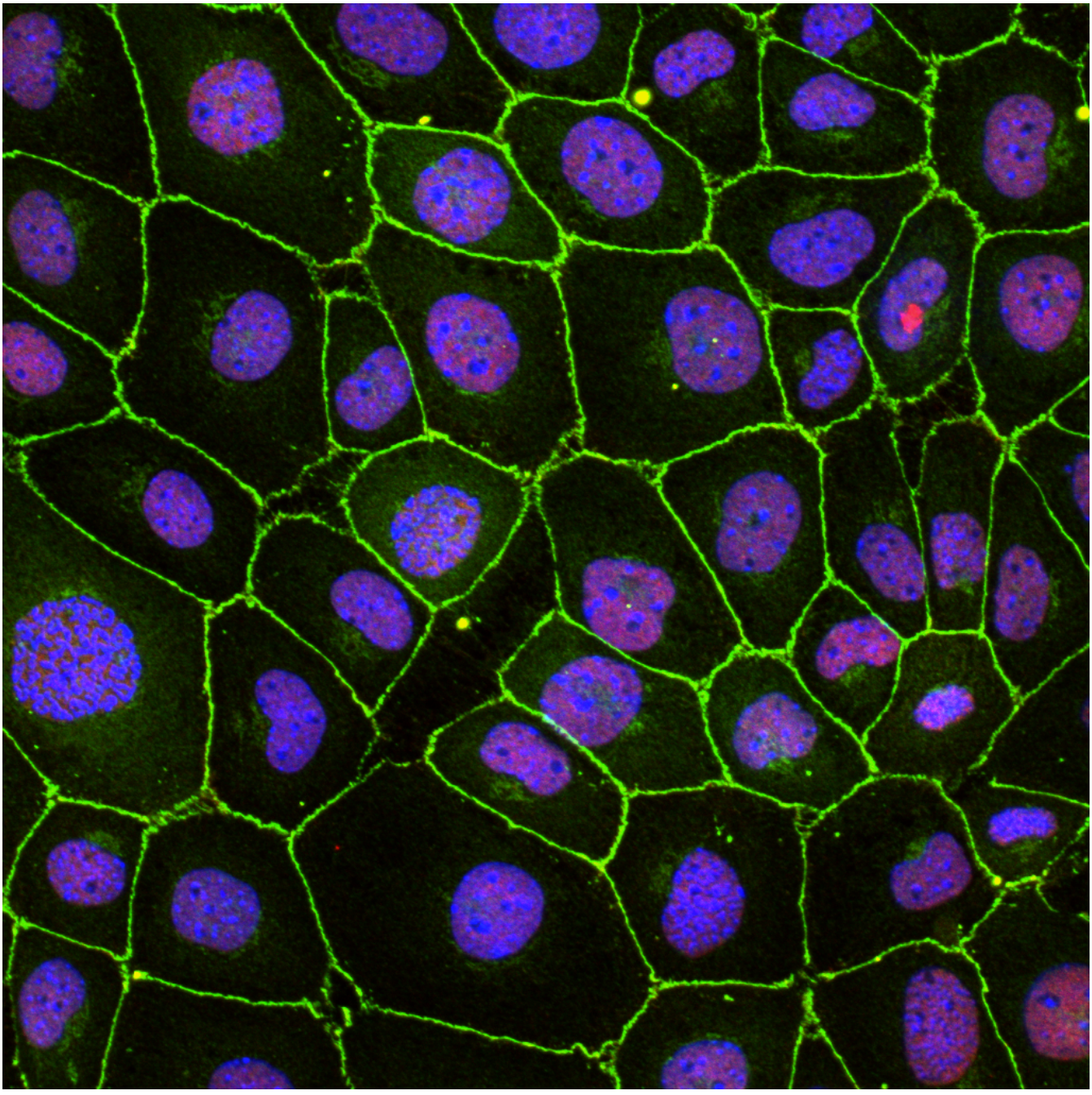
Aab Cardiovascular Research Institute
University of Rochester Medical Center



Annual Report
July 2018

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A. Overview

From the Director

This has been a year of program building for the Aab Cardiovascular Research Institute. We are forming new collaborative teams that connect researchers to our patients. We've developed three interdisciplinary teams organized around clinically relevant areas, and we plan for two more. 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. Eric Small. 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. Charles Lowenstein, examining endothelial interactions with cardiac fibroblasts,
- Dr. Peng Yao, determining the translational regulation of fibroblast activation.
- Dr. Jeffrey Alexis, an advanced heart failure cardiologist who studies cardiac fibrosis in patients with mechanical assist devices.

(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. Scott Cameron, a vascular cardiologist who treats patients with thrombosis and studies how myocardial infarction alters platelets in humans,
- Dr. Charlie Lowenstein, who studies the genetics of patients with thrombosis.

(3) The **CVRI Program in Angiogenesis** is led by Dr. Zheng-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.

We now plan to build two new programs to complement our established programs.

(4) The **CVRI Program in Neurovascular Inflammation** is led by Dr. Berk & Dr. Halterman. Ischemic stroke can damage the blood-brain barrier, leading to inflammation, thrombosis, and neuronal injury. We will study cellular pathways leading to neurovascular inflammation. Members of our Neurovascular Inflammation Team include:

- Dr. Mark Halterman, a neurologist who explores inflammatory signaling networks in global brain ischemia,

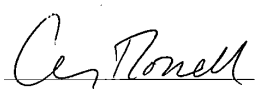
- Dr. Brad Berk, who studies endothelial mediators of vascular inflammation,
- Dr. Joseph Miano, who characterizes the transcriptional response of smooth muscle cells to vascular injury,
- Dr. Craig Morrell, who studies how platelet mediators activate monocytes and increase brain inflammation.
- Dr. Scott Cameron, who studies ischemic alterations of platelet function

(5) The **CVRI Program in Arrhythmias** will be led by Dr. Coeli Lopes and Dr. Ilan Goldenbeg. The University of Rochester is the world leader in clinical research into sudden cardiac death. We have run 5 large multi-center clinical trials testing the effect of cardiac devices upon sudden death in high-risk patients. We will use animal and cellular models to study how regulators of ion channels cause electrical instability leading to sudden death. Members of our Arrhythmia Studies Team include:

- Dr. Coeli Lopes, who explores the effect of genetic variants upon ion channel function,
- Dr. David Auerbach, who studies genetic diseases caused by mutations in ion channels which lead to seizures and sudden death,
- Dr. Doug Anderson, who has identified novel micro-peptides that regulate ion channel function,
- Dr. Ilan Goldenberg, who runs large-scale clinical trials of patients at risk for sudden cardiac death.

Our new programs in Neurovascular Inflammation and Arrhythmias will lead to more collaborations and training opportunities within the CVRI and the University as a whole. Our established programs in Cardiac Fibrosis, Thrombosis and Angiogenesis are leading to 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



Craig N. Morrell, DVM, PhD
Associate Director,
Aab Cardiovascular Research Institute

CVRI Facts Fiscal Year 2018

Personnel

Faculty	13
Research Faculty	3
Postdoctoral Fellows	14
Adjunct/Visiting Faculty	3
Graduate Students	12
Technical & Administrative Staff	21

Finances

NIH Grant Funding	$3.2M
Other Funding	$3.1 M
Total Operating Revenue	$6.3 M
Salary & Benefits	$4.7 M
Supplies & Equipment	$1.6 M
Total Expenses	$6.3 M

Scientific Publications

Publications Academic Year 2018:	41

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

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

Craig N. Morrell, D.V.M., Ph.D.
Associate Professor, Department of Medicine,
Associate Director, Aab Cardiovascular Research Institute

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

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

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

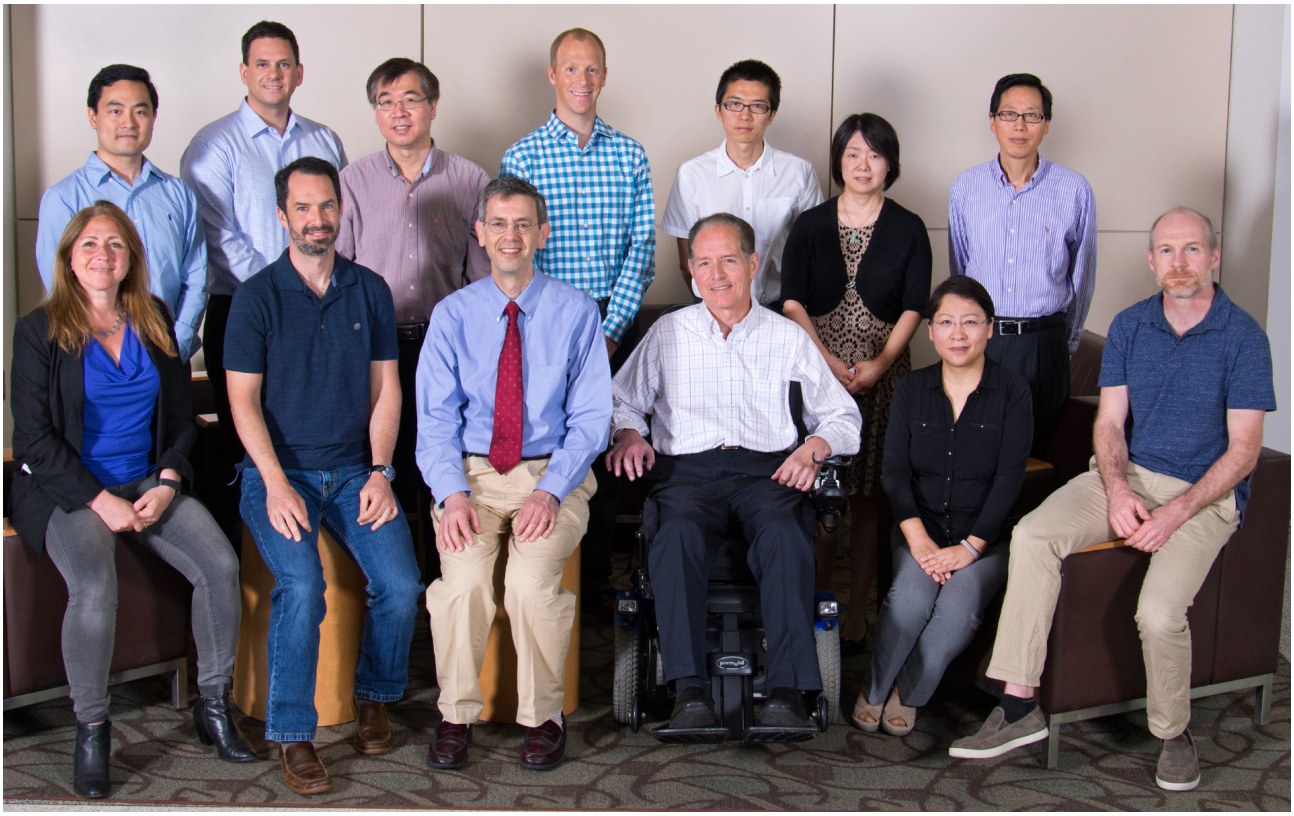
Xu, Suowen, PhD
Research Assistant Professor, Department of Medicine, Aab CVRI

