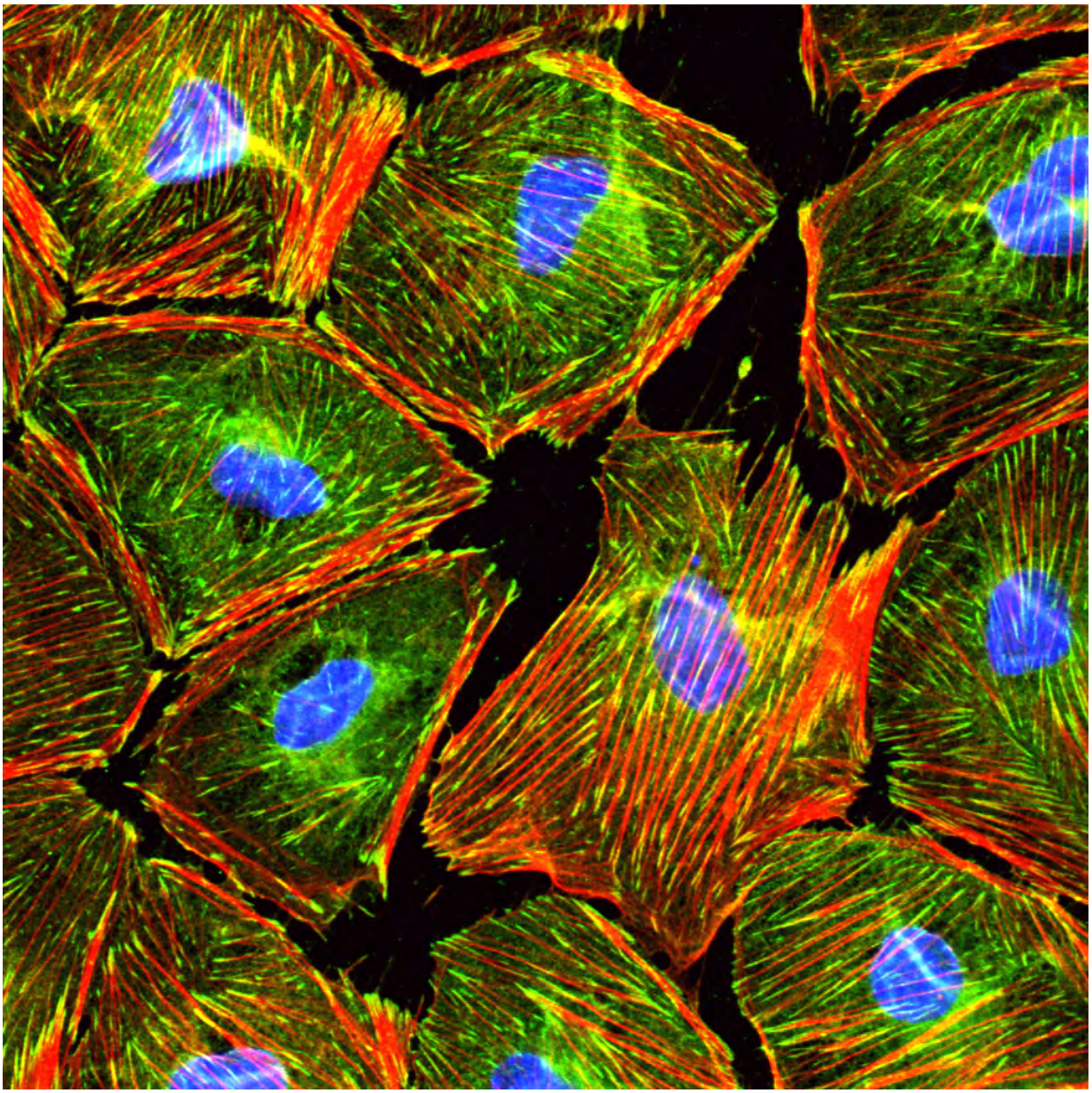
Aab Cardiovascular Research Institute
University of Rochester Medical Center



Annual Report
July 2017

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A. Overview

From the Director

This has been an exciting year for the Aab Cardiovascular Research Institute. We are building new collaborative programs that connect researchers to our patients. We've developed three interdisciplinary teams organized around clinically relevant areas. Our teams now plan to expand their programs, producing program project grants and co-authored publications.

(1) The **CVRI Program in Cardiac Fibrosis** is led by Dr. Chen Yan. Human hearts respond to injury such as myocardial infarction by inflammation and fibrosis – and fibrosis can lead to heart failure and sudden cardiac death. We study how cardiovascular cells activate cardiac fibroblasts, triggering abnormal proliferation and excess production of matrix molecules. Members of our Cardiac Fibrosis Team include:

- Dr. Eric Small, studying genes that control fibroblast activation,
- Dr. Chen Yan, exploring how injured cardiac myocytes communicate with fibroblasts,
- Dr. Craig Morrell, investigating how platelets drive fibroblast proliferation,
- Dr. Doug Anderson, who studies micropeptides that regulate cardiac contraction in heart failure,
- Dr. Jeffrey Alexis, a heart failure cardiologist who studies patients with end stage cardiomyopathy.

(2) The **CVRI Program in Thrombosis is** led by Dr. Craig Morrell. Thrombosis is the final stage in life-threatening cardiovascular diseases such as myocardial infarction and pulmonary embolism. We study how platelets interact with the vessel wall to drive thrombosis. Members of our Thrombosis Team include:

- Dr. Craig Morrell, who studies how platelets accelerate inflammation,
- Dr. Peng Yao, who studies platelet expression of inflammatory proteins,
- Dr. Charlie Lowenstein, who studies the genetics of patients with thrombosis,
- Dr. Scott Cameron, a vascular cardiologist who treats patients with thrombosis and studies how myocardial infarction activates platelets in humans.

(3) The **CVRI Program in Angiogenesis** is led by Dr. Zhang Gen Jin. Arterial disease of the heart leads to ischemia and tissue injury – and the host responds by developing new blood vessels. We study new regulators of angiogenesis. Members of our Angiogenesis Team include:

- Dr. Gen Jin, who explores transcription factors regulating new blood vessels,
- Dr. Brad Berk, who studies the mechanisms that control vascular remodeling,
- Dr. Joseph Miano, who studies how smooth muscle cells respond to vascular injury,
- Dr. Jinjiang Peng, who investigates endothelial cell signaling during angiogenesis,
- Dr. Peng Yao, who studies macrophage expression of signals that control angiogenesis.

Our new programs in Cardiac Fibrosis, Thrombosis and Angiogenesis are leading to new collaborations, new training opportunities and new discoveries which will ultimately benefit our patients at the University of Rochester Medical Center and throughout the nation. Thank you for taking the time to learn about the work we have conducted this past year.

Charles Lowenstein, MD
Director, Aab Cardiovascular Research Institute

CVRI Facts Fiscal Year 2017

Personnel

Faculty	12
Research Faculty	3
Postdoctoral Fellows	11
Graduate Students	11
Technical & Administrative Staff	20

Finances

NIH Grant Funding	$3.6 M
Other Funding	$2.8 M
Total Operating Revenue	$6.4 M
Salary & Benefits	$4.3 M
Supplies & Equipment	$2.1 M
Total Expenses	$6.4 M

Scientific Publications

Publications Academic Year 2017:	34

Seminar Series Speakers

Richard Aab Cardiovascular Seminar Series: 12

B. Faculty Appointments

Faculty

Douglas Anderson, Ph.D.
Assistant Professor, Department of Medicine, Aab CVRI

David Auerbach, Ph.D.
Research Assistant Professor, Department of Medicine, Aab CVRI

***Bradford C. Berk, M.D., Ph.D.**
Distinguished University Professor in Medicine/Cardiology,
Neurology, Pathology, and Pharmacology & Physiology
Director, University of Rochester Neurorestoration Institute

Scott James Cameron, Ph.D., M.D.
Assistant Professor, Department of Medicine, Aab CVRI

***Zheng-Gen Jin, Ph.D.**
Associate Professor, Department of Medicine, Aab CVRI

Vyacheslav (Slava) Korshunov, Ph.D.
Associate Professor, Department of Medicine, Aab CVRI and Department of Biomedical Genetics

Coeli Lopes, Ph.D.
Research Associate Professor, Department of Medicine, Aab CVRI

***Charles J. Lowenstein, M.D.**
Paul N. Yu Professor in Cardiology, Department of Medicine
Chief, Division of Cardiology and Director, Aab Cardiovascular Research Institute

***Joseph M. Miano, Ph.D.**
Professor, Department of Medicine, Aab CVRI and Pathology and Laboratory Medicine
Associate Director, Aab CVRI

Craig N. Morrell, D.V.M., Ph.D.
Associate Professor, Department of Medicine, Aab CVRI

Jinjiang Pang, B. Med., Ph.D.
Assistant Professor, Department of Medicine, Aab CVRI

Eric M. Small, Ph.D.
Assistant Professor, Department of Medicine, Aab CVRI and Department of Pharmacology and Physiology

***Chen Yan, Ph.D.**
Professor, Department of Medicine, Aab CVRI

Peng Yao, Ph.D.
Assistant Professor, Department of Medicine, Aab CVRI

**With tenure*

Research Faculty

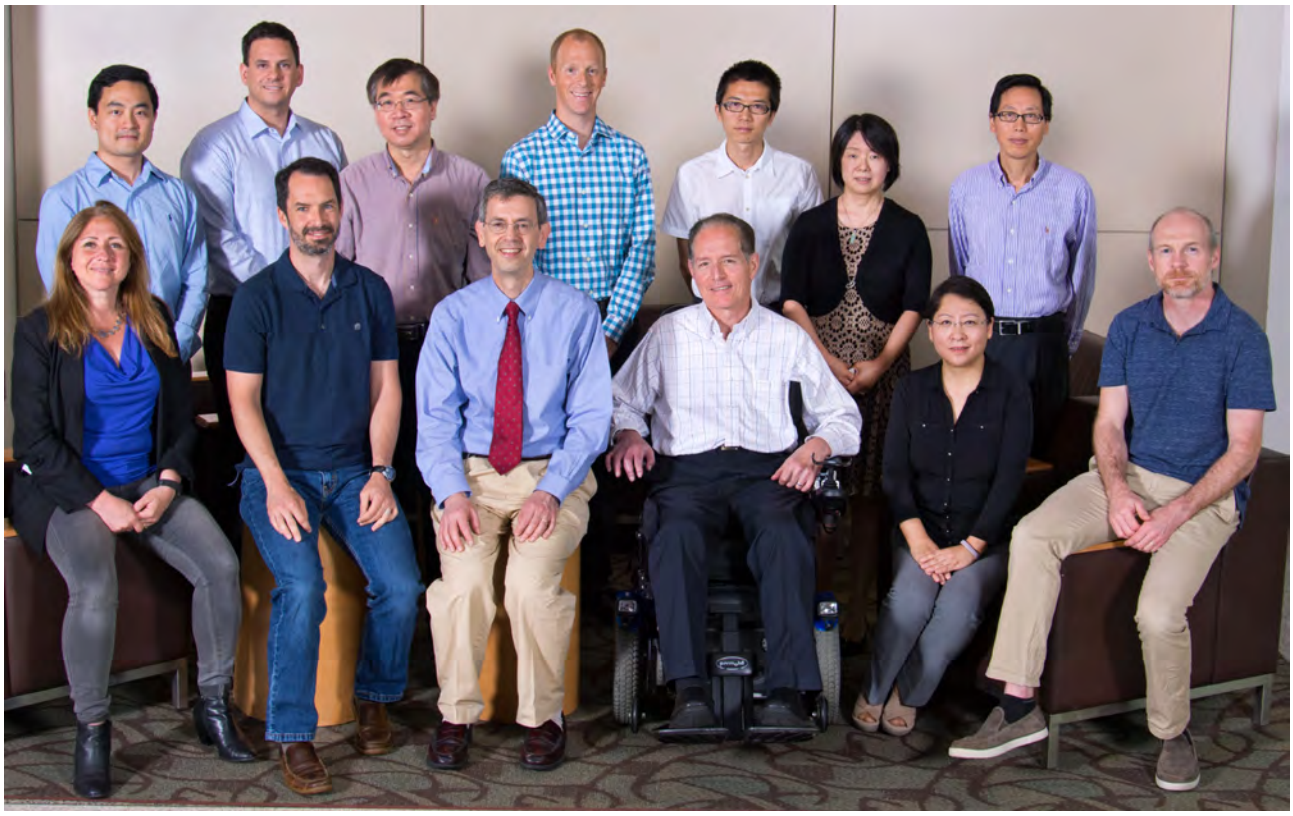
Mark Sowden, Ph.D.
Research Associate Professor, Department of Medicine, Aab CVRI

Jian Fu, B. Med., Ph.D.
Research Assistant Professor, Department of Medicine, Aab CVRI

Xu, Suowen, PhD
Research Assistant Professor, Department of Medicine, Aab CVRI

Adjunct Faculty

Guoyong Yin, B. Med., Ph.D.
Professor, First Affiliated Hospital of Nanjing Medical University



The faculty of the CVRI. Standing in back: Peng Yao, Doug Anderson, Jian Fu, David Auerbach, Suowen Xu, Chen Yan, and Zheng-Gen Jin. Sitting in front: Coeli Lopes, Eric Small, Charles Lowenstein, Brad Berk, Jinjiang Pang, and Craig Morrell.

C. Our Research Labs

Douglas M. Anderson, PhD

Synopsis

My lab is focused on deciphering the regulatory pathways that control striated muscle function and how errors in those pathways can give rise to human disease. Striated muscle diseases are among the leading causes of morbidity worldwide and arise from multiple genetic factors, many of which are yet to be discovered.

