwon, S., Rasmussen, J.C., Litorja, M., and Sevick-Muraca, E.M., "Comparison of NIR versus SWIR fluorescence image device performance using working standards calibrated with SI units," *IEEE Transactions on Medical Imaging*, 39(4):944-951, 2020.



Normal lymphatic vessels in a healthy volunteer (left); abnormal, segmented lymphatics in a patient with C3 and C5 venous disease (center); and abnormal dermal lymphatic backflow in C4 venous disease (right). Adapted from Rasmussen, *et al.*, J. of Vas. Surg.: Ven. and Lymph. Dis., 2020.

Banghe Zhu, PhD
Assistant Professor

NIR optical imaging of brain network dysfunction and CSF outflow

Brain network dysfunction from cerebral palsy, a birth-related stroke, or epilepsy contributes to developmental delays in childhood. Although fMRI can diagnose brain network dysfunction, the complexities and general anesthesia needed to obtain motion-free BOLD fMRI data limit their practical use in young children. Recently, we report a transcranial near infrared (NIR) optical imaging system, called Cap-based Transcranial Optical Tomography (CTOT) able to image whole brain hemodynamic activity in an awake child. With recent advances to couple fast read-out scientific CMOS (sCMOS) devices and with optical switching of detector fiber optics, rapid dynamic CTOT mapping should be possible, which would then enable evaluation of functional connectivity in awake infants.

The lymphatic vasculature is an essential highway for the immune system, enabling local resolution of innate and mounting of adaptive immune responses in regional draining lymph nodes (LNs). In the brain, CSF drains from the subarachnoid space into the newly rediscovered meningeal and cribriform lymphatics that drain via gravity to cervical LNs. In addition more recent work suggests CSF drains and exchanges with interstitial fluid through perivascular channels (via glymphatics) before draining presumably through basement membrane channels and into cervical LNs. Currently, we seek to non-invasively visualize CSF outflow and peripheral lymphatic function as a function of time in transgenic Alzheimer models.

RESEARCH PROJECTS

- Develop fast CTOT imaging system for functional brain mapping
- Develop NIR fluorescence tomographic imaging system for CSF mapping
- Investigate the relationship between CSF and peripheral lymphatic function

KEY PUBLICATIONS

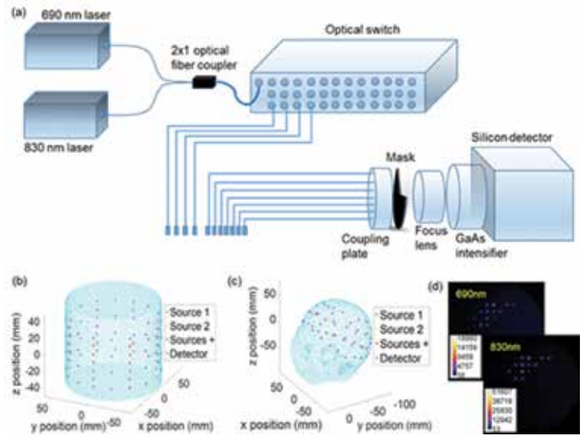
Rasmussen, J.C., Zhu, B., John R. Morrow, Melissa B. Aldrich, Aaron Sahihi, Stuart A. Harlin, Sheila Coogan, Caroline E. Fife, Thomas F. O'Donnell Jr, and Eva M. Sevick-Muraca, "Degradation of Lymphatic Anatomy and Function in Early Venous Disease," *Journal of Vascular Surgery: Venous and Lymphatic Disorders doi.*org/10.1016/j.jvsv.2020.09.007 (2020).

Zhu, B., and Sevick-Muraca, E. M., Nguyen R., and Shah, M. N., "Cap-based Transcranial Optical Tomography in an Awake Infant," *IEEE Transactions on Medical Imaging;* DOI:10.1109/TMI.2020.2990823 (2020).

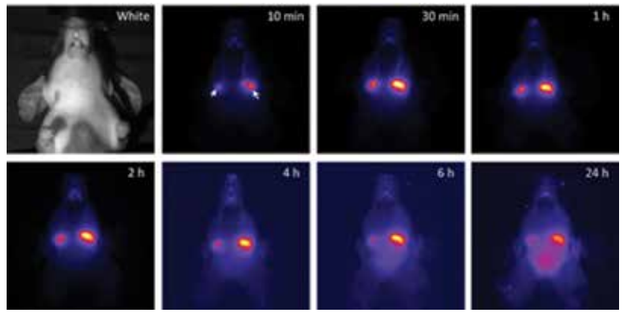
Zhu, B., Kwon, S., Rasmussen, J. C., Litorja, M., and Sevick-Muraca, E. M., "Comparison of NIR versus SWIR fluorescence imaging of indocyanine green using SI-derived metrics of image performance," *IEEE Transactions on Medical Imaging,* DOI: 10.1109/TMI.2019.2937760 (2019).

LAB MEMBER

Research assistant: Janelle Morton



(a) A schematic of the CTOT imaging system. The source and detector arrangements are depicted on surface meshes on a cylindrical phantom (b) and infant head (c). (d) Typical images of the coupling plate depict the fluence from the array of collection fibers following illumination at two different wavelengths.



NIRF images in the ventral view of a typical mouse 10 min, 30 min, 1 h, 2 h, 4 h, 6 h and 24 h after i.t. injection of ICG. Inset, white light images. Arrow, submandibular LNs (SMLNs).

The faculty, research staff, and trainees of the Center for Stem Cell and Regenerative Medicine (CSCRM) are focused on experimental studies of the biological properties of stem cells in both health and disease. The interest in healthy stem cells is motivated by their essential role in both normal development as well as in maintenance of tissues and organs throughout life. One of the hopes of regenerative medicine is that this fundamental understanding of stem cells may be effectively translated into therapies in which healthy stem cells, or their derivatives, can be employed to replace cells and tissues lost as a consequence of normal aging, injury, or disease.

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

For patients presenting with genetically inherited disease, Center faculty are utilizing recently developed gene editing technologies to correct the disease-causing mutations in either tissue-resident stem cells or iPSCs. The goal of these studies is development of therapies that include correcting the mutations in a patient's own stem cells, then delivering either the corrected stem cells or cells/tissues derived from them back into the same patient.

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

In the pages following you will find examples of Center faculty exploring the potential therapeutic value of stem cells for repairing tissues such as spinal cord, brain, muscle, lung, and blood, as well as elucidating the role of stem cells in cancer. If I may provide any additional information, please do not hesitate to contact me.

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

Brian R. Davis, PhD
Professor and Director
C. Harold and Lorine G. Wallace Distinguished University Chair

Genetic correction of stem cells for treatment of inherited lung and blood diseases

Our laboratory has as its primary objective the sequence-specific genetic correction of mutations in the chromosomal DNA of induced pluripotent stem (iPS) cells and/or tissue-specific stem cells derived from patients with inherited disorders affecting the lung or blood system. This is being pursued with the ultimate goal of developing stem cell-based therapeutic approaches.

We have utilized DNA sequence-specific nuclease-mediated homology directed repair to correct the most common genetic mutations in iPS cell lines derived from patients with cystic fibrosis – and have demonstrated genetic and functional correction in lung epithelial cells derived from these corrected iPS cells. We have introduced lung-specific fluorescent reporters into iPS cells and utilized to specifically isolate early lung progenitors and then airway basal stem cells for purposes of molecular and functional characterization. Significantly, we have now demonstrated that our iPSC-derived airway basal cells (iBCs) correspond closely to adult airway basal cells; furthermore, the iBCs are functionally able to engraft and yield airway epithelium in an *in vivo* model. One of our objectives is to employ CF patient-specific iPS cell-derived lung epithelium for testing sensitivity to specific CF drugs -- in order to facilitate a personalized therapeutic approach. We are also presently utilizing the fore-mentioned gene correction methodologies to correct the CF mutations in tissue-specific stem cells directly obtained from CF patients. We have now demonstrated highly efficient correction of the CF airway basal cells with functional restoration of CFTR channel activity. The second major project in the laboratory focuses on the site-specific correction of gene mutations responsible for inherited blood disorders such as the Wiskott-Aldrich Syndrome (WAS), a primary immune deficiency. Again, we are seeking to correct the disease-causing mutations in patient-specific blood stem cells. In both the CF and WAS projects, the ultimate objective is the delivery back to patients of their own lung or blood stem cells, only differing from the original stem cells by the genetic correction of the relevant mutation.

RESEARCH PROJECTS

- Correction of airway basal stem cells from cystic fibrosis patients *in vitro* and *in vivo*
- Derivation and expansion of airway basal stem cell from cystic fibrosis patient-specific iPS cells.
- Correction of blood stem cells from Wiskott-Aldrich Syndrome patients

KEY PUBLICATIONS

N. King, S. Suzuki, C. Barilla, F.J. Hawkins, S.H. Randell, S.D. Reynolds, B.R. Stripp, B.R. Davis. Correction of airway stem cells: genome editing approaches for the treatment of Cystic Fibrosis. *Human Gene Therapy* 2020 31: 956-972.

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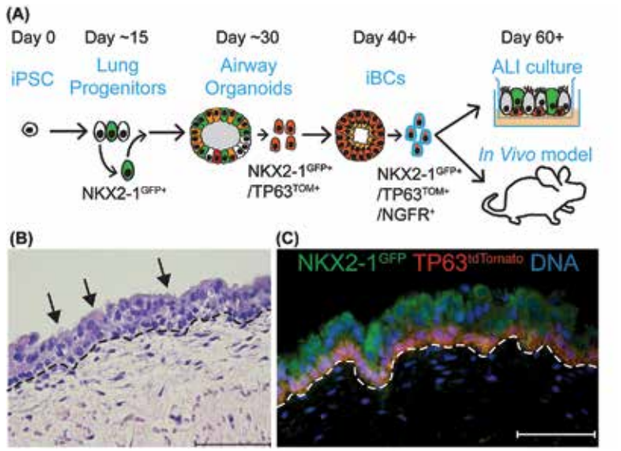
LAB MEMBERS

Post-doctoral fellows: Dr. John M. Avila, Dr. Cristina Barilla, Dr. Shingo Suzuki
Research staff: Dr. Bailiang Wang, Haipeng Xue



Derivation of airway basal stem cells from induced pluripotent stem cells (iPSCs). (A) Summary of protocol with critical steps highlighted. (B) iPSC-derived airway basal cells are able to generate airway epithelium. (C) Detection of NKX2.1^{GFP} and TP63^{tdTomato} in iPSC-derived airway epithelium.

Laura A. Smith Callahan, PhD
Assistant Professor

Tissue engineering approaches for the treatment of CNS injuries

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

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

In order to advance biomaterial cell support matrices to wide spread clinical use, protocols for the expansion and differentiation of clinically relevant cell sources, also, need to be optimized. Human induced pluripotent stem cells (hiPSC) offer a potentially autologous cell sources for the treatment of traumatic injuries to the central nervous system. However, the number of viable cells for transplant produced from current differentiation protocols is extremely low. Both biochemical and mechanical properties of the cell culture surface have been shown to significantly affect cellular differentiation but have not been studied significantly in respect to hiPSC differentiation. The laboratory seeks to extend our knowledge of three dimensional culture systems to optimize two dimensional cell culture surfaces for differentiation of neural stem cells and oligodendrocyte progenitor cells from hiPSC. Preliminary studies have focused on the covalent tethering of proteins to the surface of hydrogels with containing a Young's Modulus gradient to study the effect of mechanical properties on hiPSC lineage choice.

RESEARCH PROJECTS

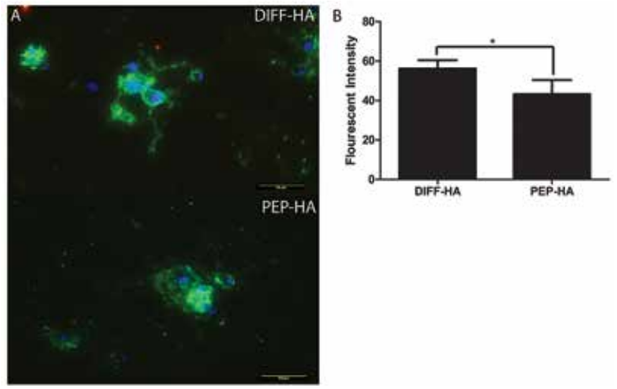
- Optimization of substrates and matrices to direct human induced pluripotent stem cells to neural progenitor cells to therapeutic linages using combinatorial approaches.
- Modulation of cellular environment *in vivo* to promote cell therapy survival, integration with the host and maturation toward functional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

KEY PUBLICATIONS

Perera TH, Lu X, Howell SM, Kurosu YE, Smith Callahan LA. Combination of IKVAV, LRE and GPQGIWGQ Bioactive Signaling Peptides Increases Human Induced Pluripotent Stem Cell Derived Neural Stem Cells Extracellular Matrix Remodeling and Neurite Extension. *Advanced Biosystems.* 8: e2000084, 2020.

Perera TH, Lu X, Smith Callahan LA. Effect of laminin derived peptides IKVAV and LRE tethered to hyaluronic acid on hiPSC derived neural stem cell morphology, attachment and neurite extension. *Journal of Functional Biomaterials.*11: 15, 2020.

Perera TH, Howell SM, Smith Callahan LA. Manipulation of Extracellular Matrix Remodeling and Neurite Extension by Mouse Embryonic Stem Cells using IKVAV and LRE Peptide Tethering in Hyaluronic Acid Matrices. *Biomacromolecules* 20: 3009-3020, 2019.



Interaction of optimized laminin derived peptides signaling (IKVAV and LRE) on extracellular matrix secretion by human induced pluripotent stem cell derived neural stem cells (hNSC). A) Fibornectin staining (green) surrounds hNSC nucleus (blue) on hyaluronic acid matrices with (PEP-HA) and without (DIFF-HA) peptide signaling. Scale bars= 50µm. b) Quantification of fibronectin staining intensity. Fibronectin is associated with inflammation and fibrotic scarring, so reduced expression is beneficial to establishing new neural concentration during regenerative therapies.

Qi Lin Cao, MD
Professor

Stem cells for neurological diseases

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

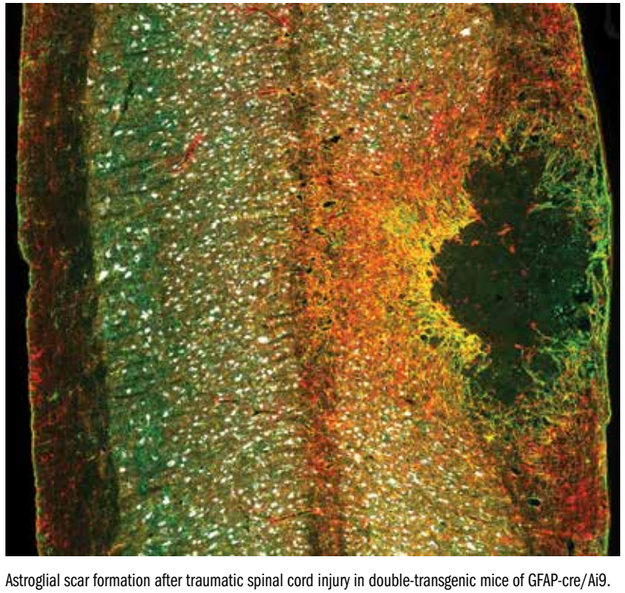
Liu, Y.; Zheng, Y.Y.; Li, S.L.; Xue, H.P.; Schmitt, K.; Hergenroeder, G.W.; Wu, J.Q.; Zhang, Y.Y.; Kim, D.H.; and Cao, Q.L. (2017). Human neural progenitors derived from integration-free iPSCs for SCI therapy. *Stem Cell Res.* 9:55-64. doi: 10.1016. PMID: 28073086

Cuevas-Diaz Duran, R.; Yan, H.; Zheng, Y.Y.; Huang, X.F.; Grill, R.; Kim, D.H.; Cao, Q.L.;* and Wu, J.Q.* (2017). The systematic analysis of coding and long non-coding RNAs in the sub-chronic and chronic stages of spinal cord injury. *Sci Rep.* 7:41008. doi: 10.1038. PMID: 28106101 (* co-corresponding authors).