Adjunct/Visiting Faculty

Wenting Du, Ph.D.
Resident Physician, Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine

Jinping Wang, Ph.D.
Clinical Pharmacist, Shenzhen Second People's Hospital

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



The faculty of the Aab 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

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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*

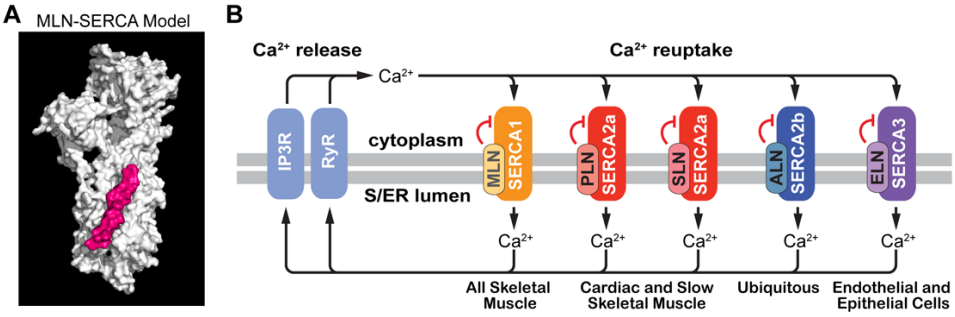

Figure 1. A family of calcium-regulatory micropeptides. Putative lncRNAs can encode peptides translated from small open reading frames, called micropeptides. (A) We identified Myoregulin (MLN), a 46 amino acid transmembrane micropeptide (pink) related to Phospholamban (PLN) and Sarcolipin (SLN), that 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.

Biological Chemistry. 287(42):35351-9. PMID: 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.
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David S. Auerbach, PhD

Synopsis

We are a translational research group that utilize cellular, animal, and patient database approaches to investigate the co-prevalence, risk, and mechanisms for neuro-cardiac electrical disturbances and Sudden Unexpected Death in Epilepsy (SUDEP.)

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. Our lab takes a multi-system approach to explore the implications of genetic ion channel diseases on neuro-cardiac electrical function. For example, in severe genetic forms of epilepsy, in addition to seizures, there are alterations in cardiac electrical function, cardiac arrhythmias, and sudden death (Auerbach DS et al. 2013.)

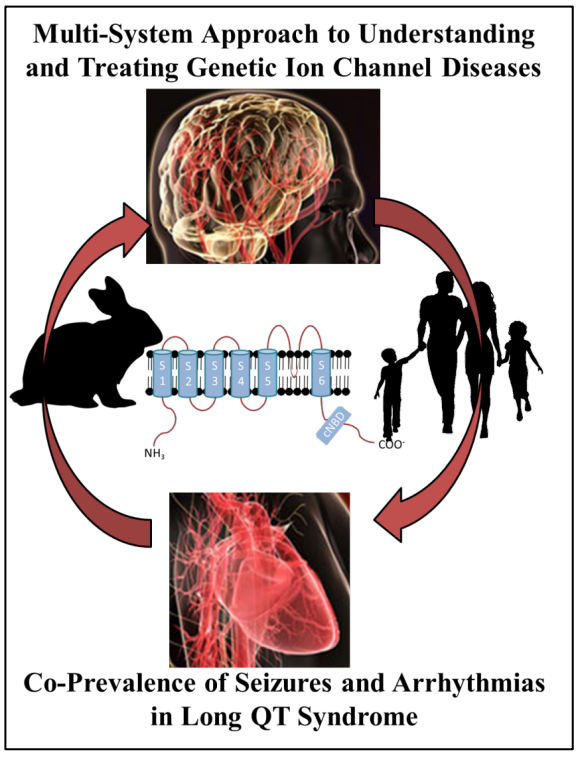
Next, approaching these neuro-cardiac investigations in the opposite direction, we are assessing the co-prevalence and severity of seizures and cardiac arrhythmias in a classically studied cardiac disease, called Long QT Syndrome (LQTS, Auerbach et al. 2016.) LQTS is a genetic disease, characterized by cardiac electrocardiographic (ECG) pathologies, arrhythmias, and a high risk of sudden death. Mutated genes in LQTS1-3 are expressed in the heart and brain, and anti-seizure drugs alter the risk of arrhythmias in LQTS patients (Auerbach et al. 2018.) Using both LQTS patient registries and animal/cellular models of LQTS we are exploring the mechanisms for 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.

Using cellular and animal models of LQTS with the same mutations as seen in LQTS patients with seizures, plus state of the art *in vivo/in vitro* techniques (e.g., radiotelemetry ECGs & single cell electrophysiology), we are examining the 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: Molecular, 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 events are one mechanism for SUDEP. Cardiac ECG abnormalities and arrhythmias are reported surrounding seizures and preceding SUDEP. Also, many genetic forms of epilepsy, especially SUDEP cases, are associated with mutations in genes expressed in both the brain and heart.

An algorithm that integrates biomarkers for each of the proposed mechanisms for SUDEP will revolutionize our ability to predict and prevent SUDEP. Starting with the heart, we are testing whether alterations in cardiac and autonomic nervous system measures are associated with a high risk of SUDEP. Using the NIH funded Center for SUDEP Research database of multi-day recordings from multiple systems in ~1000 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.

Project 3: Novel Cardiac ECG-Based Methods to Distinguish Epileptic vs. Psychogenic Seizures

Seizures are classically thought to be due to electrical disturbances in the brain (epileptic seizures, ES), and recorded on the electroencephalogram (EEG). Yet, 30-40% of patients who are not controlled by anti-seizure medications do not have ES. The seizures have physical and behavioral manifestations indistinguishable from ES, but there are no EEG abnormalities. These non-epileptic seizure events are likely a response to emotional or social distress, and are called psychogenic non-epileptic seizures (PNES). The risks, treatments, and prognosis in ES vs. PNES differ substantially. Long-term inpatient video/EEG/ECG monitoring is the gold standard diagnostic test for discriminating between ES and PNES events. However, it is burdensome, expensive, often inconclusive, and not available in many affected populations worldwide. Thus, using in-house patient recordings and analytical tools, we are developing cost effective outpatient methods (wearable technology) and cardiac/autonomic measures to distinguish ES from PNES.

Lab Members

- Joshua Brown – University of Rochester Undergraduate (Biochemistry)
- Ahmed Selmi – University of Rochester Undergraduate (Biomedical Engineering)
- Matthew Wang – John Hopkins Undergraduate (Biomedical Engineering)

Publications

1. Wang M, Szepietowska B, Polonsky B, McNitt S, Moss AJ, Zareba W, **AUERBACH DS***. Risk of

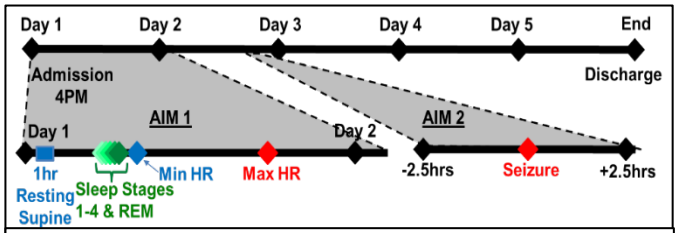


Fig. 2: Study design & timepoints for assessing repolarization measures at baseline & preceding/following a seizure.

Cardiac Events Associated With Antidepressant Therapy in Patients With Long QT Syndrome. Am J Cardiol. 2018 Jan 15;121(2):182-187. **Corresponding author.*

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4. Carrell ST, Carrell EM, **AUERBACH D**, Pandey SK, Bennett CF, Dirksen RT, Thornton CA. Dmpk gene deletion or antisense knockdown does not compromise cardiac or skeletal muscle function in mice. Hum Mol Genet. 2016 Aug 13
5. **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 Oct 18;87(16):1660-1668. **Corresponding author.*
6. Stables CL, **AUERBACH DS**, Whitesall SE, D'Alecy LG, Feldman EL. Differential impact of type-1 and type-2 diabetes on control of heart rate in mice. Auton Neurosci. 2016 Jan;194:17-25.
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10. Noujaim SF, Kaur K, Milstein M, Jones JM, Furspan P, Jiang D, **AUERBACH DS**, Herron T, Meisler MH, Jalife J. A null mutation of the neuronal sodium channel Nav1.6 disrupts action potential propagation and excitation-contraction coupling in the mouse heart. FASEB January 2012 26:63-72.
11. **AUERBACH DS**, Jalife J. "Substrates and Triggers for the Initiation of Arrhythmias" Physiology News. Winter 2011. 85: 15–17
12. **AUERBACH DS**, Grzeda KR, Furspan PB, Sato PY, Mironov S, Jalife J. Structural heterogeneity promotes triggered activity, reflection and arrhythmogenesis in cardiomyocyte monolayers. J Physiol. 2011 May 1;589(Pt 9):2363-81.

