Journal of Molecular and Cellular Cardiology 85 (2015) S1–S56

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Abstracts from the 2015 ISHR-NAS Annual Meeting, *June 7-10, 2015, Seattle, WA, USA*

001

Serum metabolomics reveal a distinct fingerprint of heart failure with preserved ejection fraction

Beshay Zordoky[a], Miranda Sung[a], Justin Ezekowitz[a], Rupasri Mandal[a], Beomsoo Han[a], Trent Bjorndahl[a], Souhaila Bouatra[a], Todd Anderson[b], Gavin Oudit[a], David Wishart[a], Jason Dyck[a]

[a]*University of Alberta, Edmonton, Alberta, Canada*
[b]*University of Calgary, Calgary, Alberta, Canada*

Background: Heart failure (HF) with preserved ejection fraction (HFpEF) is increasingly recognized as an important clinical entity. Preclinical studies have shown differences in the pathophysiology between HFpEF and HF with reduced ejection fraction (HFrEF). Therefore, we hypothesized that a systematic metabolomic analysis would reveal a novel metabolomic fingerprint of HFpEF that will help understand its pathophysiology.

Methods and Results: Ambulatory patients with clinical diagnosis of HFpEF (n = 24), HFrEF (n = 20), and age-matched non-HF controls (n = 38) were selected for metabolomic analysis as part of the Alberta HEART (Heart Failure Etiology and Analysis Research Team) project. 181 serum metabolites were quantified by LC-MS/MS and ^{1}H-NMR spectroscopy. Compared to non-HF control, HFpEF patients demonstrated higher serum concentrations of short-chain, medium-chain, and long-chain acylcarnitines, carnitine, creatinine, betaine, and several amino acids; and lower levels of phosphatidylcholines, lysophosphatidylcholines, and sphingomyelins. Long-chain acylcarnitines, 2-hydroxybutyrate, 3-hydroxybutyrate, and acetate were found to be higher in the HFpEF group than the HFrEF group, while sphingomyelin (C24:1), some phosphatidyl cholines and lysophosphatidyl cholines were found to be lower in the HFpEF group than the HFrEF group

Conclusions: The metabolomics approach employed in this study identified a unique metabolomic fingerprint of HFpEF that is distinct from that of non-HF controls and from patients with HFrEF.

002

Cardiac ion channel changes in response to the ER stress

Man Liu, Guangbin Shi, Hong Liu, Samuel C. Dudley

Rhode Island Hospital and Brown University, Providence, RI, USA

Table 1
Whole-cell cardiac ion channel currents of mouse ventricular cardiomyocytes altered in response to the ER stress or in heart failure (HF) mice. *P < 0.05 vs untreated or sham; n, averaged cell number.

	Testing potential (mV)	**Untreated**		**TM-induced ER stress**		
		pA/pF	n	pA/pF	n	Ratio to untreated
I_{Na}	-30	-26.7 ± 1.3	17	-12.8 ± 1.2*	20	0.48 ± 0.05*
I_{to}	+50	8.3 ± 1.2	15	4.2 ± 0.5*	9	0.51 ± 0.09*
I_{K1}	-100	-1.2 ± 1.2	20	6.5 ± 0.5*	9	0.58 ± 0.8*
$I_{K,slow}$	+50	11.2 ± 1.2	19	7.3 ± 0.7*	9	0.65 ± 0.09*
		Sham		Heart failure		
		pA/pF	n	pA/pF	n	Ratio to sham
I_{Na}	-30	-36.6 ± 2.1	15	-22.0 ± 3.4*	20	0.60 ± 0.10*
I_{to}	+50	8.3 ± 1.2	15	3.4 ± 0.4*	11	0.41 ± 0.08*
I_{K1}	-100	-11.2 ± 1.2	20	-4.8 ± 0.5*	11	0.43 ± 0.06*
$I_{K,slow}$	+50	11.2 ± 1.2	19	6.6 ± 0.8*	10	0.59 ± 0.10*

Introduction: Heart failure is associated with electrical remodelling and endoplasmic reticulum (ER) stress causing activation of the unfolded protein response (UPR). We hypothesized that UPR could contribute to the electrical remodelling seen in heart failure.

Methods: Tunicamycin (TM) was applied to healthy myocytes to induce the ER stress at 10 μg/ml for 22-24 h. Nonischemic cardiomyopathy was induced in C57BL/6 mice 6-7 weeks after unilateral nephrectomy, deoxycorticosterone acetate (DOCA) pellet implantation, and salt water substitution. Sham operated mice were used as controls. Isolated ventricular myocytes were utilized for whole-cell patch clamp recording.

Results: With TM treatment, the action potential duration (APD) was prolonged (225 ± 21 ms of TM group vs. 108 ± 16 ms of untreated/sham, P < 0.05), and the maximum upstroke velocity dV/dt_{max} of phase 0 was slowed (119.0 ± 7.0 mV/ms of TM group vs. 174.0 ± 9.7 mV/ms of untreated, P < 0.05). The APD of heart failure myocytes was prolonged to 173 ± 23 ms (P < 0.05 vs sham). Among the major cardiac ion channels, the peak I_{Na} and three types of K^+ currents were decreased significantly by TM treatment or in heart failure group (Table 1), while L-type Ca^{2+} current and some other types of K^+ current were not affected.

Conclusion: The changes induced by TM were similar to those in heart failure. The reductions of I_{Na}, I_{to}, I_{K1} and $I_{K,slow}$ under both conditions suggests that $Na_v1.5$, $K_v4.3$, Kir2.1, and $K_v1.5$ are downregulated by the ER stress. Therefore, UPR seems to contribute

http://dx.doi.org/10.1016/j.yjmcc.2015.06.017
0022-2828/

to electrical remodeling in heart failure and targeting the UPR may be a novel antiarrhythmic strategy.

003
Cellular Mechanisms of Endogenous Cardiomyocyte Regeneration in Injured Hearts

Yiqiang Zhang, Yonggang Liu, Avin Mehri, Yi Lin, Amy Martinson, Michael Chin, Charles Murry, William MacLellan

University of Washington, Seattle, WA, USA

Background: Despite of the controversial roles of adult progenitor cells in regenerating the myocardium of injured hearts, increasing evidence demonstrated that cardiomyocytes (CMs) can be renewed in adult hearts but at a low rate. Due to the limitations of current methodology and the low CM turnover rate, it has been challenging to accurately quantify CM renewal and determine the exact underlying cellular mechanism(s). **Objective**: To quantify the rate of CM renewal in injured mouse hearts and to decipher the underlying cellular mechanisms. **Methods and Results**: 1) We generated a bi-transgenic aMHC-MerCreMer;RFPfl/GFP mouse, with superior inducible GFP genetic tagging (>97%) in adult CMs. We also created a novel CM nucleus-specific BFP reporter mouse Tg(αMHC-H2BBFP6xHis). The tri-transgenic mouse enabled us to unambiguously investigate the potential cellular mechanisms of endogenous CM renewal, including: (i) direct CM proliferation, (ii) dedifferentiation and proliferation of pre-existing CM, and (iii) the proliferation and cardiac differentiation of progenitor cells or cardioblasts. 2) Using high-throughput analyses, we observed a ~5 fold increase of BFP + EdU + nuclei in BFP transgenic mouse hearts 3 weeks post-myocardial infarction (MI) compared to Sham, indicating the augmentation of cycling CMs in injured myocardium. 3) Our MI experiments revealed no significant increase in RFP + CM after MI, consistent with a minimal role of adult progenitor cells, if any, for the regeneration of adult myocardium. 4) Comparing BrdU + cells from tri-transgenic mouse hearts, the increment in GFP + BFP + or GFP + BFP- was similar; The GFP + BFP- cells in post-MI heart had lower FSC and FSC indices; In the cardiomyocyte-depleted cardioblast preparation, GFP + BFP- cells accounted only a very minor population. **Conclusion and Prospect**: CM regeneration in the injured heart is achieved by CM dedifferentiation/proliferation and but unlikely by progenitor cell differentiation. We will use this model to dissect the cellular and epigenetic mechanisms underlying cardiomyocyte dedifferentiation and proliferation, thereby cardiac regeneration.

004
Effect of SERCA inhibition on sarcoplasmic reticulum Ca alternans in intact rabbit hearts

Lianguo Wang[a], Rachel Myles[b], Donald Bers[a], Crystal Ripplinger[a]

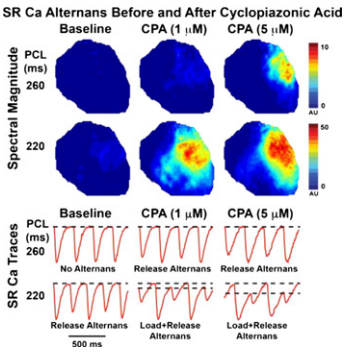
[a]*UC Davis, Davis, CA, USA*
[b]*University of Glasgow, Glasgow, UK*

Introduction: Reduced sarco-endoplasmic reticulum Ca-ATPase (SERCA) function occurs in heart failure and is known to potentiate intracellular Ca alternans, presumably due to insufficient Ca reuptake during diastole and subsequent alternation of sarcoplasmic reticulum (SR) Ca load. However, recent studies have also implicated alternation of ryanodine receptor (RyR) refractoriness as a contributor to SR Ca alternans. To address the role of reduced SERCA function in contributing to SR Ca and subsequent arrhythmogenic action potential duration (APD) alternans, we utilized novel imaging of free intra-SR Ca concomitantly with transmembrane potential (V_m) in intact hearts.

Methods: Simultaneous optical mapping of V_m (with RH237) and SR Ca (with Fluo-5 N AM) was performed in isolated rabbit hearts ($n = 10$). Alternans was induced by progressively decreasing the pacing cycle length (PCL). SERCA was inhibited with cyclopiazonic acid (CPA; 1-5 μM).

Results: SERCA inhibition by CPA caused a dose-dependent increase in SR Ca reuptake time (tau, baseline 70.8 ± 3.5 ms; 1 μM 85.5 ± 6.6 ms; 5 μM 129.9 ± 20.7; $p < 0.05$ for all). CPA also caused a dose-dependent increase in the magnitude of SR Ca and APD alternans and an increase in the PCL at which alternans occurred (FIG). Interestingly, even with reduced SERCA function, alternation of SR Ca release still occurred prior to alternation of SR Ca load under all conditions.

Conclusions: As expected, SERCA inhibition potentiates SR Ca and subsequent APD alternans. However, alternation of SR Ca release still occurred prior to alternation of SR Ca load, even under conditions of SERCA inhibition, indicating complex interactions between SR Ca reuptake and RyR refractoriness.



Therefore, H_2S-mediated PostC appears to elicit its protective effect in large part through an increase of NO/SNO signaling, suggesting that signaling crosstalk is involved in the cardioprotection induced by pharmacological PostC with H_2S and NO donors.

005
Synergistic cardioprotection induced by pharmacological post-conditioning with H_2S and NO donors: S-sulfhydration (SSH) *vs* S-nitrosylation (SNO)

Junhui Sun[a], Angel Aponte[a], Sara Menazza[a], Marjan Gucek[a], Charles Steenbergen[b], Elizabeth Murphy[a,b]

[a]*Systems Biology Center, NHLBI/NIH, Bethesda, MD, USA*
[b]*Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, USA*

Two gaseous signaling molecules, hydrogen sulfide (H_2S) and nitric oxide (NO), play important roles in postconditioning (PostC)-induced cardioprotection. Emerging data suggest that both H_2S and NO regulate protein function through redox-based protein post-translational modification on cysteine residue(s), i.e., S-sulfhydration (SSH) and S-nitrosylation (SNO), respectively. In this study, we examined whether

there is a synergistic protective effect in pharmacological PostC mouse hearts with H_2S and NO donors using Langendorff perfused heart model.

After 20 min of equilibrium perfusion and 20 min of no-flow global ischemia, hearts were subjected to pharmacological PostC at the beginning of reperfusion for 7 min with either 100 μmol/L NaHS (H_2S donor), 10 μmol/L SNAP (NO/SNO donor), or both, followed by reperfusion with regular perfusion buffer for total 90 min. Compared to control, PostC with either NaHS or SNAP significantly reduced post-ischemic contractile dysfunction, the post-ischemic heart rate pressure product (RPP) recovery was 52.3 ± 4.8% for PostC-NaHS, 51.7 ± 3.9% for PostC-SNAP *vs* 36.4 ± 2.5% for control (n = 8 in each group). The post-ischemic myocardial infarction was decreased from 49.9 ± 1.4% for control to 34.6 ± 2.9% for PostC-NaHS and 35.2 ± 2.9% for PostC-SNAP. Interestingly, PostC simultaneously with two donors together had a synergistic protective effect, post-ischemic RPP recovery was 72.2 ± 4.2% and infarct size was 19.7 ± 3.0%. We used iodoacetyl tandem mass tag (iodoTMT) reagents to identify SSH/SNO-modified proteins. Consistent with our previous study, PostC with SNAP significantly increased SNO signaling. Surprisingly, however, only a few SSH modification were detected in PostC-NaHS hearts. Instead, PostC-NaHS led to an increase of SNO level comparable to that in Post-SNAP hearts. Furthermore, PostC with both donors together caused additional increase in SNO.

Therefore, H_2S-mediated PostC appears to elicit its protective effect in large part through an increase of NO/SNO signaling, suggesting that signaling crosstalk is involved in the cardioprotection induced by pharmacological PostC with H_2S and NO donors.

006

Proteomic approaches to identify the cardiac prolyl hydroxalome and to analyze protein stability

Andrea Stoehr, Alicia Evangelista, Sajni Patel, Yongshun Lin, Angel Aponte, Marjan Gucek, Elizabeth Murphy

NIH/NHLBI, Bethesda, MD, USA

Prolyl hydroxylases (PHD) are oxygen dependent enzymes that hydroxylate proline residues. Although HIF-1α was identified years ago as a substrate for PHD proteins, only a few proteins have been shown to undergo prolyl hydroxylation. The goal of this study was to (I) identify new targets of prolyl hydroxylation, and (II) test whether inhibition of prolyl hydroxylation alters protein stability. Tryptic digests of rat heart extracts were immunoprecipitated with an antibody against prolyl hydroxylation and the samples were run on an LTQ Orbitrap Elite LC-MS/MS. 45 prolyl hydroxylated peptides from 18 unique proteins were identified. To address whether prolyl hydroxylation influences protein stability and to transfer the results to human, we used an *in vitro* cell culture model of iPS cell-derived cardiomyocytes with Stable Isotopic Labeling with Amino Acids (SILAC). Culture medium was switched to heavy amino acids ($^{13}C_6$ L-Lysine-2HCL, $^{13}C_6^{15}N_4$ L-Arginine-HCl) and cells were divided into two treatment groups: vehicle or dimethyloxalylglycine (DMOG, 1 mM), a PDH inhibitor. After 2, 6 and 18 hours, tryptic digests were analyzed using an Orbitrap Fusion. We identified 96 proteins that had a significant difference in light peptides between DMOG and vehicle 18 hours after switching to heavy media ($p < 0.05$). To measure protein turnover, the rate of decay of the light peptides was measured. Rates for vehicle and DMOG degradation were calculated by a multi-point first-order fit equation and statistically compared. Targets such as serpin H1, polyadenylate-binding protein 1 and SRSF1 were confirmed as being significantly stabilized with DMOG treatment. Panther protein class analysis revealed that nucleic acid binding proteins such as proteins involved in mRNA processing were the largest group of proteins stabilized by DMOG, suggesting a potential effect on alternative splicing during PHD inhibiting conditions such as hypoxia in cardiomyocytes.