Recent deep sequencing of vertebrate genomes has revealed a vast number of novel RNA transcripts, many of which have been annotated as long noncoding RNAs (lncRNAs). Acting through diverse mechanisms, we have found that some cardiac-enriched lncRNAs function as essential components of the gene regulatory networks required for cardiovascular development and survival. Unexpectedly, we've also found that many annotated lncRNAs, in fact, encode small functional proteins, called micropeptides. By investigating the function of lncRNAs and micropeptides, my lab aims to uncover novel mechanisms and insights into the regulatory pathways that control muscle biology.

Current projects in my lab focus on the role of muscle-enriched lncRNAs and micropeptides as novel regulators of cardiovascular development, function and disease. To understand the molecular mechanisms and pathways by which these factors act, we utilize and generate a variety of biochemical, cell-based and loss- and gain-of-function mouse strains.

Project 1: Micropeptide control of calcium signaling in the cardiovascular system.

During my postdoctoral work in the laboratory of Dr. Eric Olson, we discovered a 46 amino acid micropeptide, named Myoregulin (MLN), within a skeletal muscle-specific RNA believed to be non-coding. (Figure 1). MLN shares structural and functional similarity with Phospholamban (PLN) and Sarcolipin (SLN), two cardiac micropeptides that inhibit SERCA, the membrane pump that controls muscle relaxation by regulating calcium uptake into the sarcoplasmic reticulum (SR). MLN similarly interacts with SERCA and impedes calcium uptake into the SR (Figure 1B and C). Since PLN and SLN are expressed predominantly in the adult heart, MLN functions as the dominant regulator of SERCA in adult skeletal muscles. Consistent with this finding, genetic deletion of MLN in mice resulted in enhanced calcium handling and improved exercise performance.

In addition to the essential role that SERCA plays in regulating striated muscle contractility, SERCA plays an important role in regulating calcium signaling across diverse cell types, which do not express MLN, PLN or SLN. We have recently identified two additional transmembrane micropeptides, that we named Endoregulin (ELN) and Another-regulin (ALN), that overlap with the expression of SERCA isoforms in non-muscle cell types. ELN overlaps with SERCA3 in endothelial and epithelial cells of vascular and visceral organs and ALN overlaps with the broadly expressed isoform SERCA2b. These findings reveal a general mechanism for the control of calcium handling across diverse cell types by a family of structurally and functionally related micropeptides. Considering that intracellular calcium dynamics are essential for many cellular processes (muscle relaxation, cardiac hypertrophy, smooth muscle relaxation, platelet activation, etc.), projects in my lab will focus on the role these micropeptides play in the function of the cardiovascular system.

Project 2: Role of lncRNAs in cardiovascular development, function and disease.

Many of the RNA transcripts identified by deep sequencing are *bon fide* lncRNAs and do not appear to generate stable proteins. While challenging to study, recent advances in RNA probing techniques have allowed us to elucidate their function. Interestingly, many lncRNA transcripts are found near essential cardiac-specific transcription factors, where together they are required for normal development and survival. Using novel knockout approaches that prematurely stop transcription of these

transcripts, we are able to assess their role *in vivo*. Current projects include the study of lncRNAs that are enriched in the heart (Figure 2).

Project 3: Enhancing CRISPR/Cas9 DNA editing.

The CRISPR/Cas9 revolution has made genome editing increasingly simplified, however significant challenges remain towards optimizing editing efficiencies as both a basic research or therapeutic tool. My lab utilizes a number of targeted nuclease technologies to generate loss-of-function models for lncRNA and micropeptides in the mouse. Toward the goal of improving and quickly measuring the effectiveness of these technologies, we have developed a programmable reporter system to quickly determine gene editing efficiency by targeted nucleases, called Prospector (Figure 3). Using this system, we have additionally screened a small FDA approved small molecule library and identified a group of related DNA intercalating molecules that enhance repair through non-homologous end joining (NHEJ). Future projects will focus on the discovery of other small molecules that enhance genome editing through NHEJ or through homology directed repair (HDR).

Lab Members

- Kelly Anderson, Postdoctoral Associate

Publications

1. Anderson DM, Arredondo J, Hahn K, Valente G, Martin JF, Wilson-Rawls J and Rawls A.(2006). Mohawk is a novel homeobox gene expressed in the developing mouse embryo. *Developmental Dynamics* 235:792-801. PMID: 16408284.
2. William DA, Saitta B, Gibson JD, Traas J, Markov V, Gonzalez DM, Sewell W, Anderson DM, Pratt SC, Rappaport EF and Kusumi K. 2007. Identification of oscillatory genes in somitogenesis from functional genomic analysis of a human mesenchymal stem cell model. *Developmental Biology* 305:172-86. PMID: 17362910.
3. Book chapter: Anderson, DM (Author), Rawls, JA (Author) ,Rhee, JM (Author) . Development of Muscle and Somites. In: Inborn Errors of Development: The Molecular Basis of Clinical Disorders of Morphogenesis. Oxford University Press (2008). ISBN: 9780195306910
4. Anderson DM, Beres BJ, Wilson-Rawls J and Rawls A. 2009. The homeobox gene Mohawk represses transcription by recruiting the Sin3A/HDAC co-repressor complex. *Developmental Dynamics* 238:572-580. PMID: 19235719.
5. Burnett LA, Anderson DM, Rawls A, Bieber AL and Chandler DE. 2011. Mouse sperm exhibit chemotaxis to allurin, a truncated member of the cysteine-rich secretory protein family. *Developmental Biology* 360:318-328. PMID: 22008793.
6. Anderson DM, George R, Noyes MB, Rowton M, Liu W, Jiang R, Wolfe SA, Wilson-Rawls J and Rawls A. (2012). Characterization of the DNA-binding properties of the Mohawk homeobox transcription factor. *Journal of Biological Chemistry*. 287(42):35351-9. PMID:

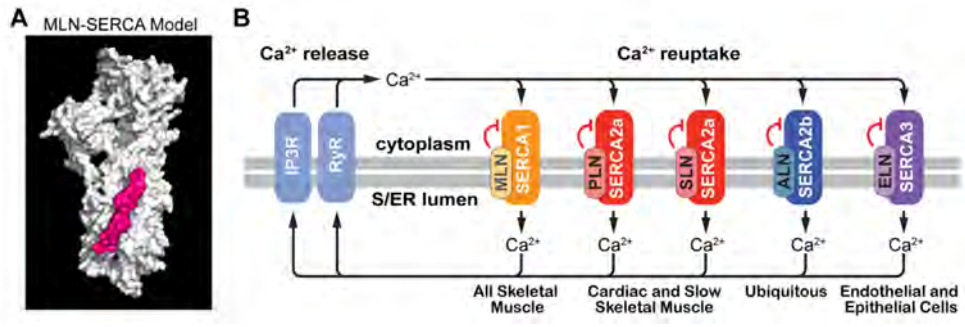


Figure 1. A family of calcium-regulatory micropeptides. Putative lncRNAs can encode peptides translated from small open reading frames, called micropeptides. (A) We identified Myoregulin (MLN), a 46 amino acid transmembrane micropeptide (pink) related to Phospholamban (PLN) and Sarcolipin (SLN), that directly regulates the activity of SERCA (white), the membrane pump that controls striated muscle contractility. (B) We've since discovered two other related members of this family, Endoregulin (ELN) and Another-regulin (ALN), which altogether regulate calcium signaling across diverse cell types of the cardiovascular system.

22923612.
7. Rowton M, Ramos P, Anderson DM, Rhee JM, Cunliffe HE and Rawls A. (2013). Regulation of mesenchymal-to-epithelial transition by PARAXIS during somitogenesis. *Developmental Dynamics* 242(11):1332-44. PMID: 24038871.
8. Nelson BR, Wu F, Liu Y, Anderson DM, McAnally J, Lin W, Cannon SC, Bassel-Duby R and Olson EN. (2013). Skeletal muscle-specific T-tubule protein STAC3 mediates voltage-induced Ca2+ release and contractility. *Proceedings of the National Academy of Sciences* USA 110(29):11881-6. PMID: 23818578.
9. Nelson BR, Anderson DM and Olson EN. (2014). Small open reading frames pack a big punch in cardiac calcium regulation. *Circulation Research* 114(1):18-20. PMID: 24385504.
10. Anderson DM, Anderson KM, Chang CL, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R and Olson EN. (2015). A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell* 160(4), 595-606. PMID: 25640239.
11. Carroll KJ, Makarewich CA, McAnally J, Anderson DM, Zentilin L, Liu N, Giacca M, Bassel-Duby R and Olson EN. (2015). A mouse model for adult cardiac-specific gene deletion with CRISPR/Cas9. *Proceedings of the National Academy of Sciences* USA 113(2):338-343. PMID: 26719419.
12. Nelson BR*, Makarewich CA*, Anderson DM, Winders BR, Troupes CD, Wu F, Reese AL, McAnally J, Chen X, Kavalali ET, Cannon SC, Houser SR, Bassel-Duby R and Olson EN. (2016). A micropeptide encoded by a transcript annotated as long noncoding RNA enhances SERCA activity in muscle. *Science* 351(6270):271-275. PMID: 26816378.
13. Anderson DM*, Cannavino J*, Li H, Anderson KM, Nelson BR, McAnally JR, Bezprozvannaya S, Liu Y, Lin W, Liu N, Bassel-Duby R and Olson EN. (2016). Severe muscle wasting and denervation in mice lacking the RNA binding protein ZFP106. *Proceedings of the National Academy of Sciences* USA 113. E4494-4503. PMID: 27418600
14. Anderson KM, Anderson DM, McAnally JR, Shelton JM, Bassel-Duby R and Olson EN. (2016). Transcription of the non-coding RNA upperhand controls Hand2 expression and heart development. *Nature* 539: 433-436. PMID: 27783597
15. Anderson DM*, Makarewich CA*, Anderson KM, Shelton JM, Bezprozvannaya S, Bassel-Duby R and Olson EN. (2016). Widespread control of calcium signaling by a family of SERCA-inhibiting micropeptides. *Science Signaling* 9: ra119. PMID: 27923914

David S. Auerbach, PhD

Synopsis

Project 1: Electrical Disturbances in the Brain & Heart in Long QT Syndrome – Dangerous Combination

Patients with genetic ion channel diseases develop electrical disturbances in the brain (seizures) and heart (arrhythmias) that can lead to sudden death. I explore the mechanisms for multisystem genetic ion channel diseases. I previously showed that in severe genetic forms of epilepsy, in addition to seizures, there are alterations in cardiac electrical function, with a high rate of cardiac arrhythmias. Arrhythmias provide one potential mechanism for the high rate of sudden death in epilepsy.

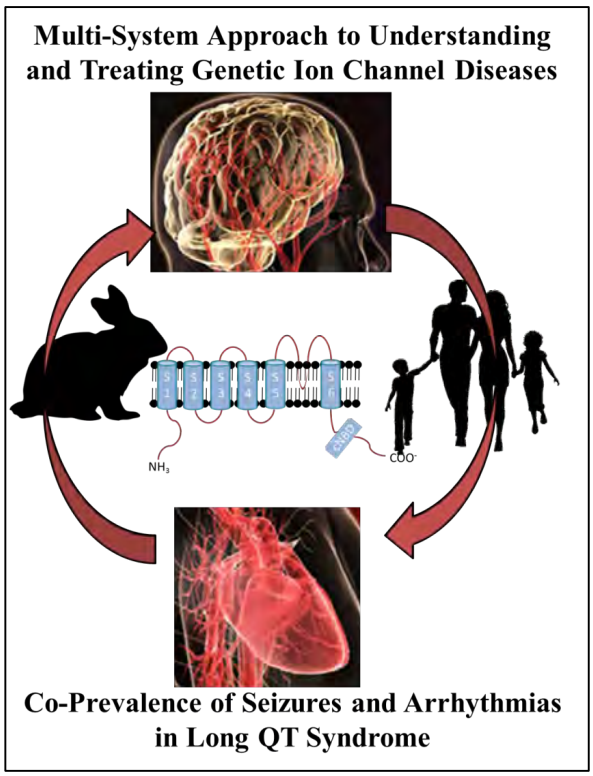
Now approaching these neuro-cardiac investigations in the opposite direction, I am assessing the co-prevalence and severity of seizures and cardiac arrhythmias in a classically studied cardiac disease, called Long QT Syndrome (LQTS.) LQTS is a genetic disease, characterized by cardiac electrocardiographic pathologies, arrhythmias, and a high risk of sudden death. Mutated genes in LQTS1-3 are expressed in the heart and brain, and seizures have been reported in LQTS patients. Ongoing studies using both LQTS patient registries and animal models of LQTS are being used to establish new clinical and mechanistic insights into this dual system disease, and the potential intricate crosstalk between the brain and heart.

The LQTS registry provides a unique and powerful resource to advance LQTS and epilepsy research. The Rochester-based LQTS Registry is the largest (>18,000 subjects) and most deeply annotated LQTS database in the world. It contains detailed clinical and genetic information from LQTS patients, plus affected and unaffected family members. It offers temporal resolution of the disease progression, including the dates of seizures, syncopes, arrhythmias, start/end of medications, sudden death, and records/results from clinical and genetic tests.

Rabbits carrying the same mutation as LQTS2 patients with seizures, and state of the art *in vivo/in vitro* techniques (e.g., radiotelemetry ECGs & single cell electrophysiology), provide excellent tools to interrogate direct vs. indirect mechanisms for the neuro-cardiac disease manifestations and progression.

Translational insights into the mechanisms for neuro-cardiac pathologies in LQTS.