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

Synopsis

The major goal of my laboratory is to understand the molecular mechanisms of cardiovascular disease (CVD), especially atherosclerosis, hypertension, and stroke. The broad scope of my research is to study the roles of oxidative stress and inflammation in the early development and progression of CVD. Among the cells present in the vessel wall, we have focused on endothelial cells (EC), macrophages, and vascular smooth muscle cells (VSMC). We have developed both in vitro and in vivo models to simulate known human risk factors such as high cholesterol, smoking (oxidative stress), and disturbed blood flow (areas where atherosclerosis develops earliest such as vessel branch points). We use contemporary methods: genetic screens, RNA–Seq, transgenic mice, in vitro flow simulators, and mass spectrometry for metabolism and identification of protein modifications.

Project 1 (R01 HL049192): 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 EC. Second, it binds to a unique receptor on the membranes of EC and VSMC. A key pathogenic mechanism is that it participates in a positive feedback loop in VSMC 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 VSMC. 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. Mechanistic analysis using cultured mouse pulmonary microvascular EC and human pulmonary microvascular EC showed that eCypA and AcK-CypA stimulated EC inflammatory signals: increased VCAM1 (vascular cell adhesion molecule 1) and ICAM1 (intercellular adhesion molecule 1), phosphorylation of p65, and degradation of IkB. Extracellular CypA and AcK-CypA increased EC apoptosis measured by TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) staining, Apo-ONE assay, and caspase 3 cleavage. Oxidative stress stimulated CypA and AcK-CypA secretion, which further promoted EC oxidative stress. AcKCypA, compared with CypA, stimulated greater increases in apoptosis, inflammation, and oxidative stress. MM284, a specific inhibitor of eCypA, attenuated EC apoptosis induced by CypA and AcK-CypA. In conclusion, EC-derived CypA (especially AcK-CypA) causes PAH by a presumptive mechanism involving increased EC apoptosis, inflammation, and oxidative stress. Our results suggest that inhibiting secreted eCypA is a novel therapeutic approach for PAH.

Project 2: Mechanisms of vascular remodeling – PDE10A, PNPT1, and RPL17.

A long-term goal in treating atherosclerosis and hypertension is to understand the mechanisms that regulate the structure of blood vessels; a process termed "vascular remodeling." An important predictive phenotype for human cardiovascular disease is vascular remodeling in the carotid artery represented by the measurement termed intima-media thickening (IMT). IMT is mediated by endothelial cell (EC) dysfunction, vascular smooth muscle cell (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.) To identify novel genes responsible for intima growth, we used a genetic approach in a panel of mouse-inbred strains. Briefly, carotid remodeling was induced by partial ligation in 17 inbred strains. Genome-wide association analysis was performed on mouse carotid exposed to d-flow. 17 SNPs and 7 new genes associated with intima growth were discovered, including phosphodiesterase 10A **(**PDE10A), polyribonucleotide nucleotidyltransferase 1 (PNPT1), and ribosome protein-like 17 (RPL17). Transcriptomic and bioinformatic analyses revealed significant differences in inflammation, cell cycle and RNA degradation.

Project 2A (R01 HL134910): Phosphodiesterase 10A (PDE10A): There is strong human clinical data as well as mouse genetic and biochemical data to support a key role for PDE10A in atherosclerosis. The Stockholm Atherosclerosis Gene Expression (STAGE) study in patients with coronary and carotid atherosclerosis found a significant increase in PDE10A expression in atherosclerotic arteries. Furthermore, we found increased expression of PDE10A in human carotid lesions, especially in plaque cells with morphologic features of VSMC and inflammatory cells. Our major hypothesis is that increased PDE10A expression, by inhibiting cAMP signaling, promotes synthetic VSMC phenotype transition and macrophage inflammasome expression/activation; and thus, stimulates intimal growth. The overall objective is to investigate the mechanisms that regulate expression of PDE10A, and PDE10A's specific enzymatic role in the processes responsible for intimal hyperplasia. PDE10A promotes macrophage inflammatory response by facilitating inflammatory mediator expression, and NLRP3 inflammasome expression and activation. Specifically, we will study the effect of dopamine binding to the dopamine D1 receptor in VSMC and macrophages, thereby increasing cAMP on inflammatory gene expression through NF-kB and inflammasome pathways.

Project 2B (R01 HL140958): Polyribonucleotide nucleotidyltransferase 1 (PNPT1): PNPT1 is a 3'-5' exoribonuclease that is required for import and processing of RNA in mitochondria. High level PNPT1 expression correlated with decreased IMT and inflammation in the carotid ligation model suggesting it was protective. The goal of this research program is to understand how PNPT1 restricts inflammation and atherosclerosis, focusing on novel transcriptional programs and mechanisms that link d-flow-mediated signaling through mitochondrial homeostasis, mitophagy/autophagy and cellular RNA processing pathways to EC dysfunction and cardiovascular disease.

Project 2C: Ribosome-protein-like 17 (RPL17): RPL17 was highly linked to intima growth in an inverse manner in our initial screen. RPL17 is an important protein because it is located at the exit tunnel of the large ribosome subunit, so it is strategically situated to influence the rate of protein elongation and termination. It is also located on the surface of the ribosome so it is likely to assemble protein complexes that play a role in regulating the processing and function of newly synthesized proteins. This gene was highly expressed in cells with low proliferation rates and in vessels with little intima. The research goal for RPL17 is to characterize its role in the ribosome to study its effect on protein translation and to identify pathways by which flow pattern regulates its expression. Future work on RPL17 will focus on its role in regulating ribosome composition, translational efficiency, binding of other proteins to the ribosome (especially since it is located on the surface) and its role in regulating protein folding since it is part of the exit tunnel.

Lab Members

- Chia (George) Hsu, Postdoctoral Fellow
- Chen Qiang, Graduate Student
- Sharon Senchanthisai, Technical Assistant
- Mark Sowden, Research Assoc. Professor
- Mary Wines-Samuelson, Research Asst. Professor
- Chao Xue, Graduate Student

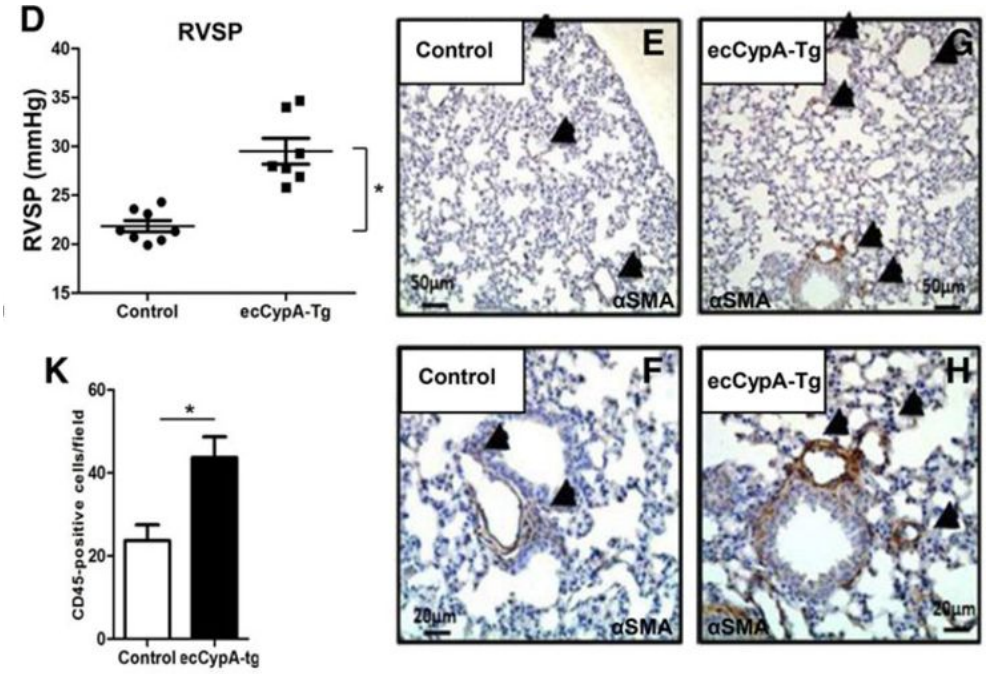

Figure shows (D) increased right ventricular systolic pressure (RVSP) in mice that overexpress CypA in endothelial cells (ecCypA-Tg); (E&F, 40x and 100x) control mice lungs show low expression of VSMC as measured by alpha smooth muscle actin expression (αSMA), brown stain indicated by arrowheads; while (G&H, 40x and 100x) ecCypA-Tg show high αSMA expression.

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Project 1: Cyclophilin A and Pulmonary Hypertension

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Project 2: Flow responsive endothelial Pnpt1

Project 2A: Phosphodiesterase 10A (PDE10A):

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Project 2B: Polyribonucleotide nucleotidyltransferase 1 (PNPT1)

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Project 2C: Ribosome-protein-like 17 (RPL17)

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

Synopsis

Platelets are blood cells involved in thrombosis (blood clotting) and inflammation. Our laboratory studies signaling pathways in platelets as they relate to thrombotic diseases. We are specifically interested in how platelet responses differ in males and females, and how platelets may contribute to adverse remodeling of the heart after a heart attack. We also study how platelets remodel blood vessels in aneurysmal disorders of arteries and veins. We utilize contemporary cell biology, biochemistry, molecular biology, and animal models to address these questions. Our overall goal is to evaluate gaps in clinical care, identify viable signaling pathways in human tissue for drug intervention, and then finally to utilize animal models to test the working hypothesis.

Project 1: The role of gender in platelet responsiveness during myocardial infarction

Using a mouse model of heart attack and enrolling patients at our hospital with heart attacks, we have shown that platelet RNA and the platelet proteome as well as platelet drug responsiveness are all different in males and females. This urged us to consider a more personalized approach to anti-platelet drug use to improve patient outcomes.

Project 2: The role of platelet proteins in aortic aneurysmal disease.

Using a mouse model of abdominal aortic aneurysm (AAA) and, along with our colleagues in vascular surgery and cardiac surgery at the University of Rochester, we study platelet function both as a consequence of aortic disease and in promoting aortic disease progression. We use genetic approaches to identify platelet targets useful in modulating vascular health.

Project 3: The role of platelets in the progression of peripheral vascular disease (PAD).