007

The novel Cyclophilin-D interacting protein FASTKD1 protects cells against oxidative stress-induced death

Kurt Marshall[a], Maike Krenz[b,c], Christopher Baines[a,c]

[a]*Department of Biomedical Sciences, University of Missouri-Columbia, Columbia, MO, USA*
[b]*Department of Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, MO, USA*
[c]*Dalton Cardiovascular Research Center, University of Missouri-Columbia, Columbia, MO, USA*

Mitochondria-dependent mechanisms play critical roles in cell death induced by noxious stimuli. The purpose of this study was to identify novel mitochondrial modulators of cell death. We hypothesized that one of the known major regulators of mitochondrial-dependent cell death, cyclophilin D (CypD), interacts with other so far unidentified cell death modulators. Utilizing a yeast-two hybrid system, we searched for novel proteins associated with CypD and identified the mitochondrial protein Fas-activated serine/threonine phosphoprotein kinase domain-containing protein 1 (FASTKD1). Due its interaction with CypD, we first tested whether FASTKD1 modulates mitochondrial permeability transition (MPT) pore opening and therefore cell death. Surprisingly, neither FASTKD1 overexpression nor knockdown altered mitochondrial Ca^{2+}-retention capacity (CRC) in mouse embryonic fibroblasts (MEFs), an index of MPT. Consistent with this, manipulation of FASTKD1 levels did not affect Ca^{2+}-ionophore-induced cell death, which is MPT-dependent. However, overexpression of FASTKD1 protected both MEFs and neonatal rat cardiac myocytes against oxidative stress-induced cell death. Importantly, FASTKD1 was still able to confer protection against oxidative stress in CypD-deficient cells. Conversely, knockdown of FASTKD1 sensitized MEFs to oxidative stress. These effects were independent of any changes in antioxidant expression and capacity. Additionally, we observed that overexpression of FASTKD1 induced mitochondrial fragmentation in both MEFs and myocytes, whereas knockdown of FASTKD1 had the opposite effect. Finally, we developed an inducible, cardiac specific overexpression FASTKD1 mouse model. The hearts from these mice are morphologically normal and show no alterations in mitochondrial respiration or MPT pore opening as assessed by CRC or expression of proteins associated with these processes. In conclusion, our data indicate that FASTKD1 is a novel CypD binding protein that protects cells against oxidative stress-induced death and modulates mitochondrial morphology. FASTKD1 appears to act independently of the MPT pore, suggesting that FASTKD1 could be part of a novel cytoprotective mechanism.

009

A Human S10F-Hsp20 Mutant Abrogates the Contractile and Protective Effects of Hsp20 in the Heart

Guan-Sheng Liu[a], Wen Zhao[a], Elizabeth Vafiadaki[b], George Adly[a], Chi Keung Lam[a], Wen-Feng Cai[c], Persoulla Nicolaou[a], Min Jiang[d], Jack

Rubinstein[d], Guo-Chang Fan[a], Gerald W. DornII[e], Evangelia G. Kranias[a,b]

[a]*Department of Pharmacology & Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH, USA*
[b]*Molecular Biology Division, Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, Greece*
[c]*Department of Pathology & Lab Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA*
[d]*Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, USA*
[e]*Center for Pharmacogenomics, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA*

The small heat shock protein 20 (Hsp20) has emerged as a novel cardioprotector against ischemia/reperfusion-induced injury and β-agonist-mediated remodelling. In addition, Hsp20 enhances cardiac Ca-cycling and contractility by inhibiting the phospholamban-associated protein phosphatase-1 (PP1). Thus, Hsp20 has dual benefits in the heart and may hold promise as a therapeutic target. We recently identified a human S10F-Hsp20 mutant in dilated cardiomyopathy patients. The frequency of S10F was 2.8% in dilated cardiomyopathy (470 patients screened), while there were no normal subjects carrying this mutation (screened over 282 normals). Adenoviral-mediated expression of S10F-Hsp20 in cardiomyocytes resulted in depressed contractile parameters and Ca-kinetics, while WT-Hsp20 significantly increased contractility and Ca-cycling. Furthermore, S10F was associated with increased apoptosis under prolonged β-adrenergic stimulation in contrast to the protective effects by WT-Hsp20. In agreement with these *ex vivo* studies, cardiac-specific overexpression of S10F in the mouse resulted in significant decreases in contractile parameters and Ca-cycling. In contrast, overexpression of WT-Hsp20 at similar levels as S10F exhibited increased contractility and Ca-kinetics. The mechanisms underlying the depressive effects of mutant Hsp20 involved reduced ability to interact with PP1 and inhibit it. The depressed function in S10F hearts induced remodelling, which progressed to dilated cardiomyopathy and S10F hearts exhibited both interstitial fibrosis and cardiomyocyte apoptosis by 14 months of age. By 16 months, 13 out of 16 (80%) S10F-Hsp20 mice died, compared with 1 out of 15 (6%) WTs. The main mechanisms associated with the detrimental remodelling in mutant hearts included decreased interaction of S10F-Hsp20 with Beclin1. As a result, Beclin1 and the LC3-II/LC3-I ratio were reduced, contributing to suppressed autophagy activity and increased cell death. These findings indicate that both the contractile and cardioprotective effects, elicited by WT-Hsp20, are abrogated by the S10F mutation and suggest that human carriers may be intrinsically compromised in coping with cardiac stress conditions.

010
HAX-1 is a new regulator of cyclophilin-D expression and the mitochondria permeability transition pore in the heart

Chi Keung Lam, Wen Zhao, Guansheng Liu, Wenfeng Cai, George Gardner, Evangelia Kranias

University of Cincinnati, Cincinnati, OH, USA

In cardiac ischemia/reperfusion injury, oxidative stress and calcium overload induce the opening of the mitochondria permeability transition pore (mPTP), which disrupts mitochondria membrane integrity and causes massive cell death. Studies in human lymphocytes suggest that the hematopoietic substrate-1 associated protein X-1 (HAX-1) may regulate mitochondrial membrane integrity. However, its role in controlling mPTP opening remains to be elucidated. We recently showed that HAX-1 associates with both SR/ER and mitochondria in the heart. To examine its role in regulation of mitochondrial membrane potential, we used cardiomyocytes from genetically altered mice with cardiac overexpression (HAXOE) or heterozygous ablation of HAX-1 (HAX+/-; the homozygous mice die at young age) and subjected them to hydrogen peroxide challenge. HAXOE cells were resistant to oxidative stress, while heterozygous cardiomyocytes showed exacerbated membrane potential loss. Moreover, over-expression of HAX-1 reduced the sensitivity of mPTP opening, rendering mitochondria resistant to swelling induced by calcium overload. The protective effect of HAX-1 on mPTP opening was attributed to specific regulation of cyclophilin-D (Cyp-D) levels without any effects on the expression of the other proposed mPTP components. Over-expression of HAX-1 reduced Cyp-D levels, while HAX-1 heterozygous ablation had opposite effects. The elevated Cyp-D levels in heterozygous hearts were associated with increased infarction upon ischemia/reperfusion injury. To confirm the role of Cyp-D in the increased injury of heterozygous hearts, we crossed the HAX+/- mouse with the Cyp-DKO mouse. Indeed, Cyp-D ablation alleviated the increases in myocardial infarction elicited by HAX-1 heterozygosity, suggesting the crucial role of mPTP regulation in the HAX-1 cardioprotective effects. Mechanistically, the alterations in Cyp-D levels appeared to involve the ubiquitin-proteosomal degradation pathway, as over-expressing HAX-1 enhanced Cyp-D ubiquitination and proteosomal inhibition restored Cyp-D levels. Taken together, our studies demonstrate a novel role of HAX-1 in regulating Cyp-D levels and preventing mPTP activation, resulting in protection from ischemia/reperfusion injury.

011
Network-based Approaches to Identify Novel Regulators of Heart Failure

Christoph Rau, Milagros Romay, Jessica Wang, Shuxun Ren, Yibin Wang, Aldons Lusis

University of California, Los Angeles, Los Angeles, CA, USA

Heart failure is a highly heterogeneous disorder characterized by the interactions of multiple environmental and genetic factors. While reductionistic approaches have made significant inroads into characterizing the pathophysiology of the syndrome, they are unable to properly dissect the complex interactions between sets of genes and pathways which result in the emergent phenotypes . Systems genetics offers a means by which these interactions may be identified and explored. We have developed a resource, the Hybrid Mouse Diversity Panel (HMDP) to perform systems-level analyses in mice. Nine week old female mice from 93 unique inbred lines of the HMDP were give 30 ug/g/day of isoproterenol through an abdominally implanted Alzet micropump. After three weeks, mice were sacrificed along with age-matched controls. A portion of the left ventricle was arrayed on an Illumina Mouse Ref 8.0 platform.

Maximal Information Component Analysis was used to construct gene networks, and a module of 41 genes was identified which shows strong correlation to a number of important phenotypic traits, including heart weight and cardiac fibrosis. This module

contains a number of genes of interest, including *Lgals3*, a diagnostic marker for heart failure. Through the use of structural equation modeling, we identified several key genes within the module for further analysis, the most important of which is the metalloprotease *Adamts2*.

We have performed a series of *in vitro* analyses demonstrating the important role of *Adamts2* in this module using neonatal rat ventricular myocytes. Knockout of *Adamts2* results in an amelioration of the hypertrophic response to catecholamine stimulation as well as a reduction of hypertrophic markers such as *Nppa* and *Nppb*. Furthermore, we observe that other genes in the module no longer respond to catecholamine stimulation after knockdown of *Adamts2*. Further analysis of this module and others will reveal further regulators of heart failure

012

Human Cardiac, Endothelial and Blood Lineages are Controlled by Gradients of Activin A, BMP4, and Wnt/B-Catenin Signaling

Nathan Palpant, Lil Pabon, Meredith Roberts, Brandon Hadland, Daniel Jones, Christina Jones, Randall Moon, Walter Ruzzo, Irwin Bernstein, Ying Zheng, Charles Murry

University of Washington, Seattle, WA, USA

During development, dosage of morphogens like Wnt/β-catenin, activin/nodal and BMPs are critical for defining the patterning mesoderm and specifying down-stream derivatives including cardiomyocyte and endothelial fates. We used activin A and BMP4 dosage to pattern head-to-tail mesoderm polarization from hESC-as it occurs in the developing embryo. **Results:** We found that Wnt/β-catenin signalling increased as cells transitioned from an anterior to posterior mesoderm with concomitant changes in gene expression that reflect polarized mesoderm states *in vivo*. When cells were differentiated under anterior-mesoderm conditions, cardiomyocytes developed robustly (90% $cTnT^+$) compared to posterior mesoderm (14% $cTnT^+$) reflecting *in vivo* lineage bias for cardiogenesis in anterior mesoderm. We found that endothelial cells (ECs) could be generated with equal efficiency (90% $KDR^+/CD34^+$) from all mesodermal origins but had unique physiological functions that reflect their development ontogeny. Anterior endothelial cells expressed genes involved in endocardial development, responded to TGFβ stimulation to undergo EMT, and had poor blood forming potential. In contrast, posterior endothelium expressed markers of hemogenic endothelium and showed robust blood forming activity. Interestingly, we found that inhibiting Wnt/β-catenin signalling in anterior-mesoderm derived endothelium activated the cardiac gene program resulting in a fate switch to cardiomyocyte development with greater than 90% efficiency. The global transcriptional profile of EC-derived cardiomyocytes was indistinguishable from cardiomyocytes generated under standard cardiac differentiation conditions. However, posterior-mesoderm derived hemogenic endothelium failed to convert to the cardiac fate under the same conditions. **Conclusion:** These studies show that modulating activin A and BMP4 conditions at the onset of differentiation is sufficient to direct polarized mesoderm from hESCs that recapitulates embryonic gastrulation *in vivo*. This fate specification is required to establish lineage biases for cardiogenic vs. hemogenic mesoderm and downstream definitive derivatives. We further show the potential for fate inter-conversion between the endothelial and cardiac fates on the basis of manipulating canonical Wnt/β-catenin signalling.

013

Non-nuclear estrogen receptor activation reduces cardiac ischemic-reperfusion injury in mice with cardiac specific ablation of ER-alpha

Sara Menazza[a], Swathi Appachi[a], Junhui Sun[a], Benita Katzenellenbogen[b], John Katzenellenbogen[c], Philip Shaul[d], Elizabeth Murphy[a]

[a]*Systems Biology Center, NHLBI, NIH, Bethesda, MD, USA*
[b]*Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
[c]*Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
[d]*Department of Pediatrics, Division of Pulmonary and Vascular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Introduction: Steroid hormone receptors, ER and ER, classically function as transcription factors regulating gene expression. Recent data indicate that estrogen can also elicit effects by binding to estrogen receptors (ER, ER and GPR30) at the plasma membrane and initiate kinase signaling. We investigate the hypothesis that that non-nuclear ER activation reduces cardiac I-R injury in mice.

Methods and Results: We used an estrogen-dendrimer conjugate (EDC), which has been demonstrated in mice to be a non-nuclear selective ER modulator. We treated ovariectomized wild type mice with EDC, estradiol or dendrimer control for two weeks using Alzet osmotic minipumps. Isolated hearts were perfused in the Langendorff model and subjected to 30 minutes ischemia and 90 minutes reperfusion. Two weeks of treatment with estradiol significantly decreased infarct size and improved post-ischemic contractile dysfunction (40.4±2.5% vs. 62.9±5.8% for infarct and 44.7±4.0% vs. 27.0±2.7% for post-ischemic functional recovery). Similarly, EDC treatment significantly decreased infarct size (40.9 ±3.6% for EDC vs 63.8±4.7% vehicle) and increased post-ischemic functional recovery (48.8±3.0% EDC vs. 28.6±2.5% vehicle) compared to vehicle-treated hearts. Uterine weight was significantly increased by estrogen treatment but not by EDC. To better understand which ER was involved in cardioprotection, we generated cardiac-specific ERα knockout mice. In these mice, EDC treatment significantly decreased infarct size (20.1±1.9% vs. 51.2 ±7.8% dendrimer) and improved functional recovery (65.8±4.2% vs. 36.8±5.2% dendrimer) compared to the vehicle-treated ERα knockout mice.

Conclusion: These results indicate that EDC is effective in providing cardioprotection during ischemia-reperfusion injury in mice, indicating that non-nuclear ER actions play a major role in this protection. Moreover, non-nuclear ER and/or GPR30 (or ER- in other cell types) are likely candidates to mediate cardioprotection during ischemia-reperfusion injury. Thus, EDC could be utilized clinically to provide cardiovascular benefit without the classical steroid hormone side effect, such as uterine and breast cancer.

014

Recapitulating human myocardial aging and regeneration using wild mice

Nirmala Hariharan[a], Jacqueline Emathinger[b], Nathalie Nguyen[b], Pearl Quijada[b], Hazel Salunga[b], Donald Bers[a], Mark Sussman[b]

[a]*University of California at Davis, Davis, CA, USA*
[b]*San Diego State University, San Diego, CA, USA*

Background: Myocardial regeneration following injury is severely limited by aging. Understanding the impact of age-associated factors such as telomeric shortening is critical to enhance the regenerative potential of cardiac progenitor cells (CPCs). Common inbred mouse strains (such as FVB, C57) possess long and hypervariable telomeres vastly different from human telomeric biology, challenging the relevance of standard mouse lines for studying the molecular phenotypic characteristics of human cardiac aging. Instead, we hypothesize that effects of aging upon cardiac molecular profile and regenerative potential are more relevant to the human condition in Mus musculus castaneus (CAST). CAST is a naturally occurring, inbred wild mouse strain possessing critically short telomeres from birth, obviating the need for generations of breeding or genetic engineering to uncover the impact of telomeric shortening.

Results: Young CAST mice exhibit clinical manifestations of human aging, evidenced by reduction in cardiac output (-50%, $p<0.01$), diastolic dysfunction (E/A ratio: 0.6, $p<0.01$), telomere shortening, elevation of plasma catecholamines ($p<0.05$), increased fibrosis and hypertrophy ($p<0.01$), accompanied by accumulation of senescence markers, p53 and p16 (2 and 2.5 fold) in the myocardium and the CPC pool. CPCs isolated from FVB, C57 and CAST mice were compared to understand the effect of aging on the cardiac stem cell population. Consistent with acquisition of a senescent phenotype, CAST CPCs have slower proliferation rate (-80%) and increased expression of p53, p21 and senescence associated b-galactosidase activity. Surprisingly, CAST CPCs also exhibit increased lineage commitment as determined by expression of differentiation markers and decline in stemness marker c-kit, suggestive of loss of self-renewability.

Conclusions: CAST mice exhibit early cardiac aging which significantly impacts stem cell function. Short telomeres are associated with senescence and differentiation in CPCs. Therefore CAST mice with their critically short telomeres from birth provide a novel approach to link telomere length, cardiac aging, and regeneration.

015

Atrial-selective targeting of arrhythmogenic phase-3 early afterdepolarizations in human myocytes

Stefano Morotti[a], Andrew McCulloch[b], Donald Bers[a], Andrew Edwards[c,d], Eleonora Grandi[a]

[a]*University of California Davis, Davis, Ca, USA,*
[b]*University of California San Diego, San Diego, Ca, USA*
[c]*Oslo University Hospital, Oslo, Norway*
[d]*Simula Research Laboratory, Lysaker, Norway*

Background. We have previously shown that Na current (I_{Na}) non-equilibrium reactivation drives isoproterenol-induced phase-3 early afterdepolarizations (EADs) in failing mouse ventricular myocytes. EAD initiation is secondary to potentiated sarcoplasmic reticulum Ca release and enhanced Na/Ca exchange (NCX), and is abolished by the I_{Na} blocker tetrodotoxin, but not the selective (in ventricles) late I_{Na} blocker ranolazine.