Cheng, X.X.; Zheng, Y.Y.; Bu, P; Qi, X.B.; Fan, C.L.; Li, F.Q.; Kim, D.H.; and Cao, Q.L. (2018). Apolipoprotein E as a novel therapeutic neuroprotection target after traumatic spinal cord injury. *Experimental Neurology* 299: 97-108.

LAB MEMBERS

Post-doctoral fellow: Yiyan Zheng
Graduate student: Chrystine Gallegos
Research associate: Haipeng Xue
Undergraduate student: Matthew Carey



Astroglial scar formation after traumatic spinal cord injury in double-transgenic mice of GFAP-cre/Ai9.

Charles Cox, Jr., MD
Professor
George and Cynthia Mitchell Distinguished University Chair

Cellular therapies for neurological injury

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

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

Liao, G.P.; Aertker, B.A.; Kota, D.J.; Prabhakara, K.S.; Smith, P.A.; Hetz, R.A.; Xue, H.; Bedi, S.; Olson, S.D.; Cox, C.S. Assessing blood brain barrier permeability in traumatic brain injury research. *ADMET & DMPK*. 3(3):182-189, 2015.

Cox, C.S.; Hetz, R.A.; Liao, G.P.; Aertker, B.M.; Ewing-Cobbs, L.; Juranek, J.; Savitz, S.I.; Jackson, M.L.; Romanowska-Pawliczek, A.; Triolo, F.; Dash, P.K.; Pedroza, C.; Lee, D.A.; Worth, L.; Aisiku, I.; Choi, H.A.; Holcomb, J.B.; Kitagawa, R. Treatment of severe adult traumatic brain injury using bone marrow mononuclear cells. *Stem Cells* 35:1065-1079, 2016. PMID: 27800660, PMCID: PMC5367945

LAB MEMBERS

Steven Kosmach, MSN, RN, CCRC-TBI clinical
Joiya Arrington, MSN, RN, TBI clinical
Yidao Ca, -programmer analyst
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Supinder Bedi, PhD, assistant professor
Amit Srivastava, PhD, assistant professor
Naama Toledo-Furman, PhD, flow cytometry/ innate immunity
Hasen Xue, MD, research asssociate
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Sufira Kiran, GMP, QA director
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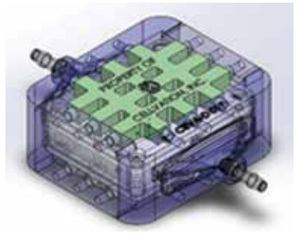
Matteo Costantini, research assistant
Kevin Aroom, scientific programmer
Tushar Sharma, scientific programmer
Max Skibber, research assistant
Christina Willingham, program manager
Stephanie Baca, program manager



Development of a novel bioreactor for stem cell production.

Radbod Darabi, MD, PhD
Associate Professor

Human induced pluripotent stem cells (iPSCs) to treat skeletal muscle disorders

Skeletal muscle disorders consist of a diverse and heterogeneous group of disorders affecting patient's function and mobility. Common disorders include muscular dystrophies and muscle injuries. Muscular dystrophies are hereditary and genetic disorders of the skeletal muscles. In these group of disorders due to a mutated gene, a structural protein of the skeletal muscle becomes defective, which leads to progressive muscle inflammation and degeneration. Depending on the affected gene, patients may show different degrees of progressive muscle weakness with early or late onsets. Another major group of muscle disorders are volumetric muscle mass loss (VML) injuries and defects, which are very common in traumatic patients, such as car accidents or combat injuries or after tumor resection in cancer patients. These also often lead to a sizable muscle defect and different levels of disabilities. Skeletal muscle disorders are often incurable and are a major cause of disabilities and create a big burden on the health system.

Here at the IMM and stem cell center, we are interested in using induced pluripotent stem cells (iPSCs) for skeletal muscle repair. iPSCs can be easily reprogrammed from an adult skin or blood cell and can generate a source of stem cells capable of unlimited differentiation to all cell types in the human body. In addition, since iPSCs are derived from same patients, they are fully compatible with the patient with minimal immune rejection risk. Therefore, iPSCs are considered as the top candidate for stem cell therapy in degenerative disorders.

Our lab uses cutting edge technologies to create iPSCs from muscle disorder patients and use them for generation of large quantities of muscle cells useful for engraftment applications. So far, we have generated patient iPSCs from novel types of muscular dystrophy patients (LGMDR21) due to a defect in a novel gene (POGLUT1) and use them to study disease mechanism and pathophysiology. We also use advanced gene correction methods, such as CRISPR, to design strategies for correction of defective genes in these disorders. In addition, we use different mice models for muscular dystrophies (such as *mdx* mouse which is a common model for Duchenne muscular dystrophy/DMD) and muscle loss injury mouse models (to model muscle injuries after trauma or combat injury) to validate engraftment and regenerative potential of human iPSCs.

So far, our lab has pioneered new methods for derivation of engraftable muscle cells from human iPSCs and demonstrated their application for skeletal muscle repair in these models. The longterm goal of our lab is to pave the way toward clinical application of human iPSCs to treat skeletal muscle disorders. Our research program is currently funded by two NIH R01 grant awards from National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) to support these exciting and novel projects.

RESEARCH PROJECTS

- Evaluation of the engraftment and functional recovery potential of human iPSCs in the mice models for Duchenne muscular dystrophy (DMD)
- Therapeutic application of human iPSCs for volumetric muscle mass loss injuries (VML) and evaluation of their innervation and functional recovery
- Gene correction of muscular dystrophies using CRISPR/Cas9 system

KEY PUBLICATIONS

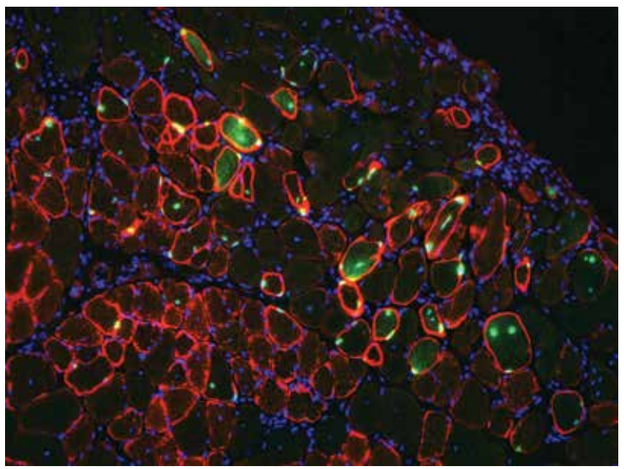
Wu J, Hunt SD, Matthias N, Servián-Morilla E, Lo J, Jafar-Nejad H, Paradas C, Darabi R. Generation of an induced pluripotent stem cell line (CSCRMi001-A) from a patient with a new type of limb-girdle muscular dystrophy (LGMD) due to a missense mutation in POGLUT1 (Rumi). *Stem Cell Research*, 2017 Sep. 24: 102-105. 2017

Wu J, Matthias N, Lo J, Ortiz-Vitali JL, Shieh AW, Wang SH, Darabi R. A Myogenic Double-Reporter Human Pluripotent Stem Cell Line Allows Prospective Isolation of Skeletal Muscle Progenitors. *Cell Reports*. 2018 Nov. 25/7: 1966-1981. 2018

Wu J, M. N., Bhalla S, Darabi R, Evaluation of the Therapeutic Potential of Human iPSCs in a Murine Model of VML. *Molecular Therapy* 2020, DOI: https://doi.org/10.1016/j.ymthe.2020.09.012.

LAB MEMBERS

Instructor: Jianbo Wu
Research assistant: Nasa Xu



Engraftment of human iPSCs in a mouse model for volumetric muscle loss (VML) injury. Red and green colors mark human iPSCs expressing specific markers of dystrophin and lamin A/C.

Pramod Dash, PhD
Professor and Chair, Department of Neurobiology and Anatomy
Nina and Michael Zilkha Distinguished Chair, Neurodegenerative Disease Research

Concussion and stress-related disorders

Concussion (also known as mild traumatic brain injury, mTBI) has emerged as a major health problem, striking not only athletes participating in contact sports, but persons of all ages and sexes. According to the Centers for Disease Control, approximately 2.6 million Americans sustain a brain injury each year, of which 87% can be classified as concussion. Recently, due to the increase in longevity and the number of falls in our older population, the incidence of concussion is on the rise in older Americans. As a person can sustain a concussion without ever losing consciousness, and many of these people never seek medical attention, the above statistics may only represent a fraction of actual concussion cases. Currently, there is no objective way to assess if brain injury has occurred after a concussion.

It has been recently appreciated that concussion is not a singular event but rather is a progressive disease with long-lasting consequences. It remains unknown when, or if, the brain returns to its pre-injury state. As the brain remains vulnerable to a second injury, continued research is required to understand the molecular, cellular, and structural changes that occur following concussion in order to develop treatments, which can offer functional improvement. To this end, we have been examining the influence of repeated brain injury in both humans and in animal models.

One of the consistent pathologies associated with both clinical and experimental traumatic brain injury is axonal injury, especially following concussion. Several lines of experimental evidence have demonstrated a role for NAD+ metabolism in axonal degeneration. One of the enzymes that metabolizes NAD+ in axons is Sarm1 (Sterile Alpha and TIR Motif Containing 1), and its activity is thought to play a key role in axonal degeneration. We have been examining the role of Sarm1 in axonal injury and cognitive outcome after repeated mild closed head injury (rmCHI). Our results indicate that rmCHI elicited white matter damage is markedly reduced in mice lacking the Sarm1 protein (Sarm1-/- mice). Further, we have found that the activation of astrocytes and microglia is also attenuated in the areas with white matter damage, suggesting a reduction in inflammation. Associated with these improvements, injured Sarm1-/- mice were found to perform significantly better in both motor and cognitive tasks.

RESEARCH PROJECTS

- To identify how concussion alters neural communication.
- To investigate neurovascular function after concussion.
- To investigate the consequences of mitochondrial plasticity and altered brain energy metabolism after concussion.

KEY PUBLICATIONS

Underwood E, Redell JB, Zhao J, Moore AN, Dash PK. A method for assessing tissue respiration in anatomically defined brain regions. *Sci Rep*. 10:13179, 2020.

Vedantam A, Brennan J, Levin HS, McCarthy JJ, Dash PK, Redell JB, Yamal JM, Robertson CS. Early versus Late Profiles of Inflammatory Cytokines after Mild Traumatic Brain Injury and Their Association with Neuropsychological Outcomes. *J Neurotrauma*, 2020.

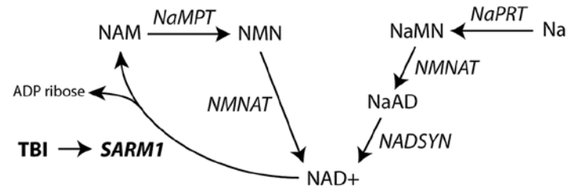
Maynard ME, Redell JB, Zhao J, Hood KN, Vita SM, Kobori N, Dash PK. Sarm1 loss reduces axonal damage and improves cognitive outcome after repetitive mild closed head injury. *Exp Neurol.* 327:113207, 2020.

Maynard ME, Redell JB, Kobori N, Underwood EL, Fischer TD, Hood KN, LaRoche V, Waxham MN, Moore AN, Dash PK. Loss of PTEN-induced kinase 1 (Pink1) reduces hippocampal tyrosine hydroxylase and impairs learning and memory. *Exp Neurol*. 323:113081, 2020.

Broussard JI, Redell JB, Zhao J, Maynard ME, Kobori N, Perez A, Hood KN, Zhang XO, Moore AN, Dash PK. Mild Traumatic Brain Injury Decreases Spatial Information Content and Reduces Place Field Stability of Hippocampal CA1 Neurons. *J Neurotrauma* 37:227-235, 2020.

COLLABORATORS/LAB MEMBERS

Dr. James McCarthy: chairman of Emergency Medicine; medical director, Emergency Center at Memorial Hermann Hospital-TMC
Dr. Paul Schulz: associate professor of Neurology; director, Dementia and Memory Disorders group
Dr. Summer Ott: associate professor of Orthopedic Surgery; director, Concussion Program at Ironman Sports Medicine Institute
Dr. Cameron Jeter: assistant professor of Diagnostic and Biomedical Sciences
Dr. John Redell: assistant professor-research of Neurobiology and Anatomy
Dr. Jing Zhao: assistant professor-research of Neurobiology and Anatomy
Dr. Nobuhide Kobori: assistant professor-research of Neurobiology and Anatomy
Dr. Erica Underwood: instructor for Neurobiology and Anatomy
Post-doctoral fellows: Dr. Rebecca West
Research assistants: John Broussard, PhD, Kimberly Hood, MS, Anthony Moore, BS



Simplified pathway for NAD metabolism. NAD+ is synthesized from two metabolic pathways: a de novo synthesis pathway from Na (and amino acids) or a recycling pathway. Sarm1 is a NAD+ consuming enzyme. Our results indicate that in the absence of SARM1, axonal injury is reduced suggesting that depletion of NAD+ contributes to axonal damage after repeat concussion.

Sarah Xuelian Huang, MBBS, PhD
Assistant Professor

Human pluripotent stem cells for lung regeneration and disease modeling

My laboratory is interested in applying human pluripotent stem cells to study the molecular mechanisms of lung cell fate specification in the context of both normal and pathological conditions. The long-term goal is translation of the acquired knowledge into prevention and treatment of currently not curable lung diseases. Lung diseases are among the leading causes of death globally. Lower respiratory infections, chronic obstructive pulmonary disease and lung cancer together account for approximately 9 million deaths annually worldwide. Despite the huge lung disease burden, we still have very limited understanding of the pathogenic mechanisms responsible for these diseases, and consequently there is a lack of successful therapeutic approaches.

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

Realization of stem cell therapy in lung diseases relies on the successful generation of clinically applicable cell types. As a first, critical step in this direction, we have previously developed a step-wise differentiation strategy that directs human pluripotent stem cells to become different types of upper (airway) and lower (alveoli) respiratory lung epithelial cells at large quantities (Huang et al. Nat Biotechnol 2014, Nat Protoc 2015). As a proof of principle, the generated cells have been applied for lung development or disease studies by us and other research groups. Currently, we are working on culture conditions that can direct the human pluripotent stem cell-derived early lung progenitors toward an enriched population of either airway epithelial cells or distal alveolar cells. The availability of each of these enriched airway- and alveolar- fated cells provides a valid platform for studying lung diseases originate in both airway and alveolar. Examples include influenza virus infection induced severe infection and acute respiratory destress syndrome that affects the lower respiratory of the lung; and lung cancers that can arise in both the airway and alveoli cells depending on the subtype.