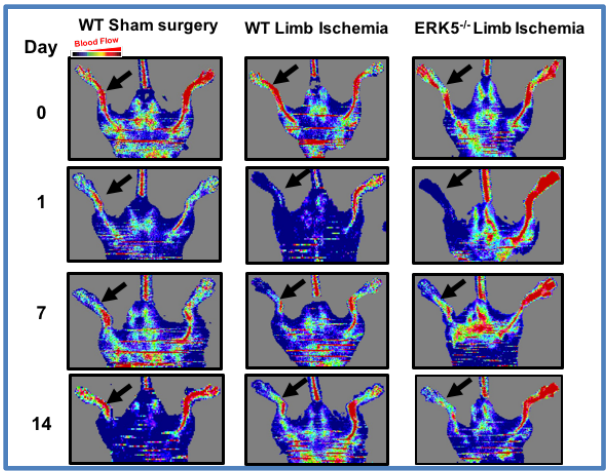
Using a mouse model of extreme peripheral artery disease (PAD) called critical limb ischemia (CLI) we have identified important signal transduction mechanisms which lead to dysregulated platelet behavior, and re-growth of diseased blood vessels. CLI is sometimes considered to be a 'heart attack of the leg' and is responsible for amputation and death in patients afflicted with advanced PAD. We recently extended our studies to human platelets from PAD patients with our colleagues in vascular surgery at the University of Rochester and in the Department of Pathology at the Johns Hopkins Hospital.

Project 4: Mechanisms of venous thromboembolism

Using our existing database of patients with venous thromboembolism (VTE), we are studying various clinical and translational aspect of high risk pulmonary embolism (blood clots in the lung) locally and as part of the National Pulmonary Embolism Response Team (PERT) consortium.

Project 5: Chronic vein disease and platelet reactivity

Using publicly-available human population data, we have shown that varicose veins and chronic vein disease are risk factors for heart attack and stroke. We are examining the role that circulating platelets play in these diseases. This work is conducted with our colleagues in vascular surgery.



Lab Members

- Dr. Doran Mix (postdoctoral fellow in surgery)
- Hamza Sadhra (UR medical student)

Publications

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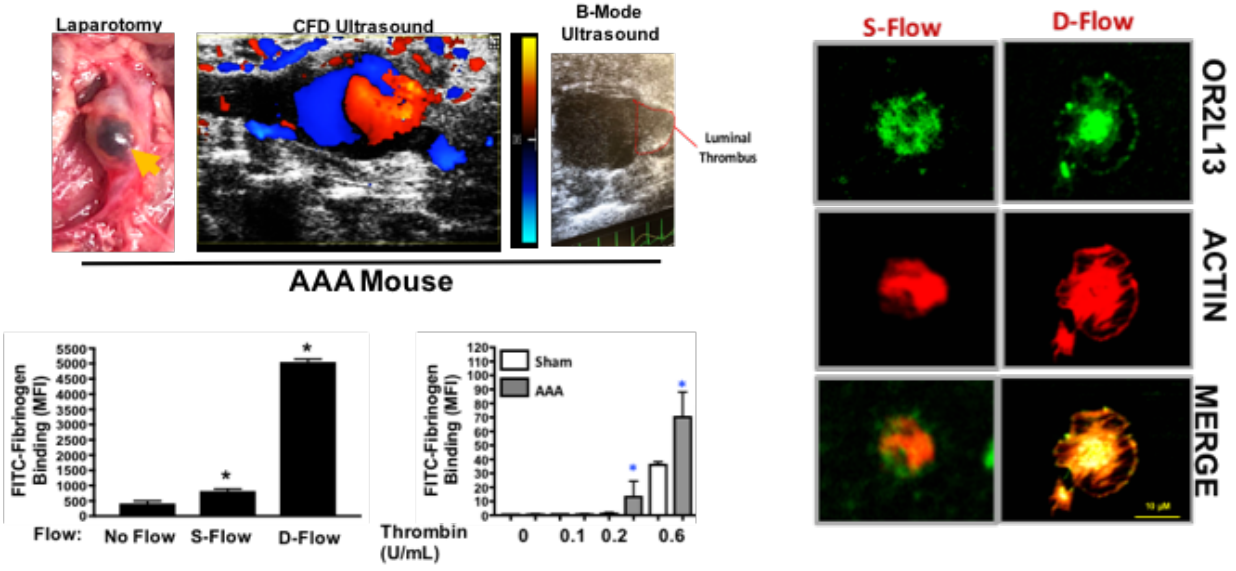

Disturbed flow in aortic aneurysmal disease mechanically activates platelets
C57BL/6J mice with infrarenal abdominal aortic aneurysms (AAA, yellow arrow indicates mural thrombus) or sham operation. Color Flow Doppler (CFD) ultrasound with disturbed flow (D-flow) indicated by alternating directions of blood flow (red<>blue), with intramural luminal thrombus. Graphs: *Ex vivo* or *in vivo* platelet exposure to steady laminar flow (S-flow) or disturbed flow (D-flow) followed by *ex vivo* platelet stimulation with thrombin shows augmented platelet activation (fibrinogen binding to activated GPIIb/IIa receptor). Right: following spreading on a fibrinogen matrix, platelets were doubled stained with a FITC-tagged antibody to mechanosensor OR2L13 (green) and rhodamine-tagged phalloidin for actin (red). The yellow bar is 20 µm.

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.
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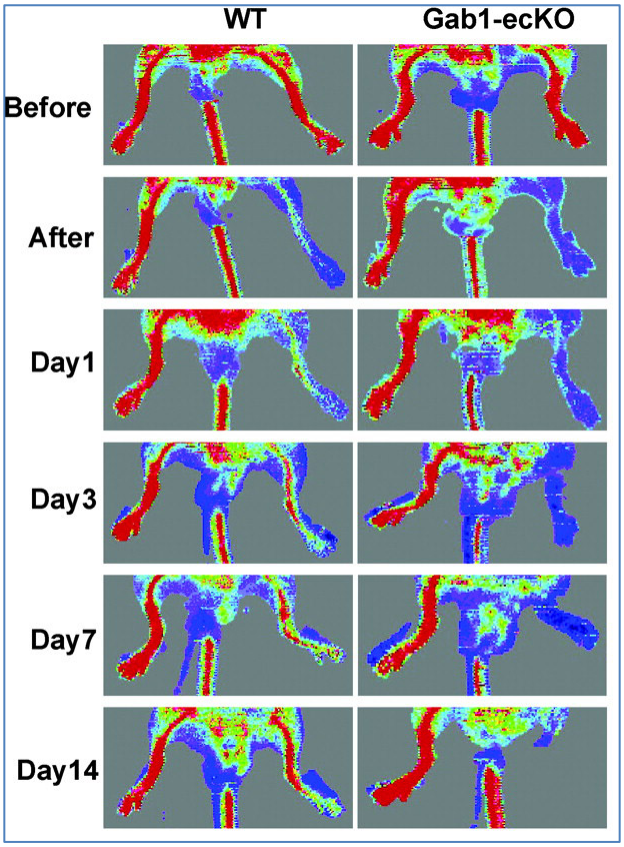

Figure: Hind limb ischemia is more severe in the absence of Gab1 from endothelial cells.

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-dependent hypertension in mice. We showed that Axl regulates reactive oxygen species production in kidney's leukocytes in hypertension (brown staining, Figure).

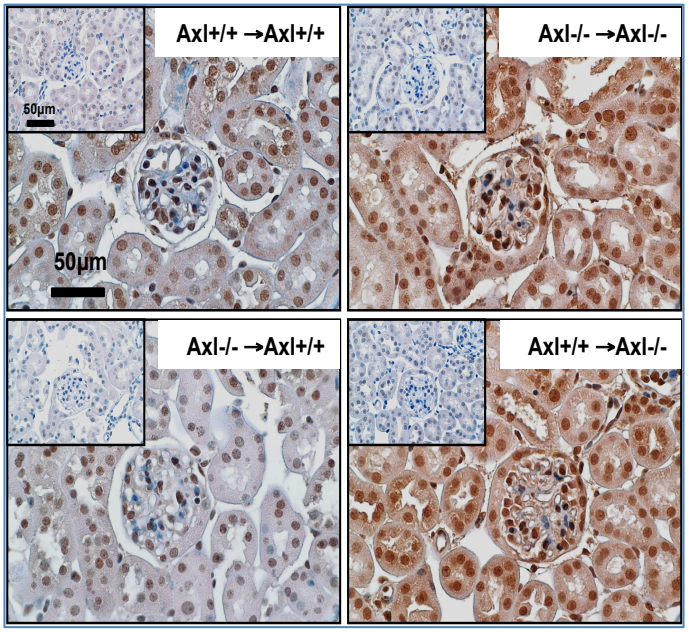

Figure. Representative images represent the levels of reactive oxygen species in kidneys from Axl chimeras during salt-dependent hypertension. Positive cells are dark brown. Negative controls as shown as insets. Magnification bar is 50μm. Adapted from *Batchu et al., Hypertension 2013*

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

1. Batchu SN, Hughson A, Wadosky KM, Morrell CN, Fowell DJ, Korshunov VA. Role of Axl in T-lymphocyte survival in salt-dependent hypertension. *Arterioscler Thromb Vasc Biol* 2016;36(8):1638-46.
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Project 2 publications

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2. Smolock EM, Machleder DE, Korshunov VA, Berk BC. Identification of a genetic locus on chromosome 11 that regulates leukocyte infiltration in mouse carotid. *Arterioscler Thromb Vasc Biol* 2013;33:1014-1019.
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

The Lopes lab research generally focus on the dysregulation of cardiac ion channels and consequent cardiac arrhythmia susceptibility caused by either genetic and/or cardiac disease factors. In particular we have published many translational studies on mutation-specific treatment efficacy and cardiac risk susceptibility involving the Long QT patient population, the most common form of inherited cardiac arrhythmias. These include high profile work in collaboration with computer modelers to risk stratify patient risk (*Sci Transl Med*. (2011) and *J Am Coll Cardiol (2012))*. I have funding to study regulation of cardiac ion channels by cardiac stress stimulus (NIH funding, Lopes PI); and the drug effects in cardiac arrhythmias (AHA Lopes PI and Geneen Foundation, Lopes PI). In addition, I have been for many years collaborating with clinical research group and the University of Rochester on studying genetic determinants of arrhythmia susceptibility (NIH funding, Moss PI, Lopes co-I).