Aim. Since repolarization in human atrial myocytes is relatively rapid and potently modulated by Ca (as in mouse ventricle), we investigated whether the same EAD mechanism may occur in human atria. Indeed, phase-3 EADs have been suggested to underlie re-initiation of atrial fibrillation (AF) after termination upon autonomic stimuli - well recognized AF triggers.

Methods. We integrated a Markov model of I_{Na} gating in our human atrial myocyte model. To recapitulate experimental results, we simulated rapid cell pacing (10 Hz) in the presence of acetylcholine (0.1 μM) and isoproterenol (1 μM), and assessed EAD occurrence upon return to sinus rhythm (1 Hz).

Results. Cellular Ca overload during fast pacing results in a transient period of hyper-contractility after return to sinus rhythm. Here, fast repolarization and enhanced NCX facilitate I_{Na} reactivation via the canonical gating mode (i.e., not late I_{Na} burst mode), which drives EAD initiation. Simulating ranolazine administration reduces atrial peak I_{Na} and leads to faster repolarization, during which I_{Na} fails to reactivate.

Conclusions. Non-equilibrium I_{Na} reactivation can critically contribute to arrhythmias in human atrial myocytes. Ranolazine might be beneficial in this context by blocking peak (not late) atrial I_{Na}.

016

β-arrestin Signaling as a Novel Therapeutic Approach to Familial Dilated Cardiomyopathy

David M. Ryba[a], Conrad L. Cowan[b], David F. Wieczorek[c], Beata M. Wolska[a], R. John Solaro[a]

[a]*University of Illinois at Chicago, Chicago, IL, USA*
[b]*Trevena, Inc., King of Prussia, PA, USA*
[c]*University of Cincinnati, Cincinnati, OH, USA*

Dilated cardiomyopathies (DCM) are the most common form of cardiomyopathy, frequently ending in heart transplant or death. There are no targeted therapies for DCM and current treatment aims to prevent progression of maladaptation via β-blockers, angiotensin receptor blockers (ARB) and angiotensin converting enzyme inhibitors. Compounds coined "biased ligands" are able to act as ARBs, but differ from traditional ARBs in that they are able to preserve and promote β-arrestin signaling. Previously, we have shown that biased agonism of the angiotensin II type 1 receptor (AT1R) with the biased ligand, TRV120023, is able to prevent angiotensin II-mediated hypertrophy while preserving an increased myofilament response to Ca^{2+}. Therefore, we hypothesized that biased agonism of the AT1R would improve cardiac function in DCM compared to the unbiased ARB, losartan. We treated non-transgenic (NTG) mice and mice expressing a mutant tropomyosin (Tm-E54K), which display a phenotype similar to human DCM, with TRV120023 or losartan for three months. Echocardiography revealed that TRV120023 improved ejection fraction, fractional shortening, and systolic wall dimensions in Tm-E54K mice, whereas losartan had no effect. TRV120023 and losartan had no effect on cardiac function of NTG mice as assessed by echocardiography. Detergent-extracted fiber bundles revealed that TRV120023 restored maximum tension generation to NTG-levels and significantly improved Ca^{2+}-sensitivity, as measured by the pCa of half-maximal tension (pCa_{50}), of Tm-E54K mice compared to control and losartan-treated Tm-E54K mice. Fibers isolated from losartan-treated Tm-E54K mice showed no change, but decreased maximum tension generation and pCa_{50} in NTG mice. TRV120023 significantly increased myosin light chain 2 (MLC2) phosphorylation in Tm-E54K myofilaments compared to control and losartan-treated Tm-E54K mice. Taken together, these results indicate that biased agonism of the AT1R is able to improve long-term cardiac function in DCM by improving Ca^{2+}-sensitivity and maximum tension generation through increasing MLC2 phosphorylation.

017

Type 3 p90 ribosomal S6 kinase is required for concentric myocyte hypertrophy in a mouse model for Noonan syndrome

Catherine L. Passariello, Eliana C. Martinez, Jinling Li, Michael S. Kapiloff

University of Miami, Miami, FL, USA

BACKGROUND: Noonan syndrome (NS) is a developmental disorder caused by germline mutations in genes that are components of the Ras/MAPK pathway. This "Rasopathy" is characterized by craniofacial abnormalities, short stature and skeletal malformations, and variable cardiac defects. The two most commons types of cardiac defects in NS are pulmonary valve stenosis and hypertrophic cardiomyopathy. A knock-in mouse for the NS mutation Raf1 L613V has been generated that has a NS-like phenotype including cardiac hypertrophy. p90 ribosomal S6 kinases are effectors for extracellular signal-regulated kinases (ERK) that can be activated by Raf1-MEK signaling. We have published that type 3 RSK is required for concentric myocyte hypertrophy in response to pressure overload. **METHODS:** In order to test whether RSK3 signaling also contributes to NS-related hypertrophy, we have crossed the Raf1 L613V mouse to a RSK3 constitutive knockout mouse. **RESULTS:** RSK3 knock-out attenuated the concentric growth of ventricular myocytes in the Raf1 L613V mouse. Pathological cardiac gene expression induced by Raf1 L613V mutation was also attenuated by RSK3 knock-out. However, the decreased cardiac function and mild pulmonary edema associated with the Raf1 L613V mutation were not improved by RSK3 knockout. **CONCLUSION:** Together these results show a requirement for RSK3 in the concentric hypertrophy induced by Raf1 mutation, while implying the importance of other Raf1-dependent signaling pathways in the induction of heart failure in NS.

018

Cardiac Myosin Binding Protein-C R495Q and R502W Mutations Slow Contractile Kinetics in an Engineered Cardiac Tissue Model

Dan Smelter, Sumit Kar, Willem de Lange, Carter Ralphe

University of Wisconsin, Madison, WI, USA

Cardiac myosin binding protein C (cMyBP-C) is a regulatory protein of the cardiac sarcomere that contributes to contractile reserve. Mutations in cMyBP-C are a leading cause of hypertrophic cardiomyopathy (HCM), a disease that affects about 1 in 500 people worldwide. Truncation mutations in cMyBP-C cause HCM primarily through haploinsufficiency; however, the pathogenesis of disease-causing missense mutations is not well understood. While the physiologic function of the cMyBP-C C3 domain remains unknown, it nonetheless contains the greatest number of HCM-causing mutations. Recent structural studies of domain C3 identified a highly-conserved, positively charged region that is a proposed protein-protein interaction interface. Two highly prevalent and pathogenic mutations, R495Q and R502W, occur in this positively charged region.

We hypothesized that the R495Q and R502W mutations may affect contractility through altered ligand binding, without affecting domain stability.

We used adenovirus to express human R495Q and R502W mutant cMyBP-C in neonatal mouse cardiomyocytes deficient in endogenous cMyBP-C, followed by molecular and functional characterization using a 3D engineered cardiac tissue (ECT) model.

Exogenous expression of the mutant proteins results in stable mRNA and protein expression with normal incorporation in the cardiac sarcomere. Functional assessment of the R495Q and R502W mutations revealed no change in twitch force amplitude. However, the R495Q mutation's time to peak contraction was $9\pm2\%$ slower ($p<0.05$) and the time to 50% relaxation was $22\pm1\%$ slower ($p<0.05$; $n=14$) than the wild-type controls. The R502W mutation's time to 50% relaxation trended slower than wild type by $17\pm5\%$ ($p=0.079$; $n=7$). Altered contractility was independent of extracellular calcium sensitivity. These data contrast the accelerated kinetics observed in both cMyBP-C ablated ECT and models of haploinsufficiency. Ongoing work will focus on defining the mechanism through which mutations in the C3 domain of cMyBP-C alter cardiac contraction kinetics.

019

Enhancing Myocardial Repair with CardioChimeras

Pearl Quijada[a], Hazel Salunga[a], Nirmala Hariharan[b], Farid El-Sayed[a], Maryam Moshref[a], Roberto Alvarez[a], Natalie Gude[a], Mark Sussman[a]

[a]*San Diego State University, San Diego, USA*
[b]*University of California, Davis, Davis, USA*

Dual cell transplantation of cardiac progenitor cells (CPCs) and mesenchymal stem cells (MSCs) after infarction enhances myocardial repair and performance in large animal models relative to delivery of either cell population individually. However, a single stem cell to support both direct and indirect mechanisms of myocardial repair has yet to be identified. CardioChimeras (CCs), a progenitor cell formed by fusion between CPCs and MSCs were analysed for reparative potential after myocardial infarction (MI) relative to individual parents cell or combined parent cell delivery. Two representative CCs, CardioChimera 1 (CC1) and CardioChimera 2 (CC2) were used for this study. CC1 and CC2 improved left ventricular anterior wall thickness (AWT) at 4 weeks, but only CC1 treatment preserved AWT at 18 weeks relative to no cell treatment (PBS). Ejection fraction was enhanced at 6 weeks post injury in CC1 and CC2 groups, which was maintained in CC1, CC2 and CPC + MSC combined groups up to 18 weeks. Infarct size was decreased by 5% in CC1 and CC2 hearts, whereas CPC + MSC and CPC parent groups remained unchanged when comparing 4 to 12 week change in scar size. MSC and PBS groups displayed marked increases in infarct size (10-15%). CC1 and CC2 showed enhanced engraftment potential by 3-fold relative to CPC + MSC and CPC hearts. In contrast, MSCs were detected at low levels (0.04%). CC1 and CC2 discovered within the myocardium expressed early commitment marker cardiac troponin T relative to controls. CC1 and CC2 treatment increased capillary density within the infarct, indicating that cell persistence facilitates paracrine mediated vasculature stabilization and/or formation. CCs merge the application of distinct cells into a single entity for cellular therapeutic intervention in the progression of heart failure. CCs represent a tractable cellular system that improves upon combinatorial cell therapy approaches and supports myocardial regeneration.

020

Enhanced Na^+ - glucose cotransport causes Na^+ overload in diabetic hearts

Rebekah Lambert[a], Sarah Srodulski[a], Xiaoli Peng[a], Kenneth Margulies[b], Florin Despa[a], Sanda Despa[a]

[a]*University of Kentucky, Lexington, KY, USA*
[b]*University of Pennsylvania, Philadelphia, PA, USA*

Rationale: Intracellular Na^+ concentration ($[Na^+]_i$) critically regulates cardiac Ca^{2+} cycling, contractility, metabolism and electrical stability. Myocyte $[Na^+]_i$ is elevated in heart failure (HF), leading to arrhythmias and oxidative stress.

Objective: To test the hypothesis that myocyte $[Na^+]_i$ is increased in type-2 diabetes (T2D) due to enhanced activity of the Na^+-glucose cotransporter (SGLT).

Methods and Results: We found increased SGLT expression in failing hearts from patients with T2D compared to non-diabetic individuals (by 73±13%) and in hearts from rats with late-onset T2D (HIP rats) *versus* wild-type (WT) littermates (by 61±8%). $[Na^+]_i$, measured with the fluorescent indicator SBFI, was increased in HIP rat myocytes, both at rest (14.7±0.9 *versus* 11.4±0.7 mmol/L in WT) and during electrical stimulation at 2 Hz (17.3±0.8 *versus* 15.0±0.7 mmol/L). However, the Na^+/K^+-pump function (measured as the rate of pump-mediated $[Na^+]_i$ decline in intact myocytes) was similar in HIP and WT cells, suggesting that higher $[Na^+]_i$ is due to elevated Na^+ entry in HIP myocytes. Indeed, Na^+ influx, assessed as the rate of $[Na^+]_i$ rise upon Na^+/K^+-pump inhibition with 10 mM ouabain, was significantly larger in myocytes from HIP compared to WT rats (1.77±0.11 *versus* 1.29±0.06 mmol/L/min). SGLT inhibition with 250 μM phlorizin or glucose-free solution greatly reduced Na^+ influx in HIP rat myocytes (to 1.20±0.16 mmol/L/min), while it had no effect in WT hearts. Phlorizin also significantly decreased glucose uptake in HIP myocytes (by 33±9 %) but not in WT, indicating an increased reliance on SGLT for glucose uptake in T2D hearts.

Conclusions: Myocyte Na^+-glucose cotransport is enhanced in T2D, which increases Na^+ influx and causes Na^+ overload. While elevated $[Na^+]_i$ and Na^+ influx are common to other pathological conditions (e.g., HF), the major underlying mechanism in diabetic hearts is specific to T2D.

021

Gene delivery into rat myocardium using Cardiac Isoform of alpha-2 macroglobulin – a new Cardiac Biomarker (CA2M)

Ponnambalam Annapoorani

V.V. Vanniaperumal College For Women, Virudhunagar, Tamil Nadu, India

Gene therapy is emerging as a realistic addition to the therapeutic arsenal in heart failure, the search for suitable vectors for cardiac transfection is still ongoing. Cardiac isoform of alpha 2 macroglobulin-CA2M, has been shown to be an early marker in cardiac hypertrophy in rat and human. Direct injection of full length c DNA of the CA2M induce cardiac hypertrophy in rat heart.

Aim: the CA2M specificity towards muscle prompt us to explore the possibility of using this protein to deliver genes into myocardium

Method: the purified CA2M was coupled with Poly L Lysine (PLL) by crosslinker N-Succinimidyl 3-(2pyridyldithio) –propionate (SPDP) and green fluorescent protein(GFP) reporter gene and standardized DNA-Protein Complex(DPC) were used for injection studies. Histological analysis and RT- PCR were carried out to identify and quantify the reporter gene (GFP) expression.

Results: successful in-vivo gene delivery into myocardium by DPC was obtained though unspecific traces of expression of GFP were also documented in spleen, liver, reproducti ve organs and kidney. However predominant expression in myocardium and induction of fetal genes, growth factor genes, oncogenes and cardiac specific genes including b MHC,(beta myosin heavy chain) ,TGFβ1 (Transforming growth factor beta 1), ANG (Angiotensinogen) ,ANF (Artrial Natriuretic Factor) and c-fos can be taken into consideration to suggest this protein as a vehicle for gene transfer into myocardium. Collectively these data along with the induction of P13 kinase /AKT pathway argues in favour of using this protein to transfer desirable genes into myocardium. Results and postulated mechanism from other studies predominantly suggests receptor – mediated uptake of DPC by heart. Recent analysis and characterization of c DNA of CA2M revealed that the specificity of CA2M towards cardiac tissue was due to variation of amino acids clustering at the region of receptor binding domain that comprises the residues between 776-1399(Gen Bank accession nos AY919611,AY921651 and AY887133), and this support s the receptor mediated uptake of DPC into myocardium .

Conclusion: our attempt to know the possibility of gene transfer using CA2M towards myocardium has shown to be successful.

022

Development of Tissue Engineered Small Diameter Vascular Grafts

Harveen Lamba[a], Yakov Elgudin[a], Gary Wnek[b], Steven Emancipator[a]

[a]*Louis Stokes Veterans Affairs, Cleveland, OH, USA*
[b]*Case Western Reserve University School of Engineering, Cleveland, OH, USA*

Background: There is an increasing need for vascular grafts in the field of surgical revascularization. However, smaller vascular grafts made from synthetic biomaterials, particularly those <5 mm in diameter, are associated with a high incidence of thrombosis. We evaluated in vitro and in vivo characteristics of small-diameter vascular grafts made of Polycaprolactone (PCL), a biodegradable polymer, alone or integrated with either vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (B-FGF).

Methods and Results: 2 mm PCL grafts were evaluated in the rat abdominal aorta replacement model for 6 weeks (n=5). Patency and integrity were evaluated by ultrasound. All animals survived until the end of follow-up and all grafts were patent. The explanted graft was investigated histologically and results showed reactive endothelial coverage, robust neointima formation along the entire length of the graft, and transmural cellular ingrowth throughout the thickness of the graft, also along its entire length. We also developed 2 mm PCL grafts integrated with either VEGF or B-FGF, using a novel biphasic electrospinning method, and compared them to non-modified PCL grafts in vitro via dynamic mechanical analysis (DMA) and scanning electron microscopy (SEM). Results showed improved tensile strength and decreased fiber diameter and pore size in the VEGF and B-FGF modified PCL grafts. VEGF and B-FGF integrated PCL grafts were also compared to control PCL grafts in vivo via subcutaneous implantation in rats (n=5 per group). These

grafts were explanted after 3 weeks and analyzed histologically. The cytokine modified PCL grafts demonstrated considerably more extracellular matrix than control grafts, less conspicuous macrophage infiltrate, more retained polymer fibrils and better giant cell profile.