RESEARCH PROJECTS

- Use human pluripotent stem cell-derived lung epithelium to model small cell lung cancer
- Use patient hiPSC differentiated lung and airway epithelial cells to study normal development and pathogen infection
- Understanding the basic mechanisms of lung lineage specification from NKX2.1+SOX2+SOX9+ NKX2.1+SOX2+P63+ human lung and airway progenitors using molecular, genetic and epigenetic approaches

KEY PUBLICATIONS

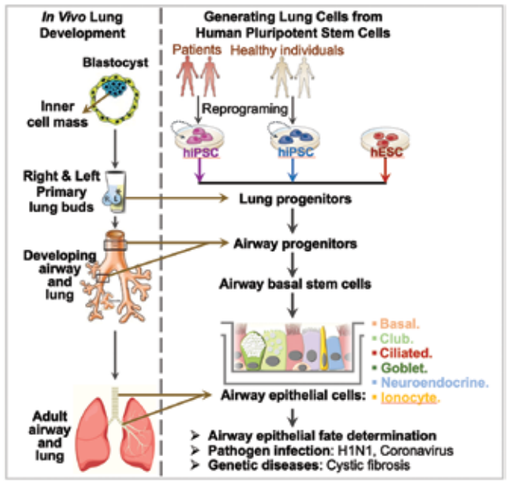
Edwin J. Ostrin, Danielle R. Little, Kamryn N. Gerner-Mauro, Elizabeth A. Sumner, Ricardo Ríos-Corzo, Elizabeth Ambrosio, Samantha E. Holt, Nicolas R. Forcioli-Conti, Haruhiko Akiyama, Sam M. Hanash, Shioko Kimura, Sarah X.L. Huang, Jichao Chen. Beta-Catenin maintains lung epithelial progenitors after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

Huanhuan Joyce Chen, Asaf Poran, Arun M. Unni, Sarah Xuelian Huang, Olivier Elemento, Hans-Willem Snoeck, Harold Varmus. Generation of pulmonary neuroendocrine cells and SCLC-like tumors from human embryonic stem cells. *J Exp Med*. 2019 Mar 4;216(3):674-687.

Hye Kyung Lim, Sarah X.L. Huang, Jie Chen, Gaspard Kerner, Olivier Gilliaux, Paul Bastard, Kerry Dobbs, Nicholas Hernandez, Nicolas Goudin, Mary L. Hasek, Eduardo Javier García Reino, Fabien G. Lafaille, Lazaro Lorenzo, Priya Luthra, Tatiana Kochetkov, Benedetta Bigio, Soraya Boucherit, Flore Rozenberg, Catherine Vedrinne, Michael D. Keller, Yuval Itan, Adolfo García-Sastre, Marie Celard, Jordan S. Orange, Michael J. Ciancanelli, Isabelle Meyts, Qian Zhang, Laurent Abel, Luigi D. Notarangelo, Hans-Willem Snoeck, Jean-Laurent Casanova, Shen-Ying Zhang. Severe influenza pneumonitis in children with inherited TLR3 deficiency. *J Exp Med*. 2019 Sep 2;216(9):2038-2056.

LAB MEMBERS

Post-doctoral fellow: Nicolas Focioli-Conti



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

Dong Kim, MD
Professor and Chair, Vivian L. Smith Department of Neurosurgery
Director, Mischer Neuroscience Institute
Memorial Hermann–TMC
Clive and Nancy Runnels Chair in Neurosurgery

Advancing the field of neuroscience

Professor and chair of the Department of Neurosurgery at McGovern Medical School at UTHealth, I also lead the clinical neuroscience efforts for the Memorial Hermann Healthcare System as the director of the Mischer Neurosciences Institute. Our group includes over 100 faculty and residents/fellows.

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

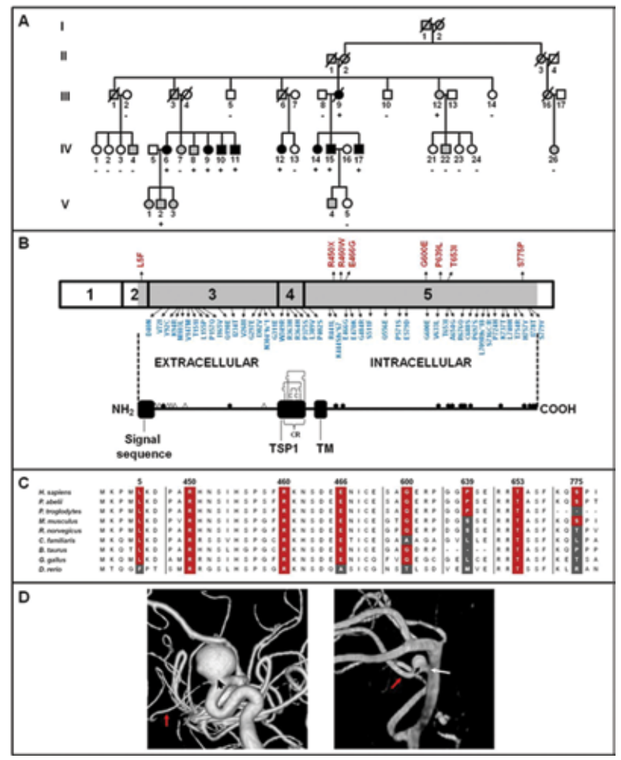
Santiago-Sim T, Fang X, Hennessy M, Nalbach S, DePalma S, et al. THSD1 (Thrombospondin Type 1 Domain Containing Protein 1) Mutation in the Pathogenesis of Intracranial Aneurysm and Subarachnoid Hemorrhage. *Stroke.* 2016 Dec;47(12):3005-3013. Epub 2016 Nov 15.

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

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

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

Levi AD, Okonkwo D, Park P, Jenkins A, Kurpad S, Parr A, Ganju A, Aarabi B, Kim D, Casha S, Fehlings M, Anderson KD, Gage A, Hsieh J, Huhn S, Curt A, Guzman R. Emerging safety of intramedullary transplantation of human neural stem cells in chronic cervical and thoracic spinal cord injury. *Neurosurgery.* 24 May 2017.



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

Momoko Yoshimoto, MD, PhD
Associate Professor

Development of hematopoietic stem cells and innate-like B cells in the mouse embryo

The hematopoietic stem cells (HSCs) that produce all types of blood cells in the body are first generated in the aortic region of the mouse embryo at embryonic day (E) 10-11. Interestingly though, there are multiple waves of blood cell production prior to the emergence of the first HSC from endothelial cells (referred to as hemogenic endothelial cells: HECs), and these blood cells include erythro-myeloid, T- and B- lymphoid cells. We have recently found that innate-like B-1 lymphocytes and the first HSCs are produced simultaneously from HECs. We are elucidating 1) what molecular signals determine the divergent point between innate-like B-1a biased and multi-potent HSCs, 2) how embryo-derived B-1 progenitors contribute to postnatal peritoneal B-1 cell pool, and 3) how HSC-precursors mature into adult-repopulating HSCs in a limited time window of embryonic development.

B-1 cells are unique murine innate immune cells that are distinguished from conventional adoptive B cells (B-2 cells). B-1 cells localize in the peritoneal and pleural cavities and secrete natural antibodies without T cell help, displaying important roles in the first line of defense against various infections, atherosclerosis, and autoimmunity. It has been postulated for decades that B-1 cells are derived from fetal progenitor cells, not from adult bone marrow HSCs, based on the results of transplantation assays. Our aim is to identify the main source of HSC-independent B-1 progenitor cells and evaluate its real contribution to postnatal B-1 cell pool, utilizing various lineage tracing mouse models.

B-1 biased progenitors and precursor of HSCs are produced from hemogenic endothelial cells simultaneously in the embryo. However, it is still unknown what molecules determine the cell fate of hemogenic endothelial cells into these two types progenitors. By utilizing transplantation assays, lineage tracing mouse models, and single-cell RNA-sequencing, we are elucidating the biological and molecular mechanisms that are responsible for this cell fate decision and maturation.

Knowledge obtained from above projects will help us to improve the system where HSCs are produced from human iPSCs in vitro, which may be utilized for cell therapy to the patients with hematological disorders and leukemias.

RESEARCH PROJECTS

- Lineage tracing for HSC-independent and/or HSC-dependent B-1 cell development from embryos to adults.
- Identify important molecules for HSC maturation in the mouse embryo utilizing single-cell RNA-sequencing.
- Examining the contribution of fetal-derived B cells to IgA secreting cells in the lamina propria of intestine.
- Producing human B-1 cells from human iPSCs..

KEY PUBLICATIONS

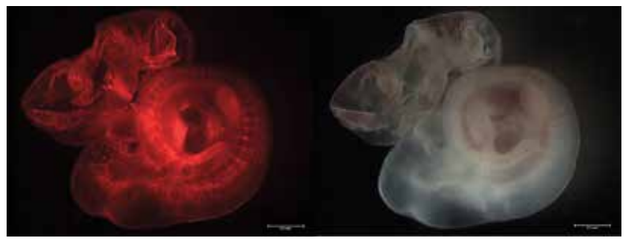
Kobayashi M, Lin Y, Mishra A, Shelly C, Gao R, Reeh CW, Wang PZ, Xi R, Liu Y, Wenzel P, Ghosn E, Liu Y, Yoshimoto M. Bmi1 Maintains the Self-Renewal Property of Innate-like B Lymphocytes. J Immunol. 204. 3262-3272, (2020) PMC7293378 10.4049/*jimmunol*.2000030

Kobayashi M, Tarnawsky SP, Wei H, Mishra A, Azevedo Portilho N, Wenzel P, Davis B, Wu J, Hadland B, Yoshimoto M. Hemogenic Endothelial Cells Can Transition to Hematopoietic Stem Cells through a B-1 Lymphocyte-Biased State during Maturation in the Mouse Embryo. *Stem Cell Reports*. 13 (1): 21-30, 2019.

Lin Y, Kobayashi M, Azevedo Portilho N, Mishra A, Gao H, Liu Y, Wenzel P, Davis B, Yoder MC, Yoshimoto M. Long-Term Engraftment of ESC-Derived B-1 Progenitor Cells Supports HSC-Independent Lymphopoiesis. *Stem Cell Reports*. 12(3):572-583, 2019.

LAB MEMBERS

Assistant professor: Michihiro Kobayashi MD, PhD
Research assistants: Chika Nishida MD., Samuel A. Cornelius, Noemi Valiente



A lineage tracing mouse model
Cdh5CreERT2: Rosa-Tomato embryos. Tamoxifen was injected into day 9 pregnant mother and cdh5+ endothelial cells were labeled with Tomato+ on the following day.

Dung-Fang Lee, PhD
Assistant Professor

Familial cancer syndromes in a dish

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

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

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

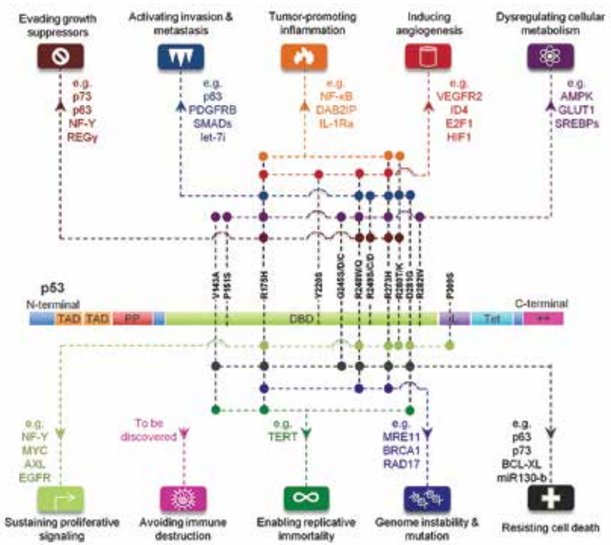
Huensuk Kim, Seungyeul Yoo, Ruoji Zhou, An Xu, Jeffrey M. Bernitz, Ye Yuan, Andreia M Gomes, Michael G Daniel, Jie Su, Elizabeth G. Demicco, Jun Zhu, Kateri A. Moore, Dung-Fang Lee, Ihor R Lemischka, Christoph Schaniel. Oncogenic role of SFRP2 in p53-mutant osteosarcoma development via autocrine and paracrine mechanism. *Proc Natl Acad Sci* U S A. 2018 Nov 20:115(47):E11128-E11137.

ie Su, Dandan Zhu, Zijun Huo, Julian A. Gingold, Yen-Sin Ang, Jian Tu, Ruoji Zhou, Yu Lin, Haidan Luo, Huiling Yang, Ruiying Zhao, Christoph Schaniel, Kateri A. Moore, Ihor R. Lemischka, Dung-Fang Lee. Genomic integrity safeguards self-renewal in embryonic stem cells. *Cell Rep*. 2019 Aug 6;28(6):1400-1409.e4

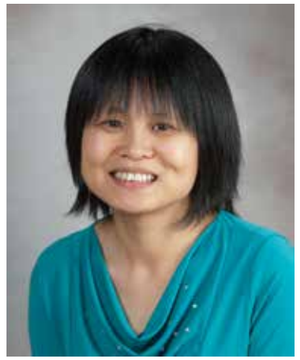
Aimin Li, Saurav Mallik, Haidan Luo, Peilin Jia, Dung-Fang Lee†, Zhongming Zhao†. H19, a Long Non-coding RNA, Mediates Transcription Factors and Target Genes through Interference of MicroRNAs in Pan-Cancer. *Mol Ther Nucleic Acids*. 2020 Sep 4; 21:180-191. (†Corresponding author)

LAB MEMBERS

Post-doctoral fellows: An Xu, Mo Liu, Dandan Zhu
Students: Brittany E. Jewell
Technicians: Ying Liu



Mutant p53 gain-of-function driver cancer through cancer hallmarks. Different mutations on p53 protein arm p53 with new weapons (downstream targets indicted in the figure) to drive cancer development and progression. Each color-coded node indicates gain-of-function of specific mutation of *TP53*, which further drives cancer through various cancer hallmarks.



Ying Liu, MD, PhD
Assistant Professor

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

Our research focuses on dissecting the neural developmental pathways and the corresponding pathogenesis in CNS injury and neurodegenerative diseases. Our long-term goal is to identify therapeutic targets for the treatment of CNS diseases.

Human induced pluripotent stem cells (iPSCs) provide autologous materials for patients, which theoretically omit the need for immune suppression. We have optimized the more clinically relevant, integration-free hiPSC generation protocol and performed directed differentiation of patient-specific iPSCs into neural stem cells, neuronal and glial progenitors, as well as mature cell types for disease modeling, transplantation studies, neural regeneration and repair, and drug screening and testing. The highly efficient CRISPR gene editing tool adapted in the lab allows for quick creation of neural lineage reporters and multigene activation for lineage induction. These neural lineage specific cells are applied to in-depth study of signal transduction in disease and development.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

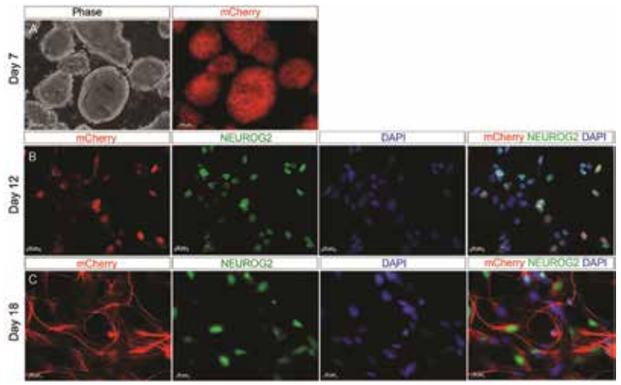
Liu, Y.*, Zheng, Y., Li, S., Xue, H., Schmitt, K., Hergenroeder, G.W., Wu, J., Zhang, Y., Kim, D.H., Cao, Q*. (2017) Human neural progenitors derived from integration-free iPSCs for SCI therapy. Stem Cell Res. 2017 Jan 5;19:55-64. doi: 10.1016/*j. scr.*2017.01.004. [Epub ahead of print] (*corresponding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

Li, S., Zhang, A., Xue, H., Li, D., Liu, Y. (2017) One-step piggyBac transposon-based CRISPR/Cas9 activation of multiple genes. Mol Ther Nucleic Acids. 15 September 2017. 8:64–76, doi. http://dx.doi.org/10.1016/*j. omtn.*2017.06.007. PMID: 28918057 PMCID:PMC5485764

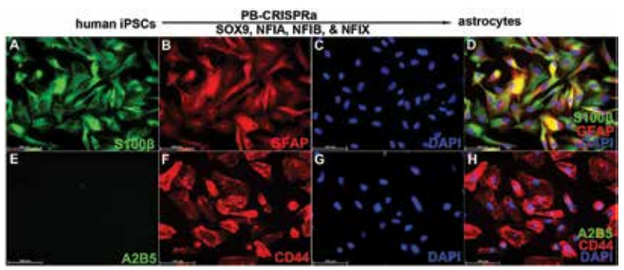
Li D, Li S, Xue AZ, Smith Callahan LA, Liu Y. (2020) Expression of SREBP2 and cholesterol metabolism related genes in TCGA glioma cohorts. *Medicine.* 2020 Mar;99(12):e18815. doi: 10.1097/MD.0000000000018815. PMID: 32195924

LAB MEMBERS

Research scientist: Shenglan Li
Research associate: Haipeng Xue



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



Rapid generation of astrocytes from human iPSCs by endogenous activation of astrocyte lineage specific transcription factors with the *piggyBac*-CRISPR activation system. Human iPSCs cell line was transfected with all-in-one vectors expressing guide RNAs that activate SOX9-NFIA-NFIB-NFIX transcription factors. Fourteen days post transfection, nearly all cells expressed astrocyte markers S100B (A), GFAP (B) and CD44 (F), while did not express glial progenitor marker A2B5 (E). Nuclei are revealed by DAPI (C, G). (D) and (H) are overlapped images.