Our previous research was seminal in the understanding of risk stratification and treatment differentiation of Long QT syndrome patients regarding their mutation dysfunction. This include work that show beta-adrenergic impairment caused by cytoplasmic-loop mutations in KCNQ1 gene correlated with higher risk, adrenergic triggers and higher effectiveness of beta-blocker treatment. In addition, we were the first to use computer models to risk assessment of cardiac risk in LQT patients. We have made important contributions to the field by studying changes in cardiac ion channel function and membrane expression after acute and prolonged stress stimulus, in particular relating to protein kinase C regulation of ion channels. These include work on calcium channel changes in membrane expression in response to prolonged PKC/PKD stimulus via Rem1 phosphorylation and regulation of the IKs channels voltage-dependence of activation by calcium dependent PKCs.

Project 1: Phospholipid regulation of ion channel localization.

Currently a major line of research in the lab is the study of the role of phospholipids on channel membrane localization. Although the role of the phospholipid $PI(4,5)P_2$ (or PIP_2) in stabilizing I_{Ks} function is well established, little is known about the role of the PIP_2 precursor PI4P or other phospholipids in channel function and localization. PI4P has been shown to bind both to the KCNQ1 subunit and to PKCβII, but the roles of these interactions on cardiac electrophysiology are unknown. Our data suggest that mutations linked to Long QT shown to affect channel interactions with phospholipids, increase sensitivity to PKCβII-mediated internalization, leading to decrease in membrane expression that can be rescued by PKCβII inhibition. We showed that patients carrying mutations in putative phospholipid-interacting sites are at higher risk for cardiac arrhythmias. Our studies suggest PKCβII-PI4P signaling leads to channel internalization that has a major role on QT prolongation in inherited and acquired Long QT syndrome. Our research aims to identify possible harmful pro-arrhythmic drug combinations and novel antiarrhythmic drug targets.

Project 2: Statins and ion channel regulation.

Another focus of the Lopes Lab research has been to understand the effect of statins in cardiac electrophysiology. Statins are among the most commonly prescribed drug classes, and their use is expected to increase due to recent changes in therapy guidelines. Although statins have an overall favorable safety profile in the general population, the effect of this drug class on cardiac electrophysiology, in particular for non-heart-failure patients, has been incompletely studied. Understanding the molecular effect of statins will allow tailoring of therapy to patients who would benefit the most and to avoid drug combinations or particular patient population for which statins may be harmful. Our preliminary clinical data in Long QT syndrome patients suggest that statins may be detrimental to patients with mutations in the KCNH2 gene, increasing their cardiac risk, but may be protective to LQT patients with mutations in other genes. Our main goal is to understand

the molecular mechanism of action of statins in combination with either naturally occurring mutations associated with Long QT syndrome or Long QT prolonging drugs by studying the effect of statins at the cellular level. The ultimate goal of this research project is to tailor statin therapy to the patient genetic profile and avoid detrimental drug-interaction.

Project 3: Interactions of sex hormones and ion channels in the long QT syndrome.

Additionally, our lab collaborations with the clinical Long QT research group has led to recent studies investigating the role of sex hormones in propensity to QTc prolongation and cardiac events in LQTS females by genotype. We are currently studying the effect of sex hormones on the ion channel function to investigate whether these changes will translate to changes in QTc prolongation and dynamics and will modify propensity to cardiac events in LQTS females undergoing long-term ECG monitoring and serial sex hormone assessment. Finally, currently collaboration with the Anderson Lab has focus on studding the role of a novel accessory ion channel subunit in cardiac ion channel function. These studies of a previous unrecognized protein may lead not only to a fuller understanding of physiology of cardiac rhythm, but be an important novel target for diagnosis and therapeutic of cardiac arrhythmias.

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.
4. Mathias A, Moss AJ, Lopes CM… and Goldenberg I. Prognostic implications of mutation-specific QTc standard deviation in congenital long QT syndrome. *Heart Rhythm*. 2013 May;10(5):720-5.
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6. Hoefen R, Reumann M,… and Lopes CM. In silico cardiac risk assessment in patients with long QT syndrome: type 1: clinical predictability of cardiac models. *J Am Coll* Cardiol. 2012 Nov 20;60(21):2182-91.
7. Couderc JP, Xia … and Lopes CM. Genotype- and Sex-Specific QT-RR Relationship in the Type-1 Long-QT Syndrome. *J Am Heart Assoc*. 2012 Apr;1(2):e000570.
8. Barsheshet A, Goldenberg I… and Lopes CM. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to β-blocker therapy in type 1 long-QT syndrome. *Circulation*. 2012 Apr 24;125(16):1988-96.
9. Costa J, Lopes CM… and Goldenberg I. Combined assessment of sex- and mutation-specific information for risk stratification in type 1 long QT syndrome. *Heart Rhythm*. 2012 Jun;9(6):892-8.

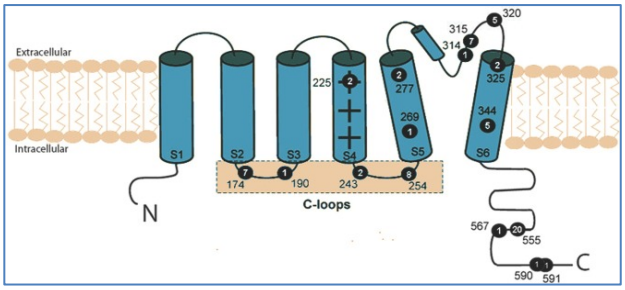

Figure: Schematic of mutations in KCNQ1 ion channel.

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.

One goal of my lab is to understand pathways of exocytosis in the human vasculature, and how they relate to human cardiovascular disease. 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.

We are also collaborating with the CHARGE Consortium of genetic epidemiologists to identify new proteins in platelets that accelerate thrombosis.

These approaches will increase our understanding of endothelial and platelet 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

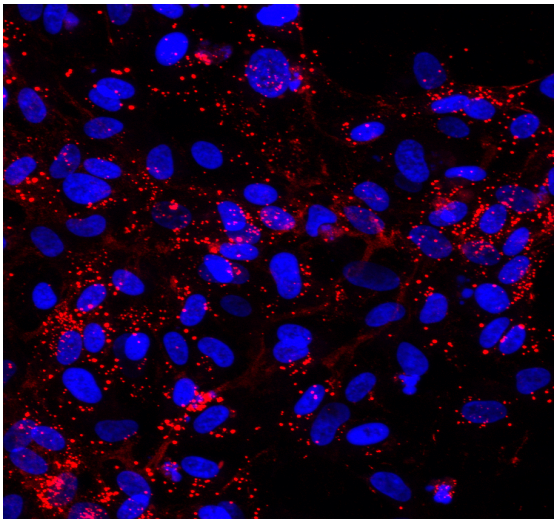

Figure: Endothelial cells grown on a defined matrix express granules containing VWF (red).

Publications

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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 deletion 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

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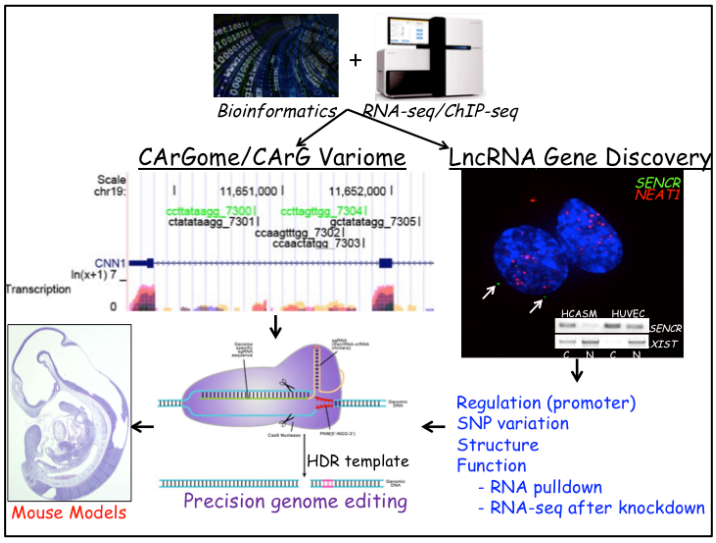

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Craig Morrell, D.V.M., Ph.D.

Synopsis

Research in our lab is focused on the complex interface between platelets, the vasculature, and immune cells. Platelets are not only the cellular mediators of thrombosis, but also have important roles in inflammatory and immune cell responses. Ongoing and past studies by our group have examined platelet and platelet derived inflammatory mediator interactions with both vascular and immune cells, and the impact of these interactions on tissue injury responses in disease relevant contexts, such as myocardial infarction, transplant rejection, and cerebral malaria. These studies demonstrated that platelets are regulators of all aspects of inflammatory responses, including the induction of acute phase responses, innate immune cell activation and trafficking, platelet regulation of T helper cell differentiation, and B cell development. Our lab regularly uses advanced mouse models to dissect molecular interactions and vascular inflammatory signaling pathways both *in vitro* and *in vivo*, establishing the lab as leaders in understanding how platelets regulate vascular and immune cell homeostatic and inflammatory responses. Taken together this work has shown that platelet mediated inflammatory and tissue injury responses have broad implications in many vascular and inflammatory disease processes.