Conclusions: In sum, these results demonstrate strong potential for cytokine modified PCL grafts as small diameter vascular replacements because of their better Improved tensile strength, endothelialization and extracellular matrix formation as compared to non-modified PCL grafts.

023

Reciprocal regulation of cardiac chromatin by HMGB and CTCF: implications for transcriptional regulation in pathologic hypertrophy

Emma Monte[a], Manuel Rosa-Garrido[a], Elaheh Karbassi[a], Haodong Chen[a], Christoph Rau[a], Jessica Wang[a], Yong Wu[a], Enrico Stefani[a], James N. Weiss[a], Aldons J. Lusis[a], Siavash Kurdistani[a], Sarah Franklin[b], Thomas M. Vondriska[a]

[a]*UCLA, Los Angeles, CA, USA*
[b]*Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, USA*

Transcriptome remodeling in heart failure is coordinated through changes in the abundance of transcription factors, histone modifications and other chromatin features localized at these genes. However, it remains unknown if, and to what extent, chromatin reorganization on a genome-wide scale contributes to the gene expression remodeling seen in the pathologic stress response in the heart. In this study, we examined the roles of two chromatin structural proteins, CTCF and HMGB2, to regulate pathologic gene expression. Our data demonstrate a reciprocal relationship between HMGB2 and CTCF in regulating myocyte hypertrophy and many aspects of chromatin structure and gene expression. HMGB2, but not CTCF, is positively correlated with heart mass across a panel of inbred mouse strains in the basal setting—in contrast, the response of HMGB2 (but not CTCF) expression to hypertrophic stress is strongly influenced by common genetic variation. Both proteins regulate each other's expression as well as the transcription of fetal genes and ribosomal RNA in cardiac myocytes: however, only HMGB2 does so in a manner that involves targeted reprogramming of chromatin accessibility. To our knowledge, these studies (using a combination of micrococcal nuclease digestion, chromatin fragment analysis and PCR) are the first to directly measure chromatin structure in cardiac cells. Lastly, while both proteins share gene targets, HMGB2 and CTCF neither bind these genes simultaneously nor do they physically co-localize in myocyte nuclei. Our study uncovers a previously unknown relationship between these two ubiquitous chromatin proteins and provides a mechanistic explanation for how HMGB2 regulates gene expression and cellular phenotype. Furthermore, we demonstrate direct evidence for hierarchical remodeling of chromatin (at a scale above the level of a single gene) on a genome-wide scale in the setting of cardiac disease.

024

TRPV2 stimulation diminishes peripartum cardiomyopathy through increased ejection fraction and decreased dilation

Evan Onusko, Guansheng Liu, Sheryl Koch, Min Jiang, Evangelia Kranias, Jack Rubinstein

University of Cincinnati, Cincinnati, OH, USA

Background

Peripartum cardiomyopathy (PPCM) is a potentially life-threatening disorder in which dilated cardiomyopathy and heart failure with reduced ejection fraction develop in the mother in the months surrounding the end of pregnancy. Recent data have shown that an S10F mutation in the cardiac heat shock protein 20 (Hsp20) disrupts its function and produces a phenotype strikingly similar to that of PPCM. TRPV2, a channel known to regulate contractility via modulation of calcium handling, is an effective therapeutic target for HFrEF through stimulation by an agonist, probenecid. Our preliminary data demonstrated that the Hsp20 mutation is associated with decreased expression of TRPV2, therefore we hypothesized that TRPV2 stimulation will preserve cardiac function and provide a novel therapeutic option.

Methods

Female wild type and S10F mutant mice of similar ages were split into two breeding groups, one of which received water containing probenecid after its first pregnancy (the typical period of diagnosis), while the other served as a control. Each mouse was allowed to progress through several pregnancies, and echocardiograms were obtained using the Vevo2100 ultrasound system before, during, and after each pregnancy to evaluate for changes in cardiac function and structure. After the third pregnancy, the mice were euthanized and their hearts were collected to determine the extent of dilated cardiomyopathy through changes in heart failure protein markers, cell size, hypertrophy and apoptosis.

Results

Initial results demonstrated that treated S10F have smaller LV volume at both systole and diastole ($<.05$). The cardiac function and structure of the treated mice was not significantly different from the WT mice.

Conclusion

These data support our hypothesis that TRPV2 stimulation prevents the deterioration of cardiac function in a model of PPCM.

025

Phosphorylation of cardiac myosin binding protein C is a dominant determinant of diastolic function in engineered cardiac tissue

Sumit Kar, Dan Smelter, Willem de Lange, Daniel Fitzsimons, Richard Moss, J. Carter Ralphe

University of Wisconsin-Madison, Madison, WI, USA

Cardiac myosin binding protein-C (cMyBP-C) is a sarcomeric protein that regulates contractility in response to increased circulatory demand. Phosphorylation of cMyBP-C during adrenergic stimulation increases cardiac contractility by attenuating inhibitory interactions of cMyBP-C with myosin. Reduced cMyBP-C phosphorylation due to reduced adrenergic signaling in heart failure contributes to decreased cardiac contractility. In addition, mutations in cMyBP-C linked to hypertrophic cardiomyopathy (HCM) may contribute to the development of the disease by altering phosphorylation. cMyBP-C contains three phosphorylatable serines at the N-terminus that exist in a range of phosphorylation states in vivo. However, the functional effects of these partial phosphorylation states are not known. We tested the hypothesis that the phosphorylation profile of cMyBP-C modulates cardiac function in mouse engineered cardiac tissue (ECTs). Constitutively phosphorylated ($n=7$) and constitutively non-phosphorylated

(n=2) cMyBP-C were expressed in ECTs lacking endogenous cMyBP-C by adenoviral-mediated gene transfer to determine the primary contractile effects of phosphorylation in the absence of confounding remodeling events. Constitutively non-phosphorylated ECTs exhibited similar contractile kinetics to WT ECTs but ablated the kinetic response to adrenergic stimulation, as expected. Constitutive phosphorylation did not alter contractile kinetics but significantly reduced time to 50% relaxation (0.026±0.01 sec vs. 0.037±0.01 sec in WT, P<0.05). Interestingly, constitutive phosphorylation of cMyBP-C exhibits faster late relaxation kinetics than after ablation of cMyBP-C (0.024±0.01 sec vs. 0.028±0.01 sec in KO, P<0.05). Our data suggest HCM mutations that affect phosphorylation of cMyBP-C modulate diastolic function via mechanisms that are distinct from those due to mutations that ablate cMyBP-C. Studies to characterize the binding of cMyBP-C to myosin and actin following phosphorylation are underway to determine these mechanisms. These results indicate that ECTs are a valuable pure expression model to study the unique phosphorylation states of cMyBP-C.

026

CARD9 knockout reduces myocardial ischemia and reperfusion injury in mice

Xing Qin, Li Cao, Tiantian Zheng, Sreejayan Nair, Jun Ren, Guanglong He

School of Pharmacy, University of Wyoming, Laramie, USA

Despite advances in the development of new strategies for treating ischemic heart disease, myocardial ischemia and reperfusion (I/R) injury remains a major cause of cardiac dysfunction and associated morbidity and mortality in the United States. Neutrophil infiltration and tissue damage-associated inflammatory responses have been reported to play a critical role in the cellular damage following myocardial infarction. Caspase recruitment domain containing protein 9 (CARD9) that regulates innate and adaptive immune responses is highly expressed in neutrophils. We hypothesized that CARD9 activates myocardial infarction-associated pro-inflammatory signaling and contributes to I/R injury.

Male wilt-type C57/BL6 (WT) and CARD9 knockout ($CARD9^{-/-}$) mice (12 weeks of age) were anesthetized with intraperitoneal injection of ketamine (55 mg/kg) and xylazine (15 mg/kg), followed by intubation and ventilation with room air at a tidal volume of 220 μL and respiratory rate of 120 breath/min. Mice were then subjected to 45 min of LAD occlusion followed by 24 h of reperfusion. The sham group was without the I/R procedures. Area at risk (AAR) and infarct size were determined with Evans blue and TTC staining. Following reperfusion, left ventricular tissue was excised and subjected to Western immunoblotting analyses to assess the phosphorylation levels of p38 MAPK, a pro-inflammatory transcriptional factor associated with myocardial injury. The infarct size of $CARD9^{-/-}$+I/R mice was significantly reduced compared to the WT+I/R mice, with no significant difference of AAR between the two groups. CARD9 protein levels were significantly higher in the WT+I/R group compared to the sham group. Further, the phosphorylation level of p38 MAPK in the WT+I/R mice was significantly increased compared to the sham group, and was significantly attenuated in the $CARD9^{-/-}$+I/R group compared to the WT+I/R group.

In conclusion, CARD9 knockout protected the heart from ischemia and reperfusion injury via down-regulation of p38 MAPK phosphorylation in the infiltrated neutrophils.

027

Myocardial accumulation of amylin induces oxidative stress through sarcolemmal lipid peroxidation

Miao Liu[a], Nirmal Verma[a], Xiao Li Peng[a], Andrew Morris[a], Kenneth Margulies[b], Sanda Despa[a], Florin Despa[a]

[a]*University of Kentucky, Lexington, KY, USA*
[b]*University of Pennsylvania, Philadelphia, PA, USA*

Background: Chronic hypersecretion of pancreatic hormone amylin leads to infiltration of oligomeric amylin in cellular membranes, which contributes to β-cell dysfunction and development of type-2 diabetes (T2D). We recently showed that oligomeric amylin also accumulates in myocardium of patients with obesity or T2D. Oligomeric amylin may infiltrate the sarcolemma exposing membrane lipids to peroxidation by ROS, thus exacerbating myocardial oxidative stress.

Methods: Accordingly, we investigated the relationship between incorporated amylin and lipid peroxidation in ventricular myocyte from obese patients (BMI≥30) with heart failure (HF) (O-HF; N=6), obese humans without HF (O-NF; N=6) and healthy people with BMI<30 (L-NF; N=4). The proposed mechanism was also tested in an animal model of myocardial amylin accumulation (HIP rat) and control rat myocytes incubated with preformed amylin oligomers.

Results: Compared to L-NF, the myocyte level of oligomeric amylin is increased in both O-HF (10-fold; P<0.01) and O-NF (3.5-fold; P<0.05). 4-HNE, an aldehydic product of lipid peroxidation, is also increased in O-NF (4-fold; P<0.01) and even further in O-HF (6-fold; P<0.001). Confocal microscopy analysis of lipid peroxidation probes C_{11}-BODIPY and Liperfluo indicates increased lipid peroxidation in HIP rat myocytes (2-fold; P<0.001). Significantly lower levels of lipid peroxide were found in age- and glucose-matched UCD rats which do not accumulate amylin in the heart. Moreover, incubation of control rat myocytes with exogenous amylin oligomers (50 μM; 2 hours) increased lipid peroxidation and ROS production. In contrast, incubation for the same duration with 400 mg/dl glucose had no effect on lipid peroxidation. Reducing ROS in vitro, by pre-incubation with 5mM NAC, or amylin oligomer-mediated sarcolemmal damage, by 50 μM membrane sealant P188, blocked lipid peroxidation.

Conclusions: Together, these findings support the hypothesis that oligomerized amylin infiltrates cardiac myocytes in humans and exacerbates lipid peroxidation. Strategies to mitigate myocardial deposition of amylin oligomers should be pursued.

028

Role of miR-181 Family in Heart Failure: A Tale of Two Intracellular Cardiomyocyte Compartments

Samarjit Das[a], Mark Kohr[a], Brittany Dunkerly[a], Djahida Bedja[a], Oliver Kent[b], Anthony Leung[a], Jorge Henao-Mejia[c], Richard Flavell[c], David Kass[a], Charles Steenbergen[a]

[a]*Johns Hopkins University, Baltimore, MD, USA*
[b]*University of Toronto, Toronto, ON, Canada*
[c]*Yale University School of Medicine, New Haven, CT, USA*

MicroRNA is a type of small non-coding RNA that can repress the expression of target genes by post-transcriptional regulation. miRNAs play an important role in physiologic and pathologic processes of cardiovascular health. We demonstrated that miR-181c

exists in heart-mitochondria, but nuclear encoded, and more importantly affects mitochondrial function by regulating mitochondrial gene. To investigate how miR-181c leads to cardiac injury, we designed miR-181-sponges, RNA molecules with ten repeated complimentary miR-181 "seed" sequences, and generated a set of stable-H9c2 cells by transfecting either a scrambled- or the miR-181-sponge-sequences. Sponge-H9c2 showed a significant decrease in ROS production and reduced basal mitochondrial respiration, and significant protection against Doxorubicin-induced oxidative stress. However, chronic down-regulation of the entire miR-181 family by miR-181-sponge may alter other gene(s). We found that miR-181a/b targets PTEN, and thus the sponge decreased PI3K signaling. Thus, protection against Doxorubicin goes even higher when we treated sponge-H9c2 with siRNA against PTEN. We hypothesized that miR-181a/b targets PTEN in the cytosol and miR-181c targets mt-COX1 in the mitochondria. To extend this finding, we have used two sets of knock-out mice (miR-181a/b$^{-/-}$ and miR-181c/d$^{-/-}$), and subjected them to ischemia-reperfusion injury. miR-181c/d$^{-/-}$ show a significant decrease, while miR-181a/b$^{-/-}$ show a significant increase in infarct size compared to WT. Isolated mitochondria study showed miR-181c/d$^{-/-}$ is more protective compare to WT; whereas, miR-181a/b$^{-/-}$ failed to show this phenotype. Taken together, the miR-181 family regulates important signaling pathways in oxidative stress, notably with detrimental results by targeting mt-COX1 (miR-181c), or with protection by targeting PTEN (miR-181a/b).

029

Mitochondrial Ca^{2+} Uniporter inhibition induces unsolicited Ca^{2+} waves by increasing sarcoplasmic reticulum Ca^{2+} content

Jesús Roberto Garza López, Gerardo de Jesús García Rivas

Tecnologico de Monterrey, Nuevo León, Mexico
Cátedra de Cardiología de la Escula de Medicina del Tecnológico de Monterrey, Nuevo León, Mexico

Background

Sudden death is a main cause of mortality among cardiovascular pathologies, mainly caused by ventricular arrhythmias. Modulating Ca^{2+} release from the sarcoplasmic reticulum (SR), Ca^{2+} uptake to the SR or Ca^{2+} removal to extracellular space, are potential approaches to reduce this events. Recently, it has been described that mitochondria seems to be a participant of this dynamic. Is thought to play this role by buffering Ca^{2+} signalling in the cardiomyocytes and with the recent molecular identification of mitochondrial Ca^{2+} uniporter (MCU), genetic engineering strategies shows that cytosolic Ca^{2+} peaks are reduced or enhanced by MCU overexpression and siRNA silencing, respectively. But the integral contribution of MCU on Ca^{2+} dynamics is still under discussion, and moreover its implication on physiopathology is not concluded.

Methods

Isolated adult male rat cardiomyocytes were used to determine the Ca^{2+} transient, caffeine induced Ca^{2+} transient and spontaneous Ca^{2+} release (SCR) under confocal microscopy with Fluo 3-AM as fluorescent Ca^{2+} indicator, in a line scan mode. SCR events were determined at rest after a 5 pulse electric field training during the modulation of MCU with Ru_{360}.

Results

Transient amplitude is 4.24 ± 0.28 vs. 5.71 ± 0.34 $\Delta F/F_0$ with an EC_{50} 1.12 μM. The time at which 50% of Ca^{2+} is removed is 146.5 ± 5.6 vs. 177.5 ± 6.8 milliseconds. The number of spontaneous Ca^{2+} events is 1.75 ± 1.04 vs. 2.11 ± 0.49. Data Control vs.1μM Ru_{360}.

Conclusion

Cytosolic Ca^{2+} is increased by 34% when inhibiting MCU; further, this has an impact delaying Ca^{2+} removal. This shows that the mitochondria could partially buffer the ion during the Ca^{2+} transient and could indirectly modulate the activity of SR Ca^{2+} ATPase. Moreover, the effect of MCU inhibition on Ca^{2+} dynamics is related with positive tendency of apparition of SCR possibly due to an increase of SR Ca^{2+} content, which could represent a possible substrate for ventricular arrhythmia.