Nami McCarty, PhD
Associate Professor
Annie and Bob Graham Distinguished Chair in Stem Cell Biology

Deciphering mechanisms of human cancer cell survival within the bone microenvironment

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

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

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

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

RESEARCH PROJECTS

- Survival mechanisms of dormant multiple myeloma cells and their microenvironment in the bone marrow: We conducted microarray analyses to identify genes expressed in quiescent multiple myeloma cells from the different niches of the bone marrow. We will continue to characterize functions of these genes in the multiple myeloma interaction with bone marrow microenvironment to delineate how dormant multiple myeloma cells evade chemotherapies.
- Development of small molecule inhibitors to target drug-resistant lymphomas: We have conducted high throughput chemical screening to identify the compounds that selectively target mantle cell lymphoma cells that develop drug resistance. We will further develop and test these compounds in animal models for pre-clinical studies and plan to test their efficacies in the patients.
- Protein homeostasis is orchestrated by coordinated protein synthesis, folding, transport, and degradation. Inappropriate protein assembly or modification promotes protein misfolding, which can lead to not only disruptions to protein homeostasis but also to normal cellular functions. We focuses on delineating functions of protein homeostasis control in cancer progression.

KEY PUBLICATIONS

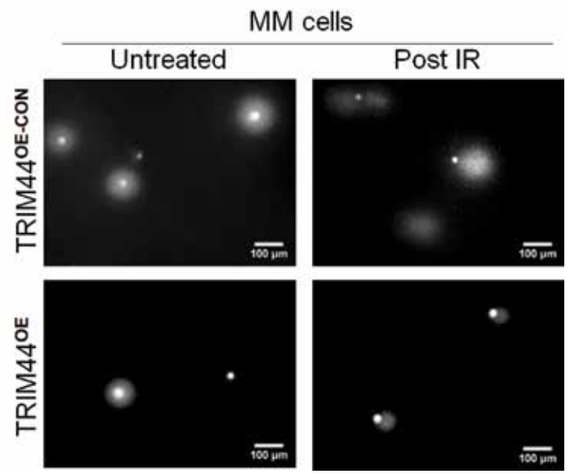
Zhang, H., Chen, Z., Miranda, R.N., Medeiros, L.J., and McCarty, N. Bifurcated BACH2 control coordinates mantle cell lymphoma survival and dispersal during hypoxia. *Blood* 130:763-776. 2017. This article was featured in "this week in Blood" as an Editor's pick.

McCarty, N. (2018) Battling quiescence for tumor eradication, is too good to be true? *Oncotarget* editorial 9:37276-37277. PMID: 30647863

Chen, Z., Lin, T-C., Bi, X., Lu, G., Dawson, B.C., McNiece, I., McCarty, N. (2019) TRIM44 in quiescent multiple myeloma cells stabilizes HIF-1α via deubiquitination for niche control. *Leukemia* 33:469-486. PMID: 30089913

LAB MEMBERS

Post-doctoral fellow: Lyn Liu, PhD, Lili Chen, MD, PhD
Graduate student: Parnaz Merikihan
Research associate: Rao Raksha, PhD



COMET assay confirms TRIM44 enhances DNA damage repair in MM cells. Cells were treated with irradiation and, COMET images were captured using fluorescence microscopy after a couple of hours. Tail moment was calculated as tail length multiplied by tail DNA percentage in at least 100 cells.

Pamela Wenzel, PhD
Associate Professor
Director of Immunology Program, MD Anderson

Effects of flow on stem cell potential and immune function

Our lab studies how biomechanical force generated by the flow of blood in the circulatory system impacts cell fate and behavior. One of our primary research projects addresses how frictional force caused by blood flow promotes emergence of blood stem cells during embryo development. We are interested in how we might use this information in the laboratory to expand improved sources of these stem cells for treatment of hematologic disorders and cancers, such as bone marrow failure syndromes and leukemias.

Complex signaling occurs in response to flow that potentiates stem cell potential, including activation of integrins, mechanosensitive ion channels, and primary cilia (Fig. 1). In our prior published work, we have shown that fluid frictional force in biomimetic microfluidics that matches the intensity of blood flow present in the developing embryo can stimulate calcium sparks within the cytoplasm, thus triggering the cell to produce prostaglandin E2. Elevated prostaglandin synthesis is key to forming hematopoietic stem cells that later will supply the body with blood and immune cells into adulthood. We have additionally shown that the force generated by this flow activates classic developmental signaling, including Notch and Wnt. Both of these signal transduction pathways are known regulators of blood development and must be tightly modulated in order to direct differentiation of certain immune cell lineages, including T lymphocytes. Lastly, in work spanning various model systems, evidence has begun to emerge that implicate focal adhesion kinase and the Src family kinases in regulation of transcription factors such as Yap and Taz downstream of fluid force. We are currently pursuing both collaborative and independent studies aimed at better understanding the mechanosensors and intracellular signaling that are central to dictating how blood stem cells respond to biomechanical cues to ensure proper self-renewal and differentiation.

Another related area of research in our lab includes the study of how flow alters bioenergetics and, specifically, how the powerhouses of the cell – the mitochondria - adapt to meet the changing metabolic needs of stem cells. We are finding that these organelles change shape and move differently within the cell depending upon the biophysical cues in the environment. This is particularly relevant during fate commitment of hematopoietic stem cells in the embryo, but also could be important in the adult. Mitochondria are critical in both hematopoietic stem cells and mesenchymal stem cells of the adult bone marrow, the latter of which are known to be capable of promoting repair of damaged tissues by mitochondrial transfer to injured cells when administered as a cellular therapeutic. Ongoing studies are directed at determining how mitochondria contribute to ensuring that hematopoietic stem cells are properly specified in the embryo and how we might modify mitochondrial behavior to enhance stem cell activity in bone marrow transplantation.

RESEARCH PROJECTS

- Effects of flow on hematopoietic stem cell fate and the bone marrow niche
- Biomechanical force in modulation of mitochondrial dynamics

KEY PUBLICATIONS

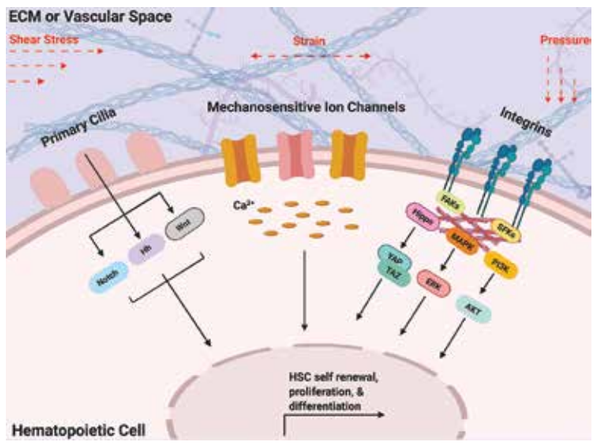
Horton, P.D., Dumbali, S., & Wenzel, P.L. Mechanoregulation in hematopoiesis and hematologic disorders. *Current Stem Cell Reports.* Sep;6(3):86-95. doi: 10.1007/s40778-020-00172-4. https://link.springer.com/article/10.1007/s40778-020-00172-4, 2020.

Mohammadalipour, A., Diaz, M.F., Pareek, S., & Wenzel, P.L. Ex vivo modeling of hematopoietic stem cell homing to the fetal liver. *Methods in Molecular Biology.* Jun 12. doi: 10.1007/7651_2020_293. https://link.springer.com/protocol/10.1007%/2F72651_2020_293

Diaz, M.F., Horton, P.D., Kumar, A., Livingston, M., Mohammadalipour, A., Xue, H., Skibber, M.A., Ewere, A., Toledano Furman, N.E., Aroom, K.R., Zhang, S., Gill, B.S., Cox, C.S., & Wenzel, P.L. Injury intensifies T cell mediated graft-versus-host disease in a humanized model of traumatic brain injury. *Scientific Reports.* 10(1):10729. doi: 10.1038/s41598-020-67723-x, 2020.

LAB MEMBERS

Graduate student: Paulina Horton
Post-doctoral fellows: Amina Mohammadalipour, Sandeep Dumbali
Senior research associate: Miguel Diaz



Integrins, mechanosensitive ion channels, and primary cilia sense mechanical features of the hematopoietic niche. Activation of mechanotransduction pathways alter gene expression and cell behavior critical for homeostasis and response to stress.

Jiaqian Wu, PhD
Associate Professor

Gene transcription and regulation of stem cell differentiation and neural injuries

I also serve as an associate professor with tenure in the Vivian L. Smith Department of Neurosurgery. During my graduate study at Baylor College of Medicine, I led the NIH Mammalian Gene Collection effort and cloned thousands of mammalian genes, which are publicly available through GE Dharmacon now. I published extensive work in transcriptome complexity, which revealed large amount of non-coding sequence transcription in the mammalian genomes. During my postdoctoral training at Yale University and Stanford University, I was closely involved in the ENCODE project and employed interdisciplinary approaches to study gene expression, transcription factor regulation, and regulatory networks of stem cell self-renewal and differentiation. I was one of the first using RNA-Seq to characterize stem cell neural differentiation process. Our lab has carried out unprecedented transcriptome profiling of eight highly purified neuron, glia, and vascular cells from brain by RNA-Seq. The lab identified a large number of novel long non-coding RNAs, and functional and genetic experiments substantiated the role of lncRNA in oligodendrocyte precursor cell (OPC) formation and astrogliosis. One of the neurological disorders that we are focusing on is spinal cord injury (SCI). The lab has already published RNA-Seq studies for acute and chronic SCI phases in mouse and rat contusive injury models. The lab provided unprecedented data source and a powerful analysis framework for functional investigations of coding and long non-coding RNAs in CNS cell types and SCI. Our work has been recognized with prestigious honors and awards, including the National Institutes of Health Ruth L. Kirschstein National Research Service Award for Individual Postdoctoral Fellows, the International Society for Stem Cell Research (ISSCR) Annual Meeting Travel Award, the National Institute of Health Pathway to Independence (PI) Award (K99/R00), R01s, R21s and the Senator Lloyd and B.A. Bentsen Investigator Award. A reviewer for NIH, New York State Department of Health-Spinal Cord Injury Research Board, MRC, Wellcome Truest, ANR, and many journals. I have presented invited talks and lectures on stem cell biology, neuroscience, and functional genomics at international conferences, Lawrence Livermore National Laboratory, the University of Florida, and the Multiple Sclerosis Research Center of New York, etc. I have developed a patent, authored two books, and written many articles that have appeared in *Nature, PNAS, the Journal of Neuroscience, Plos Genetics, Genome Research*, and *Scientific Reports* among others.

RESEARCH PROJECTS

- Investigate gene expression and regulatory mechanisms during stem cell differentiation; pinpoint key transcription factors and regulatory RNAs, and modulate key regulators to steer the direction of stem cell differentiation and improve efficiency
- Characterize molecular signatures and identify therapeutic targets for spinal cord injury and neurological diseases
- Network analysis of stem cell differentiation and global network integration of multiple types of omic data

KEY PUBLICATIONS

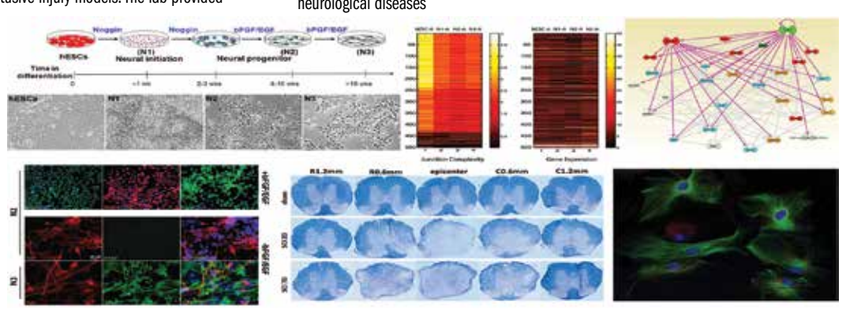
K.Lakshmi Narayanan, Wu X, Wei H and Wu J. Evolving Roles of Long Noncoding RNAs. *The Chemical Biology of Long Noncoding RNAs*. 2020. Springer Publishing. Netherlands.

Bhoi JD, Zhang Z, Janz R, You Y, Wei H, Wu J, Ribelayga CP. Bhoi JD, Zhang Z, Janz R, You Y, Wei H, Wu J, Ribelayga CP. *The SNARE Regulator Complexin3 is a Target of the Cone Circadian Clock* [published online ahead of print, 2020 Aug 12]. J Comp Neurol. 2020;10.1002/cne.25004.

Jin, N., Zhang, Z., Keung, J., Youn, S.B., Ishibashi, M., Tian, L-M., Marshak, D. W., Solessio, E., Umino, Y., Fahrenfort, I., Kiyama, T., Mao, C-A., You, Y., Wei, H., Wu, J., Postma, F., Paul, D. L., Massey, S. C., Ribelayga, C. P. Molecular and functional architecture of the mouse photoreceptor network. *Science Advances.* 2020 Jul; (6):28.

LAB MEMBERS

Post-doctoral fellows: Haichao Wei, PhD; Xizi Wu, MD, MS
Research associate:Bo Hai, DDS.
Resident physician: Michael Monterey
Undergraduate students: Tanuj Prajapati, Neha Tallapragada



Wu Lab uses interdisciplinary approaches including molecular biology, genetics, genomics, proteomics, and bioinformatics to study gene expression and transcriptional regulation in stem cells and the nervous system.

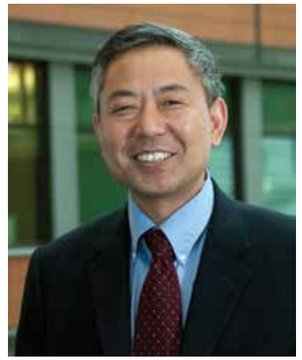
Translational cancer research aims to identify novel drug targets followed by the discovery and development of drug candidates as potential cancer therapeutics. The goal is to translate discoveries made in basic cancer research to potential drugs that could be tested in human patients. It relies on a plethora of information and data on cancer origination, progression, metastasis, drug-resistance, and disease relapse to uncover the driving mechanisms of tumor growth and invasion. Technologies such as next generation sequencing of DNA and RNA in cancer and non-cancer cells of tumor tissues, CRISPR screening, proteomics, imaging, patient-derived tumor models, drug candidate discovery, and bioinformatics are utilized to reveal drug targets and validate potential drug candidates.

The current research in the Center for Translational Cancer Research emphasizes several areas, including the application of cutting edge bioinformatic and experimental technologies to identify and validate novel drug targets in several major types of solid tumors, the discovery of specific molecules against the targets with a focus on antibody/protein-drug conjugates, the development of targeted contrast agents for disease visualization, and the study of proteome alterations to elucidate disease mechanism and discover biomarkers.