Project 1: Platelet Regulation of Monocyte Responses:

Monocytes are circulating immune cells that differentiate into longer lived macrophages upon extravascular trafficking. Environmental cues and stimuli have differential effects on monocyte functions that can be generally classified as: i) pro-inflammatory monocytes that are phagocytic and produce cytokines that stimulate other inflammatory immune cells; ii) pro-reparative monocytes that produce cytokines that promote would healing and fibrosis. We have discovered that platelets regulate the differentiation of monocytes to either pro-inflammatory or pro-reparative phenotypes, in part through platelet derived beta-2 microglobulin (β2M). β2M is a molecular chaperone for the major histocompatibility class I (MHC I) complex, but β2M may also have less well understood immune functions, as elevated plasma β2M is a risk factor for adverse cardiovascular events. We have now found that platelet derived β2M is a mediator of monocyte pro-inflammatory differentiation. Circulating monocytes from mice lacking β2M only in platelets (Plt-β2M$^{-/-}$) had a more pro-reparative monocyte phenotype, in part dependent on increased platelet derived TGFβ signaling in the absence of β2M. Using a mouse myocardial infarction (MI) model, Plt-β2M$^{-/-}$ mice had limited post-MI pro-inflammatory monocyte responses, and instead demonstrated early pro-reparative monocyte differentiation, pro-fibrotic myofibroblast responses, and a rapid decline in heart function compared to WT mice. These data demonstrate a novel means by which platelets mediate monocyte phenotypes that may be important in tissue injury responses that has to potential to be therapeutically modulated.

Project 2: The Platelet/Megakaryocyte Regulation of Acquired Immune Responses

Megakaryocytes (Mks) are typically defined as bone marrow resident platelet progenitors. However, Mks are also present in the lung and recent work by other groups has shown that lung Mks contribute to platelet production. We have now found that compared to bone marrow Mks, lung Mks are differentiated to an immune regulatory phenotype, including lung Mk specialization to present antigen to T cells. Aided by unique expertise and research tools we have developed, we now seek to discover mechanisms that regulate Mk functions as professional antigen presenting cells (APCs) in the lung environment. This involves the use of Mk-specific MHC I$^{-/-}$ and MHC II$^{-/-}$ to directly determine how lung Mks participate in adaptive immunity. Outcomes of this work will impact our understanding of many immune disease processes, including immune responses to lung pathogens such as Influenza virus and the pathogenesis of asthma and allergy.

Project 3: ERK5 Regulation of Platelet Protein Expression and Activation in Ischemic Tissue Environments:

We recently published studies demonstrating the role of ERK5 in platelet function following acute and chronic ischemic pathologies, including myocardial infarction and peripheral artery disease. We discovered that select platelet proteins have altered expression in both humans and mouse models of these vascular diseases and demonstrated that the regulation of platelet protein expression occurs at least in part at the level of the platelet itself. We now seek to define the platelet 'translatome' in response to ischemic vascular disease and how ERK5 regulates the activation of the platelet translational machinery in a disease context.

Lab Members

- Zachary T. Hilt– Graduate Student
- Daphne N. Pariser – Graduate Student
- Denisse Vega Ocasio – Graduate Student
- Sara Ture – Laboratory Technician

Publications

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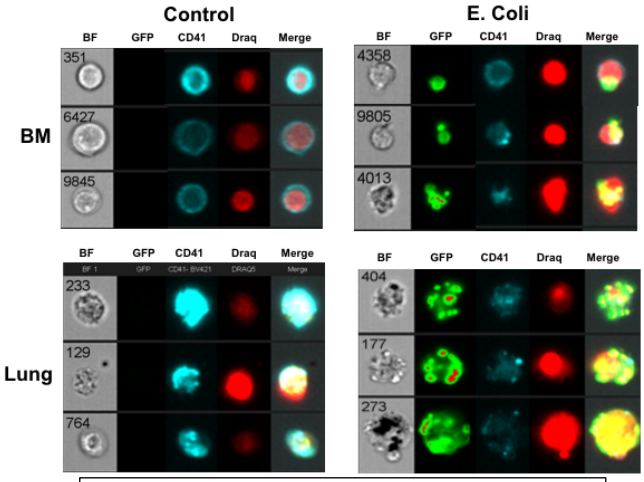

Bone Marrow (BM) and Lung Derived Megakaryocyte Phagocytosis of E. Coli.

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

Synopsis

Blood vessels are responsible for transportation of nutrients, oxygen and immune cells throughout the whole body. Changes of oxygen level, metabolites or immune cell function lead to vascular network modifications, including angiogenesis (the formation of new blood vessels from existing ones), vascular trimming (also called vessel pruning) or regression and vascular remodeling. These procedures are essential steps for establishing the functional and hierarchical mature vascular networks to meet the needs of various organs and also critical events 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 my lab is to identify the critical targets that regulate vascular structure and function under physiological and pathological conditions.

Project 1: Define the role of Notch signaling in postnatal lung vasculature development and in lung vascular diseases.

Project 2: Determine the mechanisms of vascular pruning and regression under physiological and pathological conditions.

Project 3: Elucidate the communication between immune cells and endothelial cells in pulmonary artery hypertension.

Lab Members

- Jordan Rhen, Laboratory Technician
- Shumin Wang, Postdoctoral Research Associate

Publications

1. Jiang D, Zhuang J, Peng W, Lv Y, Liu H, Zhao Q, Chi C, Li X, Zhu G, Xu X, Yan C, Xu Y, Ge J, **Pang J*.** Phospholipase C gamma-1 mediates intima formation through Akt-Notch1 signaling. J Am Heart Assoc. 2017 Jul 11;6(7).
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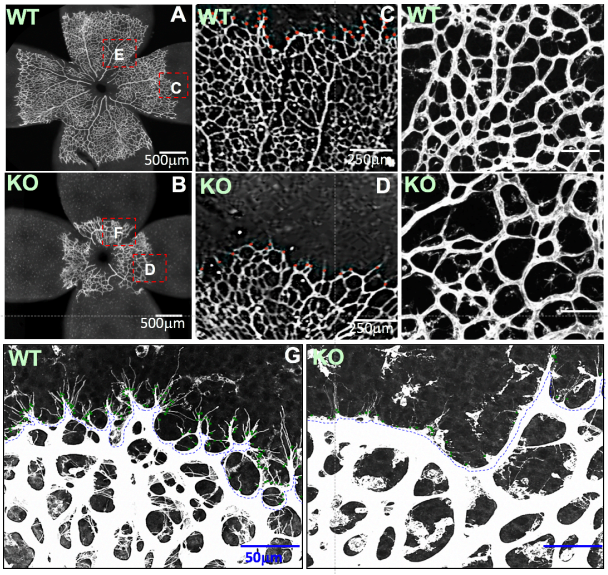

Eric Small, Ph.D.

Synopsis

The overall goal of the Small lab is to better understand the mechanisms that control cell identity and lineage commitment by studying the transcriptional regulation and function of cardiac tissue-restricted genes. Our motivation is to decipher how disruption of cardiac gene expression programs in heart disease contributes to cellular pathophysiology and the decline in cardiac function. There are two major themes of research within the lab that we study using mouse genetics, cell biology, advanced imaging, bioinformatic and biochemical approaches:

Project 1: Cardiac fibroblast plasticity and the development of cardiac fibrosis.

The transition to heart failure following cardiac insult is the result of irreversible cardiomyocyte loss and the development of cardiac fibrosis, which impedes contractility and can initiate lethal arrhythmias. 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. We utilize gene expression profiling in animal models of heart disease and human heart failure patient samples to identify novel regulators of cardiac fibroblast accumulation and myofibroblast activation in health and disease. We also utilize high-throughput screening and pre-clinical animal studies to develop novel pharmacological and gene-targeting strategies to block or reverse cardiac scarring and the progression of heart failure.

Project 2: Epicardium-derived progenitor cell mobilization.

Epicardium-derived progenitor cells (EPDCs) can differentiate into cardiac fibroblasts and coronary blood vessels, and secrete signals that stimulate cardiac growth during embryonic development. We are striving to understand how EPDCs interpret developmental signals and differentiate into the appropriate cell type based upon their location within the heart. We have identified a mechanosensitive gene program that is essential for EPDC migration and subsequent differentiation into fibroblasts and perivascular cells. Ongoing studies are aimed at evaluating whether disruption of mechanosensitive transcriptional programs within the epicardium might contribute to cardiomyopathy. Our long-term goal is to harness the regenerative potential of the epicardium to improve cardiac repair.

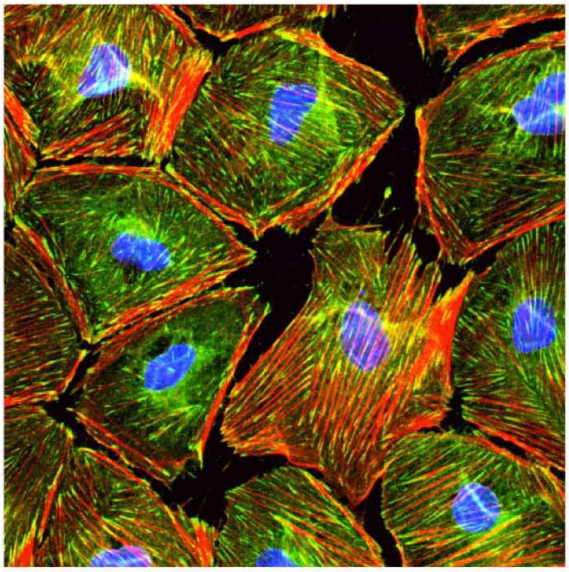

Figure: Epicardium-derived cells undergoing epithelial- mesenchymal transition (EMT) exhibit Vinculin-positive focal adhesions (green) and smooth muscle actin-positive stress fibers (red).

Lab Members

- Alan Brooks, MD/PhD – Cardiology Research Fellow
- Ryan Burke, PhD, Postdoctoral Fellow
- Ron Dirkx – Technical Associate
- Brian Kang – Technical Assistant
- Janet Lighthouse, PhD – Staff Scientist
- Adwiteeya Misra – MSTP Student
- Pearl Quijada, PhD – Postdoctoral Fellow

Publications

1. *Trembley M.A., Quijada P.*, Agullo-Pascual E., Tylock M.S., Colpan M., *Dirkx R.A.*, Myers J.R., Mickelsen D.M., Bentley K., Rothenberg E., Moravec C.S., Alexis J.D., Gregorio C.C., Dirksen R.T., Delmar M. and **Small E.M.*** (2018) Mechanosensitive Gene Regulation by Myocardin-Related Transcription Factors is Required for Cadrdiomyocyte Integrity in Load-Induced Ventricular Hypertrophy. *Circulation* (In press).
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10. *Trembley M.A., Velasquez L.S.*, de Mesy Bentley K.L., and **Small E.M.*** (2015). Myocardin-related transcription factors control the motility of epicardium-derived cells and the maturation of coronary vessels. *Development* 142:21-30. PMID:25516967. **Feature article**.
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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:

Project 1: Vascular smooth muscle cell phenotypic modulation and vascular disorders, such as hypertension, intima/media thickening, atherosclerosis and aortic aneurysms.