030

Chaer lncRNA negatively regulates PRC2 during cardiac hypertrophy

Zhihua Wang[a], Xinghua Wang[b], Iris Chen[a], Chen Gao[a], Tomohiro Yokota[a], He Wang[a], Shuxun Ren[a], Ashley Cass[c], Xinshu Xiao[c], Guangping Li[b], Yibin Wang[a]

[a]*Division of Molecular Medicine, Departments of Anesthesiology, Physiology and Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, USA*
[b]*Department of Cardiology, Tianjin Institute of Cardiology, Second Hospital of Tianjin Medical University, Tianjin, China*
[c]*Molecular Biology Institute, Department of Integrative Biology and Physiology, College of Life Sciences, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, USA*

Long non-coding RNAs (lncRNAs) emerge to be critical regulators of cellular processes, but only a few out of thousands have been functionally characterized. We identified a novel heart-specific lncRNA, named cardiac hypertrophy associated epigenetics regulator (Chaer), which was both necessary and sufficient for hypertrophy of neonatal rat ventricular cardiomyocytes. RNA deep-sequencing revealed that Chaer contributed to the global transcriptome reprogramming during phenylephrine (50 microM)-induced hypertrophy, and regulated imprinted gene H19 expression independent of DNA methylation but dependent on histone tri-methylation at H3K27 (H3K27me3). RNA immunoprecipitation assay found that Chaer directly interacting with and negatively regulating PRC2 function on H3K27me3. Tagged RNA pull-dwon and RNA EMSA assays confirmed that Chaer directly bound to the catalytic subunit Ezh2 with a conserved 66-mer motif near its 5' end in competition with and functionally interrupting other PRC2-binding lncRNAs. Interestingly, Chaer-PRC2 interaction was transiently enhanced at the onset of hypertrophy and responsible for hypertrophy fetal gene induction which was sensitive to Ezh2 inhibitor GSK126 (1 microM). Moreover, mTOR inhibitor rapamycin (20 nM) completely blocked the enhanced Chaer-PRC2 interaction, reversed the decrease of global H3K27me3, and abolished phenylephrine-induced expression of hypertrophy fetal genes. Finally, Chaer silence *in vivo* using chemically modified siRNA and nanoparticle transfection reagents significantly reversed the development of cardiac hypertrophy, pathological remodeling and H3K27m3-modification-mediated fetal gene induction under transaortic-constriction-induced pressure overload. The findings unveil Chaer as an epigenetic determinant of cardiac hypertrophy, and shed a light into the early molecular events under cardiac stress.

031

Comparable calcium handling of human iPSC-derived cardiomyocytes generated by multiple laboratories

Tromondae K. Feaster[a], Hyun Seok Hwang[a], Dmytro O. Kryshtal[a], Verónica Sánchez-Freire[b], Jianhua Zhang[c], Timothy J. Kamp[c], Charles C. Hong[a], Joseph C. Wu[b], Björn C. Knollmann[a]

[a]*Vanderbilt University School of Medicine, Nashville, TN, USA*
[b]*Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, USA*
[c]*Department of Cell and Regenerative Biology, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA*

Cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs) are being increasingly used to model human heart diseases. hiPSC-CMs generated by earlier aggregation based methods (i.e., embryoid body) often lack functional sarcoplasmic reticulum (SR) Ca stores characteristic of mature mammalian CMs. Newer monolayer-based cardiac differentiation methods (i.e., Matrigel sandwich or small molecule-based differentiation) produce hiPSC-CMs of high purity and yield, but their Ca handling properties have not been comprehensively investigated. Here, we study Ca handling and cytosolic Ca buffering properties of hiPSC-CMs generated independently from multiple hiPSC lines at Stanford University, Vanderbilt University and University of Wisconsin. hiPSC-CMs were cryopreserved at each university. Frozen aliquots were shipped, recovered from cryopreservation, plated at low density and compared 3-5 days after plating with acutely-isolated adult rabbit and mouse ventricular CMs. Cytoplasmic Ca buffering was not significantly different between hiPSC-CMs and rabbit-CMs. hiPSC-CMs from all three laboratories exhibited robust caffeine-releasable SR Ca stores. SR Ca content and height of field-stimulated (0.5 Hz) Ca transients were not significantly different from rabbit-CMs. Ca transport rates by sarcoendoplasmic reticulum Ca ATPase (SERCA) and Na/Ca exchanger (NCX) were comparable in all hiPSC-CM lines, but significantly slower compared to rabbit- CMs. However, the relative contribution of SERCA and NCX to Ca transport of hiPSC-CMs was comparable to rabbit-CMs. Ca handling maturity of hiPSC-CMs increased significantly from 15 to 21 days post-induction. We conclude that hiPSC-CMs generated independently from multiple iPSC lines using monolayer-based methods can be reproducibly recovered from cryopreservation and exhibit comparable and functional SR Ca handling.

032

ImageStream analysis of dispersed human cardiomyocytes: A high through-put assay for proliferation

Jun Luo, Stephen Farris, Deri Helterline, April Stempien-Otero

University of Washington, Seattle, WA, USA

Background: Accurate measurement of human cardiomyocyte (CM) proliferation is critical to assessing the success of regenerative therapies. To develop an unbiased approach for sampling large numbers of adult human CM, we developed protocols to dissociate CM from human heart and analyse the dispersed cells using ImageStream® flow cytometry.

Methods: Ventricular tissues were obtained from six subjects undergoing primary cardiac transplant. Subjects had preserved left ventricular systolic function with intractable angina (Group1, $n = 2$) or end-stage systolic dysfunction (Group2, $n = 4$). Individual cardiomyocytes were enzymatically isolated and stored in ethanol at - 80 °C until FACS analysis. Cardiomyocytes (7 ± 4 x10^3 per subject) were identified by presence of α-Actinin and DNA content was gauged by Draq5 staining using Image-Stream® FACS. Cell cycle events were examined using antibodies to Ki-67, phospho-histone3 (H3P) and mitotic kinesin-like protein-1 (MKLP-1).

Results: Failing hearts from Group2 had increased left ventricular mass and individual CM were larger. Group2 had more multi-nucleated CM ($73.9 \pm 6.5\%$ vs $81.5 \pm 1.1\%$ mononuclear, $17.9 \pm 3.8\%$ vs $16.4 \pm 1.1\%$ binuclear, $4 \pm 1.8\%$ vs $0.5 \pm 0.1\%$ trinuclear, $P < 0.05$ for each comparison). Furthermore, mononucleated CM's from failing hearts have significantly higher DNA content than those with normal function ($P < 0.05$, vs Group1). No positive cell cycle or cytokinesis events were captured form a combined total of 5.6x10^4 CMs in the current study. Control cardiac fibroblasts were positive: 12% Ki-67^+, 9% H3P^+ and 4% MKLP1^+.

Conclusions: Combined fluorescent microscopy and FACS provides a high throughput method to sample cell cycle events in human CM. Our preliminary data show similar trends as histologic studies: hypertrophy is associated with increase nucleation and DNA content. However, we detected different absolute levels of nucleation and DNA content than previously reported. This finding could alter the interpretation of other studies of CM proliferation.

033

Deletion of Diacylglycerol:acyltransferase 1 (DGAT1) selectively reduces incorporation of 16:0 fatty acid into triglyceride and alters cardiac metabolism

Nathan Roe, Rong Tian

University of Washington, Seattle, WA, USA

Triglyceride (TG) metabolism in heart failure has been well established to be impaired in both human and animal models however its impact on severity and progression of heart failure has not been investigated. We found that patients with heart failure displayed decreased mRNA levels of diacylglycerol:acyltransferase 1 (DGAT1), the rate limiting enzyme in TG synthesis (0.56 ± 0.06 fold of NF, $p = 0.0001$) and a lower myocardial TG content (3.65 ± 0.41 vs. 8.27 ± 1.48 mg/g tissue in NF, $n = 8$-11, $p = 0.019$). Therefore we hypothesized that altered TG synthesis caused metabolic remodeling in heart failure. In mice with tamoxifen-inducible cardiac specific deletion of DGAT1 (iKO), ^{13}C NMR spectroscopy in perfused hearts revealed a 30% reduction in labeled fatty acid (FA) incorporation into TG in iKO (40 minute peak area 7.86 ± 0.26 vs. 5.73 ± 0.50) with no changes in total TG content. In depth investigation of TG pool labeling by GCMS revealed that iKO mice had reduced labeling of 16:0 FA (42.85 ± 2.83 vs. 33.09 ± 4.75 $\%^{13}C$, $P = 0.014$) with no change in 16:1 18:0, 18:1, 18:2 labeling or overall TG FA composition were observed ($n = 4$). Reduced 16:0 incorporation reduced overall fatty acid TG incorporation rate into TG (34.18 ± 5.05 vs. 24.20 ± 3.77 nmol/min/g, $p = 0.16$) albeit not significantly. Fats not incorporated into TG appear to be oxidized directly as iKO hearts showed increased oxidation of exogenous FA ($67.0 \pm 4.1\%$ vs. $48.5 \pm 5.3\%$) and reduced glucose oxidation ($12.9 \pm 4.2\%$ vs. $27.4 \pm 4.5\%$, $n = 5$-7). Using linear regression analysis, a strong correlation between the rate of TG turnover and FA oxidation in both

groups was observed ($R^2 = 0.82$, $P = 0.002$, $n = 8$). These observations suggest that DGAT1 preferentially incorporates 16:0 FA into TG and that fatty acids not incorporated into the TG pool are diverted towards oxidative metabolism. Therefore reduced DGAT1 expression in failing hearts whose oxidative metabolism is compromised, may promote lipotoxicity by reducing incorporation of toxic lipids into TG.

034

Reduced autophagic flux contributes to accumulation of fragmented mitochondria in heart failure: Impact of exercise training

Juliane Campos[a], Bruno Queliconi[b], Luiz Bechara[a], Paulo Jannig[c], Paulo Dourado[d], Kátia Gomes[a], Vanessa Zambelli[e], Cibele Resende[f], Silvia Guatimosim[f], Alicia Kowaltowski[b], Daria Mochly-Rosen[g], Patricia Brum[c], Julio Ferreira[a]

[a]*Institute of Biomedical Sciences - University of Sao Paulo, Sao Paulo, SP, Brazil*
[b]*Instituto de Química - Universidade de São Paulo, São Paulo, SP, Brazil*
[c]*School of Physical Education and Sport - University of Sao Paulo, Sao Paulo, SP, Brazil*
[d]*Heart Institute, Sao Paulo, SP, Brazil*
[e]*Butantan Institute, Sao Paulo, SP, Brazil*
[f]*Physiology and Biophysics - Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*
[g]*Department of Chemical and Systems Biology - Stanford University School of Medicine, Stanford, CA, USA*

We previously reported that facilitating mitochondrial autophagy [mitophagy] protects against acute myocardial infarction. Here we characterized the impact of exercise training, an important and safe strategy for prevention and treatment of cardiovascular disease, on cardiac autophagy and its contribution to mitochondrial bioenergetics profile and oxidative stress in a post-myocardial infarction-induced heart failure animal model. We showed that failing hearts display reduced autophagic flux, characterized by accumulation of autophagy-related markers as well as loss of responsiveness to chloroquine treatment at 4 and 10 weeks after myocardial infarction. These changes were accompanied by accumulation of fragmented mitochondria with reduced oxygen consumption, elevated H_2O_2 release and increased calcium-induced mPTP opening. Further supporting an autophagic mechanism for accumulation of dysfunctional mitochondrial, disruption of autophagic flux by either genetic ablation ($ATG5^{-/-}$) or pharmacological intervention (chloroquine) decreased mitochondrial bioenergetics and increased oxidative stress. Excessive mitochondrial fragmentation *per* se seen in embryonic fibroblasts lacking MFN1 did not affect mitochondrial bioenergetics. Importantly, 8 weeks of aerobic exercise training, starting 4 weeks after myocardial infarction at a time when autophagic flux is reduced already, improved cardiac autophagic flux. These changes were followed by re-established mitochondrial number:size ratio, increased mitochondrial bioenergetics, reduced oxidative stress and better cardiac function. Collectively, our findings uncover the potential contribution of reduced autophagic flux to the accumulation of dysfunctional mitochondria in heart failure and highlight a new benefit of exercise training in restoring cardiac autophagy flux, which is associated with a better mitochondrial bioenergetics profile and cardiac function.

035

The E487K Variant of Aldehyde Dehydrogenase 2 Protects Cardiac Mitochondrial Metabolism In Heart Failure In Mice

Vanessa Lima, Ivson Silva, Cintia Ueta, Julio Ferreira

University of São Paulo, São Paulo, São Paulo, Brazil

The aldehyde dehydrogenase 2 (ALDH2) located in the mitochondrial matrix is crucial for the maintenance of cellular redox balance. Its main role is to metabolize reactive aldehydes produced during oxidative stress. We, recently demonstrated that pharmacological inhibition of ALDH2 results in accumulation of cytotoxic aldehydes and increased myocardial damage during ischemic stress. Currently, it is estimated that 14% of the world population have a point mutation in the ALDH2 gene (E487K) which reduces its enzymatic activity by 95%. We assess the impact of the E487K variant of ALDH2 on cardiac mitochondrial metabolism in myocardial infarction-induced heart failure in wild-type (WT), heterozygous and homozygous ALDH2 E487K knock-in mice. We evaluated the oxygen consumption and H2O2 release of isolated cardiac mitochondria of wild type (WT) and mutant hetero- and homozygous mice. We also evaluate whether the knock-in animal were more likely to develop heart metabolic damages induced myocardial infarction.

Our results indicate that both ALDH2 hetero- and homozygous mice display reduced basal oxygen consumption compared to WT (14781 ± 876 vs. 15674 ± 563 vs $17508 \pm 354 mLO_2/kg/min$). These differences are followed by decreased cardiac mitochondrial oxygen consumption and increased mitochondrial hydrogen peroxide release in ALDH2 knock-in mice compared to WT, highlighting a scenario of mitochondrial dysfunction during baseline conditions. However, when these animals develop myocardial infarction-induced heart failure, they present increased survival rate compared to WT littermates. Of interest, the E487K variant of ALDH2 induces improved mitochondrial function during heart failure, characterized by higher oxygen consumption (79.5 ± 7.3 vs. 59.3 ± 8.3 vs. $41.1 \pm 4.5 nmolO_2/min/mg$) and lower mitochondrial H2O2 release in both ALDH2 hetero- and homozygous mice compare to WT. Our findings suggest that the E487K variant of ALDH2 induces a compensatory metabolic remodeling capable of protecting mitochondria dysfunction against myocardial infarction-induced heart failure.

036

Determining I_{KS} β-Subunit Stoichiometry Using Unnatural Amino Acid Mutagenesis

Christopher Murray, Maatje Westhoff, Robert Emes, Jodene Eldstrom, David Fedida

University of British Columbia, Vancouver, BC, Canada

Background: The slow delayed rectifier current (I_{Ks}) is a key repolarizing potassium current in the cardiac action potential. I_{Ks} is composed of KCNQ1 which forms the tetrameric voltage-gated ion channel and KCNE1, a single transmembrane domain β-subunit. KCNE1 resides in the channel's exterior clefts and dramatically delays opening. While this channel complex was characterized almost 20 years ago, the stoichiometry of the β-subunits remains controversial. Several conflicting studies have reported either a strict ratio of 4 KCNQ1s:2 KCNE1s or a variable ratio up to 4:4. Here, we sought to clarify this issue using the UV-crosslinking unnatural amino acid, *p*-

benzoyl-L-phenylalanine (Bpa). **Methods:** Bpa was genetically incorporated into KCNE1-GFP at residue F57 using the amber stop codon (TAG) suppression system in TSA cells. KCNE1-Bpa was co-expressed with either KCNQ1 or fusion constructs where KCNE1 was linked to one KCNQ1 (EQ - 4:4) or two KCNQ1s (EQQ - 4:2). Activation kinetics and response to UV were evaluated using the whole-cell patch clamp technique. **Results and Conclusions**c KCNQ1 + KCNE1-Bpa crosslinking was induced by applying a 300 ms flash of UV light at rest (-90 mV) followed by a 4 s activation step (+60 mV). This resulted in a rapid decrease in current indicating the permanent trapping of channels in the closed state. To determine if 4:2 is the maximum complex stoichiometry, EQQ, which has two out of four occupied clefts, was coexpressed with KCNE1-Bpa. Analysis of the crosslinking rates indicates that KCNE1 is able to associate with EQQ and crosslink at ~0.5 the rate when all four exterior clefts are available. No crosslinking was observed with EQ + KCNE1-Bpa indicating the independent KCNE1 subunits are not displacing the tethered KCNE1s. These findings demonstrate that there is no intrinsic mechanism limiting the association of independent β-subunits with the channel complex, confirming the variable stoichiometry model.