1. LQTS2 mutant gene expressed in the brain and the heart: Biochemical and electrophysiological approaches are employed to investigate the effects that a LQTS2 mutation has on cardiac and neuronal electrical function.
2. Cardiogenic seizures & Neurogenic arrhythmias: Conscious *in vivo* simultaneous video/EEG/ECG recordings to investigate the incidence, dynamics, concordance, and types of seizures and arrhythmias in LQTS2 rabbits.
3. Pro- vs. anti-arrhythmic effects of anti-seizure and anti-depressant drugs in LQTS. Cellular and animal pharmacogenomics studies will indicate the mechanisms for LQTS genotype and class of drug specific differences in the risk of arrhythmias when LQTS patients are taking vs. off the medications.
4. Autonomic nervous system connects the brain and heart: Heart rate and QT variability parameters provide biomarkers for arrhythmias and seizures in LQTS patients and animals.



Project 2: Cardiac Ventricular Repolarization Abnormalities in Patients at Risk for SUDEP

Millions of Americans have epilepsy. They have a 24-fold higher risk of sudden death. Unfortunately, the cause of death is often unknown and termed Sudden Unexpected Death in Epilepsy (SUDEP). Thus, we are unable to predict a person's risk of SUDEP. Cardiac arrhythmias are a proposed mechanism for SUDEP, and have been recorded prior to sudden death in patients with epilepsy. Many genetic forms of epilepsy and SUDEP cases are associated with mutations in genes expressed in the brain and heart.

Using the NIH funded Center for SUDEP Research database of multi-day recordings from multiple systems in 760 patients, we are investigating whether alterations in cardiac electrical and autonomic function serve as biomarkers associated with a high risk of future SUDEP.

1. Investigate whether patients at a high vs. low risk of SUDEP develop alterations in cardiac electrical and autonomic function during a 24-hour seizure-free baseline period.
2. Assess the temporal evolution of cardiac electrical and autonomic function preceding and following seizures in patients at a high and low risk of future SUDEP.

The development of ECG biomarkers associated with a high risk of SUDEP will enable physicians to identify and combat the manifestations of these life threatening diseases.

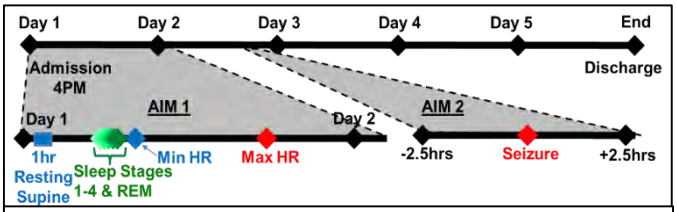


Fig. 2: Study design & timepoints for assessing repolarization measures at baseline & preceding/following a seizure.

Lab Members

- Joshua Brown – UR Undergraduate
- Ahmed Selmi – UR Undergraduate

Publications

3. Auerbach DS, McNitt S, Gross RA, Zareba W, Dirksen RT, Moss AJ. Genetic biomarkers for the risk of seizures in long QT syndrome. Neurology. 2016;87(16):1660-8. doi: 10.1212/WNL.0000000000003056. PubMed PMID: 27466471; PMCID: PMC5085072.
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7. Lin X, O'Malley H, Chen C, Auerbach D, Foster M, Shekhar A, Zhang M, Coetzee W, Jalife J, Fishman GI, Isom L, Delmar M. Scn1b deletion leads to increased tetrodotoxin-sensitive sodium current, altered intracellular calcium homeostasis and arrhythmias in murine hearts. J Physiol. 2015;593(6):1389-407. doi: 10.1113/jphysiol.2014.277699. PubMed PMID: 25772295; PMCID: PMC4376420.
8. Auerbach DS, Jones J, Clawson BC, Offord J, Lenk GM, Ogiwara I, Yamakawa K, Meisler MH, Parent JM, Isom LL. Altered Cardiac Electrophysiology and SUDEP in a Model of Dravet Syndrome. Plos One. 2013;8(10):15.

doi: 10.1371/journal.pone.0077843. PubMed PMID: WOS:000325887300110.

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11. Auerbach DS, Grzda KR, Furspan PB, Sato PY, Mironov S, Jalife J. Structural heterogeneity promotes triggered activity, reflection and arrhythmogenesis in cardiomyocyte monolayers. J Physiol. 2011;589(Pt 9):2363-81. doi: 10.1113/jphysiol.2010.200576. PubMed PMID: 21486795; PMCID: 3098708.
12. Auerbach D, Jalife, J Substrates and Triggers for the Initiation of Arrhythmias. Physiology News. 2011;85:15-7.
13.

Bradford C. Berk, M.D., Ph.D.

Synopsis

My laboratory investigates fundamental and clinical mechanisms of signal transduction in blood vessels that contribute to cardiovascular diseases (CVD) such as atherosclerosis, aneurysms, hypertension and stroke. Current projects include:

Project 1: The role of Cyclophilin A (CypA) in pulmonary arterial hypertension (PAH).

We have previously established that CypA is secreted from all cell types present in vessels. We term secreted CypA as extracellular CypA (eCypA) because it has two very important properties that differentiate it from intracellular CypA. First, it exists preferentially as an acetylated protein which has 6-fold greater activity for stimulation of pro-inflammatory and pro-apoptotic signals in endothelial cells (EC). Second, it binds to a unique receptor on the membranes of EC and SMC. A key pathogenic mechanism is that it participates in a positive feedback loop in smooth muscle cells (SMC) generating reactive oxygen species (ROS) by binding p47phox and translocating it to the membrane. Most importantly, we used several mouse models of CypA deletion and over-expression to demonstrate a pathogenic role for CypA in atherosclerosis and aneurysm formation. The most likely pathogenic mechanism is the generation of ROS that stimulate inflammation and apoptosis of EC, as well as proliferation and migration of SMC. Based on these results, it is logical that CypA would be a potential pathogenic mediator of PAH. As anticipated, we found significant increases in CypA levels in plasma and lungs of PAH patients. To strengthen our hypothesis that CypA is a novel mediator of PAH, we generated cell-specific CypA over-expressing transgenic mice (ecCypA-tg and smcCypA-tg). The exciting result was that EC specific ecCypA-tg mice developed pulmonary hypertension at 3 months of age. We are now investigating the mechanisms by which eCypA causes PAH based on two mechanisms: vascular remodeling and inflammation. Furthermore, we are testing the ability of a novel eCypA inhibitor to prevent PAH in both mouse and rat PAH models.

Project 2: Flow responsive endothelial Pnpt1: an exoribonuclease that regulatesmitochondrial function and vascular disease.

This project aims to delineate molecular mechanisms that link laminar flow mediated signaling with gene expression, mitochondrial homeostasis and EC function. Carotid intima-media thickening (IMT) is caused by intima growth, and is a significant risk factor for cardiovascular diseases (CVD). Intima growth is mediated by EC dysfunction, VSMC growth as well as inflammatory cell accumulation and activation. These pathological processes are stimulated by a disturbed flow pattern (d-flow), while being minimized by steady (s-flow). We performed genetic, transcriptomic and bioinformatic analyses of IMT in the mouse carotid exposed to d-flow; and showed significant differences in inflammation, cell cycle and RNA degradation. Further analyses of expression QTL studies, human GWAS, and congenic mice strains demonstrated that high level expression of polyribonucleotide nucleotidyltransferase 1 (Pnpt1), correlated with decreased intima growth and inflammation in the carotid ligation model. This finding suggests that Pnpt1 is protective against IMT, like a tumor suppressor gene. Pnpt1 is a 3′-5′ exoribonuclease that is required for import and processing of RNA in mitochondria. We hypothesize that Pnpt1 is a mechanoresponsive enzyme that is critical to mitochondrial homeostasis and acts as a negative regulator of vascular inflammation and intima growth, thereby limiting CVD. To prove this, we will study changes in vascular remodeling and atherosclerosis in transgenic mouse models; determine the transcriptional program regulated by Pnpt1 focusing on the TFAP2b/c transcription factor; and the mechanisms by which flow regulates Pnpt1 function assayed by expression and enzyme activity. This proposal will characterize for the first time the role of Pnpt1, a major enzyme for mitochondrial RNA import and processing, in mouse models of atherosclerosis

and vascular remodeling, and in human carotid endarterectomy specimens.

Lab Members

- Chao Xue, Graduate Student
- Chen Qiang, Graduate Student
- Mark Sowden, Research Assoc. Professor
- Mary Wines-Samuelson, Research Asst. Professor
- Sharon Senchanthisai, Technical Assistant
- Martha Zettel, Technical Associate

Publications

Project 1: Cyclophilin A and Pulmonary Hypertension

1. Satoh, K., Nigro, P., Matoba, T., O'Dell, M.R., Cui, Z., Shi, X., Mohan, A., Yan, C., Abe, J., Illig, K.A. & Berk, B.C. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. Nat Med 2009; 15:649-656.
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Project 2: Flow responsive endothelial Pnpt1

1. Smolock EM, Korshunov VA, Glazko G, Qui X, Gerloff J, and Berk BC. Ribosomal protein L17, RpL17, is an inhibitor of vascular smooth muscle growth and carotid intima formation. Circulation. 2012; 126:2418-2427. PMID: 23065385.
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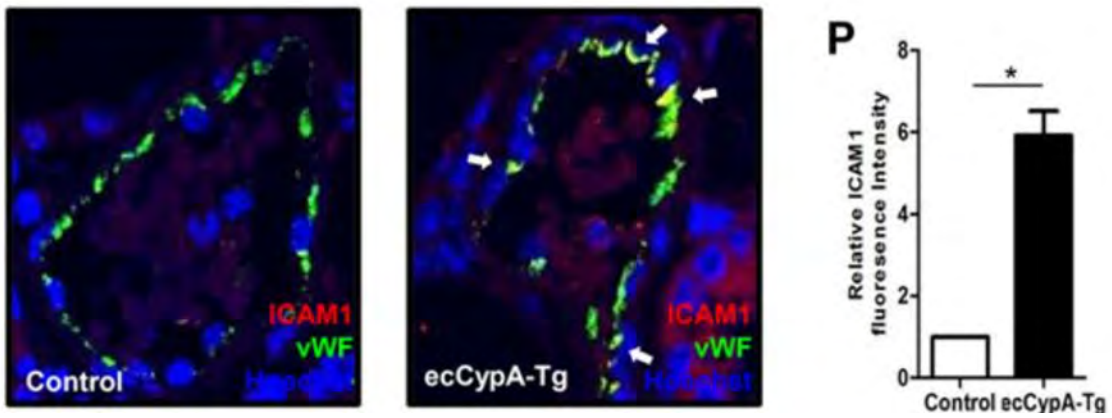


Figure: Increased ICAM1 expression in mice over-expressing cyclophilin A.

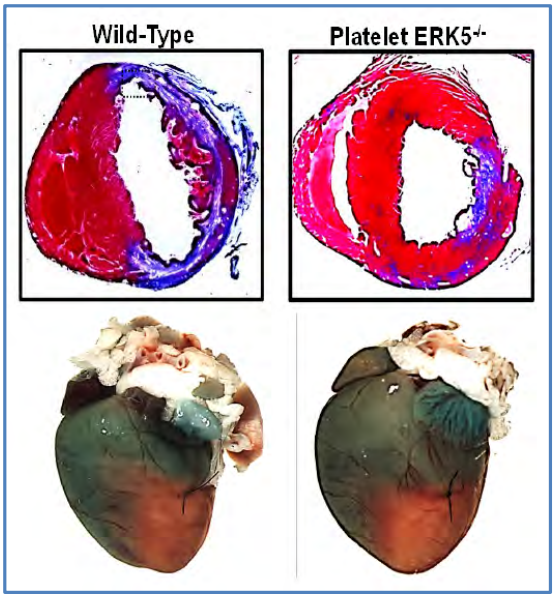
Scott Cameron, M.D., Ph.D.

Synopsis

Platelets are small anucleate blood particles which play an important role in thrombosis, hemostasis, and inflammation. Patients with ischemic and thrombotic disease of the coronary and peripheral vasculature are treated with anti-platelet drugs, yet some patients do not derive benefit from these drugs or they experience unexpected, off-target adverse events. We aim to better define platelet function in disease states, paying particular attention to post-receptor signal transduction pathways. Our overall goal is to evaluate gaps in clinical care, then identify viable signaling pathways in human tissue for drug intervention, and finally to utilize animal models to test the hypothesis.

Project 1: The role of platelet ERK5 in myocardial infarct expansion.

We recently identified a novel role for the protein ERK5 (also known as Big Map Kinase 1), a MAPK family member which is expressed in human and murine platelets. We discovered that ERK5 regulates platelet activation, heart function, and scar size after a heart attack. We found that ERK5 changes the expression of platelet proteins during ischemia. We began enrolling patients with ST-segment myocardial infarction (STEMI) and Non-ST-segment myocardial infarction (NSTEMI) and showed that platelet ERK5 is likely involved in dysregulated platelet activity, with changes in platelet receptor signaling and platelet activation. This project implies that the platelet phenotype and therefore function is fundamentally different in STEMI and NSTEMI, and this may directly affect patient care. We lastly have identified two platelet-derived biomarkers that predict heart attack better than the current test available, and we are filing patents for these.