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.

Project 2: Pathological cardiac remodeling and heart failure.

Cyclic nucleotide phosphodiesterases (PDEs), by catalyzing the breakdown of cyclic nucleotides, play essential roles in the regulation of the magnitude, duration, and compartmentation of individual cyclic nucleotide pools. However, little is known about the causal relationship between alterations of PDE expression/activity and cardiac dysfunctions. The objective of this project is to understand the regulation and function of PDE isoforms in cardiac gene expression and the progression of heart failure.

Lab Members:

- Si Chen, Graduate Student
- Wenting Du - Postdoctoral Fellow
- Lingfeng Luo - Graduate Student
- Chongyang Zhang - Graduate Student
- Yishuai Zhang – Postdoctoral Fellow
- Qian Zhou - Staff Scientist Qian Zhou – Staff Scientist

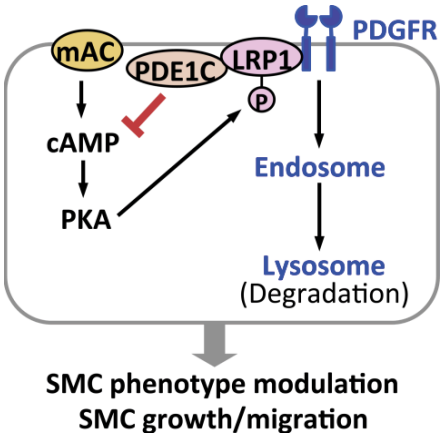

Figure: A mAC-derived cAMP-PKA signaling is critical in promoting PDGFR internalization and endocytosis. PDE1C activation antagonizes the mAC-cAMP-PKA signaling and thus suppresses PDGFR degradation, which facilitates SMC phenotype modulation and accelerates SMC growth/migration. PKA-dependent phosphorylation of LRP1 is important in PDE1C-cAMP regulation of PDGFR protein degradation

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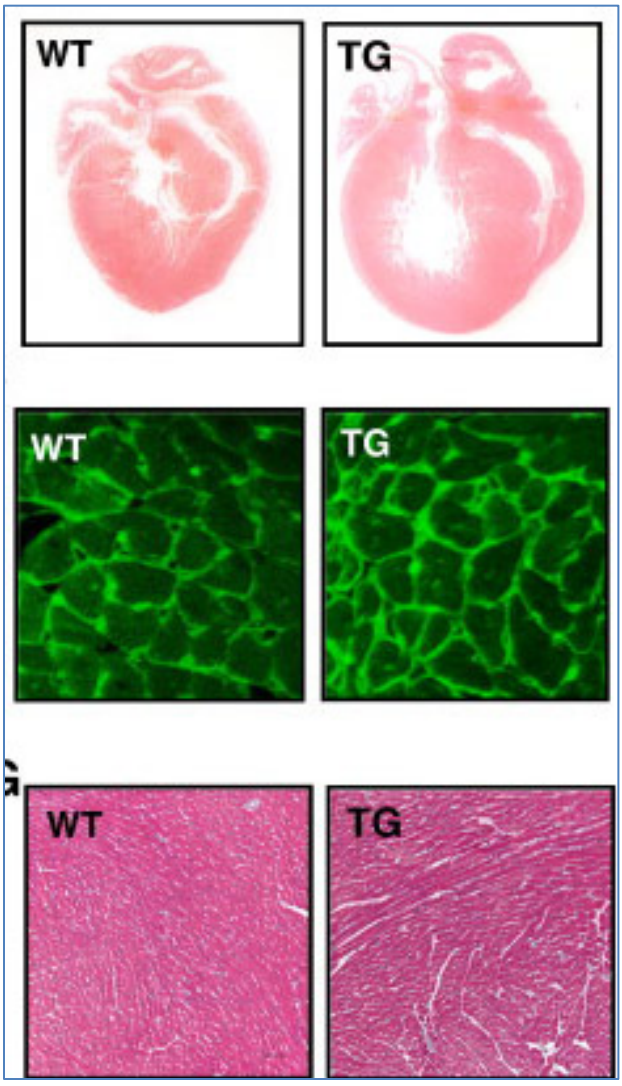


Figure: Transgenic mouse with cardiac expression of PDE3A1 have myocardial hypertrophy with larger cardiac myocytes and larger hearts.

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.

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.

Project 1: Pathophysiological function and regulatory mechanism of miRNA (or mRNA 3'UTR) and RNA-binding proteins in cardiac disorders.

Project 2: Riboswitch-like RNA switch mechanisms of gene regulation in mammalian systems

Project 2: The role of translation machinery and translational control in cardiac health and disease and therapeutic applications

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.

Publications

1. Halawani D, Gogonea V, DiDonato JA, Pipich V, **Yao P**, China A, Topbas C, Vasu K, Arif A, Hazen SL, Fox PL. Structural control of caspase-generated glutamyl-tRNA synthetase by appended noncatalytic WHEP domains. *Journal of Biological Chemistry* 2018 293(23): 8843–60
2. **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
3. **Yao P**, Eswarappa SM, Fox PL. Translational control mechanisms in angiogenesis and vascular biology. *Current Atherosclerosis Reports* 2015 17(5): 30
4. **Yao P**, Potdar AA, Ray PS, et al. The HILDA complex coordinates a conditional switch in the 3´-untranslated region of the VEGFA mRNA. *Plos Biology* 2013 11(8): e1001635
5. **Yao P**, Fox PL. Aminoacyl-tRNA synthetases in medicine and disease. *EMBO Molecular Medicine* 2013 5(3): 332-43
6. **Yao P**, Potdar AA, Arif A, et al. Coding region polyadenylation generates a truncated tRNA synthetase that counters translation repression. *Cell* 2012 149(1): 88-100

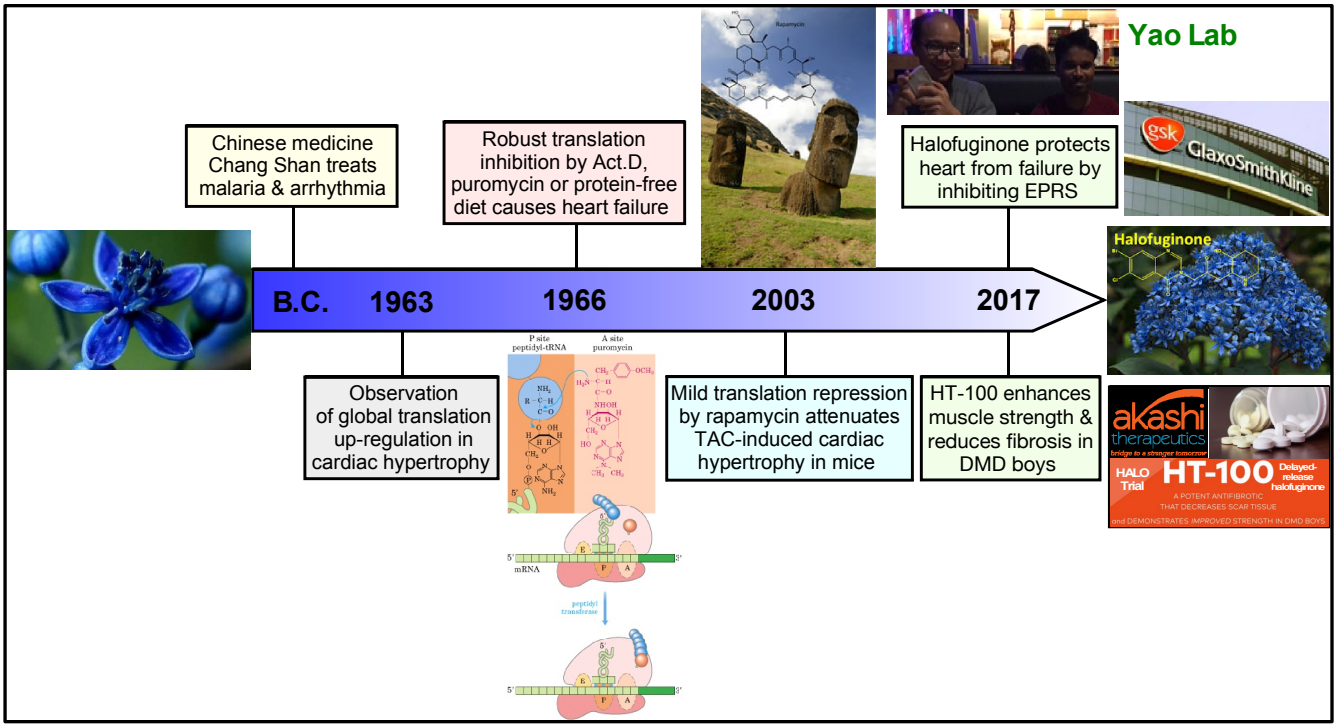


Figure: The human history of intervention of protein translation for treatment of cardiac hypertrophy and heart failure. In 2017, our laboratory discovered that halofuginone, a chemical compound from Chinese herbal medicine Chang Shan, could protect murine hearts from isoproterenol- and TAC-induced failure. 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.