037

Silencing of the mitochondrial calcium uniporter improves post-ischemic cardiac dysfunction and attenuates mitochondrial Ca^{2+} overload and apoptosis in cardiac myoblast and cardiomyocytes

Yuriana Oropeza-Almazán, Alberto Marbán-González, Eduardo Reyes-Alvarez, Gerardo García-Rivas

Tecnológico de Monterrey, Monterrey, Mexico

Ischemic heart disease is characterized by the reduction or absence of oxygen supply to the myocardium primarily by occlusion of a coronary artery. Acute ischemia induces cell death during myocardial infarction. However, restoration of coronary flow also precipitates cell death during the first minutes of reperfusion. This ischemia-reperfusion injury is produced by multiple interrelated mechanisms. It is well characterized that ionic imbalance initiated during ischemia promotes a significant increase in $[Ca^{2+}]_I$ during reperfusion that induces a mitochondrial Ca^{2+} overload; the increase in $[Ca^{2+}]_m$ contributes to the opening of a nonspecific pore known as the mitochondrial permeability transition pore (mPTP), which leads to a disruption in the membrane potential ($\Delta\Psi_m$) and to cellular energy collapse. Therefore, the opening of the mPTP is one of the causes of reperfusion injury, inducing myocyte cell death by both necrosis and apoptosis. The aim of this work is to study the role of mitochondrial calcium uniporter channel (MCU) in post-ischemic injury as a regulator of $Ca^{2+}{}_m$ overload that induces cell death in myoblast and cardiomyocytes. Specific small interfering RNA (siRNA) targeting MCU was used to silence MCU expression. The mRNA level of MCU was measured using qRT-PCR, and the protein levels of MCU and regulator proteins were determined using Western blot analysis. MCU expression decreased by 80% with a consequent decrease in mitochondrial Ca^{2+} transport. MCU silencing effects against hypoxia/reoxygenation injury in cardiomyocytes reduces apoptosis by 50%, measured with caspases 3,7 activity. These results indicates that MCU has a main role in post-ischemic cardiac dysfunction. Thus, the chemical inhibition of MCU or MCU knockdown could be a therapeutic approach used to prevent Ca^{2+} overload, which induces injury in several pathologies such ischemia/reperfusion, cardiac arrhythmias and heart failure.

038

Nrf2 Dependent Rescue of Protein Aggregation Mediated Hypertrophic Cardiomyopathy

Madhusudhanan Narasimhan[a], Cory Miller[b], Sakthivel Ramasamy[a], Jennifer Hong[b], Asokan Devarajan[c], Nancy Atieno[b], Christopher Davidson[b], Kevin Whitehead[b], John R. Hoidal[b], Namakkal S. Rajasekaran[a]

[a]*University of Alabama at Birmingham, Birmingham, USA*
[b]*University of Utah, Salt Lake City, USA*
[c]*University of California at LA, Las Angeles, USA*

Background: Progressive increase of misfolded/unfolded proteins represents impaired homeostasis of proteins and lead to proteotoxicity. Our recent discovery using a mouse model of familial human cardiac disease displayed prominent swing in the redox state towards reductive stress (RS) in association with accumulation of toxic protein aggregates. Further, sustained activation of nuclear erythroid-2 like factor-2 (Nrf2) causes RS and hypertrophic cardiomyopathy (HCM) in TG hearts. Therefore, we hypothesized that whether disrupting Nrf2-antioxidant signaling prevents RS and that could delay proteotoxic cardiac disease.

Methods: Non-transgenic (NTG), hR120G-CryAB transgenic (TG) with HCM and TG:Nrf2-deficient (Nrf2-def) mice were used. The effects of Nrf2 diminution on RS and development of myopathy in TG mice were evaluated at 25-30 and 42 weeks of age. Cardiac function, redox biochemistry, cellular and molecular mechanisms in relation to progression of protein aggregation (PA) and cardiomyopathy were investigated.

Results: The diminution of Nrf2 prevented RS and prolonged the survival of TG mice (>60 weeks) by an additional 20-25 weeks as compared to TG mice exhibited >80% mortality at ~40 weeks of age. In TG mice, upregulation of Nrf2/antioxidants signaling at transcriptional/translational levels resulted in RS and caused HCM at 6 months of age. Interestingly, the cytosolic repressor of Nrf2, Keap1 was predominantly sequestrated in protein aggregation suggesting that sustained activation of Nrf2 may be a contributing mechanism for RS. The TG:Nrf2-def mice did not exhibit cardiac hypertrophy at even 60 weeks, while the TG mice developed pathological hypertrophy and heart failure starting at 24-28 weeks of age. PA was significantly reduced in TG:Nrf2-def when compared with TG mice at 28-30 weeks. Preventing RS and maintaining redox homeostasis in the TG:Nrf2-def mice lessened PA and ameliorated ubiquitination of proteins and proteostasis mechanisms.

Conclusion: Nrf2 deficiency rescues redox homeostasis, which reduces aggregation of mutant proteins, thereby delaying the proteotoxic cardiac remodeling.

039

Interdependence of mitochondrial fission and mitophagy in adult mouse hearts

Moshi Song[a], Yan Burelle[b], Gerald Dorn[a]

[a]*Washington University School of Medicine, Saint Louis, MO, USA*
[b]*University of Montreal, Montréal, QC, Canada*

The role of Drp1-mediated mitochondrial fission in normal hearts is controversial. Cardiomyocyte mitochondria are hypo-dynamic, yet

cardiac-specific Drp1 gene deletion (KO) provokes mitochondrial enlargement, MPTP-dependent cardiomyocyte loss, and dilated cardiomyopathy (Song et al Cell Metab, 2015). We postulated that Drp1-dependent mitochondrial fission is essential for triage and elimination of damaged cardiomyocyte mitochondria by Parkin-mediated mitophagy. Others described no benefit of Parkin KO in mice with perinatal cardiac Drp1 KO (Kageyama et al EMBO J, 2014), but these Parkin KO mice have little basal phenotype due to germline compensation. Here, to dissect the individual and interactive roles of Parkin and Drp1 in adult mouse hearts we conditionally ablated each gene (Cre-Lox at 8 wks), separately and in combination. Parkin KO hearts appear normal. As reported, Drp1 KO caused cardiomyocyte dropout and lethal cardiomyopathy after 6-7 wks. Cardiac-specific KO of Parkin concomitant with Drp1 KO ameliorated the underlying cardiomyopathy by: 1. Increasing 6 wk survival (94% vs 56%; $P < 0.0001$); 2. Enhancing cardiac performance (LV FS 36.2 $\pm$ 4.0 vs 23.2 $\pm$ 1.7%; $P = 0.01$); 3. Decreasing adverse remodeling (LV r/h 5.0 $\pm$ 0.5 vs 6.3 $\pm$ 0.4; $P = 0.05$); 4. Reducing cardiomyocyte necrosis (1.9 $\pm$ 0.5 vs 5.0 $\pm$ 1.2%; $P = 0.05$) and replacement fibrosis (33.3 $\pm$ 5.3 vs 13.0 $\pm$ 1.5%; $P = 0.02$); 5. Attenuating mitochondrial deficiency (5.7 $\pm$ 0.4 vs 4.7 $\pm$ 0.6 g/mg; $P = 0.20$). Deleting Parkin did not affect typical mitochondrial enlargement in Drp1 KO cardiomyocytes. Mitochondrial respiration was unaffected by any of the genetic manipulations. Benefits of Parkin KO were linked to normalization of mitochondrial-associated LC3 and p62, mitophagy markers increased in cardiac Drp1 KO. These studies show how Drp1-mediated mitochondrial fission and Parkin-mediated mitophagy interact to maintain the quality of cardiac mitochondria: Interrupting mitochondrial fission (Drp1 KO) prevents segregation of damaged mitochondrial components into daughter organelles normally targeted for mitophagy; mitophagy thus ultimately consumes fission-defective parent organelles. Parkin KO suppresses generalized mitophagy, postponing (but not preventing) the Drp1 KO cardiomyopathy.

040

Alternative splicing of NOX4 in the failing human heart

Zoltán Varga[a,b], Márton Pipicz[c], Przemyslaw Leszek[d], Júlia A. Baan[e], Luca Mendler[e,f], Tamás Baranyai[a], Enrique Lara-Pezzi[g], Pál Pacher[b], Tamás Csont[c], Péter Ferdinandy[a,h]

[a]*Cardiometabolic Research Group, Dept. of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary*
[b]*Laboratory of Physiological Studies, National Institute on Alcohol Abuse and Alcoholism, National Institute of Health, Bethesda, MD, USA*
[c]*Metabolic Diseases and Cell Signaling Group, Dept. of Biochemistry, University of Szeged, Szeged, Hungary*
[d]*Institute of Cardiology, Warszawa, Poland*
[e]*Muscle Adaptation Group, Dept. of Biochemistry, University of Szeged, Szeged, Hungary*
[f]*Institute of Biochemistry II, Goethe University, Faculty of Medicine, Frankfurt, Germany*
[g]*Centro de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain*
[h]*Pharmahungary Group, Szeged, Hungary*

Oxidative stress is a major contributor to the development of heart failure. NADPH oxidases (NOX) are enzymes solely responsible for production of reactive oxygen species (ROS). NOX activity is increased in failing human hearts, however, neither NOX1 nor NOX2 was demonstrated to be increased. The role of NOX4 is controversial in mouse models of heart failure. Targeting ROS production by selective inhibition of disease-associated NOX is an intriguing field, accordingly selective NOX inhibitors are under extensive investigation. To shed light on the role of NOX4 in the development of human heart failure, we aimed to characterize and assess the expression of NOX4 in failing human heart samples.

Human heart samples were obtained from patients undergoing heart transplantation. Deep RNA sequencing of the cardiac transcriptome indicated extensive alternative splicing of the NOX4 gene in heart failure. Long distance PCR analysis (amplification of long PCR products with an universal 5`-3` end primer pair and a high fidelity enzyme) confirmed the transcription of the splice variants. Among spliced transcripts the most abundant variants are the ~1500 bp NOX4A, a slightly shorter ~1400 bp NOX4B, a ~1000 bp NOX4C, a ~600 bp NOX4D and a ~500 bp NOX4E. To further assess the relevance of alternatively spliced transcripts we determined the protein expression of NOX4 by using a specific antibody recognizing a conserved region in all transcript variants (C-terminal region). Western blot analysis showed up-regulation of the full-length NOX4 in failing hearts and confirmed the presence of shorter isoforms.

We conclude that NOX4 undergoes extensive alternative splicing in human heart failure giving rise to the expression of different enzyme isoforms. The full length NOX4 is significantly upregulated suggesting a central role for NOX4 in increased ROS production in heart failure, however, to explore the relevance of smaller isoenzymes further investigations are required.

041

Novel roles for Chromogranin A peptide catestatin in cardiac metabolism and physiology

Jan M. Schilling[a,b], Teresa Pasqua[b], Venkat R. Chirasani[c], Nilima Biswas[b], Ennio Avolio[b], Hongqiang Cheng[d], Suvajit Sen[e], Alice Zemljic-Harpf[a,b], John P. Headrick[f], Heidi N. Fridolfsson[a,b], Adam Kassan[a,b], Kevin Yei[g], Sumana Mahata[g], Gautam Bandyopadhyay[b], David M. Roth[a,b], Nicholas J.G. Webster[a,b], Sanjib Senapati[c], Hemal H. Patel[a,b], Sushil K. Mahata[a,b]

[a]*VASDHS, San Diego, CA, USA*
[b]*UCSD, La Jolla, CA, USA*
[c]*India Institute of Technology, Chennai, India*
[d]*Zhejiang University School of Medicine, Hangzhou, China*
[e]*UCLA, Los Angeles, CA, USA*
[f]*Griffith Health Institute, Griffith, Australia*
[g]*California Institue of Technology, Passadena, CA, USA*

More than 50 million people suffer from cardiometabolic syndrome in the US. Catestatin (CST), a peptide derived form chromogranin A acts as an antihypertensive, cardioprotective, pro-angiogenic, and insulin-sensitizing peptide. The mechanistic role of CST in cardioprotection and substrate utilization under normal and stressed conditions is not well understood. We hypothesized that CST is a key regulator of cardiac metabolism and stress adaptation. We investigated wild-type (WT) and CST KO mice to evaluate cardiac function and metabolism. We utilized *in vivo* physiology, substrate metabolism, gene array, electron microscopy, affinity binding and molecular dynamics to illustrate the possible mechanisms underlying CST regulation of cardioprotection. CST-KO mice display hypertension and are hyperadrenergic. Comparison of injuries after ischemia-reperfusion and ischemic preconditioning showed that

CST-KO mice had diminished ischemic tolerance and could not be protected. Fatty acid uptake and incomplete fatty acid oxidation was higher in left ventricles of CST-KO mice. Ultrastructural studies revealed decreased sarcomere length and altered mitochondrial morphology in CST-KO mice. Phospho AMPK as well as insulin-stimulated phosphorylation of Akt and GSK-3β were decreased in CST-KO hearts. Additionally, we used proteomics and molecular simulations to identify CST as a binding partner for ATP synthase and describe possible binding sites as well as potential conformational changes within the ATP-synthase complex upon CST binding. Taken together, we show that KO of CST leads to decreased ischemic tolerance and loss of cardioprotective signaling and this appears to be dependent on altered structure of the contractile apparatus and impaired energy utilization by mitochondria.

042

The phosphodiesterase-5 inhibitor vardenafil protects against diabetic cardiomyopathy in a type-2 diabetic animal model

Csaba Mátyás[a], Balázs Tamás Németh[a], Attila Oláh[a], Mihály Ruppert[a], Dalma Kellermayer[a], Marianna Török[a], Lilla Szabó[a], Gábor Szabó[b], Béla Merkely[a], Tamás Radovits[a]

[a]*Heart and Vascular Center, Semmelweis University, Budapest, Hungary*
[b]*Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany*

Purpose: Diabetes mellitus (DM) is associated with impaired cyclic guanosine monophosphate (cGMP) signaling which leads to the development of diabetic cardiomyopathy. The second messenger cGMP, broken down by the phosphodiesterase-5 enzyme (PDE5), has been shown to exert cytoprotective effects. We investigated the cardiac effects of chronic inhibition of PDE5 by vardenafil in type-2 DM.

Methods: For type-2 DM Zucker Diabetic Fatty (ZDF; homozygous recessive (*fa/fa*)) rats were used. Heterozygous (*fa/+*) or homozygous dominant (+/+) ZDF Lean (ZDFL) rats served as controls. Animals received vehicle (ZDFL, ZDF) or 10 mg/kgBW vardenafil per os (ZDFLVard, ZDFVard) from 7 to 32 weeks of age. Cardiac morphology was followed by echocardiography. Left ventricular (LV) function was assessed using a pressure-volume (PV) conductance microcatheter system. Gene expression analysis of atrial natriuretic factor (ANF; qRT-PCR), cardiomyocyte diameter/tibia length (CD/TL) and Masson's staining (fibrosis score (FS)) were used to prove pathological myocardium hypertrophy.

Results: Cardiac hypertrophy (echocardiography: LV anterior wall thickness in systole (LVAWs): 2.81 ± 0.1 mm; relative wall thickness (RWT): 0.49 ± 0.02; LVmass/TL: 0.30 ± 0.01 g/cm; CD/TL: 3.53 ± 0.02 μm/cm; ANF: 3.04 ± 0.26 vs ZDFL (LVAWs: 2.53 ± 0.04 mm; RWT: 0.43 ± 0.02; LVmass/TL: 0.23 ± 0.004 g/cm; CD/TL: 3.09 ± 0.02 μm/cm; ANF: 0.92 ± 0.17);$p < 0.05$) and fibrotic remodelling (FS: 1.05 ± 0.09 vs ZDFL (0.57 ± 0.13);$p < 0.05$) have been observed in ZDF. Drug treatment significantly decreased myocardial hypertrophy and fibrosis (LVAWs: 2.47 ± 0.05 mm; CD/TL: 3.15 ± 0.02; ANF: 1.39 ± 0.21; FS: 0.59 ± 0.08 vs ZDF;$p < 0.05$) in DM. PV analysis showed impaired diastolic function and increased cardiac stiffness (time constant of LV pressure decay (τ): 9.17 ± 0.25 ms; slope of end-diastolic pressure volume relationship (EDPVR): 0.078 ± 0.002 mmHg/μl vs ZDFL (τ: 8.18 ± 0.13 ms; EDPVR: 0.045 ± 0.003 mmHg/μl);$p < 0.05$) while contractility parameters and blood pressure remained unchanged in ZDF. Vardenafil improved diastolic parameters (τ: 8.62 ± 0.34 ms, EDPVR: 0.062 ± 0.006 mmHg/μl vs ZDF;$p < 0.05$). Vardenafil did not have effect in ZDFL.