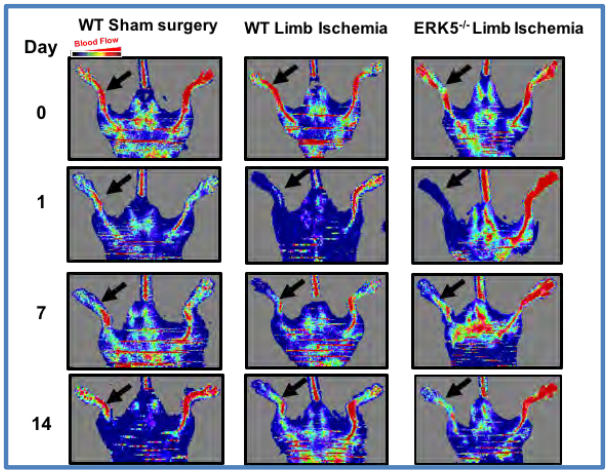


Project 2: The role of platelets in the progression of peripheral vascular disease (PAD).

We have discovered that atherosclerosis and ischemia in mice and in humans dramatically changes platelet protein expression, altering the activation state of the platelet and the risk for thrombosis. We have characterized the platelet signaling pathways in patients with PAD using a proteomic approach with our colleagues at Johns Hopkins. We found that platelet ERK5 may contribute to 'reprogramming' of the platelet in an adverse manner, increasing the risk for blood clotting. This may be an explanation for the higher risk of heart attack and stroke in patients with PAD and gives insight into potential therapeutics.

Project 3: The role of platelet proteins in aortic aneurysmal disease.

Acute aortic syndromes are emergent conditions and include aortic dissection (AD), intramural hematoma and penetrating atherosclerotic ulcerations. AAA has potentially



fatal consequences, often preceded by asymptomatic abdominal aortic aneurysms (AAA). Published studies suggest a potentially beneficial effect of anti-platelet agents on AAA growth, presenting the tantalizing possibility that platelet-derived mediators or microvascular thrombosis promotes aneurysmal development and rupture in animal models and humans. We have data in platelets isolated form humans with AAA showing platelet function is fundamentally different, and expression of certain genes which ultimately regulate platelet function may be key. We have a mouse model of AAA under development for which we will test our candidate genes for promoting dysregulated platelet behavior and aneurysmal growth in AAA. The findings from this investigation offer the potential for rapid translation back to clinical care in a bedside-to-bench and back again approach.

Lab Members

- Rachel Schmidt, Laboratory Technician

Publications

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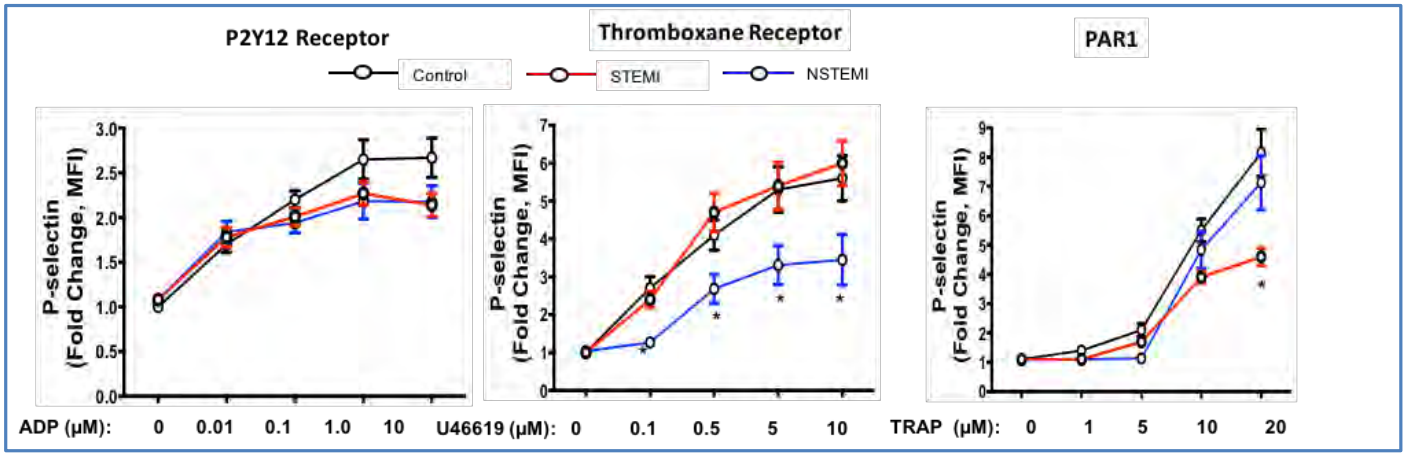
8. Cameron SJ, Ture SK , Modjeski KL, Mickelsen D, Chakrabarti E, Seaberry M, Field DJ , Le N, Abe J, Morrell, CN. Platelet ERK5 is a Redox Switch and Triggers Maladaptive Platelet Responses and Myocardial Infarct Expansion. *Circulation*. 2015 Jul 7;132(1):47-58



Jian Fu, B. Med., Ph.D.

Synopsis

My research focuses on the role of SM20, the ortholog of EGLN3, with a specific emphasis on deciphering the effect of SM20 on the development of skeletal muscle. Towards this end, we have been using pharmacological inhibitors to repress the activity of the prolyl hydroxylase, siRNAs to knock down SM20 expression level, and transient transfection strategy to overexpress SM20 to reveal a role for SM20 in the differentiation of cultured skeletal myoblasts. Strikingly, our preliminary results suggest that SM20 is involved in myogenesis.

Myogenic differentiation is orchestrated by a family of muscle regulatory factors including MyoD, myogenin, Myf-5, and MRF4. We are now exploring the impact of SM20 on these master muscle-specific molecules. We are creating SM20 knock-out mice to understand how SM20 regulates myogenesis. Our study will generate novel insights into the regulation of myogenic differentiation. Current projects include:

Project 1: Role of EGLN3 in Vascular Inflammation and Remodeling. EGLN3 was first cloned from vascular smooth muscle cells.

It is also expressed in endothelial cells and macrophages. Our previous studies showed that EGLN3 is an inhibitor of NFκB, a master regulator of inflammation. However, it remains to be determined whether EGLN3 is involved in vascular inflammation. Our ongoing project is to uncover the role of EGLN3 in vascular inflammation and remodeling.

Project 2: EGLN3 Signaling Pathways. EGLN3 (also known as PHD3, HPH1, and SM-20) belongs to the C.

Elegans gene egl-9 (EGLN) family of prolyl hydroxylases that require oxygen, iron and α-ketoglutarate for their enzymatic activity. EGLN hydroxylases are best known for the ability to catalyze the hydroxylation of HIF1α (hypoxia-inducible factor 1α). However, the role and mechanism for EGLN3 in other signaling pathways are poorly understood. We are now identifying novel vascular targets of EGLN3.

Project 3: Protein Ubiquitination and Vascular Inflammation. Protein ubiquitination is a post-translational modification involved in all facets of cell signaling and cell function.

Ubiquitination not only influences the fate of the substrate but also has non-degradative functions such as signaling. We are exploring the role of protein ubiquitination in vascular inflammation.

Lab Members

- Ying Jin, Staff Scientist

Publications

1. Fu J. Catalytic-independent inhibition of cIAP1-mediated RIP1 ubiquitination by EGLN3. *Cellular Signaling* 2016, 28:72-80.
2. Fu J, Taubman MB. EGLN3 inhibition of NF-κB is mediated by prolyl hydroxylase-independent inhibition of IκB kinase γ ubiquitination. *Molecular and Cellular Biology* 2013, 33: 3050-61
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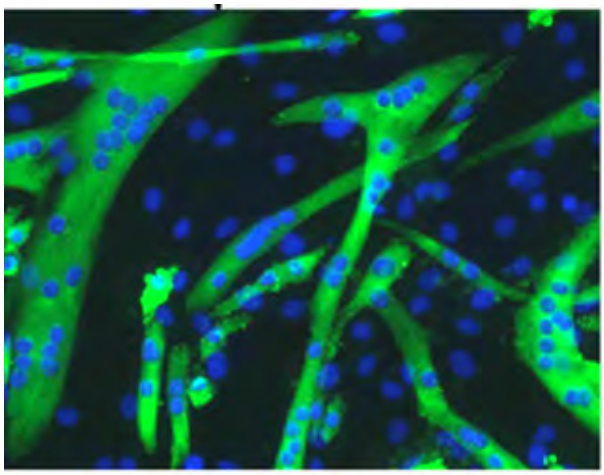

Zheng-Gen Jin, Ph.D.

Synopsis

Atherosclerosis, the formation of plaque inside arterial wall, is the leading cause of death and disability in the United States and throughout the world. Atherosclerotic lesions develop in the regions of curvature, bifurcation, and branching of vessels, where fluid shear stress is low. In contrast, steady laminar flow associated with high fluid shear stress within the large straight arteries is atheroprotective.

Our research goal is to elucidate the molecular mechanisms of atherosclerosis and to identify the key molecules and signal pathways in the atheroprotective programs of laminar flow. Our recent studies have demonstrated that histone deacetylase 5 (HDAC5) plays an important role in regulation of laminar flow-sensitive genes. Current projects focus on exploring the mechanisms by which HDAC5 and other chromatin-modifying enzymes control gene transcription in vascular endothelial cells in response to laminar flow. Our studies may provide insights into the pathogenesis of atherosclerosis and lead to the development of new therapies to prevent/treat atherosclerotic disease.

Lab Members

- Marina Koroleva – Laboratory Technician
- Suowen Xu – Research Assistant Professor
- Shu-ya Zhang – Postdoctoral Associate

Publications

1. Xu S, Ha CH, Wang W, Xu X, Yin M, Jin FQ, Mastrangelo M, Koroleva M, Fujiwara K, Jin ZG. PECAM1 regulates flow-mediated Gab1 tyrosine phosphorylation and signaling. *Cell Signal*. 2016 Mar;28(3):117-24.
2. Zhao J, Yin M, Deng H, Jin FQ, Xu S, Lu Y, Mastrangelo MA, Luo H, Jin ZG. Cardiac Gab1 deletion leads to dilated cardiomyopathy associated with mitochondrial damage and cardiomyocyte apoptosis. *Cell Death Differ*. 2015 Oct 30.
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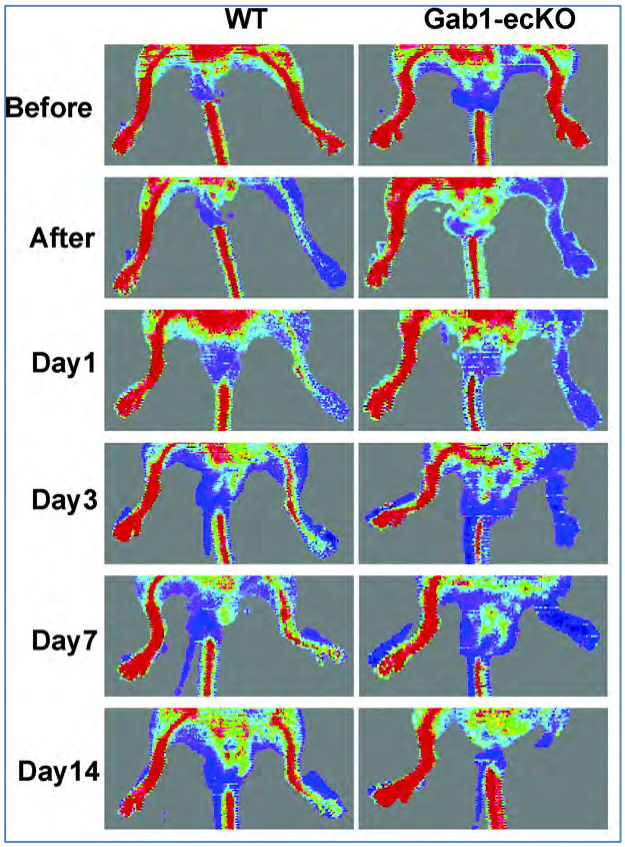

Figure: Hind limb ischemia is more severe in the absence of Gab1 from endothelial cells.

Vyacheslav Korshunov, Ph.D.

Synopsis

Understanding the mechanisms that regulate the structure of blood vessels could prevent cardiovascular morbidity and mortality in humans. My current research focuses on immune mechanisms of cardiovascular disorders that affect vascular remodeling.

My laboratory research has three directions:

1. We are working on a project that explores the role of Axl, a receptor tyrosine kinase, in regulation of the immune responses in hypertension. Using state-of-the-art flow cytometry techniques we found that expression of Axl affected accumulation of leukocytes in the kidneys and determined pathogenesis of salt-dependend hypertension in mice. We showed that Axl regulates reactive oxygen species production in kidney's leukocytes in hypertension (brown staining, Figure).

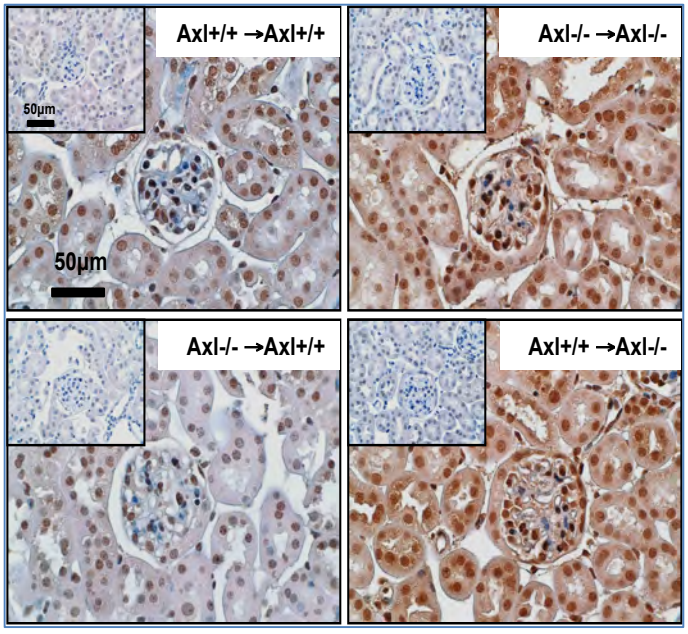

Figure. Representative images represent the levels of reactive oxygen species in kidneys from Axl chimeras during salt-dependent hypertension. Positive cells are dark brown. Negative controls as shown as insets. Magnification bar is 50μm. Adapted from *Batchu et al., Hypertension 2013*

2. We study genetic mechanisms that lead to differences between physiological and pathological carotid artery remodeling. We discovered a new candidate gene that regulates arterial rigidity and arterial stenosis; we are currently defining the biomechanical pathways through which this gene regulates pathological remodeling.
3. We used a combined genetic approach of genome-wide linkage and association analyses to identify a novel locus on mouse chromosome 7 that controls elevated heart rate and vascular remodeling. Our most recent findings suggest that autonomic dysfunction is crucial for stress-induced vascular inflammation in mice.We are studying candidate genes within chromosome 7 locus that control hemodynamic parameters and vascular inflammation.