These efforts connect us with collaborators, such as physicians, pathologists, biologists, bioinformaticians, and bioengineers, across UTHealth, institutions within the Texas Medical Center, and across Texas to enhance basic, translational and clinical research. At the IMM, we have state-of-the-art mass spectrometers that provides in-depth proteomic analysis of cells, tissues or biological fluids, with the goals to discover novel targets and biomarkers to inform the development of therapeutic treatment and early detection of diseases. We combine critical data from cancer genetics, genomics, and proteomics to identify drug targets, create targeted antibodies and peptides, and synthesize drug conjugates that are then evaluated in tumor models. We also have expertise in the development and application of novel antibody-based agents that have imaging implications in cancer as well as infectious diseases. Furthermore, the Center specializes in the development of multifunctional peptides that combine radioactive and fluorescent contrast to enable tumor identification before, during, and after surgery, thus introducing a precision surgery approach. In addition, we have an active probe development program that includes the development of new aptamers and multi-functional nanoparticle therapeutics for targeting cancer and other diseases. We also have large-scale, multi-color, high resolution state-of-the-art 3D printers for both fast prototypes and finished production level models of new surgical tools and instruments or patient-specific organ models.

Our center houses several core facilities, including the Nanochemistry Service Center, 3D-printing Service Center and Clinical and Translational Proteomics Service Center, to support many research labs through service and collaborative efforts.

Qingyun "Jim" Liu, Ph.D.
Center Director & Professor
Janice Davis Gordon Distinguished Professor for Bowel Cancer Research



Qingyun (Jim) Liu, PhD
Professor
Janice Davis Gordon Chair for Bowel Cancer Research

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

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

Our research is focused on delineating the function and mechanisms of a group of cell surface receptors called LGR4, LGR5, and LGR6 (LGR4-6) that play critical roles in the survival of normal stem cells and tumor cells. Previously, we discovered that LGR4-6 function as receptors of a group of stem cell factors called R-spondins (RSPOs) that are essential for the survival and growth of stem cells. We are now focused on understanding how RSPOs and LGRs work together to regulate the growth and migration of normal and cancer cells. We found that LGR4 and LGR5 work through a different mechanism to control the survival and expansion of intestinal stem cells, which challenges a major current paradigm that LGR4 and LGR5 works in an identical way in cell signaling. Meanwhile, we showed that drug conjugates of ant-LGR5 antibodies showed excellent anti-tumor efficacy in preclinical models of colon cancer. Recently, we have discovered a novel approach that can target all three LGR receptors for the treatment of cancers of the digestive system. We are not optimized this approach by protein engineering to increase its potency and efficacy in tumor models.

RESEARCH PROJECTS

- Delineation of signaling mechanisms of stem cell receptors.
- Determination of the function and mechanism of the receptors in the control of normal and cancer cell growth.
- Investigation of the roles of aberrant expression of the RSPOs in the control of tumor metastasis of lung and colon cancer
- Identification of lead molecules targeting the RSPO-LGR system as novel anticancer therapeutics.
- Optimization of antibody-drug conjugates targeting the RSPO-LGR system for the treatment of colorectal and other cancers with high LGR expression.
- Determination of the function of a common mutation of RNF43 found in colon, stomach and uterine cancer.

KEY PUBLICATIONS

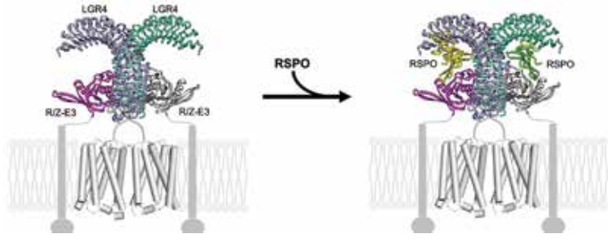
Park, S., Cui, J., Yu, W., Wu, L., Carmon, K.S., Liu, Q.J. Differential activities and mechanisms of the four R-spondins in potentiating Wnt/δ-catenin signaling. *J. Biol. Chem.* 293:9759-9769 (2018).

Tu, J., Park, S., Yu, W., Zhang, S., Wu, L., Carmon, K., Liu, Q.J. The most common RNF43 mutant G659Vfs*41 is fully functional in inhibiting Wnt signaling and unlikely to play a role in tumorigenesis. *Scientific reports*. 9(1):18557 (2019).

Park S, Wu L, Tu J, Yu W, Toh Y, Carmon KS, Liu Q.J. Unlike LGR4, LGR5 to potentiates Wnt/b-catenin signalng without sequestering E3 ligases. *Science Signaling*. In press (2020).

LAB MEMBERS

Post-doctoral fellows: Soohyun Park
Sr. research associates: Wangsheng Alice Yu, Ling Wu, and Jianghua Tu
Research scientist: Yukimatsu Toh



A schematic model illustrating how LGR4 interacts with E3 ligases RNF43 and ZNRR3 to potentiate Wnt signaling. LGR4 binds to the E3 ligases as 2:2 dimer without inhibiting ligase activity. Upon RSPO binding, E3 ligase activity is lost in the trimer and this leads to increase in the levels of Wnt receptors and therefore dramatic enhancement of Wnt signaling. Loss of function of RNF43 and ZNRF3 facilitate tumor formation. R/Z = RNF43 and ZNRF3.

Ali Azhdarinia, MS, PhD

Associate Professor
John S. Dunn Research Scholar III

Molecular imaging probe development

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

Hernandez Vargas, S., Kossatz, S., Voss, J., Ghosh, S.C., Tran Cao, H.S., Simien, J., Reiner, T., Dhingra, S., Fisher, W.E., Azhdarinia, A.* Specific targeting of somatostatin receptor subtype-2 for fluorescence-guided surgery. Clin Cancer Res. 25(14):4332-4342, 2019. PMID: 31015345.

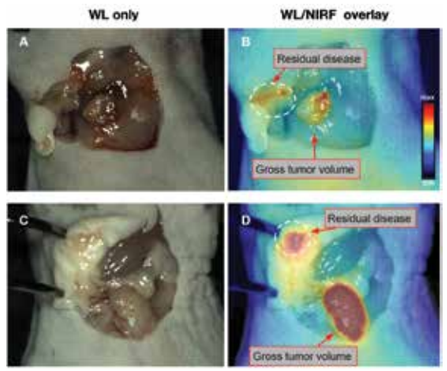
Bruno Perlatti, B., Lan, N., Earp, C.E., AghaAmiri, S., Hernandez Vargas, S., Azhdarinia, A., Bills, G.F., Gloer, J.B. Arenicolins: C-Glycosylated Depsides from Penicillium arenicola. *J. Nat. Prod.* 83(3):668-674, 2020. PMID: 31999116.

Hernandez Vargas, S., Lin, C., AghaAmiri, S., Voss, J., Ikoma, N., Tran Cao, H., Ghosh, S., Uselmann, A., and Azhdarinia, A.* A proof-of-concept methodology to validate the in situ visualization of residual disease using cancer-targeted molecular agents in fluorescence-guided surgery. *SPIE BiOS.* Vol. 11222. 2020.

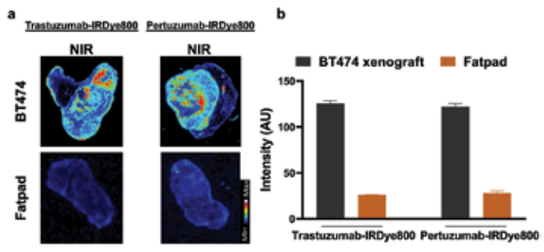
AghaAmiri, S., Simien, J., Thompson, A.M., Voss, J., Ghosh, S.C., Hernandez Vargas, S., Kim, S., Azhdarinia, A.*, and Tran Cao, H.S., Comparison of HER2-Targeted Antibodies for Fluorescence-Guided Surgery in Breast Cancer. *Mol Imaging.* 2021: p. 5540569, 2021.

LAB MEMBERS

Post-doctoral fellow: Solmaz AghaAmiri
Graduate student: Servando Hernandez Vargas
Research scientist: Sukhen Ghosh



Intraoperative visualization of residual cancer following gross tumor resection under ambient light. White light *in situ* visualization after tumor resection using direct visual inspection and palpation only (A and C). Tumor beds were then surveyed using the OnLume fluorescence-guided surgery imaging system and residual fluorescence was detected (dashed white circle) (B and D). B and D are the corresponding NIRF images overlaid on A and B, respectively. *(From Hernandez Vargas et al., SPIE BiOS. Vol. 11222. 2020).*



Fluorescence imaging (represented by the near-infrared (NIR) signal) in resected tissues shows that HER2-targeted contrasted agents are preferentially taken up by tumors (denoted as BT474) compared to normal, mammary fatpad tissue (i.e., normal tissue) (a). Quantitative analysis of the images shows was higher signal intensity in BT474 tumors (b). (From AghaAmiri et al., Mol Imaging, 2021. 2021: p. 5540569).

Kendra Carmon, MS, PhD

Assistant Professor

Therapeutic strategies for targeting colorectal tumors and cancer stem cells

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

Recent studies have established that LGR5 (Leucine-rich repeat-containing, G protein-coupled Receptor 5), a receptor expressed on normal adult stem cells, is highly upregulated in primary colorectal, tumors. Furthermore, colorectal CSCs which express LGR5 are capable of driving tumor growth and metastasis. In addition, LGR5 expression has been shown to be significantly elevated in several other major tumor types, including liver, gastric, and ovarian cancers. My previous work led to the discovery that LGR5 functions as a receptor of secreted growth factors, called R-spondins, to promote cancer cell adhesion and regulate cell signaling pathways involved in stem cell survival and tumor growth. These findings suggest that LGR5 plays an important role in cancer and could serve as a novel target for the development of innovative therapies which can eliminate CSCs.

My current research is focused on investigating the function and cell signaling mechanisms of LGR5 in colorectal CSCs using colorectal cancer cell lines and patient-derived tumors models. This work will lead to identifying the role of LGR5 in the control of tumor growth, metastasis, and drug resistance. Furthermore, we are developing innovative therapeutics called antibody-drug conjugates (ADCs) that target and destroy colorectal tumors and CSCs, similar to guided missiles. ADCs are comprised of a highly specific monoclonal antibody attached to a cytotoxic chemical "warhead" that is only released once the ADC binds and enters target tumor cells. We have successfully generated LGR5-targeted ADCs that incorporate the cytotoxin monomethyl-auristatin E (MMAE) and showed they could destroy colorectal cancer cells and tumors in mice. Currently, we are taking novel approaches to modify and improve our LGR5-targeting ADCs in order to effectively treat a larger number of tumors. Our lab is also identifying and characterizing new cancer targets for antibody and ADC development. One of these new targets is a cell receptor called GPR56, which is highly expressed in colorectal cancer and correlates with poor patient survival. We found that GPR56 can promote colorectal tumor growth and drug resistance and are currently investigating the GPR56-associated cell signaling mechanisms that drive its function. Our group is also acquiring colorectal tumor samples from patients and establishing 3D cultures called patient-derived organoids or PDOs. These PDOs can be used to study the function of our different cancer targets or to evaluate the efficacy of our ADCs before testing in animal models. Our work will lead to the elucidation of the function and mechanism of different receptors in colorectal cancer and generate innovative therapeutic leads to target CSCs for the treatment and eradication of colorectal cancer.

RESEARCH PROJECTS

- Identification of novel therapeutic targets and development of antibody-drug conjugates to target colorectal tumors and cancer stem cells
- Investigation of LGR5 function in cancer stem cells, metastasis, and drug resistance
- Elucidating the role and signaling pathways of GPR56 in colorectal cancer

KEY PUBLICATIONS

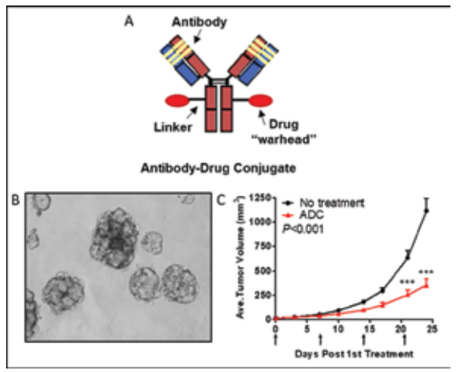
Zhang, S., Chatterjee, T., Godoy, C., Wu, L., Liu, Q.J., Carmon, K.S.* GPR56 drives colorectal tumor growth and promotes drug resistance through upregulation of MDR1 expression via a RhoA-mediated mechanism. *Mol Cancer Res.* 17(11):2196-2207, 2019.

Azhdarinia A., Voss J., Ghosh S.C., Simien J.A., Hernandez Vargas S., Cui J., Yu W.A., Liu Q., Carmon K.S.* Evaluation of Anti-LGR5 Antibodies by ImmunoPET for Imaging Colorectal Tumors and Development of Antibody-Drug Conjugates. *Mol. Pharm.* 15(6):2448-2454, 2018.

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

LAB MEMBERS

Post-doctoral fellow: Liezl Francisco Brown
Students: Joan Jacob, Tressie Posey, Treena Chatterjee
Research associate: Sheng Zhang, Zhengdong Liang



(A) Schematic of an Antibody-Drug Conjugate (ADC). (B) Patient-derived colorectal tumor organoids (PDOs) grown in 3D culture. (C) Preliminary data showing a targeted ADC treatment significantly inhibits patient-derived tumor growth in mice. Arrows indicate when treatment was administered.

Jeffrey Chang, PhD

Associate Professor
CPRIT Scholar in Cancer Research

Deciphering proteome alterations associated with diseases

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

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

1. Breast cancer metastasis. It is estimated that up to 90% of cancer deaths are due to metastasis, in part because metastatic cells do not respond to traditional therapies. To address this problem, we have used computational approaches to characterize the metastatic state and to reposition drugs to target cells that exhibit phenotypes that promote metastasis. Through these studies, we have found that metastasis is driven in part by cells that acquire a stem-like state through deregulation of cholesterol metabolism through altered expression of the ABCA1 cholesterol efflux channel. We are currently identifying therapeutic strategies to inhibit this pathway to reprogram breast cancer stem cells so that they become more amenable to therapies.

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

RESEARCH PROJECTS

- The role of cholesterol trafficking in cancer stem cell differentiation, the epithelial-to-mesenchymal transition, and cancer metastasis.
- Heterogeneity and progression of metastatic cancers.
- Intelligent computational pipelines for bioinformatic analysis.

KEY PUBLICATIONS

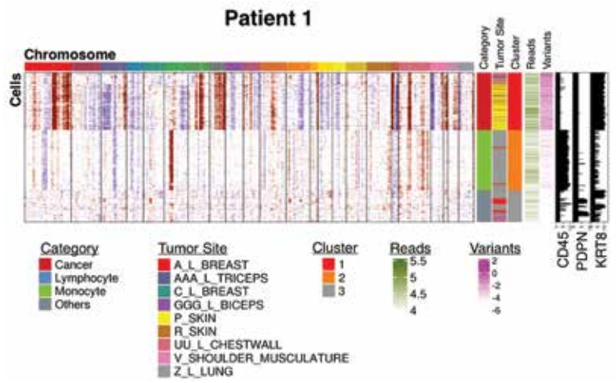
Liu X, Gosline SJC, Pflieger LT, Wallet P, Iyer A, Guinney J, Bild AH, and Chang JT: *Knowledge-based classification of fine-grained immune cell types in single-cell RNA-Seq data with ImmClassifier.* bioRxiv doi: 10.1101/2020.03.23.002758, 2020.

Zhao W., Prijic S., Urban B., Tisza M.J., Li L., Tan Z., Chen X., Mani S.A., and Chang J.T.: Candidate anti-metastasis drugs suppress the metastatic capacity of breast cancer cells by reducing membrane fluidity. *Cancer Research* 76(7):2037-49, 2016.