Conclusions: Chronic administration of vardenafil prevented DM associated myocardial complications. PDE5 inhibition might be an important target to improve the cardiovascular outcome in diabetic patients in the future.

043

MuRF1 inhibits Cardiac Thyroid Hormone Signaling by TRα Mono-Ubiquitination and Localization to CAP350

Monte Willis[a], Jessica Berthiaume[b], Kristene Wadosky[a], Wei Tang[a], Martin Gerdes[d], Michael Portman[c]

[a]*University of North Carolina, Chapel Hill, NC, USA*
[b]*Case Western Reserve University, Cleveland, OH, USA*
[c]*Seattle Children's Research Institute, University of Washington, Seattle, WA, USA*
[d]*New York College of Osteopathic Medicine at, New York Institute of Technology, Old Westbury, NY, USA*

Thyroid hormone (TH) regulates cardiac physiological hypertrophy and is protective during heart failure. TH regulates cellular processes, in part, through its receptors, TRβ and TRβ. While ubiquitination of TRβ has been described, the molecular detail of mechanisms governing TR activity remains undefined, including the identification of specific ubiquitin ligases involved. We recently demonstrated that the muscle specific ubiquitin ligase MuRF1 (Muscle Ring Finger-1) inhibits several nuclear transcription factors including serum response factor (SRF) and PPARα *in vivo*. This led us to hypothesize that MuRF1 similarly regulates TRα through ubiquitination. MuRF1 overexpression inhibited TH (T3)-induced cardiac hypertrophy in αMHC-MuRF1 cardiac Tg+ mice and cardiomyocyte-derived cells in culture. MuRF1-/- mice challenged with daily T3 exhibited an exaggerated physiological cardiac hypertrophy. Mechanistically, MuRF1 attenuated TR DNA binding and increased co-localization with centrosome-associated protein (CAP350, a novel nuclear receptor mediator) in the perinuclear region. We also identified that MuRF1 binds and catalyzes mono-ubiquitination of lysines in the ligand-binding domain (LBD) of TRα, promoting perinuclear localization. Taken together, our working model describes MuRF1 mono-ubiquitinating the T3-TRα complex, which targets it to CAP350 resulting in decreased transcriptional activity in cardiomyocytes. This work demonstrates the first ubiquitin ligase to regulate thyroid receptors and illustrates MuRF1's critical function in TH-mediated cardiac hypertrophy, underscoring the role of ubiquitin ligases in cardiac homeostasis.

044

In utero exposure to diesel exhaust promotes cardiac fibrosis through enhanced cardiac myocyte apoptosis

Jamie Goodson[a], Chad Weldy[b], Yonggang Liu[a], Jesse Tsai[a], James MacDonald[a], Theo Bammler[a], Michael Chin[a]

[a]*University of Washington, Seattle, WA, USA*
[b]*Duke University, Durham, NC, USA*

Deleterious pathological and physiological exposures during *in utero* development have been shown to increase adult susceptibility to cardiovascular disease. We have recently shown that *in utero* and early life exposure to diesel exhaust (DE) air pollution, a major source of fine particulate air pollution ($PM_{2.5}$) in urban areas, results in adult susceptibility to heart failure in mice. We have subsequently shown that *in utero* exposure to DE alone promotes placental injury, and is sufficient to confer adult susceptibility to heart failure following pressure overload. To determine the potential mechanisms, we have investigated at the tissue, cellular and molecular level. DE-exposed tissue sections show increased apoptosis and fibrosis, but no increase in myocyte cross sectional area, indicating increased susceptibility to apoptosis but not hypertrophy. Neonatal cardiomyocytes from mice exposed to diesel *in utero* have a greater apoptotic response to H_2O_2 stimulated apoptosis. RNA-seq analysis indicates dysregulation of specific target genes. As exposure to air pollution has been associated with epigenetic modifications, we hypothesize that *in utero* exposure to DE results in stable changes of the epigenetic landscape of these specific target genes, thereby promoting adult susceptibility to heart failure by increasing susceptibility to apoptotic stimuli. Understanding the molecular alterations resulting from DE exposure is necessary to identify therapeutic targets to prevent air pollution associated heart failure.

045

Chamber specific function of p38 MAP kinase during early postnatal development

Tomohiro Yokota[a], Vincent Ren[a], Susumu Minamisawa[b], Yibin Wang[a]

[a]*Department of Anesthesiology, Medicine and Physiology, University of California, Los Angeles, Los Angeles, CA, USA*
[b]*Department of Cell Physiology, Jikei University School of Medicine, Tokyo, Japan*

Background: Although a lot of studies focusing on left ventricle hypertrophy (LVH), failure, and development, little is known about right ventricular (RV). RV hypertrophy is one of the most challenging forms of heart diseases with very limited therapeutic options. There is no genetic models of RV specific heart failure available. Therefore, to generate the RVH models and unclear underlying mechanisms are important for clinical treatment. In this study, we have conducted detailed characterization of RVH model we generated.

Results and Methods: We generated cardiomyocyte specific-knockout of p38 MAP kinase alpha&beta double KO (p38ab-cdKO). Echocardiogram and histological analyses revealed RV specific hypertrophy associated with significant early lethality at postnatal day 1 (P1). p38ab-cdKO mice showed increase in both the RV wall thickness (RVW) and inner diameter of the RV (RVID) at as early as P3. However, RVID was significantly increased without changes in RVW at P1. To characterize the proliferation and apoptotic activities during early postnatal heart, we conducted p-H3 and TUNEL staining. Interestingly, apoptotic activity in the RV was suppressed in p38ab-cdKO at P1. Furthermore, proliferative activity in the RV was increased in p38ab-cdKO at P3 and P7. In addition, cross-section area of the RV was increased at as early as P1 in p38ab-cdKO, whereas that of the LV didn't show difference. Taken together with morphological change and apoptotic/proliferative activity in the RV, an increase in the RVW was associated with suppression of apoptosis, induction of proliferation, and cell growth via p38a&b suppression. In wild-type early postnatal heart, p38 activity in the RV was higher than that in LV at P3 and P7, whereas there was no difference between ventricles at P1.

Conclusion: p38 a&b MAP kinases are essential to novel normal development of RV morphology. p38ab-cdKO mice represents a unique RV-specific hypertrophy and heart failure model for further studies.

046

Restoration of NAD Redox Balance Ameliorates Heart Failure through Regulation of Cytosolic and Mitochondrial Protein Acetylation

Chi Fung Lee, Lorena Garcia-Menendez, Yongseon Choi, Juan Chavez, Jim Bruce, Rong Tian

University of Washington, Seattle, WA, USA

In a mouse model of mitochondrial dysfunction due to cardiac-specific deletion of Ndusf4 (cKO) we found that elevated NADH/NAD ratio and hyperacetylation of mitochondrial proteins led to increased mPTP sensitivity and accelerated heart failure induced by chronic pressure overload with transverse aortic constriction (TAC). Either restoring NADH/NAD pharmacologically (by supplying NAD precursor NMN) or genetically (by expressing the key enzyme in the NAD salvage pathway, NAMPT) improved HF in cKO. Comparison of protein acetylome in WT, cKO and cKO+NAMPT hearts identified a number of proteins with "NAD-sensitive" differential acetylation. Increased lysine acetylation (LysAc) of malate aspartate shuttle (MAS) proteins in cKO reduced MAS activity and increased cytosolic NADH/NAD ratio suggesting mitochondrial dysfunction altered cytosolic redox state via MAS flux. Higher LysAc of proteins involved in Ca^{2+} homeostasis and mPTP regulation in cKO was associated with higher mitochondrial Ca^{2+} content and mPTP hypersensitivity, and all could be normalized by restoring NADH/NAD. To test whether elevated NADH/NAD ratio also contributes to the pathogenesis of heart failure in animal models without prior mitochondrial defect we treated mice subjected to TAC with NMN for 4 weeks. TAC increased NADH/NAD ratio and LysAc in the heart; mitochondria isolated from these hearts showed higher sensitivity of mPTP opening during Ca^{2+} stimulation. NMN treatment normalized all the changes and furthermore, reduced pathological hypertrophy and improved contractile function in TAC-NMN compared to TAC-vehicle. Similarly, NAMPT reduced hypertrophy and contractile dysfunction induced by 2-week isoproterenol stimulation. In summary, our findings suggest that the NADH/NAD imbalance caused by impaired mitochondrial respiratory function contributes to the progression of heart failure via hyperacetylation of key regulatory proteins of stress responses.

047

Transient Mitochondrial Permeability Transition Pore Opening in Cardiac Myocytes during SR Ca release

Xiyuan Lu, Donald Bers

University of California, Davis, Davis, USA

The opening of a high-conductance and long-lasting mitochondrial permeability transition pore (mPTP) induces uncoupling of respiration from ADP phosphorylation, and causes mitochondrial injury and cell death. However, low-conductance and transient openings of mPTP may limit mitochondrial calcium load and mediate mitochondrial reactive oxygen species (ROS) signaling. Transient openings of mPTP have been proposed, and could be cardioprotective, but evidence for this in cells is indirect and not thoroughly studied. To address the cellular mechanism, we measured mitochondrial [Ca] ($[Ca]_{mito}$) with Rhod-2 AM and membrane potential ($\Delta\psi m$) in isolated single permeablized myocytes during cyclical sarcoplasmic reticulum (SR) Ca release using 2-D confocal imaging where individual mitochondria can be seen. Rapid and transient decreases in both mitochondrial [Ca] and $\Delta\psi m$ were observed during SR Ca release. The frequency of these candidate transient mPTP openings increased at higher [Ca] and with H_2O_2 (1 μM) exposure, but were typically observed in ≪1% of individual mitochondria being imaged. This suggests that both Ca and ROS modulate transient pore openings. These $[Ca]_{mito}$ and $\Delta\psi m$ oscillations and H_2O_2 effects were sensitive to mPTP inhibitor cyclosporine A (CsA, 10 μM) and we conclude that they are mediated by transient mPTP openings and closings. The duration of these openings was 57.9 ± 15 s. The size of the pore did not allow Rhod-2 or calcien (M.W. 600 Da) permeation, indicating that only small solutes can freely move across. Moreover, the frequency of transient mPTP openings increased in failing heart due to an increased oxidative stress. Our data are consistent with the idea that rare transient mPTP openings allow individual mitochondrial Ca efflux, but with minimal perturbation of global average $[Ca]_{mito}$ or $\Delta\psi m$. These events may be an important physiological protective mechanism to limit $[Ca]_{mito}$ overload.

048

Characterization of mitochondria from mice lacking the mitochondrial calcium uniporter

Randi Parks[a], Sara Menazza[a], Angel Aponte[b], Kira Holmstrom[c], Toren Finkel[c], Elizabeth Murphy[a]

[a]*Systems Biology Center, Bethesda, MD, USA*
[b]*Proteomic Core Facility, Bethesda, MD, USA*
[c]*Center for Molecular Medicine, Bethesda, MD, USA*

The mitochondrial Ca^{2+} uniporter (MCU) transports Ca^{2+} from the cytosol into the mitochondria. It was recently shown that mice lacking MCU are unable to transport Ca^{2+} into mitochondria, and do not undergo permeability transition pore opening upon addition of extra-mitochondrial Ca^{2+}. However, hearts from MCU KO mice are not protected from cell death (Pan *et al*, Nat Cell Biol.2013,15(12)). We therefore evaluated whether mitochondria from MCU KO mice have developed compensatory mechanisms. Cardiac mitochondria were isolated from MCU KO and wild-type mice by differential centrifugation. Each sample was labelled with a unique tandem mass tag (n = 5 KO, 5 WT) and mass spectrometry was used to examine total protein content. Few differences were present in the mitochondrial proteome of MCU KO versus wild-type; the only protein other than MCU that was significantly decreased following MCU KO was the essential MCU regulator (0.47-fold change). Proteins that were increased following MCU KO include peroxiredoxin-6 and glutathione-S-transferase, which could enhance antioxidant activity. As no differences were found in electron transport complexes between the two groups, we investigated whether activity may differ through supercomplex formation. Supercomplexes were isolated from mitochondria, and Blue native PAGE experiments revealed that supercomplexes were similar in MCU KO and wild-type. Complex V is known to form dimers and enhance ATPase activity; we therefore performed Blue native PAGE experiments to compare ATPase monomers and dimers in mitochondria from MCU KO and wild-type. Interestingly, the ratio of ATPase dimers to monomers was reduced in the absence of MCU. These results offer insight into proteins that are altered in response to MCU KO, and suggest that possible compensatory mechanisms may be an increase in antioxidant activity and changes in the dimer/monomer ratio of Complex V.

049

Mitochondrial complex II is a source of the reserve respiratory capacity that is regulated by metabolic sensors via sirtuin 3

Jessica Pfleger, Minzhen He, Maha Abdellatif

Rutgers University, Newark, NJ, USA

Cell survivability depends upon its ability to meet energy requirements. We hypothesized that the cells' mitochondrial reserve respiratory capacity (RRC) is a critical component of its bioenergetics that can be utilized during increased energy demand, enhancing viability. Our goal was to identify the elements that regulate and contribute to RRC development and its involvement in cell survival. Our results show that RRC is dependent on metabolic substrate availability in a cell type-dependent manner. While the neonatal rat cardiac myocytes (NRCM) utilize glucose as the main substrate, developing RRC [1.4-2.5 fold higher than basal oxygen consumption rate (OCR)] required fatty acids and glucose. Accordingly, inhibition of glucose or fatty acid oxidation separately, abrogated RRC, having little impact on basal OCR, which is sustainable with substrate or glutamate in the medium. Conversely, RRC was enhanced (1.4-1.8 fold) through increasing glucose oxidation via inhibiting pyruvate dehydrogenase kinase with dichloroacetate, or through increasing fatty acid oxidation via activation of AMP-activated kinase (AMPK). The latter was partly mediated through peroxisome proliferator-activated receptor alpha. These results suggested that RRC is an independently regulated entity of the cells' bioenergetics. An electron flow activity assay revealed that the increase in RRC correlated with a specific increase in complex II (cII) activity. Inhibiting or disassembling holo cII completely abolished RRC, accompanied by a slight decrease in basal OCR (0.82-0.9 fold), confirming it as the source of RRC. Moreover, the development of RRC required Sirtuin (Sirt)3. Functionally, we show that enhancing RRC via fatty acid oxidation with 5-Aminoimidazole-4-carboxamide1-β-Dribofuranoside in NRCM results in a burst of cII-dependent oxidative phosphorylation, accompanied by reduced superoxide production, and enhanced cell survival post-energy deprivation conditions. Thus, we show that metabolic sensors increase the cells' RRC via activating cII in a Sirt3-dependent manner, and that this can be exploited for increasing cell survival after hypoxia.

050

A Novel PKCalpha Isoform in Signaling for Cardiac Hypertrophy

Chen Gao[a], Jianli Gong[c], William Wang[c], Susan Steinberg[c], Yibin Wang[a,b]

[a]*Division of Molecular Medicine, University of California, Los Angeles, Los Angeles, CA, USA*

[b]Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA, USA
[c]Department of Pharmacology, Columbia University, New York, NY, USA

PKC is an important signal molecule with a major role in cardiac physiology and the pathogenesis of heart failure. The function of PKC is determined largely based on a previously annotated isoform detected in all tissues. In this study, using transcriptome-wide deep RNA-sequencing analysis from murine heart, we identified a novel isoform of PKC containing a previously un-annotated exon, which is named by us as -PKC-NE (Novel Exon). This novel exon encodes a 16 amino acid insert adjacent to the NFD helix in the C-terminal variable (V5) region of the enzyme. Interestingly, the PKC-NE transcript is detected only in mouse cardiac and skeletal muscle while absent from other tissues. The expression level of this splicing variant is significantly induced during postnatal maturation and reduced in pressure-overload induced failing mouse heart.