Lab Members

- Breandan Quinn – Laboratory Technician

Publications

Project 1 publications

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2. Batchu SN, Xia J, Ko KA, Doyley MM, Abe J, Morrell CN, Korshunov VA. Axl modulates immune activation of smooth muscle cells in vein graft remodeling. *Am J Physiol Heart Circ Physiol* 2015;309(6):H1048-58.
3. Batchu SN, Hughson A, Gerloff J, Fowell DJ, Korshunov VA. Role of Axl in early kidney inflammation and development of salt-dependent hypertension. *Hypertension* 2013;62(2):302-309
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Project 2 publications

1. Smolock EM, Burke R, Wang C, Batchu SN, Qiu X, Thomas T, Zettel M, Fujiwara K, Berk BC, Korshunov VA. Intima modifier locus 2 controls endothelial cell activation and vascular permeability. *Physiol Genomics* 2014;46(17):624-633.
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3. Smolock EM, Korshunov VA, Glazko G, Qiu X, Gerloff J, Berk BC. Genetic analysis of carotid intima formation identifies Rpl17 as a vascular smooth muscle growth inhibitor. *Circulation* 2012;126:2418-2427.

Project 3 publications

1. Batchu SN, Smolock EM, Dyachenko IA, Murashev AN, Korshunov VA. Autonomic dysfunction determines stress-induced cardiovascular and immune complications in mice. *J Am Heart Assoc* 2015;4:e001952.
2. Smolock EM, Ilyushkina IA, Ghazalpour A, Gerloff J, Murashev AN, Lusis AJ, Korshunov VA. Genetic locus on mouse chromosome 7 controls elevated heart rate. *Physiol Genomics* 2012;44(13):689-98.

Coeli Lopes, Ph.D.

Synopsis

We study the regulation of ion channels by diverse G-protein signaling pathways in normal and pathological states.

One major focus of our current work is the changes in function and regulation of cardiac ion channels that cause the most common form of inherited cardiac arrhythmia, Long QT syndrome. Our work translates channel dysfunction and dysregulation at the cellular level to clinical phenotype and patient's response to treatment. A second focus of our current research is the study of pathological remodeling of the slow delayed rectifier-like current (IKs) in heart failure. Our current research focus on stress signals caused by chronic stimulation of kinase signaling pathways, and their consequence for ion channel function and membrane trafficking. We explore novel antiarrhythmic treatments to reverse IKs pathological remodeling during heart failure.

Lab Members

- Xiaorong Parks, Staff Scientist
- Amanda Amoh, Graduate Student
- Chen Kaplan, Graduate Student

Publications

1. Ruwald MH, Xu Parks X… and Lopes CM. Stop-codon and C-terminal nonsense mutations are associated with a lower risk of cardiac events in patients with long QT syndrome type 1. *Heart Rhythm*. 2016 Jan;13(1):122-31.
2. O-Uchi J, Sorenson J… and Lopes CM. Isoform-specific dynamic translocation of PKC by α1-adrenoceptor stimulation in live cells. *Biochem Biophys Res Commun*. 2015 Sep 25;465(3):464-70.
3. O-Uchi J, Rice JJ… and Lopes CM. Impaired IKs channel activation by Ca(2+)-dependent PKC shows correlation with emotion/arousal-triggered events in LQT1. *J Mol Cell Cardiol*. 2015Feb;79:203-11.
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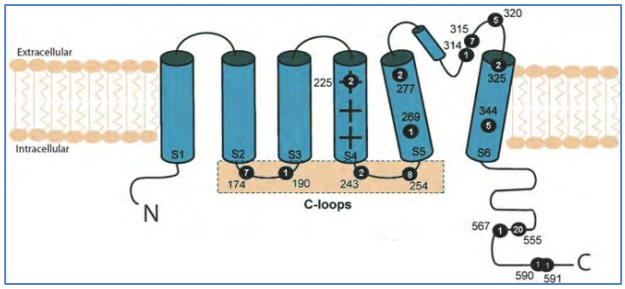

Figure: Schematic of mutations in KCNQ1 ion channel.

Charles J. Lowenstein, M.D.

Synopsis

Venous thromboembolism (VTE) is a major cause of morbidity and mortality, with an annual incidence of over 900,000 in the USA. Elevated plasma levels of Von Willebrand factor (VWF) are a risk factor for venous thrombosis, but the genetic factors that regulate VWF levels are not well understood.

VWF is a glycoprotein that mediates platelet adhesion to the vascular wall and also platelet aggregation with other platelets. VWF is synthesized by endothelial cells and platelets, stored inside intracellular granules, and then released into the blood by a process called exocytosis.

The overall goal of my lab is to understand pathways of exocytosis in the human vasculature. Our general approach is to use genetic studies of humans to identify gene products that are potential regulators of exocytosis, and then to use cells and mice to characterize the role of these candidates in exocytosis.

We identified several key components of the exocytic machinery in endothelial cells, including VAMP8, SNAP23, and STX4. We then characterized the molecular motor, NSF, that controls endothelial secretion. Next, we found that nitric oxide regulates endothelial exocytosis.

We are currently using genome-wide association studies to identify novel regulators of exocytosis. For example, a recent genome wide association of patients with altered VWF levels identified 6 novel genetic loci. We are now studying the candidate genes within these loci, and determining how mutations affect their expression and function.

These approaches will increase our understanding of endothelial pathways that increase the risk of diseases such as venous thromboembolism, and they will identify new therapeutic targets for the prevention and treatment of thromboembolic diseases.

Lab Members

- John Allen Bennett, Postdoctoral Fellow
- Michael Mastrangelo – Technical Associate

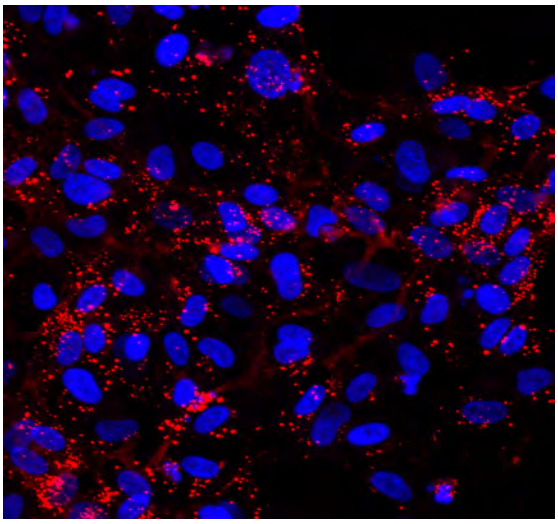

Figure: Endothelial cells grown on a defined matrix express granules containing VWF (red).

Publications

1. Zhu QM, Ko KA, Ture S, Mastrangelo MA… Morrell CN, Miano JM, Lowenstein CJ. Novel Thrombotic Function of a Human SNP in STXBP5 Revealed by CRISPR/Cas9 Gene Editing in Mice. *Arterioscler Thromb Vasc Biol*. 2017 Feb;37(2):264-270.
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3. Cameron SJ, Morrell CN, Bao C, Swaim AF, Rodriguez A, Lowenstein CJ. A Novel Anti-Inflammatory Effect for High Density Lipoprotein. *PLoS One*. 2015 Dec 17;10(12):e0144372.
4. Zhu Q, Yamakuchi M, and Lowenstein CJ. SNAP23 Regulates Endothelial Exocytosis of von Willebrand Factor. *PLoS One*. 2015 Aug 12;10(8):e0118737.
5. Zhu Q, Yamakuchi M, Ture S... and Lowenstein CJ. Syntaxin binding protein STXBP5 inhibits endothelial exocytosis and promotes platelet secretion. *The Journal of Clinical Investigation*. 2014;124(10):4503–4516.
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Joseph Miano, Ph.D.

Synopsis

The notion of "junk DNA" has been debunked with the realization that the human genome is punctuated with millions of regulatory codes and undergoes pervasive transcription, particularly with respect to the emerging class of long noncoding RNA (lncRNA) genes, which already outnumber all protein-coding genes. Most of the so called "dark matter" in our genome is, from a functional standpoint, poorly characterized. Moreover, most sequence variations associated with human diseases fall within genomic dark matter. Thus, there is urgent need to elucidate the function (or dysfunction) of the estimated 2.4 billion nucleotides of human genomic sequences once thought to be genomic refuse.

The Miano Lab uses tools in bioinformatics and genomics to study functional regulatory elements and lncRNA genes that affect transcriptional and post-transcriptional regulation of gene expression, especially as they relate to vascular smooth muscle cell (VSMC) differentiation. For example, we have computationally defined over 3.6 million regulatory elements called CArG boxes that bind the SRF transcription factor. This so-called CArGome has allowed for the discovery of over 140,000 CArG-SNPs, many of which appear to effect neighboring gene expression. We have started to validate these elements and sequence variants therein using next generation sequencing assays (RNA-seq and ChIP-seq) coupled to conventional luciferase, gel shift, and ChIP experiments. The goal of what we refer to as the CArG Variome Project is to pinpoint CArG-SNPs within haplotype blocks linked to human diseases.

The CArGome initiative has also led us to delve deep into the world of lncRNA genes. Accordingly, we have been mining the human genome for unannotated lncRNA genes, and we recently published the first novel, human vascular cell-restricted lncRNA called *SENCR*. This lncRNA appears to fine tune the program of vascular smooth muscle cell gene expression including that of Myocardin (MYOCD) which we first showed functions as a molecular switch for the smooth muscle cell differentiation program. Recent RNA-seq experiments have uncovered numerous Myocardin-dependent lncRNA genes, some of which are highly enriched in vascular smooth muscle. We are in the process of working up new and recently annotated lncRNA genes using modern tools in molecular biology, biochemistry, genetics, and cell biology.

Another focus of the lab is utilizing the revolutionary CRISPR/Cas9 system of genome editing to engineer mice carrying precision-guided mutations in key CArG elements or clinically relevant CArG-SNPs or deletions of conserved lncRNA genes.

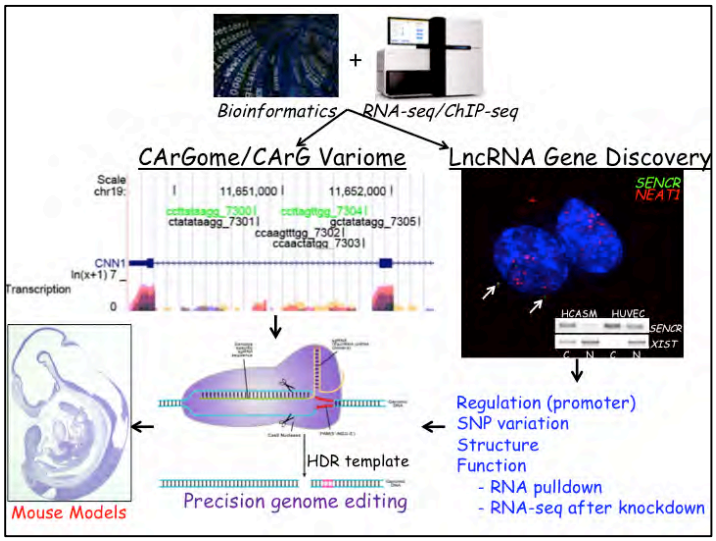
In summary, work in the Miano Lab spans the gamut from computer to cell to genetically-altered mouse models in order to understand noncoding sequences (e.g., CArG boxes and lncRNA genes) and variant sequences therein that are associated with, but not limited to, cardiovascular disease.

Lab Members

- Christine Christie, Lab Technician
- Qing (Rex) Lyu, Postdoctoral Associate
- Orazio Slivano – Technical Associate

Publications

1. Chettimada S, Joshi SR, Dhagia V, Aiezza A 2nd, Lincoln TM, Gupte R, Miano JM, Gupte SA., Vascular smooth muscle cell contractile protein expression is increased through PKG-dependent and -independent pathways by G6PD inhibition and deficiency, Am J Physiol



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Craig Morrell, D.V.M., Ph.D.

Synopsis

Platelets are best known as the cellular mediator of thrombosis, but platelets also have a major role in the initiation and regulation of inflammation and immune responses. My lab focuses on the role of platelets in vascular inflammation and platelet regulation of immune responses.

An understanding of platelets in immunity is rapidly expanding and our lab is a leader in this field. We have discovered that platelets play a central role in innate immune responses. We also found that platelets control the development of acquired immune cells, including T-helper cell differentiation and B cell development. This places platelets at the center of inflammatory processes that have a direct effect on vascular disease such as atherosclerosis, myocardial infarction and transplant rejection.