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

LAB MEMBERS

Instructor: Weina Zhao, PhD
Research scientist: Xuan Liu, PhD
Research assistant II: Jiayi Liu, MB



Profiles of cells from Patient 1. The large heatmap shows the predicted copy number profiles of cells (rows) from metastatic tumors from patient 1. Chromosomes are organized across the length of the heatmap. The colors indicate predicted copy number alterations. Colored bars to the right of the heatmap show various annotations for the cells. The Category is the cell type predicted to be cancer or other types, based on a range of criteria. The Tumor Site is the tumor that the cell came from. Cluster is based on an unbiased clustering of the copy number prediction. The Reads is the $\log_2$ of the total number of reads for the cell, and Variants is the $\log_2$ of variants per read of the cell. The black bars on the far right indicate the $\log_2$ of the counts per million of markers for immune cells (CD45), fibroblasts (PDPN), and luminal epithelial cells (KRT8).

Sheng Pan, PhD

Associate Professor / Director, the Clinical and Translational Proteomics Service Center
Rochelle and Max Levit Chair in the Neurosciences

Deciphering proteome alterations associated with diseases

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

RESEARCH PROJECTS

- Mechanistic and biomarker studies of pancreatic ductal adenocarcinoma (PDAC) and its precursors, including pancreatic intraepithelial neoplasia (PanIN) and pancreatic mucinous neoplasms (IPMN, MCN).
- Investigation of protein glycosylation, glycation and other PTMs in malignances, neurological diseases, diabetes, and chronic inflammation.
- Metaproteomic study of microbiome implicated in GI-tract malignancies and other diseases.
- Investigation of the proteome and glycoproteome alterations associated with neurodegenerative diseases.
- Innovation of proteomic technologies for basic, translational, and clinical applications.

KEY PUBLICATIONS

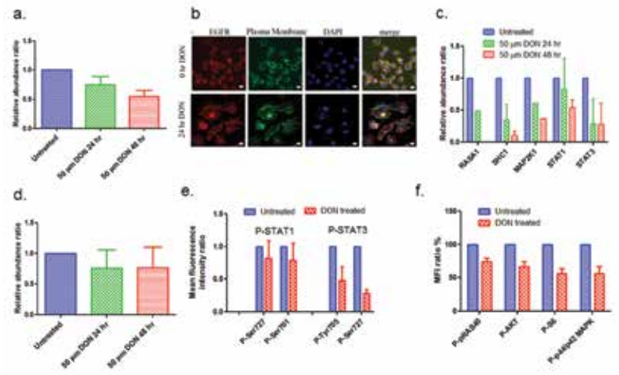
Peng H#, Pan S#, Yan Y, Brand RE, Petersen GM, Chari ST, Lai LA, Eng JK, Brentnall TA, Chen R. "Systemic proteome alterations linked to early stage pancreatic cancer in diabetic patients", *Cancers.* 2020, 12(6):1534. (# Co-first author).

Pan S, Hullar MAJ, Lai LA, Peng H, May DH, Noble WS, Raftery D, Navarro SL, Neuhouser ML, Lampe PD, Lampe JW, Chen R, "Gut microbial protein expression in response to dietary patterns in a controlled feeding study: A Metaproteomic Approach," *Microorganisms.* 2020, 8(3):379.

Pan S, Chen R, and Brentnall TA, "Proteome alterations in pancreatic ductal adenocarcinoma," *Cancer Lett.* 2020, 469:429-436.

LAB MEMBERS

Post-doctoral fellow: Lakmini Senavirathna, PhD
Research scientist: Cheng Ma, PhD
Research coordinator: Li Li
Research associate: Xin Li



Disruption of glycan synthesis pathway influenced the expression and localization of EGFR associated signaling proteins in chemoresistant pancretic cancer cells.

David Volk, PhD
Associate Professor

Targeting cancer with X-aptamers and nanoparticles

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

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

ESTA1 multistage particles directed anti-cancer siRNA to the bone marrow, reducing breast cancer metastasis and leading to increased survival rates.

Our Annexin A2 (Mangala et al., 2016) aptamer directed delivery of siRNA improves vascular maturation to enhance anti-tumor effects in ovarian cancer.

Our AXL aptamer (Kanlikilicer et al., 2017) can reduce cancer alone and enhances anti-tumor effects in combinatorial therapy.

Developed aptamers (Liu et al. 2018) targeting the endothelium of lymphoma in bone marrow.

X-aptamers can be used to develop biomarkers in schizophrenia (Walss-Bass et al, 2019)

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

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

RESEARCH PROJECTS

- Development of smart particles to attack breast and ovarian cancers.
- Developing new X-aptamers targeting other diseases.

KEY PUBLICATIONS

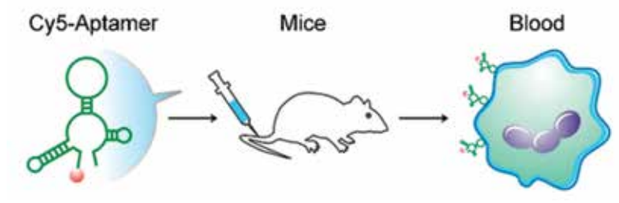
X-Aptamer Technology Identifies C4A and ApoB in Blood as Potential Markers for Schizophrenia. Walss-Bass, C, Lokesh, GLR, Dyukova, E, Gorenstein, DG, Roberts, DL, Velligan D, Volk, DE. *Molecular Neuropsychiatry*, (2019) 5:52–59.

Li, J, Mai, J, Hinkle, L, Lin, D, Zhang, J, Liu, X, Ramirez, MR, Zu, Y, Lokesh, G, Volk, DE, Shen, H. Tracking biodistribution of myeloid-derived cells in murine models of breast cancer. *Genes* (2019) 10:297.

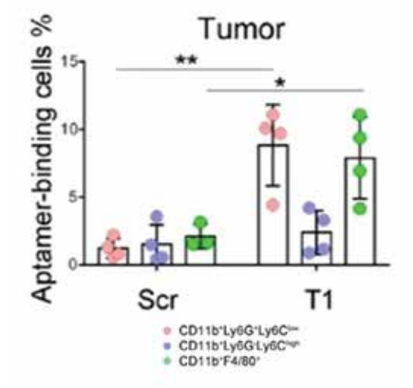
Functional Blockade of E-selectin in Tumor-associated Vessels Enhances Anti-Tumor Effect of Doxorubicin in Breast Cancer. Morita, Y., Leslie, M., Kameyama, H., Lokesh, G.L.R., Ichimura, N., Davis, R., Natalie Hills, N., Hasan, N., Zhang, R., Kondo, Y., Gorenstein, D.G., Volk, D.E., Cheveroneva, I., Hallgeir, R., and Tanaka, T. *Cancers,* (2020) 12:725.

LAB MEMBERS

Research associate: Xin Li, MS



Selecting aptamers targeting myeloid-derived cells in breast cancer.



Enhanced delivery to tumors relative to scrambled oligo.

Hongyu Wang, MD, PhD
Assistant Professor

Cancer biomarker discovery and targeted therapy

Biomarker discovery and targeted therapy are important parts of precision medicine. Aptamer mediated biomarker discovery and targeted therapy are attractive approaches for precision cancer treatment. Aptamers are single-stranded oligonucleotides with high affinity and specificity to the target molecules. DNA aptamers have many significant advantages over monoclonal antibodies in terms of feasibility, low cost, non-immunogenicity, and facile modification for various applications.

We created a systematic biology approach that combines a bead-based modified aptamer library with flow cytometry sorting and mass spectrometry to identify proteomics biomarkers. Patient's plasma was incubated with beads-X-aptamer library and sorted by flow cytometry based on fluorescence intensity (Figure 1). Using this approach, we selected a panel of prognostic biomarkers for hepatocellular carcinoma (HCC) patients under Lipiodol-based transarterial chemoembolization (TACE) treatment. In combination with quantitative imaging analysis, we will integrate blood biomarkers with quantitative imaging features to establish a non-invasive platform for precision treatment and outcome prediction for HCC patients.

Differentially expressed mRNA biomarkers have the advantage of providing dynamic insights into cellular states and regulatory processes than DNA biomarkers. The biological meaning of changes in mRNA expression is most likely mediated by corresponding changes in protein levels. We studied mRNA expression of HCC using data downloaded from TCGA to identify differentially expressed genes by the R/Bioconductor package. The associations between mRNA expression levels and overall survival of HCC patients were further analyzed using The Kaplan-Meier plotter database. A panel of best performing genes were selected as our targets. Validating protein expression of those selected mRNAs will lead to identification of the potential prognostic biomarkers for HCC.

As aptamers can serve as an important category of molecular targeting ligand, aptamer mediated targeted therapy and targeted imaging offer unique opportunity for selective delivery of therapeutic siRNA and drugs, or imaging agents. Several modified aptamers have been successfully identified in our lab for further targeted studies, such as Annexin A2, CD44, PD1, PD-L1, Vimentin, and Thy1. Those selected aptamers have great application potential in targeted drug delivery or targeted imaging. By conjugating the specific aptamer with nanoparticles that loaded with drug or siRNA, we demonstrated specific delivery and targeting to ovarian cancer after systemic administration in vivo.

RESEARCH PROJECTS

- Artificial intelligence imaging analysis with blood biomarkers for liver cancer screening and early detection
- Combined quantitative radiomic features and blood biomarkers for outcome prediction of trans-arterial chemoembolization treatment
- Proteomics biomarker discovery for hepatocellular carcinoma
- Targeted cancer therapy with aptamer mediated nanoparticle–drug delivery

KEY PUBLICATIONS

Wang H., Swaby K., Li X., Surabhi V., Bhatti Z., Patel M., Pillai A. Identification of mRNA-encoded prognostic biomarkers for hepatocellular carcinoma. *Journal of Vascular and Interventional Radiology,* Vol. 31, Issue 3, S88–S89. Published in issue: March 2020.

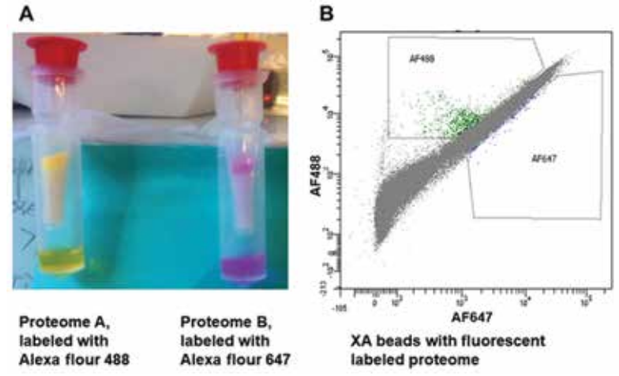
Wang H, Volk DE, Ganesh LR, Li L, Pillai AK, Gorenstein DG. Identification of Proteomic Biomarkers Utilizing a Bead-Based X-Aptamer Library and Flow Cytometry Sorting. *Molecular Therapy* 2019 27 (4), 360-360.

Wang H, Lam CH, Li X, West DL, Yang X. Selection of PD1/PD-L1 X-Aptamers. *Biochimie.* 2017. doi: 10.1016/j.biochi.2017.09.006. PubMed PMID: 28912094.

Lokesh GL, Wang H, Lam CH, Thiviyanathan V, Ward N, Gorenstein DG, Volk DE. X-Aptamer Selection and Validation. *Methods Mol Biol.* 2017; 1632:151-174. doi: 10.1007/978-1-4939-7138-1_10. PubMed PMID: 28730438.

LAB MEMBERS

Research associate: Xin Li



Protein biomarker selection using bead-based X-aptamer library. (A) Patient and health donor plasma were labeled with different color of fluoroform. (B) After incubation, proteins bound to bead-X-aptamer were sorted by flow cytometer.

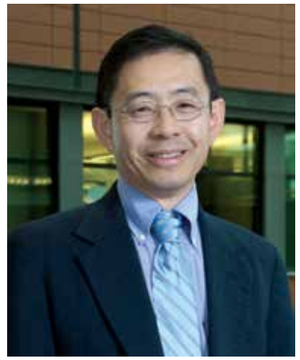
The Texas Therapeutics Institute at The Brown Foundation Institute of Molecular Medicine (TTI-IMM) was established in 2010 with funding from the Texas Emerging Technology Fund, The University of Texas System, and The University of Texas Health Science Center at Houston. TTI-IMM was created for the discovery,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

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

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

In addition to basic and translational research programs, TTI has built a major drug discovery platform for therapeutic monoclonal antibody lead discovery optimization and development. Over the last 10 years, TTI established a network of collaborators from institutions across Texas and the nation. TTI has more than 30 active drug discovery projects targeting cancer, metabolic diseases, neurodegenerative diseases, spinal cord injury, fibrosis, acute drug induced liver injury, and viral infections. Six TTI inventions have been licensed to biotech companies for drug development. Three antibody based therapeutics discovered by TTI scientists are currently in human clinical trials. In response to the COVID-19 pandemic, TTI scientists quickly discovered neutralizing antibodies targeting the SARS-CoV-2 virus. These antibodies are in development as potential therapies for the treatment of COVID-19. Licensing deals resulted in significant upfront payments, potential milestone payments, and royalties. The Texas Therapeutics Institute is recognized as the drug discovery engine of McGovern Medical School and UTHealth.

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



Zhiqiang An, PhD

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

Discovery and development of therapeutic antibodies

Our group focuses on the discovery and development of therapeutic antibodies against human diseases. Currently, we have three major research areas.

RESEARCH PROJECTS

- **Antibody response to viral infections and vaccination.** Identification of highly immunogenic vaccines that induce neutralizing antibodies against a broad range of clinical isolates is one approach to developing effective viral vaccines. We have an ongoing project to aid the design of HCMV and dengue vaccines by profiling antibody response to the experimental vaccines in rhesus and humans.
- **Cancer antibody drug resistance mechanisms.** Immune suppression is recognized as a hallmark of cancer. Our recent studies have demonstrated a new mechanism of cancer suppression of immunity. This mechanism involves impairment of antibody effector function mediated by proteolytic enzymes in the tumor microenvironment.
- **Cancer therapeutic monoclonal antibody drug discovery.** Our group has built a comprehensive antibody drug discovery platform with a focus on antibody lead optimization technologies such as antibody phage display, deep sequencing of antibody encoding genes from individual antibody expressing B cells, affinity maturation, and humanization. Currently, we have multiple collaborative antibody drug discovery projects targeting various cancer types.

KEY PUBLICATIONS

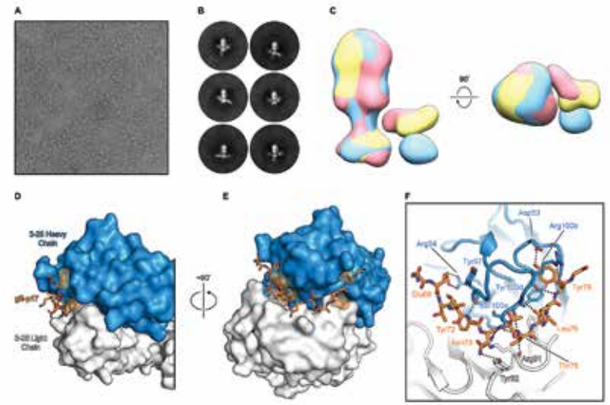
Xiaohua Ye, Hang Su, Daniel Wrapp, Daniel C. Freed, Fengsheng Li, Zihao Yuan, Aimin Tang, Leike Li, Zhiqiang Ku, Wei Xiong, Dabbu Jaijyan, Hua Zhu, Ningning Ma, Dai Wang, Jason S. McLellan, Ningyan Zhang, Tong-Ming Fu, Zhiqiang An. 2020. Recognition of a highly conserved HCMV glycoprotein B epitope by a potently neutralizing monoclonal antibody. *PLOS Pathogens* https://doi.org/10.1371/journal.ppat.1008736.