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, India. 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 graduated from The Fourth Military Medical University in China 3 years ago. Her Ph.D. Major is Biochemistry and

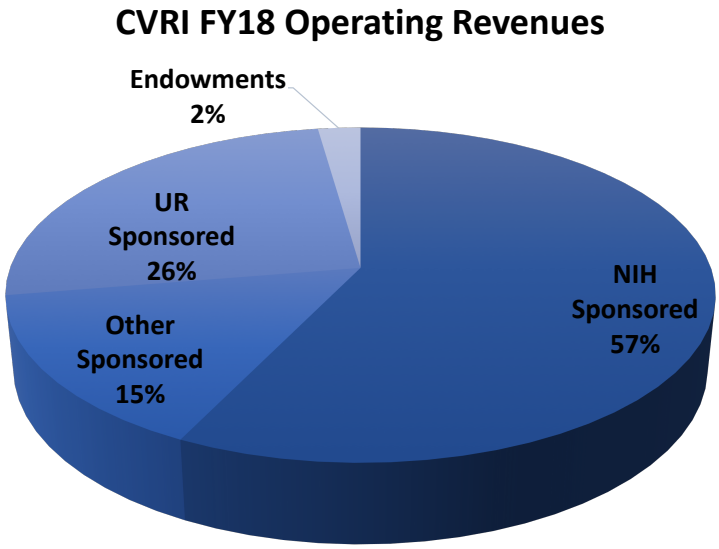
Molecular Biology and research focus on angiogenesis. She has worked previously at Ningxia medical university as an associate professor in Biochemistry and Molecular Department. Now Her research work is also focus on angiogenesis and heart valve development.

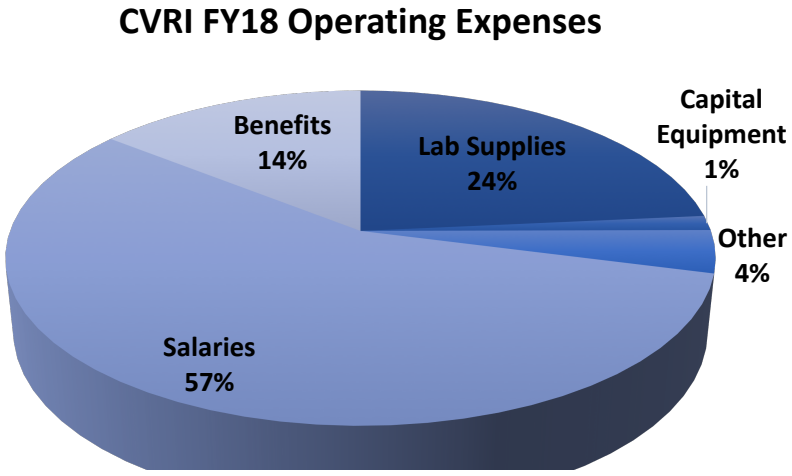
- **Dr. Yishuai Zhang** is a Postdoctoral Associate in the laboratory of Dr. Chen Yan. Dr. Zhang received his Ph.D. in cardiovascular pharmacology at the School of Pharmaceutical Science of Center South University, Changsha, Hunan, P.R. China. Dr. Zhang is exploring the underlying role and underlying 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.

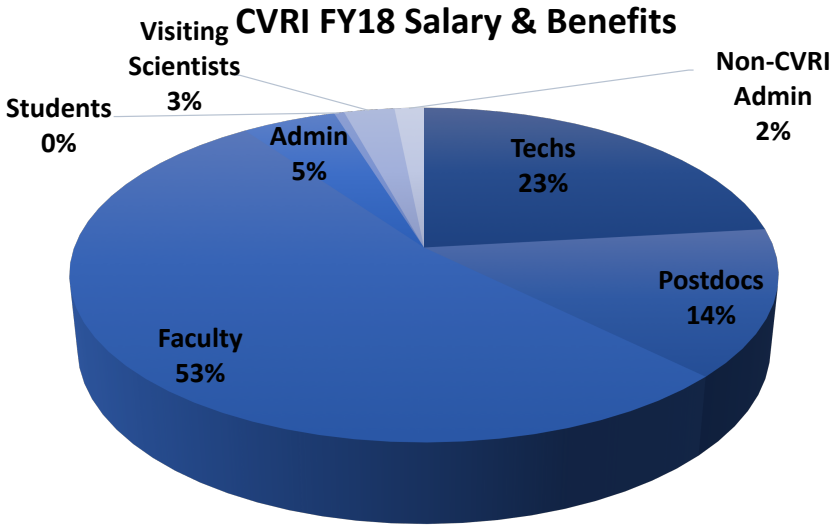
University of Rochester Graduate Students at the CVRI

- **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 the molecular basis of the Long QT Syndrome.
- **Qian Chen** is a graduate student in the lab of Dr. Bradford Berk. He received his B.S. from Nanjing Normal University in 2012. He is studying 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 studying 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 Cyclophilin A (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 University 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.

E. Financial Statement







F. Philanthropy

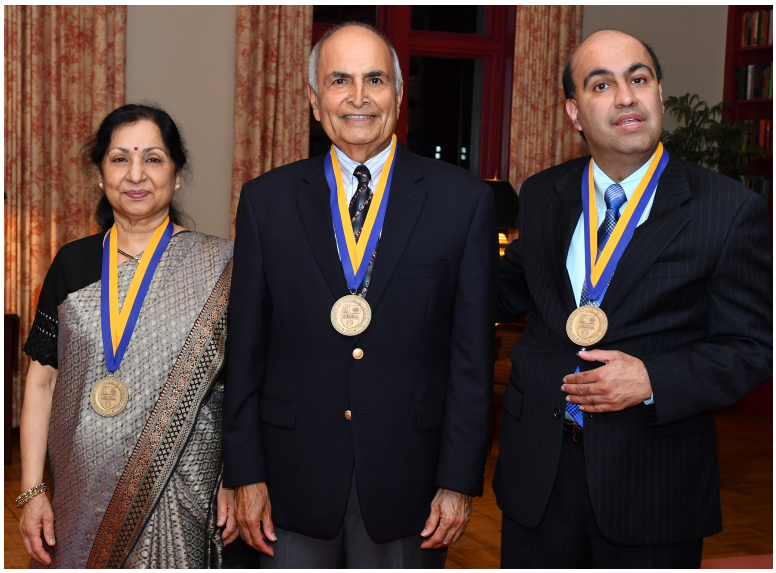
Richard T. Aab is an international entrepreneur and the chief executive officer of Idea Boxx, a company dedicated to conceptualizing, designing, and manufacturing products that improve productivity for businesses across industries. Over the last 40 years, his visionary leadership has fueled the success 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.

Aab earned his bachelor's degree in economics from Clarkson College in 1971 and began a career in finance. In 1982, Aab founded and was chairman and CEO of ACC Corp., a highly successful publicly owned telecommunications services company headquartered in Rochester, NY. ACC Corp. was acquired for over $1.1 billion in 1998 and is now part of AT&T. Later in 1996, Aab co-founded and served as chairman of US LEC Corp., a leading publicly owned telecommunications carrier. US LEC Corp. provided integrated voice, data, and internet services to medium and large businesses throughout the United States. In 2001, Aab diversified his business portfolio with the creation of 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. In 2007, when US LEC Corp. merged with PAETEC Corp., a privately owned leading telecommunications carrier, Aab served as the vice chairman and director. PAETEC was acquired by Windstream Corp. in 2011 for $2.1 billion. Today, Aab is the CEO of four start-up companies, three of which were developed from Idea Boxx including Hydra Rinse LLC, ProNatural Brands, and United Sources Sought, Inc.

A longstanding supporter of the University, Aab 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 comprehensive capital campaign which raised nearly $700 million. He has volunteered his time serving on multiple committees and his generous contributions to the University reflect his dedication to medical research. In 2007, the Aab CVRI was named in his honor in recognition of his leadership support and outstanding commitment to the Institute's mission. In 2016, the University of Rochester School of Medicine and Dentistry awarded him the Dean's Medal in recognition of his extraordinary service, philanthropy, and leadership to the School.

Aab remains an active fundraiser and advocate for numerous charities and organizations throughout the community. He lives in Fairport, New York and has two children, Melissa and Richard.

Tansukh V. Ganatra and the Ganatra Family



Tansukh V. Ganatra spent his almost 50 year career in telecommunications after migrating to the United States in 1969.

Born in Uganda, East Africa, Ganatra studied at the University of Nairobi, Kenya, East Africa and graduated in 1966 with his bachelor's degree in electrical engineering. Ganatra began a 19-year career with Rochester Telephone Corporation, now known as Frontier Communications, culminating with the position of director of network engineering. From 1987 to 1996, Ganatra held various positions with ACC Corp., including serving as its president and chief operating officer. Ganatra co-founded US LEC Corp. in 1996 where he served as president and chief operating officer from 1996-1999 and as chief executive officer and vice chairman of the Board of Directors of from 1999 until his retirement in 2001. US LEC Corp. eventually merged with PAETEC Holding Corp. in 2007. After the merger, Ganatra served as a director and audit committee member of the PAETEC Holding Corp. until its acquisition by Windstream in 2011.

The Ganatra family—Tansukh, his wife, Sarla, and their son, Rajesh—pledged a significant portion of their family estate to the Aab CVRI. The family also made a commitment to fund an endowed professorship in Pediatric Cardiac Surgery out of gratitude to the University of Rochester Medical Center doctors who have assisted numerous family members and friends. The Ganatras are dedicated to improving the lives and happiness of future generations through cardiology research at Golisano Children's Hospital and the Aab CVRI. Through their steadfast support, the family is enabling breakthrough treatments and lasting hope for pediatric cardiac patients in the greater Rochester community and around the world.

Presently, Ganatra holds advisory positions in various emerging companies and charity-based institutions. He resides in Charlotte, North Carolina with Sarla and Rajesh. The Ganatra family devotes time to spiritual, religious, educational, and health care based institutions in their commitments to give back.

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.

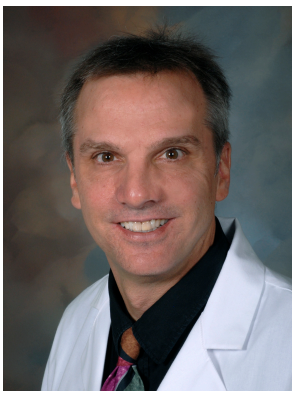
Aldons J. Lusis, Ph.D.
Vice Chair, Department of Human Genetics, Professor, Department of Human Genetics, Professor, Department of Microbiology, Immunology, and Molecular Genetics, Professor, Department of Medicine and Department of Microbiology and Molecular Genetics,
University of California, Los Angeles, 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 (Institut National de la Santé et de la Recherche) Paris, France.



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