We have recently identified a novel platelet mediated mechanism for the regulation of T-helper cell development and differentiation. Using mouse models of cardiac transplantation we have demonstrated that the platelet derived chemokine PF4/CXCL4 limits the T-helper 17 (Th17) type of CD4+ T-cell response. We are now actively exploring how platelets and PF4 interact with developing T helper cells and the signaling mechanisms involved in PF4 limiting Th17 differentiation. This work has expanded to investigate the role of platelet activation and T cell differentiation in other inflammatory diseases including inflammatory bowel disease (IBD).

We have also recently identified novel mechanisms for PF4 mediated B cell differentiation that is now being actively pursued, in addition to identifying alternative, non-megakaryocyte and platelet derived sources of PF4.

New studies in our lab are focusing on how platelets contribute to neurovascular inflammation and injury in chronic HIV infection that leads to cognitive decline. Even with current anti-retroviral therapies and undetectable viral loads there is still evidence of ongoing platelet activation and vascular inflammation that correlates with increased thrombotic risk and neurovascular injury that precedes a neurocognitive decline. We are now exploring platelet mediated mechanisms for neurovascular inflammation and the decline in memory and learning associated with chronic HIV infection.

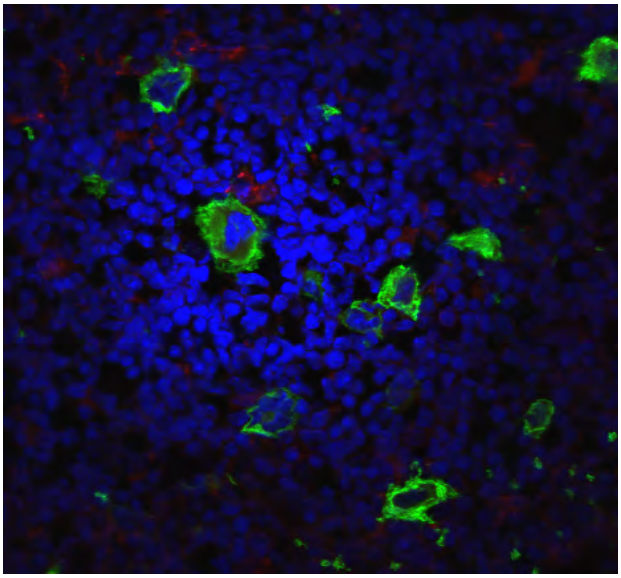

Figure: Megakaryocytes staining green in mouse bone marrow.

Lab Members

- Zachary T. Hilt– Graduate Student
- Daphne N. Pariser – Graduate Student
- Sara Ture – *Laboratory Technician*

Publications

1. Modjeski KL, Levy SC, Ture SK, Field DJ, Shi G, Ko K, Zhu Q, Morrell CN. Glutamate receptor interacting protein 1 regulates CD4(+) CTLA-4 expression and transplant rejection. Am J Transplant. 2015 Nov 25.
2. Cameron SJ, Ture SK, Mickelsen D, Chakrabarti E, Modjeski KL, McNitt S, Seaberry M, Field DJ, Le NT, Abe J, Morrell CN. Platelet Extracellular Regulated Protein Kinase 5 Is a Redox Switch and Triggers Maladaptive Platelet Responses and Myocardial Infarct Expansion. Circulation. 2015 Jul 7;132(1):47-58.
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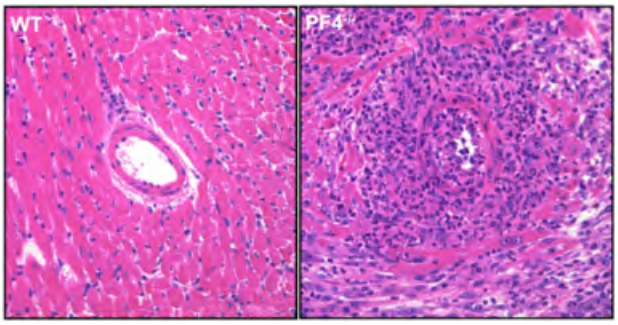

Figure. PF4$^{-/-}$ mice have severe graft vasculopathy. WT and PF4$^{-/-}$ mice were given BM12 heart allografts and transplants harvested 35 days later. PF4$^{-/-}$ mice have extensive leukocyte and neutrophil infiltrates.

Jinjiang Pang, B. Med., Ph.D.

Synopsis

Angiogenesis, the formation of new blood vessels from existing ones, is a critical event for tissue development and repair, as well as being associated with many diseases (e.g. bronchopulmonary dysplasia, pulmonary artery hypertension, ischemic cardiomyopathy, retinopathy and tumor growth).

The long-term goal of our lab is to identify the critical targets that regulate angiogenesis under physiological and pathological conditions. We also focus on molecular mechanisms involved cardiac metabolism. Current project goals are to:

1. Define the role of Dll4-Notch signaling in postnatal lung vasculature development and lung vascular diseases.
2. Determine the therapeutic effect of a cell permeable Ankyrin repeat peptides on prostate cancer.
3. Determine the molecular mechanisms involve in mitochondrial biogenesis during cardiac development and diseases.

Lab Members

- Mark Sowden, Research Associate Professor
- Jordan Rhen, Laboratory Technician
- Shumin Wang, Postdoctoral Research Associate

Publications

1. Yin G, Sheu TJ, Menon P, et al. Impaired angiogenesis during fracture healing in GPCR kinase 2 interacting protein-1 (GIT1) knock out mice. *PLoS One*. 2014;9(2):e89127.
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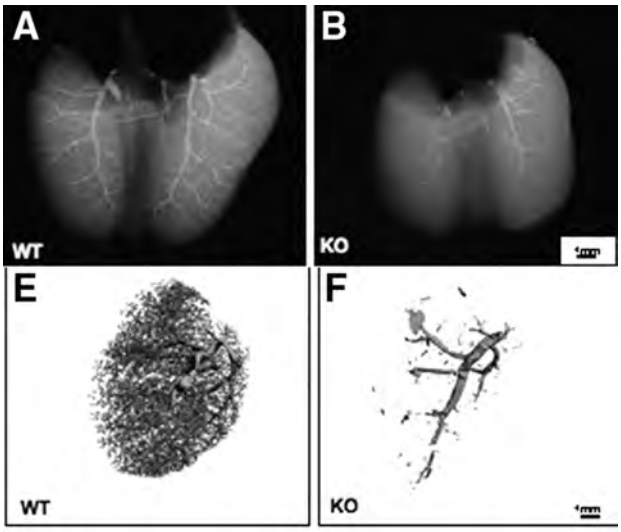
8. Figure: Imaging shows reduced vasculature in lungs of GIT1 KO mice.



Eric Small, Ph.D.

Synopsis

The transition to heart failure (HF) following an ischemic event is the result of irreversible cardiomyocyte loss and the development of cardiac fibrosis. Cardiac fibrosis arises from the aberrant and persistent stimulation of fibroblasts, the main source of extracellular matrix in the heart, in a pathological attempt to repair damaged tissue. Although current therapeutic strategies improve contractility by targeting the cardiomyocyte, without a complementary approach to block or reverse the development of fibrosis and regenerate functioning myocardium, treatment options often represent a bridge to cardiac transplantation.

The Small Lab uses mouse genetics, cell biology and biochemical approaches to define the molecular mechanisms that control fibroblast plasticity and progenitor cell differentiation during heart development and disease with the ultimate goal of developing novel therapeutic approaches to block or reverse the progression of heart failure. We have recently found that cardiac fibroblasts exhibit distinct gene expression programs (GEP) in physiological (exercise training/sustained cardiac function) versus pathological (disease states/deterioration of cardiac function) remodeling. We are currently using mouse genetics and cell biological approaches to test the hypothesis that some genes that are expressed in fibroblasts specifically during exercise might abrogate the development of cardiac fibrosis. A related project is aimed at identifying novel small molecules that might block pathological fibroblast activation and the development of cardiac fibrosis.

We have also made advances in defining the gene regulatory mechanisms leading to the mobilization and differentiation of an important population of cardiovascular progenitors, called epicardium- derived progenitor cells (EPDCs). EPDCs give rise to fibroblasts and perivascular cells in the embryo and can repopulate damaged myocardium in the adult. We have recently found that Myocardin-related transcription factors drive EPDC motility, pericyte differentiation and coronary vessel maturation. This study is expected to accelerate the development of strategies to stimulate progenitor cell mobilization for neovascularization and cardiac regeneration.

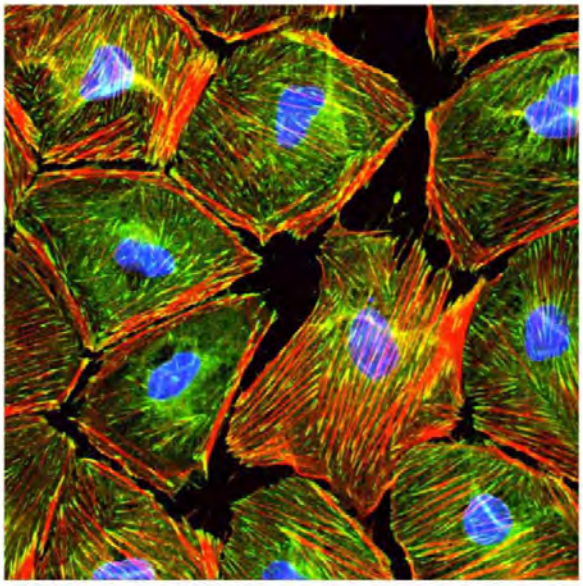


Figure: Epicardium-derived cells undergoing epithelial-mesenchymal transition (EMT) exhibit Vinculin-positive focal adhesions (green) and smooth muscle actin-positive stress fibers (red).

Lab Members

- Ryan Burke, Postdoctoral Fellow
- Ron Dirkx – Technical Associate
- Zachary Hilt – Graduate Student
- Janet Lighthouse – Postdoctoral Fellow
- Pearl Quijada – Postdoctoral Fellow
- Michael Trembley – Graduate Student

Publications

1. Trembley MA, Velåasquez LS, Small EM. Epicardial outgrowth assay and ex vivo assessment of epicardial-derived cell migration. J Vis Exp. 2016 Mar 18; (109).
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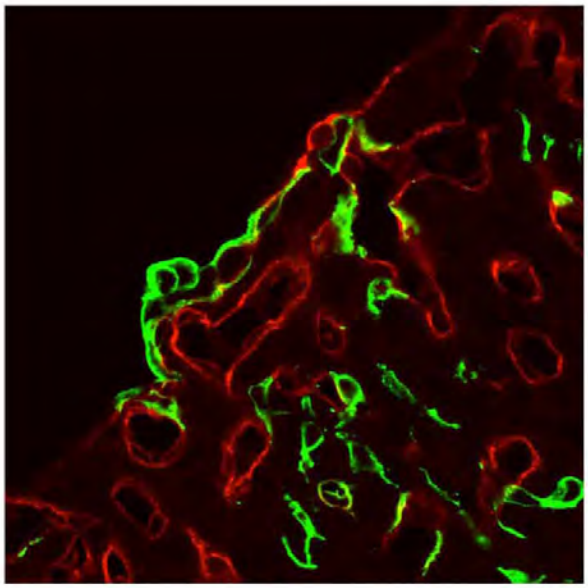

Figure: Epicardium-derived cells (green) undergoing EMT and migrating into the compact myocardium in the developing heart. Endothelial cells are stained with PECAM1 (red).

Chen Yan, Ph.D.

Synopsis

The second messengers cAMP and cGMP contribute to both normal physiological functions and cardiovascular diseases. Cyclic nucleotide phosphodiesterases (PDEs) that catalyze the degradation of cAMP and cGMP are essential for maintaining homeostasis, compartmentalization, and specificity of cyclic nucleotides. Increasing evidence has indicated that alterations in the expression and activation of different PDEs cause a number of diseases, many of which have been found to be improved by pharmacologically targeting these PDEs. PDEs are a highly promising class of therapeutic targets for drug development. Thus, defining the specific PDE isoforms responsible for the pathological pathways in cardiovascular diseases is essential for developing novel therapeutic strategies.

Our research program focuses on elucidating the roles and underlying mechanisms of PDE activation or inhibition in cardiovascular diseases, particularly from the perspective of revealing new molecular targets for pharmacologic modulation of cyclic nucleotide signaling in the treatment of cardiovascular diseases. Two primary research areas in our laboratory include:

1. Vascular smooth muscle cell phenotypic modulation and vascular disorders, such as hypertension, intima/media thickening, atherosclerosis and aortic aneurysms; and
2. Pathological cardiac remodeling and heart failure.

For example, we have recently discovered that the PDE1C isozyme is selectively induced in neointimal proliferating smooth muscle cells (SMCs) in disease vessels but not in medial contractile SMCs of normal vessels. Induction of PDE1C is essential for SMC proliferation and migration and neointimal hyperplasia by promoting growth factor receptor stability.