Dawei Bu, Clair Crewe, Christine M. Kusminski, Ruth Gordillo, Wei Xiong, Hui Deng, Xiao-Zheng Liu, Per Eystein Lønning, Nils Halberg, Adan Rios, Yujun Chang, Anneliese Gonzalez, Ningyan Zhang, Zhiqiang An, and Philipp E. Scherer. 2019. Human Endotrophin as a Driver of Malignant Tumor Growth. *Journal of Clinical Investigation Insight*

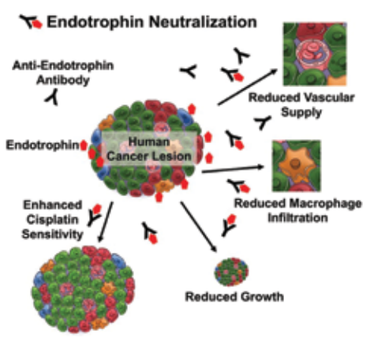
Samuel John, Heyu Chen, Mi Deng, Xun Gui, Guojin Wu, Weina Chen, Zunling Li, Ningyan Zhang, Zhiqiang An, and Cheng Cheng Zhang. A novel anti-LILRB4 CAR-T cell for the treatment of monocytic AML. *Mol Ther.* 2018 Aug 7. pii: S1525-0016(18)30372-1. doi: 10.1016/j.ymthe.2018.08.001.

LAB MEMBERS

Post-doctoral fellows: Zhiqiang Ku, Junquan (Jake) Liu, Lingxiao Tan, Xiaohua Ye, Zihao Yuan, Peng Zhao
Graduate students: Joshua W. Morse, Mason Ruiz
Research coordinator: Georgina T. Salazar
Research technician: Hannah Boyd



Structural basis for recognition of postfusion gB by 3-25. (A-C) EM analysis of the postfusion gB + 3-25 Fab complex. (D-F) The crystal structure of 3-25 Fab bound to gB-p17 peptide. PLOS Pathogens https://doi.org/10.1371/journal.ppat.1008736.



Endotrophin as a viable target for anti-tumor therapy for human breast cancer. Endotrophin (ETP) is abundantly expressed in adipose tissue and a chemoattractant for macrophages, exerts effects on endothelial cells and through epithelial-mesenchymal transition (EMT) enhances progression of tumor cells. Neutralizing monoclonal antibodies against ETP curb tumor growth and enhance chemosensitivity in a nude mouse model carrying human tumor cell lesions. *Journal of Clinical Investigation Insight*

Gerald F. Bills, PhD
Professor
Kay and Ben Fortson Distinguished Chair in Neurodegenerative Disease Research

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

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

Our lab employs genomics to interpret and predict genetically encoded chemical diversity of microorganisms using filamentous fungi as model organisms, especially for biosynthetic families relevant for pharmaceutical intervention in human diseases. For example, we have characterized biosynthetic pathways responsible for the family of echinocandin antifungal drugs, including pneumocandin B_0, the starting molecule for the antifungal drug CANCIDAS. We have re-programmed pneumocandin biosynthesis to produce new strains with improved product purity and new analogues with increased potency. In parallel, we use pathway genetics and genomic manipulation in the producing organisms to aid in supplying large quantities of these natural products to support synthesis of new derivatives and overproduce drug-precursor molecules.

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

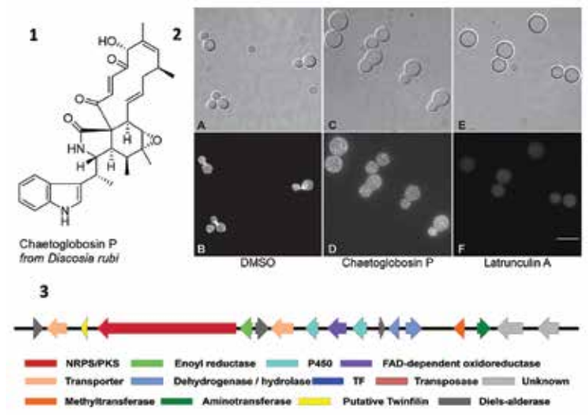
Perlatti, B., G. Harris, C.B. Nichols, D.I. Ekanayake, J.A. Alspaugh, J.B. Gloer & G.F. Bills. 2020. Campafungins: Inhibitors of *Candida albicans* and *Cryptococcus neoformans* hyphal growth. *Journal of Natural Products* 83:2718-2726.

Perlatti, B., C.B. Nichols, N. Lan, P. Weimann, C.J.B. Harvey, J.A. Alspaugh & G.F. Bills. 2020. Identification of the antifungal metabolite chaetoglobosin P from *Discosia rubi* using a *Cryptococcus neoformans* inhibition assay: Insights into mode of action and biosynthesis. *Frontiers in Microbiology* 11:1766.

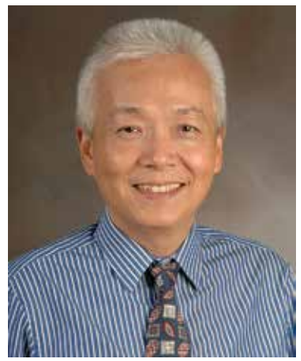
Lan, N., B. Perlatti, D.J. Kvitek, P. Wiemann, C.J.B. Harvey, J. Frisvad, Z. An & G.F. Bills. 2020. Acrophiarin (antibiotic S31794/F-1) from *Penicillium arenicola* shares biosynthetic features with both *Aspergillus*- and Leotiomycete-type echinocandins. *Environmental Microbiology* 22:2292-2311.

LAB MEMBERS

Post-doctoral fellows: Dr. Nan Lan, Dr. Bruno Perlatti, Dr. Zhuan Zhang



Chaetoglobosin P is a potent and selective inhibitor of growth of *Cryptococcus neoformans*. Fig. 1. tructure of chaetoglobosin P. Fig. 2. Visualization of actin polymerization and effects of inhibitors in cells of C. *neoformans* with TRITC-conjugated phalloidin which binds to F-actin structures. A-B. DMSO, negative control. C-D. Treatment with chaetoglobosin P. E-F. Latrunculin A, positive control. Fig. 3. Graphic representation of genes for encoding the biosynthesis of chaetoglobosin P.



Xiaodong Cheng, PhD
Professor
Walter and Mary Mischer Distinguished Professor in Molecular Medicine

cAMP - mediated cell signaling and drug discovery

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

RESEARCH PROJECTS

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

KEY PUBLICATIONS

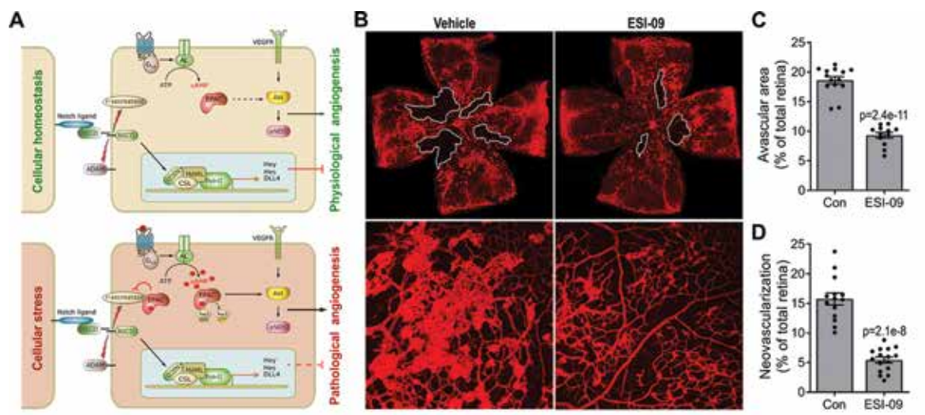
Liu, H., Mei, F. C., Yang, W., Wang, H., Wong, E., Toth, E., Luo, P., Li, Y.-M., Zhang, W. and Cheng, X. Epac1 inhibition ameliorates vasoproliferative retinopathy through coordinated activation of Notch and suppression of VEGF signaling. *Science Advances.* 6: eaay3566, 2020.

Liu, W., Ha, Y., Xia, F., Zhu, S., Shi, S., Mei, F. C., Merkley, K., Vizzeri, G., Motamedi, M., Cheng, X., Liu, H. and Zhang, W. Neuronal Epac1 mediates retinal neurodegeneration in mouse models of ocular hypertension. *J. Exp. Med.* 217: pii: e20190930, 2020.

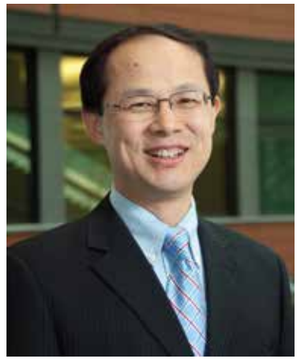
Robichaux, W. G., Mei, F. C., Wang, H., Yang, W., Sun, H., Zhou, Z., Milewicz, D. M., Teng, B. B. and Cheng, X. EPAC1 promotes foam cell formation and atherosclerosis development by upregulating the oxidized LDL scavenger receptor LOX-1 through a PKC dependent pathway in macrophages. *Arteriosclerosis, Thrombosis, and Vascular Biology.* In press.

LAB MEMBERS

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



Epac1 in pathological angiogenesis and as a therapeutic target for retinopathy. (A) Epac1 promotes pathological angiogenesis through sensitization of VEGF signaling and suppression of Notch activation via γ-secretase inhibition. (B) Pharmacological inhibition of Epac prevents neovascularization associated with oxygen-induced retinopathy (OIR). Representative retinal vasculature (upper panel) and high magnification images (lower panel) at P17 in OIR mice treated with Epac inhibitor ESI-09 or vehicle (Con). White lines outline the area of vaso-obliteration. (C, D) Graphs represent avascular and neovascularization area at P17.



Wenliang Li, PhD
Associate Professor

Studying and targeting cancer metastasis and drug resistance

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

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

Another research topic in our lab is to investigate the mechanisms of cancer cell plasticity and drug resistance. In particular, we study how prostate cancers become resistant to a new generation of androgen receptor pathway inhibitors (ARPIs), and how non-small cell lung cancers (NSCLC) become resistant to EGFR inhibitors. The common theme in this topics is to better understand and to target a process called neuroendocrine differentiation (NED), which is increasingly accepted as a critical process in cellular plasticity and drug resistance of these two major solid cancer types. Upon the acquisition of resistance to ARPIs, some AR-positive prostate adenocarcinoma cancers become AR-low/negative aggressive neuroendocrine prostate cancers. Similarly, after becoming resistant to EGFR inhibitors, some NSCLC demonstrate phenotypes of small cell lung cancer, which is neuroendocrine in nature and very aggressive. NED is still poorly understood and currently there is no effective treatments to prevent or overcome drug resistance related to NED. We investigate the underlying mechanisms of NED, cellular plasticity and drug resistance, especially the roles and mechanisms of action of several novel epigenetic regulators.

RESEARCH PROJECTS

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

KEY PUBLICATIONS

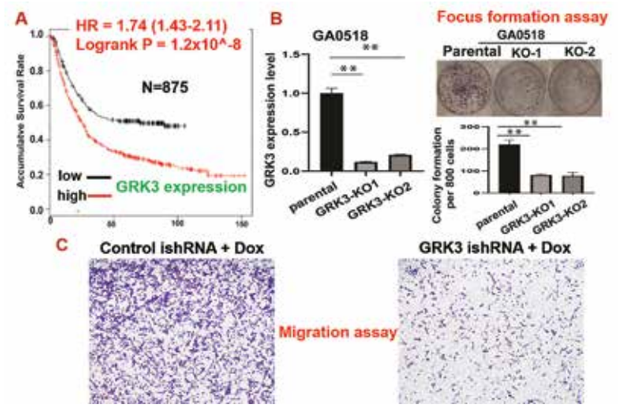
Wang Z, Zhao Y, An Z, Li W*. Molecular links between neuroendocrine differentiation and angiogenesis in prostate cancer progression. *Frontiers in Oncology*, 2020 Jan 21;9:1491. *corresponding author

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

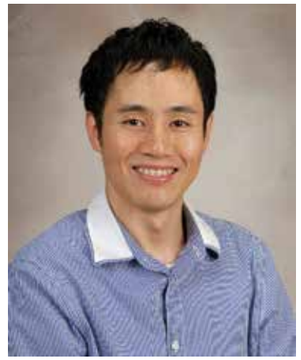
Li, L., Su, N., Zhou, T., Zheng, D., Wang, Z., Chen, H., Yuan, S., Li, W*. Mixed lineage kinase ZAK promotes epithelial-mesenchymal transition in cancer. *Cell Death & Disease* 2018 Feb 2; 9(2):143. *corresponding author

LAB MEMBERS

Post-doctoral fellow: Zheng Wang
Students: Guoliang Zhang, Samira Naderinezhad
Research assistant II: Han Yang



GRK3 is a poor prognosticator in gastric cancer patients and its silencing substantially inhibits the aggressiveness of a metastatic gastric cancer cell model. (A) Higher expression of GRK3 is significantly associated with shorter survival. (B-C) Knockout of GRK3 inhibits focus formation and migration of gastric cancer cells (manuscript under review).



Kyoji Tsuchikama, PhD
Assistant Professor

Linker and conjugation technologies for generating novel antibody-drug conjugates (ADCs) toward innovative cancer therapeutics

Antibody-Drug Conjugates (ADCs) represent a rapidly growing class of anticancer therapeutics. As demonstrated with 9 FDA-approved ADCs and more than 100 promising ADCs in clinical trials, successful clinical outcomes using ADCs have inspired scientists and clinicians to further advance this new molecular format for effective treatment of cancers. ADCs deliver anticancer drugs (payloads) selectively to blood cancer cells and solid tumors while avoiding healthy tissues, enabling the use of highly active payloads that are too toxic to be used alone. The ADC chemical linker connecting the antibody and the payload molecule needs to selectively deliver and release payloads only at the tumor sites. Thus, the use of properly designed ADC linkers is a key for successful implementation of ADC-based chemotherapy.

My research group is focused on the development of novel chemical ADC linkers by taking advantage of the power of organic chemistry, medicinal chemistry, and chemical biology. We have developed a glutamic acid-valine-citrulline tripeptide linker as a new-generation ADC linker. Unlike the conventional valine-citrulline linker that is unstable in mouse circulation, our tripeptide linker is stable in both human and mouse plasma, maximizing ADC stability and therapeutic efficacy. This is also advantageous to assure high translatability from bench to clinic. Using this technology, we recently developed highly homogeneous ADCs targeting the leukocyte immunoglobulin-like receptor subfamily B4 (LILRB4) for the treatment of acute myeloid leukemia (AML) (Figure 1A). LILRB4 is expressed at significantly higher levels on monocytic AML cells than on normal counterparts. As such, LRLRB4 represents a promising target in ADC-based AML therapy. Our anti-LILRB4 ADCs showed the capacity for LILRB4-mediated internalization, suitable physicochemical properties, and high cell killing potency against LILRB4-positive AML cells. Importantly, our data indicate that these ADCs spare normal progenitor cells. One of our homogeneous conjugates exerted a remarkable therapeutic effect and no significant toxicity in a xenograft mouse model of disseminated human AML (Figure 1B). Our findings highlight the clinical potential of anti- LILRB4 ADCs in monocytic AML therapy.

With our technology platform in hand, we are currently pursuing next-generation ADCs for treating refractory cancers, including glioblastoma multiforme (GBM), pancreatic cancers, and other solid tumors with drug resistance and/or intratumor heterogeneity. Patients with these cancers often suffer from recurrence of malignancy and exacerbated quality of life because of ineffective chemotherapy. Our lab's long-term goal is to create novel therapeutic options for overcoming such clinical issues. We envision that our novel ADC linker technology platform will help us, other researchers and clinicians in the field of oncology achieve this goal.