Lab Members:

- Si Chen, Graduate Student
- Lingfeng Lou – Graduate Student
- Chongyang Zhang – Graduate Student
- Yishuai Zhang – Postdoctoral Associate
- Qian Zhou – Staff Scientist

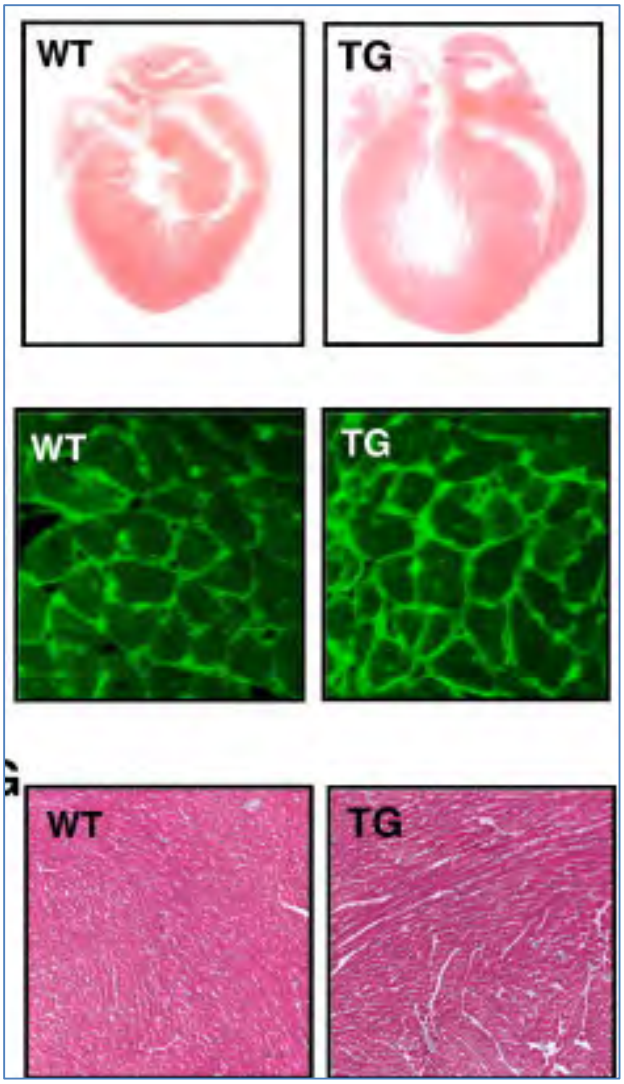

Figure: Transgenic mouse with cardiac expression of PDE3A1 have myocardial hypertrophy with larger cardiac myocytes and larger hearts.

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neointimal hyperplasia. Circ Res. 2015 Mar 27;116(7):1120-32.
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Peng Yao, Ph.D.

Synopsis

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. Better understanding of the pathological mechanisms underlying CVD will improve preventive and therapeutic interventions. Regulatory non-coding RNAs and RNA-binding proteins (RBPs) are important research areas in gene regulation and RNA biology. Our lab is interested in the understanding of pathophysiological function and molecular mechanism of new non-coding RNAs (and RBPs) and new modes of gene regulation in cardiac system and cardiovascular disease. We recently discovered a novel type of stress-responsive, protein-directed human RNA switch that regulates expression of vascular endothelial growth factor-A (VEGFA) in human monocytic cells and may play a role in ischemic cardiovascular disorders and cancers. Intriguingly, some of these RNA switches involve the interplay between microRNAs (e.g., miR-297, miR-574-3p) and RNA-binding protein hnRNP L. We also identified a novel mRNA processing mechanism, namely, the coding region alternative polyadenylation that expands human proteome by conversion of a Tyr genetic codon into a stop codon at the posttranscriptional level and regulates *VEGFA* mRNA translation in human monocytes and macrophages.

Our long-term research goals:

1. Understand the global and transcript-specific translational control mechanisms in cardiovascular system (e.g., regulation of ribosome activity, and riboswitch);
2. Investigate the role of non-coding RNAs (miRNA, mRNA 3'UTR) and RNA-binding proteins (e.g., hnRNP L) in translational control of gene expression in the heart;
3. Identify novel therapeutic targets (EPRS, and mitochondrial translation regulatory factors) in translational control pathways for treatment of cardiovascular diseases (CVD);
4. Discover new drugs (e.g., halofuginone) that target protein translation for CVD treatment.

Our current research interest includes:

1. 1) Pathophysiological function and regulatory mechanism of miRNA (or mRNA 3'UTR) and RNA-binding proteins in cardiac disorders;
2. 2) Riboswitch-like RNA switch mechanisms of gene regulation in mammalian system;
3. 3) The role of translation machinery and translational control in cardiac health and disease and therapeutic applications.

We employ various approaches of biochemistry, molecular and cellular biology, and genetic and surgical mouse models, to identify novel RNA-based molecular mechanisms that control gene expression and conduct pathophysiological function in cardiac system. In the long term, we are hoping to identify new drug targets and develop novel therapeutic approaches for the prevention and treatment of human cardiovascular diseases.

Major Methodology and techniques in Yao lab:

1. Methods for studying translational control: Polysome profiling coupled with RNA-Seq to examine global translational regulation; RiboTag-Seq using HA-tagged RPL22 transgenic mice to determine translatome in specific murine tissues; Ribosome profiling (Ribo-Seq) to map ribosome footprints of transcriptome (such as mRNA and lincRNA).
2. Mouse models of heart failure (HF): Isoproterenol s.c. injection or minipump implantation; Angiotensin II minipump implantation; Transverse aortic constriction (TAC). Phenotypic changes will be evaluated by H&E, WGA and trichrome staining. The cardiac functions will be assessed by echocardiography.
3. Construction of CRISPR-Cas9-directed gene-specific knockout mouse models.
4. Isolation of primary cardiac fibroblasts and myocytes from murine hearts for cell culture.

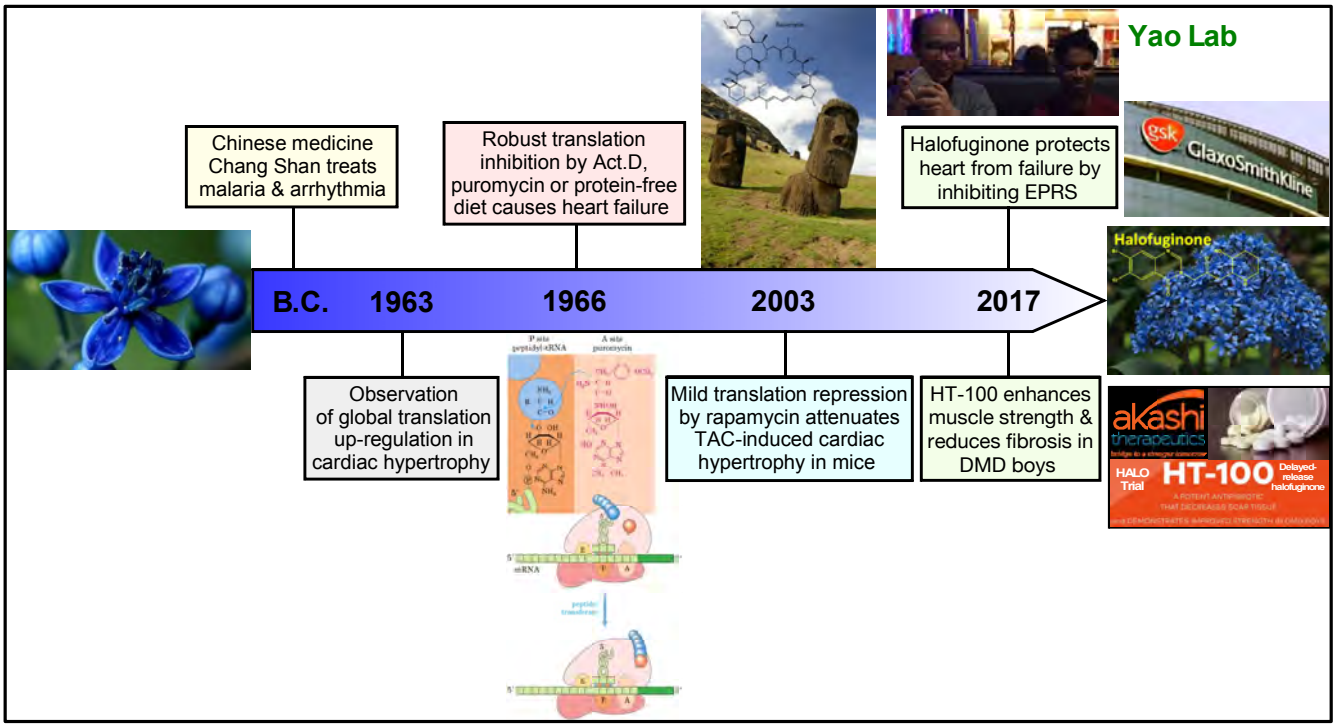

Figure: The human history of intervention of protein translation for treatment of cardiac hypertrophy and heart failure. In 2017, our laboratory discovered that halofuginone, a chemical compound from Chinese herbal medicine Chang Shan, could protect murine hearts from isoproterenol- and TAC-induced failure. GlaxoSmithKline plc (GSK) reported similar observations around the same time. We are currently investigating the molecular mechanisms of cardioprotection by halofuginone, and canonical and noncanonical functions of its target glutamyl-prolyl-tRNA synthetase (EPRS) in heart failure. This project will validate EPRS as a new drug target and establish mild translational inhibition as a novel therapeutic approach to treat CVD.

Publications

1. Yao P*, Wu JB, Lindner D, Fox PL. Interplay between miR-574-3p and hnRNP L regulates VEGFA mRNA translation and tumorigenesis. *Nucleic Acids Research* 2017 45(13): 7950-64 (*: *corresponding author*)
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5. Yao P, Poruri K, Martinis SA, Fox PL. Non-catalytic regulation of gene expression by aminoacyl-tRNA synthetases. *Top. Curr. Chem.* 2013 344: 167-87
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14. Ray PS, Jia J, Yao P, et al. A stress-responsive RNA switch regulates VEGFA expression. *Nature* 2008 457(7231): 915-19
15. Yao P, Zhou XL, et al. Unique residues crucial for optimal editing in the yeast cytoplasmic leucyl-tRNA synthetase revealed by a novel knockout yeast strain. *Journal of Biological Chemistry* 2008 283(33): 22591-600
16. Yao P, Zhu B, Jaeger S, Eriani G, Wang ED. Recognition of $tRNA^{Leu}$ by *Aquifex aeolicus* leucyl-tRNA synthetase during aminoacylation and editing steps. *Nucleic Acids Research* 2008 36(8): 2728-38

D. Training

Research Fellows at the CVRI

The CVRI faculty offer mentored research training to medical students, graduate students, and post-doctoral fellows. The CVRI is an outstanding environment for cardiovascular trainees.

- **Dr. Kelly Anderson** is a Postdoctoral Associate in the laboratory of Dr. Anderson. Dr. Anderson joined the laboratory of Dr. Douglas Anderson as a Postdoctoral Associate in November, 2016. She earned her B.S in Neuroscience at the University of Texas at Dallas and her Ph.D. in Genetics at the University of Texas Southwestern Medical School. At UT Southwestern, Kelly worked in the lab of Dr. Eric Olson to understand how long noncoding RNAs contribute to embryonic heart development.
- **Dr. John Allen Bennett** is a Postdoctoral Associate in the laboratory of Dr. Charles Lowenstein. Dr. Bennett received his Ph.D. in Pharmacology from the University of Rochester School of Medicine in 2015. He is studying the genetics of thrombosis.
- **Dr. Ryan Burke** is a Postdoctoral Fellow in the laboratory of Dr. Small. Dr. Burke joined the laboratory of Eric Small in August of 2015 as a Postdoctoral Research Associate. A native of northern California, he completed a B.S. in Biomedical Engineering in 2003, having studied at the University of California at Los Angeles and the University of Rochester. He was employed in the metabolic disease division at Merck for a year, then returned to complete a Ph.D. degree in Biomedical Engineering in the laboratory of Edward B. Brown III, focusing on nonlinear optical imaging of tumor stroma and its contributions to metastasis. After graduating in 2012, he completed a postdoctoral position in the laboratory of Bradford C. Berk at the University of Rochester, where he studied the contribution of inflammatory signaling to the inhibition of autophagy in response to altered blood flow patterns in atherosclerosis.
- **Dr. Venkata Subbaiah Kadiam Chinna** is a Postdoctoral Associate in the laboratory of Dr. Yao. Dr. Chinna received his Ph.D. in Viral pathogenesis and Therapeutics from the Dravidian University, Inda. He worked at the University of Kansas as a Postdoc and his research was on animal models and drug development of Neuro Degenerative diseases. His current research focuses on the role of noncoding RNAs in heart pathophysiology.
- **Dr. Janet Lighthouse** is a Postdoctoral Associate in the laboratory of Dr. Eric Small. Dr. Lighthouse received her Ph.D. from Stony Brook University, Stony Brook, New York. She studies novel genes involved in cardiac fibroblast plasticity during pathological remodeling and is the recipient of a 2-year postdoctoral fellowship from the American Heart Association.
- **Dr. Qing (Rex) Lyu** is a Postdoctoral Associate in the laboratory of Dr. Joseph Miano. Dr. Lyu received his Ph.D. from the School of Life Sciences, Tsinghua University, Beijing and is now studying the long non-coding RNA discovery and use of CRISPR-Cas9 for genome editing of cells and mice.
- **Dr. Pearl Quijada** is a Postdoctoral Fellow in the laboratory of Dr. Eric Small. Dr. Quijada received her Ph.D. in biology from the University of California, San Diego. She is studying the role of epicardium-derived progenitor cells in patterning the coronary vasculature during embryonic development. She is also evaluating novel signals originating from the epicardium that might stimulate regeneration of lost or damaged cardiac tissue and restore normal function after a myocardial infarction.
- **Dr. Shu-ya Zhang** is a Postdoctoral Associate in the laboratory of Dr. Jin. Shuya zhang joined the laboratory of Jin as a Postdoctoral Associate in October,2016. She graduted from The Fourth Military Medical University in China 3 years ago. Her Ph.D Major is Biochemistry and

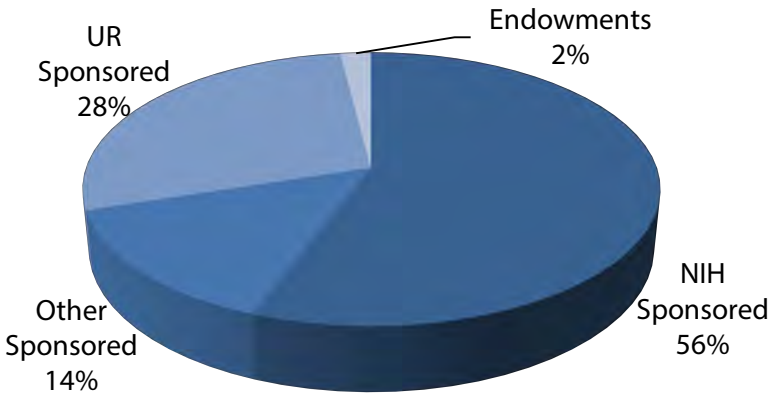
Molecular Biology and research focus on angiogenesis. She has worked previously at Ningxia medical university as a associate professor in Biochemistry and Molecular Department. Now Her research work is also focus on angiogenesis and heart valve development.