RESEARCH PROJECTS

- Design, synthesis, and evaluation of novel chemical linkers for constructing multi-loading ADCs
- Structural optimization of ADC linkers for high plasma stability, rapid drug release, and enhanced permeability to the brain
- Modulation of the ADC function by chemical modification for organ-specific delivery
- Evaluation of ADCs in refractory cancer models

KEY PUBLICATIONS

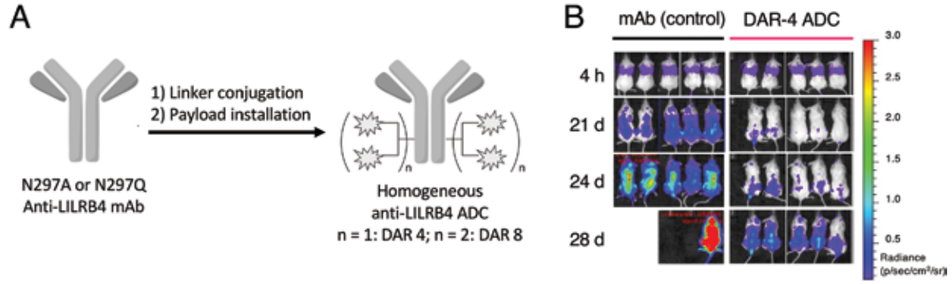
Anami, Y., Yamazaki, C. M., Xiong, W., Gui, X., Zhang, N., An, Z., Tsuchikama, K.* (2018) Glutamic acid–valine–citrulline linkers ensure stability and efficacy of antibody–drug conjugates in mice, *Nature Communications,* 9:2512.

Anami, Y., Tsuchikama, K.* (2019) Transglutaminase-Mediated Conjugations, *Methods in Molecular Biology,* 2078:71–82.

Anami, Y., Deng, M., Gui, X., Yamaguchi, A., Yamazaki, C. M., Zhang, N., Zhang, C. C.*, An, Z.*, and Tsuchikama, K.* (2020) LILRB4-Targeting Antibody–Drug Conjugates for the Treatment of Acute Myeloid Leukemia, *Molecular Cancer Therapeutics,* in press. DOI: 10.1158/1535-7163.MCT-20-0407.

LAB MEMBERS

Post-doctoral fellows: Yasuaki Anami, PhD, Chisato Tsuchikama, PhD, Aiko Yamaguchi, PhD
Yin Yuen Ha (Summer), PhD



Novel linker technologies for generating efficacious ADCs for the treatment of acute myeloid leukemia (AML). (A) Construction of anti-LILRB4 ADCs with high homogeneity using our linker technologies. (B) Our ADC provides remarkable therapeutic effect in a mouse model of disseminated THP-1 (human AML cell line) model (n = 5). All mice were treated with unmodified mAb (control) or ADC at 3 mg/kg at Day 7, 14, and 21.

Ningyan Zhang, PhD
Associate Professor

Cancer resistance mechanisms to therapeutic antibodies and modulation of anticancer immunity

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

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

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

RESEARCH PROJECTS

- Understand mechanisms of cancer immune suppression.
- Develop platform technologies for discovery of therapeutic antibodies.

KEY PUBLICATIONS

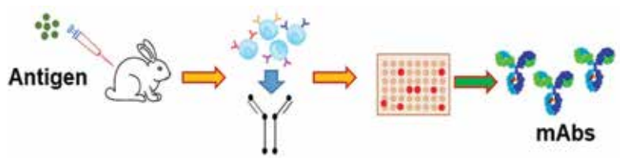
Heyu Chen, Yuanzhi Chen, Mi Deng, Samuel John, Xun Gui, Ankit Kansagra, Weina Chen, Jaehyup Kim, Cheryl Lewis, Guojin Wu, Jingjing Xie, Lingbo Zhang, Ryan Huang, Xiaoye Liu, Hisashi Arase, Yang Huang, Hai Yu, Wen-xin Luo, Ningshao Xia, Ningyan Zhang*, Zhiqiang An, and Chengcheng Zhang (2020). An antagonistic anti-LILRB1 monoclonal antibody regulates anti-tumor functions of natural killer cells. *Journal for Immunotherapy of Cancer*.

Jingnan An, Yi Du, Xuejun Fan, Y Wang, C Ivan, X Zhang, Anil K. Sood, Zhiqiang An, Ningyan Zhang* (2019) EGFL6 Promotes Breast Cancer by Simultaneously Enhancing Cancer Cell Metastasis and Stimulating Tumor Angiogenesis. *Oncogene*.

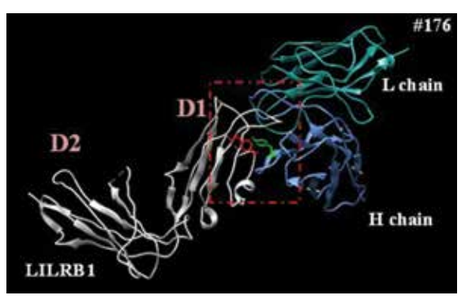
Min Deng, Xun Gui, J. Kim, X. Li, W. Chen, Z. Li, Y. Chen, H. Chen, W. Luo, Z. Lu, J. Xie, H. Churchill, Y. Xu, Z. Zhou, G. Wu, C. Yu, S. John, K. Hirayasu, N. Nguyen, H. Deng, H. Tang, Y. Zou, B. Chen, H. Arase, N. Xia, Y. Jiang, R. Collins, Y. Fu, Z. An, J. Zheng, Ningyan Zhang*, and Chengcheng Zhang (2018) Inhibitory receptor signaling in leukemia cells mediates tumor infiltration and T cell suppression. *Nature*.

LAB MEMBERS

Research associate/scientists: Hui Deng, MS; Xuejun Fan, MD, PhD; Wei Xiong, PhD; Leike Simon Li, PhD; Peng Gao, PhD



Schematic diagram for generation and screening of monoclonal antibodies (mAbs) using our established technology platform.



Structural identification of binding epitopes of a functional anti-LILRB1 monoclonal antibody for development of cancer therapeutics. From H. Chen et al. (2020) *Journal for Immunotherapy of Cancer.*

IMM Service Centers

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

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

Antibody Engineering and Expression Service Center

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

Clinical and Translational Proteomics Service Center

Proteins are the essential functional biomolecules that participate in a vast array of physiological cellular activities and are implicated in all aspects of disease mechanisms. Disease associated proteome alterations may reflect on changes in protein expression, structure, localization, polymorphism, as well as post-translational modifications (PTMs) status. Proteomics can deliver dynamic information of a protein profile in a complex system and thereby provide a vibrant picture of cellular function under biological conditions. Furthermore, quantitative proteomics can identify steady or perturbation-induced proteome alterations associated with a disease status or biological state and is highly relevant to translational and clinical applications.

Our center provides state-of-the-art proteomics services to support basic, translational and clinical research. The main services include protein profiling, label-free or label-based quantitative analysis, therapeutic protein characterization and essential PTM analysis. We have the capability to analyze a broad range of research or clinical specimens, from purified proteins to complex mixtures, including cell and tissue extracts, plasma/serum, and other biofluids or biological samples.

We also provide more advanced support through collaborative efforts, such as biomarker discovery and verification, glycoproteomics/glycomics analysis and microbiome profiling.

The center contains state-of-the-art instrumentation and well-trained personnel to provide an integrated proteomics service, including sample preparation, mass spectrometric analysis, and bioinformatics data processing.

Flow Cytometry Service Center

Flow cytometry is a single-cell analysis technology used for cell counting and fluorescent marker detection. It allows high-speed identification, and even isolation, of specific subsets within mixtures of cells. The fluorescence can be measured to determine cellular properties like relative size, complexity, cell type, and response to specific stimuli, such as drugs and genetic manipulations.

These specialized multicolor cell analysis instruments allow researchers to evaluate a large number of samples in a short time frame and gather information on very rare populations of cells and additionally isolate cell populations to be sorted. The current instrumentation allows simultaneous acquisition of more than 10 fluorescent signals from thousands of individual cells per second.

The Flow Cytometry Service Center offers FACS acquisition and analysis, cell sorting, user training, and consultation for experimental design, interpretation, and troubleshooting.

Our instruments are available on a fee-for-service charge to all research investigators from UTHealth and external organizations.

Transgenic and Stem Cell Service Center

Our Immunology and Autoimmune Diseases Center operates a Transgenic and Stem Cells service center, which was established in 1998. It has generated over 800 new transgenic and knock-out mouse animal models for all research investigators from UTHealth and external organizations on a fee-for-service basis.

The stem cell lines that have been derived in the laboratory are highly effective for the generation of knock-out/ knock-in mice and for cell differentiation studies. In addition to the production, cryopreservation, and re-derivation of genetically-engineered mice and rats, the services of the facility also include gene targeting, CRISPR/Cas9 genome editing, derivation of new cell lines, and intellectual/technical support in different aspects of microsurgery, cell culture, and stem cell research.

Nano 3D Printing Service Center

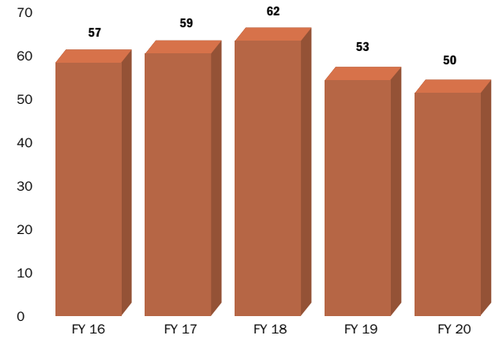
Nano 3D Printing Service Center provides state-of-the-art 3D printing services. We provide 3D printed models of human and laboratory animal organs, novel surgical tools, and custom-made laboratory supplies, in prototype or final production models.

We have both traditional FDM (Fortus 450mc) thermoplastic as well as multi-color, resin-based, high-resolution Stratasys J750 (14 micron) 3D printers with large print beds.

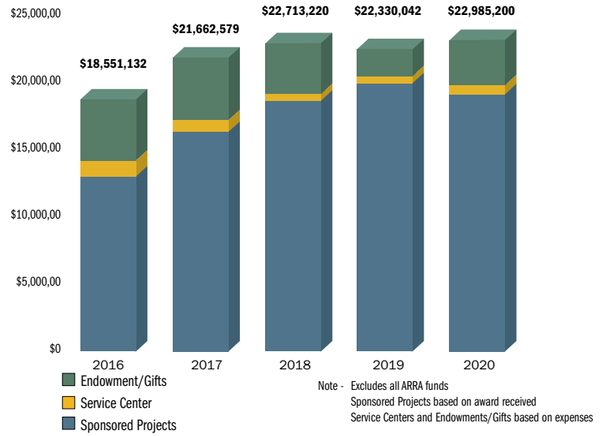
A wide range of materials with varying Shore A values (hardness) is available. STL files, SolidWorks, or medical imaging files can be used to produce the 3D models.

We are located on the 3rd floor of the Fayez S. Sarofim Research Building.

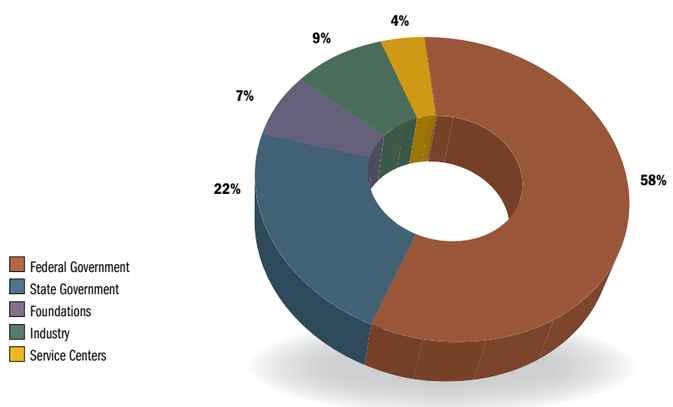
Number of Faculty



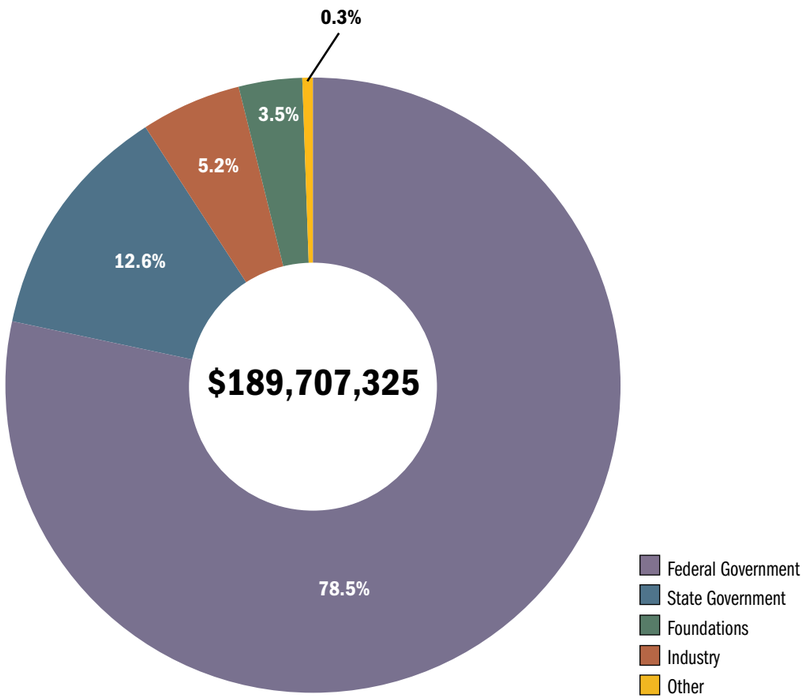
Total Funds Supporting Research



Total Expenses Supporting Research



IMM Extramural Funding Inception to Date



IMM Commercial Outcomes Inception to Date

U.S. Patents Issued
54

License & Option Agreements Executed
71

Startup Companies Formed
18

Income Generated from Intellectual Property
$17,869,290

Institute of Molecular Medicine Endowments

Becker Family Foundation Professorship in Diabetes Research
Harry E. Bovay Lecture Series in Molecular Medicine
The Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research
Cullen Chair in Molecular Medicine
John S. Dunn Research Scholars
The Laurence and Johanna Favrot Distinguished Professorship in Cardiology
Linda and Ronny Finger Foundation Distinguished Chair in Neuroimmunologic Disorders
Kay and Ben Fortson Distinguished Chair in Neurodegenerative Disease Research
Janice Davis Gordon Chair for Bowel Cancer Research
Annie and Bob Graham Distinguished Chair in Stem Cell Biology
George and Mary Josephine Hamman Foundation Distinguished Professorship in Cardiovascular Research
Mary Elizabeth Holdsworth Distinguished University Chair in Metabolic and Inflammatory Disease Research
IMM General Endowment
Jerold B. Katz Distinguished Professorship in Stem Cell Research
The Carolyn Frost Keenan Professorship in Cardiovascular Disease Research
William S. Kilroy, Sr. Distinguished University Chair in Pulmonary Disease
Nancy and Rich Kinder Distinguished Chair in Cardiovascular Disease Research
Kozmetsky Family Chair in Human Genetics
Rochelle and Max Levit Chair in the Neurosciences
George and Cynthia Mitchell Distinguished Chair in Neurosciences
Muller-Eberhard Memorial Lecture Series
Hans J. Muller-Eberhard, MD, PhD and Irma Gigli, MD Distinguished Chair in Immunology
D. Dudley and Judy White Oldham Research Fund
Marjorie B. Poyner and Herbert F. Poyner, Jr. Endowment for Medical Research in the Institute of Molecular Medicine for Prevention of Human Diseases
Dr. Edward Randall, Jr. Memorial Fund
Shavonnah Roberts Schreiber Women's Health Endowment
The Jerry and Maury Rubenstein Foundation Distinguished Professorship in Heart Disease Research
Pierce Runnells Memorial Research Fund
Rodney J. Sands New Initiatives Stem Cell Research Endowment Fund
C. Harold and Lorine G. Wallace Distinguished University Chair
Walter and Mary Mischer Distinguished Professorship in Molecular Medicine
Robert A. Welch Distinguished University Chair in Chemistry
The Welch Foundation Endowment in Chemistry and Related Sciences
James T. Willerson Distinguished Chair in Cardiovascular Research in Tribute from the Ewing Halsell Foundation
Nina and Michael Zilkha Distinguished Chair in Neurodegenerative Disease Research

Thank you to our donors,
who through the establishment of these endowments, enable the IMM to recruit and retain top scientists from around the world.