- **Dr. Yishuai Zhang** is a Postdoctoral Associate in the laboratory of Dr. Chen Yan. Dr. Zhang received his Ph.D. in cardiovascular pharmacology at the School of Pharmaceutical Science of Center South University, Changsha, Hunan, P.R. China. Dr. Zhang is exploring the underlying role and underlying mechanism of cyclic nucleotide phosphodiesterase in pathologic cardiac remodeling and dysfunction.
- **Dr. Jiangbin Wu** is a Postdoctoral Associate in the laboratory of Dr. Yao. Dr. Wu earned his BS in Biology Engineering from Xi'an Jiaotong University in 2009. He did graduate research at Tsinghua, and received his PhD in Cell and Molecular Biology and studied the function of hypoxia induced miRNAs in human cancer progression. He started his Postdoctoral at the City of Hope National Medical Center, studying molecular mechanism of DNA damage response in human.

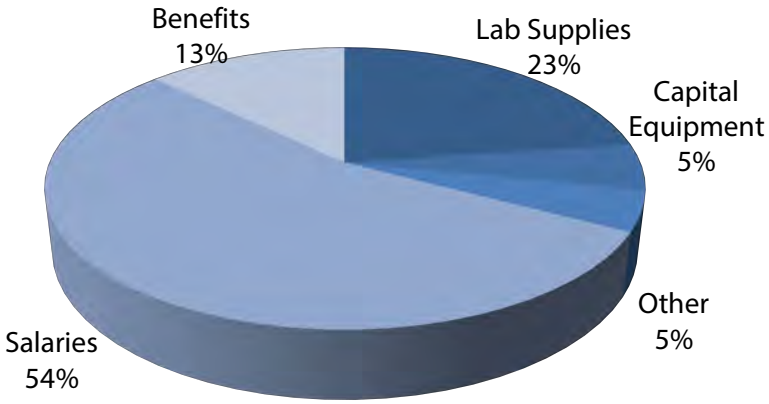
University of Rochester Graduate Students at the CVRI

- **Amanda Amoh** is a graduate student in the lab of Dr. Coeli Lopes. She received her B.S. from Connecticut College in 2015. She is studying Pharmacological Invesigation into Congenital Long QT Syndrome.
- **Qian Chen** is a graduate student in the lab of Dr. Bradford Berk. He received hs B.S. from Nanjing Normal University in 2012. He is studing the role of Pnpt1 in pathological vascular intima growth.
- **Si Chen** is a graduate student in the lab of Dr. Chen Yan. She received her B.S. from China Pharmaceutical University in 2012. She received a M.S. from SUNY Buffalo in 2014. She is studing the role of phosphodiesterase 10A on pathological cardiac hypertrophy and dysfunction.
- **Zachary Hilt** is a graduate student in the lab of Dr. Eric Small. He received his B.S. from St. John Fisher College in 2014. He is studying Platelet-derived Beta-2 Microglobulin is a Novel Regulator of Monocyte Homeostasis.
- **Chen Kaplan** is a predoctoral student in the lab of Dr. Coeli Lopes. She received her B.Sc. in biotechnology engineering from ORT Braude College, Karmiel, Israel in 2012.
- **Lingfeng Luo** is a graduate student in the lab of Dr. Chen Yan. He received his B.S. from Zhejiang University in 2012. He is studying Regulation and Function of Cyclic Nucleotide Phosphodiesterases in pathological Vascular Remodeling.
- **Daphne Pariser** is a graduate student in the lab of Dr. Craig Morrell. She received her B.S. from Flagler College in 2013. She is studying PF4 regulates T cell homeostasis.
- **Michael Trembley** is a predoctoral student in the laboratory of Dr. Eric Small. Mike received his B.S. in biochemistry from St. Lawrence University, Canton, NY. He is studying the role of epicardial cells in the regeneration of cardiac tissue.
- **Chao Xue** is a predoctoral student in the laboratory of Dr. Brad Berk. He is studying the role of CyclophilinA (CypA) and acetylated CypA in endothelial to mesenchymal transition in promoting pulmonary hypertension.
- **Chongyang Zhang** is a graduate student in Dr. Chen Yan's lab. He received his B.S. in 2015 from the State Univeristy of New York (SUNY) in 2015. He is studying the Role of PDE1C in Synthetic Smooth Muscle Cell Lysosomal Dysfunction mediated Vessel Remodeling in Atherosclerosis.

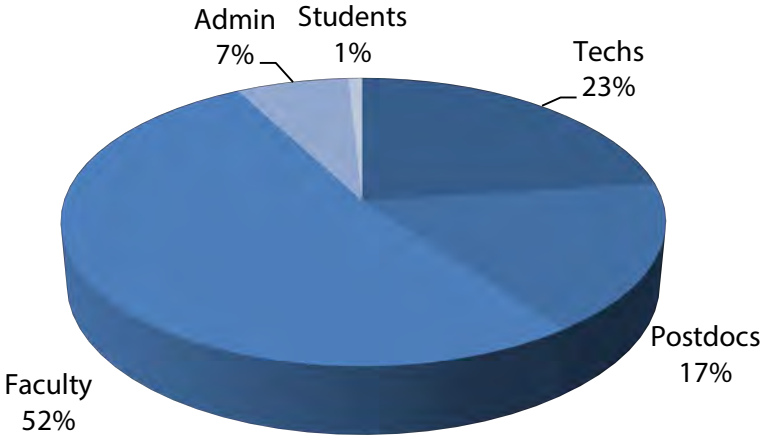
CVRI FY17 Operating Revenues



CVRI FY17 Operating Expenses



CVRI FY17 Salary & Benefits



F. Philanthropy

Mr. Richard T. Aab is an international entrepreneur and the chief executive officer of Idea Boxx, a company dedicated to conceptualizing, designing, and manufacturing products that improve and make a positive impact on productivity for businesses across industries. Over the last 40 years, his visionary leadership has fueled the success of his 15 companies, spanning a broad spectrum of industries including telecommunication services, software development, wealth advisory and management, energy consulting and resale, payroll, health care, and innovative design and development. Mr. Aab is a passionate spokesperson on behalf of medical research, and remains an active philanthropist committed to educational initiatives and our world community.

A graduate of Clarkson College, Mr. Aab earned his bachelor's degree in economics in 1971 and began his career as a financial analyst at Stromberg Carlson the following year. He joined Merrill Lynch in 1974 as an investment representative where he received world-class training in the field of wealth management. In 1979 he was recruited to become a vice president of investments at Paine Webber. In 1982, Mr. Aab founded ACC Corp., which became his first telecommunications public company in 1985. He established ACC's Canadian subsidiary and took ACC Telenterprises, Ltd. public in 1993. He later re-acquired and merged the two companies which, along with ACC's United Kingdom subsidiary, were purchased for $1.2 billion in 1998 to become part of AT&T.

Over the course of the next three decades, Mr. Aab went on to found or co-found nearly a dozen additional businesses, creating thousands of jobs and significant wealth for his employees and shareholders. In 1996, he co-founded US LEC Corp., a telecommunications company that he took public and grew to $425 million in revenues, servicing 28,000 business customers in 115 markets with more than 1,100 employees. He facilitated the merger of US LEC and PAETEC LLC, a private telecommunications company, enabling the newly combined PAETEC entity to become a NASDAQ listed company in 2007. He became vice chairman and a director of the board at PAETEC, where he remained until the company was acquired by Windstream Corp for $2.3 billion in 2011. In 2001, Mr. Aab created E-CHEX Inc., a payroll solutions company that grew to service more than 10,000 businesses nationwide. E-CHEX, now Ovation Payroll Systems, was acquired by Heartland Payment Services Inc. in 2012. Today, Mr. Aab is the CEO of four start-up companies, three of which — Hydra Rinse LLC, ProNatural Brands, and United Sources Sought, Inc. — germinated from Idea Boxx, where he also currently serves as CEO.

A longstanding supporter of the University of Rochester, Mr. Aab is an engaged volunteer for the University and its medical center. He is an active University trustee, a board member of the University of Rochester Medical Center (URMC), and served as the co-chair for URMC's successful $650 million campaign. He has volunteered his time serving on the Health Affairs Committee, the Research and Innovation Committee, and the Financial Planning Committee, among others, and his generous contributions to the University reflect his dedication to medical research, benefiting the School of Medicine and Dentistry, Golisano Children's Hospital, and Strong Memorial Hospital. In 2007, the Aab Cardiovascular Research Institute was named in his honor in recognition of his leadership support and extraordinary commitment to the Institute's mission. In addition to his University involvement, Mr. Aab has also served as a trustee of the Rochester Institute of Technology and the Greater Rochester area public broadcasting station, WXXI. He remains an active fundraiser and advocate for numerous charities and organizations throughout the community.

Mr. Aab lives in Fairport, New York and has two children, Melissa and Richard.

The Ganatra Family

As global philanthropists, the Ganatra Family exemplifies an unwavering commitment to excellence, service, and innovation. Tansukh Ganatra, now retired, was vice-chairman and CEO of North Carolina-based US LEC, which he co-founded with University Trustee Richard T. Aab. Previously, Tansukh was president and chief operating officer for ACC Corp. in Rochester.

Born in Uganda, Tansukh Ganatra met his wife, Sarla, in Kenya, and they immigrated to the U.S. in 1969. The family lived in Rochester for 21 years before moving to Charlotte, North Carolina in 1990. Sarla volunteers at the Hindu Center of Charlotte and is active in the Sanskruti Foundation. Their son, Rajesh, obtained his accounting degree at the University of North Carolina-Charlotte in 1994 and works in philanthropy, web design, and financial management.

In 2015, the family created the Tansukh, Sarla and Rajesh Ganatra Distinguished Professorship in Pediatric Cardiac Surgery out of gratitude to the University of Rochester Medical Center pediatric doctors who helped their family and loved ones. The professorship is part of the family's longtime support of pediatric cardiology at the Medical Center. Tansukh is a member of the advisory board at the Aab Cardiovascular Research Institute, where the Ganatra family has pledged a significant portion of their family estate. The Ganatra Family Atrium in the Golisano Children's Hospital is named in their honor through a generous gift by Richard Aab.

The Ganatras have also been honored for their significant philanthropic contributions in India, which include funding for rural education and medical relief, as well as for the spiritually revered Shri Hari Mandir temple in Porbandar, Gujarat, India.

G. Scientific Advisory Board

Edward A. Fisher, M.D., M.P.H., Ph.D.
The Leon H. Charney Professor of Cardiovascular Medicine, Professor of Medicine (Cardiology), Pediatrics, and Cell Biology; Director, Marc and Ruti Bell Vascular Biology and Disease Program; Director, Center for the Prevention of Cardiovascular Disease; Director of Translational Research, Clinical and Translational Science Institute, NYU School of Medicine, Adjunct Faculty, Mount Sinai School of Medicine and Rockefeller University, New York, NY.

José Jalife, M.D.
Professor of Internal Medicine and The Cyrus and Jane Farrehi Professor of Cardiovascular Research, Professor of Molecular & Integrative Physiology, Co-Director, Center for Arrhythmia Research,
University of Michigan, Ann Arbor, MI.

Aldons J. Lusis, Ph.D.
Vice Chair, Department of Human Genetics, Professor, Department of Human Genetics, Professor, Department of Microbiology, Immunology, and Molecular Genetics, Professor, Department of Medicine and Department of Microbiology and Molecular Genetics,
University of California, Los Angeles, CA.

Jeffery D. Molkentin, Ph.D.
Professor, Howard Hughes Medical Institute Investigator, Professor, UC Department of Pediatrics, Microbiology and Immunology Pulmonary, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Eric N. Olson, Ph.D.
Professor and Chair, Department of Molecular Biology, Robert A. Welch Distinguished Chair, Annie and Willie Nelson Professor, Pogue Distinguished Chair in Research on Cardiac Birth Defects,
University of Texas Southwestern Medical Center, Dallas, TX.

William C. Sessa, Ph.D.
Alfred Gilman Professor of Pharmacology and Professor of Medicine (Cardiology); Vice Chairman, Pharmacology; Director, Vascular Biology & Therapeutics Program, Yale School of Medicine, New Haven, CT.

Alain Tedgui, Ph.D.
Director, PARCC (Paris-Cardiovascular Research Center), European Editor, Arteriosclerosis, Thrombosis and Vascular Biology, Directeur de Recherche (Research Professor) Inserm, “classe exceptionnelle”, Paris, France.

Andrew S. Weyrich, Ph.D.
Professor, Internal Medicine, Department of Microbiology and Immunology
Pulmonary Adjunct Professor, Pathology, University of Utah School of Medicine, Salt Lake City, UT