In vitro enzymology studies showed that PKC-NE has a high level of basal lipid-independent catalytic activity compared with the more canonical form of PKC. Modelling studies based upon the structure of PKC suggest that the insert activates the enzyme by disrupting an autoinhibitory interface between the lipid-binding C1 domain and the C-terminal V5 domain. Immune-precipitation for individual PKC spicing variants followed by Mass Spectrometry revealed a specific interaction between the PKC-NE and the eukaryotic Elongation Factor-1 (eEF1A1). PKC-NE expression in cultured cardiomyocytes significantly increased cardiomyocyte cell size associated with increased eEF1A1 phosphorylation at a consensus PKC phosphorylation motif and elevated protein translational activity.

We also have identified DEAD-box helicase—DDX3X as a myocyte-specific regulator of PKC-NE. DDX3X interacts with PKC-NE and inactivation of DDX3X reduces protein translation activity induced by PKC-NE expression.

In conclusion, we have identified a novel muscle specific splicing variant of PKC that is dynamically regulated during development and heart failure. This novel PKC isoform has distinct biochemical property, downstream target and regulatory component different from the canonical PKC pathway. Expression of the PKC-NE isoform is sufficient to increase cardiomyocyte cell size. Therefore, PKC-NE is a novel signaling component in cardiac hypertrophy with its specific downstream effect on protein synthesis regulation. In particular, the effects of PKC-NE to increase protein synthesis and cardiomyocyte cell size implicate this enzyme as a novel signalling component of cardiac remodelling.

051

Ablation of Sirt5 in heart alters cardiac metabolism and increase ischemia-reperfusion injury

Jennifer Boylston[a], Yong Chen[b], Marjan Gucek[b], Michael Sack[c], Junhui Sun[a], Elizabeth Murphy[a]

[a]Sytems Biology Center, NHLBI, NIH, Bethesda, MD, USA
[b]Proteomics Core, NHLBI, NIH, Bethesda, MD, USA
[c]Center for Molecular Medicine, NHLBI, NIH, Betheday, MD, USA

Succinylation refers to modification of lysine residues with succinyl groups donated by succinyl-CoA. Sirtuin5 (Sirt5) is a mitochondrial NAD^+-dependent deacylase that catalyzes the removal of succinyl groups from proteins. Sirt5 and protein succinylation are conserved across species, suggesting functional importance of the modification. Sirt5 loss impacts liver metabolism but the role of succinylation in the heart is not well explored. Metabolic remodeling underlies cardiac pathology, and we expect modulation of the succinylome to impact heart function. We combined affinity enrichment with proteomics and mass spectrometry to analyze total succinylated lysine content of mitochondria isolated from WT and *Sirt5*$^{-/-}$ mouse hearts. We identified 887 succinylated lysine residues in 184 proteins. 44 peptides (5 proteins) occurred uniquely in WT samples, 289 (46 proteins) in *Sirt5*$^{-/-}$ samples, and 554 (133 proteins) were common to both groups. The 46 unique proteins in *Sirt5*$^{-/-}$ heart participate in metabolic processes such as fatty acid β-oxidation (Eci2) and branched chain amino acid catabolism, and include respiratory chain proteins (Ndufa7, 12, 13). We performed label-free analysis of the peptides common to WT and *Sirt5*$^{-/-}$ hearts. 16 peptides from 9 proteins were significantly increased in Sirt5$^{-/-}$ by at least 30%. The adenine nucleotide transporter 1 showed the highest increase in succinylation in the KO (108.4 fold). The data indicates that succinylation is widespread in the heart and enriched in metabolic pathways. *Sirt5*$^{-/-}$ mice have been shown to have elevated levels of long and medium chain fatty acids which would be expected to be detrimental in ischemia and reperfusion. Consistent with this we found an increase in infarct size in *Sirt5*$^{-/-}$ hearts compared to WT littermates ($68.5^+/_-1.1\%$ *Sirt5*$^{-/-}$ vs $39.6^+/_-6.8\%$ WT) following 30 minutes of ischemia and 90 minutes reperfusion. The data suggest that *Sirt5* loss and consequent impact on the ability to remodel metabolically underlies this phenotype.

052

Empowering cardiac progenitor cell-mediated repair of injured myocardium by overexpressing $P2Y_{14}$ nucleotide receptor

Farid El-Sayed, Mark Sussman

San Diego State University, San Diego, CA, USA

Heart failure is a leading cause of death in the US due to the limited capability of adult mammalian heart to regenerate following injury. Autologous stem cell therapy holds promise for regeneration of injured myocardium after myocardial infarction. However, stem cells derived from diseased organs exhibit impaired proliferation and migration and increased susceptibility to cell death. Empowering stem cells from diverse origins, including cardiac progenitor cells (CPCs), with pro-survival genes has been attempted. Despite the well-established roles of purinergic signaling mediated by extracellular nucleotides in regulating diverse cellular responses in cardiovascular diseases, it has not been well-defined in CPCs. Our preliminary data show, for the first time, that the majority of P2 purinergic receptors are expressed in mouse and human CPCs. The G protein-coupled UDP-sugar-sensing $P2Y_{14}$ receptor ($P2Y_{14}R$) has been shown to stimulate proliferation and migration, inhibit senescence and increase resistance to stress stimuli in a variety of experimental models. We aim to determine whether $P2Y_{14}R$ plays similar regenerative roles in cardiac tissue where the $P2Y_{14}R$-mediated physiological responses haven't been previously addressed. Our preliminary data show that the $P2Y_{14}R$ selective agonist UDP-Glucose enhances human CPC (hCPC) proliferation, migration and survival. Interestingly, hCPCs that exhibit relatively slower growth kinetics and enhanced senescence show a dramatic decrease in $P2Y_{14}R$ expression compared to fast-growing hCPCs consistent with

our hypothesis that overexpressing $P2Y_{14}R$ participates in rejuvenating hCPCs and improving their growth capabilities. This hypothesis will be tested *in vivo* by determining whether $P2Y_{14}R$ overexpression in hCPCs improves their reparative potential for injured mouse myocardium. We also introduce the novel hypothesis that $P2Y_{14}R$-induced regenerative responses in hCPCs involve the activation of Hippo signaling that is known to be regulated by different GPCRs, linking the extracellular nucleotides released during cardiac ischemia to extracellular matrix sensing and Hippo signaling that have been recently implicated in cardiac regeneration.

053
PKCepsilon is required in physical exercise-mediated cardioprotection

Lais Domingues, Cintia B. Ueta, Julio C.B. Ferreira

University of São Paulo, São Paulo, SP, Brazil

Ischemic preconditioning is able to produce cardioprotection against sustained ischemia and reperfusion (I/R) injury. This process is dependent on the translocation of protein kinase C isoform epsilon (PKCε) from cytosol to mitochondria and subsequent phosphorylation of cardiac mitochondrial aldehyde dehydrogenase 2 (ALDH2). Similar to ischemic preconditioning, previous physical exercise is able to protect the heart against I/R injury; however, the cellular mechanisms involved in this process have not been elucidated. The therefore evaluated whether PKCε is important during physical exercise-induced cardioprotection in mice. We submitted C57BL6 mice (wild type-WT) and protein kinase Cε knockout mice (PKCε KO) to a protocol of aerobic exercise on treadmill for seven following days. 24 hours after the last session of exercise hearts were excised and retrograde perfused using a *Langendorff* apparatus. Our results demonstrate that a protocol of physical exercise on treadmill for seven days protects against I/R injury by increasing ALDH2 protein levels (myocardial infarction: control 50 ± 2 vs exercised 31 ± 4). Physical exercise also increases translocation of PKCε to mitochondria, suggesting a key role of PKCε-ALDH2 axis during exercise-mediated cardioprotection. In fact, the absence of PKCε results in a loss of physical exercise-induced cardioprotection upon I/R, represented by increased myocardial infarction. These results reveal for the first time the crucial role of the axis PKCε-ALDH2 in physical exercise-induced cardioprotection during an insult of ischemia/reperfusion.

054
Reduced cardiac hypertrophy observed in females is unaffected by the ablation of cardiac-specific ERα

Josephine Harrington[a,b], Sara Menazza[a], Elizabeth Murphy[a]

[a]*NHLBI, National Institutes of Health, Bethesda, MD, USA*
[b]*University of Massachusetts Medical School, Worcester, MA, USA*

Background: Pre-menopausal females are protected from heart failure, though the mechanisms and time course over which this protection is mediated remains unclear. Cardiac hypertrophy is known to result in changes in the cardiac transcriptome, but gender-based differences are poorly understood.

Methods: 20 male and 16 female mice were given either 1.5 mg kg^{-1} day^{-1} of Angiotensin II or saline via implanted Alzet osmotic minipumps for 14-21 days. To study the effect of cardiac-specific ablation of ERα (csER-α-KO) we also studied 12 female csER-α-KO, 6 female wild-type (WT) littermates, 7 male csER-α-KO and 10 WT male littermates, that were also treated with angiotensin II or saline. After 14 or 21 days, all mice received echocardiograms. Their hearts were weighed and normalized by body weight.

Results: Male and female mice each showed significant cardiac hypertrophy after receiving angiotensin II. At 2 weeks, hypertrophy and cardiac function (measured by ejection fraction) were similar between males and females. At 3 weeks, females showed significantly less cardiac hypertrophy and significantly better cardiac function than their male counterparts, with average ejection fractions of 53.9 in females vs. 37.1% in males ($p = 0.008$), and heart weights to body weights of 0.0054 in females vs. 0.0057 in males ($p = 0.047$). The reduced hypertrophy observed in the WT females was not altered by ablation of ERα: there was no significant difference between wild-type and csER-α-KO mice when treated with either saline or angiotensin. We also evaluated differences in long non-coding RNA and miRNA between males and females that might contribute to these sex differences.

Conclusions: Our findings show that females exhibit significantly less angiotensin II-induced hypertrophy than males at 3 weeks of treatment and the reduction in hypertrophy in females is unaffected in hearts lacking ERα, suggesting that cardiac ERα is not required for the reduction in hypertrophy.

055
CARD9 Knockout Ameliorates Obesity-Associated Cardiac Dysfunction

Li Cao, Xing Qin, Tiantian Zheng, Sreejayan Nair, Jun Ren, Guanglong He

School of Pharmacy, University of Wyoming, Laramie, WY, USA

As obesity has emerged as an epidemic, strategies to cub this disease are limited. Obesity is associated with chronic inflammation with detrimental consequence on metabolism and cardiac function. The adaptor protein CARD9, which is highly expressed in immune cells, activates pro-inflammatory cytokines and regulates innate immune responses. However, whether CARD9 participates in obesity-induced chronic inflammation and cardiac dysfunction remains unknown. Here, we hypothesized that CARD9 plays a detrimental role in obesity-induced cardiac anomalies.

Male C57/BL6 wild-type (WT) and CARD9 knockout ($CARD9^{-/-}$) mice were fed normal chow (ND, 12% fat) and a Western diet (WD, 45% fat) for 5 months starting at 4 weeks of age. Cardiac geometry and function were evaluated using echocardiography. Western immunoblotting analyses were performed on heart tissues and isolated macrophages to determine inflammatory signaling pathways. At the end of 5-month WD feeding, body weight and epididymal adipose tissue weight of WD-fed WT and $CARD9^{-/-}$ mice were significantly increased compared to the respective ND-fed group with little difference between the WT and $CARD9^{-/-}$ mice. Cardiac fractional shortening and ejection fraction were significantly compromised in WD-fed WT mice and the effect of which was attenuated by CARD9 knockout. In addition, the CARD9 protein was significantly up-regulated in the heart and isolated macrophages in

WD-fed WT mice. While IKKα/β and IκBα phosphorylation levels were similar in the hearts of WD-fed WT and CARD9$^{-/-}$ mice, p38 MAPK phosphorylation level was significantly increased in WD-fed WT mice but not CARD9$^{-/-}$ mice.

In conclusion, CARD9 knockout ameliorated obesity-induced cardiac dysfunction possibly through suppression of p38 MAPK phosphorylation.

056

Molecular Mechanisms Underlie Muramyl Dipeptide-Induced Inflammation and Autophagy in Macrophages

Tiantian Zheng, Li Cao, Xing Qin, Sreejayan Nair, Jun Ren, Guanglong He

University of Wyoming, Laramie, USA

Chronic inflammation with macrophage activation and infiltration is associated with the pathogenesis of a number of diseases including obesity. The caspase recruitment domain containing protein 9 (CARD9) is highly expressed in macrophages and interacts with the intracellular receptor protein nucleotide oligomerization domain containing protein 2 (NOD2) in innate immune defense. NOD2 also participates in autophagic responses to bacterial infection via association with the autophagy protein ATG16L1. We hypothesized that CARD9 induces inflammation and down-regulates autophagy in activated macrophages.

C57 wild-type mice were injected intraperitoneally with a thioglycolate broth (4% and 2 mL/mouse). Four days later, the NOD2 specific agonist muramyl dipeptide (MDP, 300 μg/mL, 100 μL/mouse) was injected into the peritoneal cavity with PBS used as vehicle control. Peritoneal macrophages were isolated 4 hours after MDP injection prior to Western immunoblotting analyses. Experimental results showed that levels of CARD9, the phosphorylated p38 MAPK, as well as the autophagy initiation and maturation proteins LC3B and p62 were all significantly increased after MDP stimulation. However, the expression of the receptor interacting protein kinase 2 (RIP2), a partner protein of NOD2 upstream of NFκB signaling, was significantly down-regulated by MDP challenge, indicating an inhibitory role of RIP2 on MDP-induced inflammatory signaling.

In summary, our results suggested that MDP activated NOD2 with a concomitant up-regulation of CARD9, the phosphorylation of p38 MAPK, and the autophagy signaling. As p38 MAPK phosphorylation inhibits autophagy, these results also implied that activation of CARD9 and the associated p38 MAPK signaling may have prevented further up-regulation of the autophagy signaling in the activated macrophages.

057

Ethanol mediated-cardioprotection is lost in mice carrying the E487K variant of aldehyde dehydrogenase 2: Benefits of Alda-1

Cintia B. Ueta[a], Marie-Helene Disatnik[b], Che-Hong Chen[b], Daria Mochly-Rosen[b], Julio C.B. Ferreira[a]

[a]*University of São Paulo, São Paulo, SP, Brazil*
[b]*Stanford University, Stanford, CA, USA*

Background: It is well known that ethanol preconditioning protects the heart from ischemic injury. However, the molecular mechanisms involved in ethanol-mediated cardioprotection are unknown. **Methods:** In the present study we used mice carrying the E487K variant of ALDH2 (which presented reduced ALDH2 activity by 90%) to understand its role during ethanol-mediated cardioprotection. **Results:** Ethanol administration (50 mM) prior to ischemia-reperfusion (I/R) injury reduced myocardial infarction (30 ± 3: ethanol vs. 50 ± 2: I/R), and lipid peroxidation (86% of the I/R) in WT hearts compared to control. Of interest, ethanol administration potentiated I/R injury in ALDH2 knock-in mice by increasing myocardial infarction (60 ± 3: ethanol vs. 48 ± 6: I/R) and mitochondrial ROS release (0.8 ± 0.1: ethanol vs. 0.4 ± 0.1: I/R). Finally, Alda-1 (a selective ALDH2 activator) administration prior to I/R re-established the ALDH2-mediated cardioprotection in ALDH2 knock-in mice (33 ± 4: Alda-1 vs. 48 ± 6: I/R). **Conclusion:** Altogether, our results suggest that activation of ALDH2 is essential for ethanol-induced cardioprotection and highlight a new benefit of Alda-1.

058

S107 improves RBM20 deficiency-induced cardiac dysfunction

Zhiyong Yin[a,b], Chaoqun Zhu[a], Jun Ren[c], Wei Guo[a,c]

[a]*Animal Science, University of Wyoming, Laramie, WY, USA*
[b]*Department of Cardiology, Xi Jing Hospital, Fourth Military Medical University, Xi'an, China*
[c]*Center for Cardiovascular Research and Alternative Medicine, College of Health Science, University of Wyoming, Laramie, WY, USA*

Introduction

RNA binding motif 20 (RBM20) is a recently identified muscle specific splicing factor with the highest expression levels in the heart and has been recently revealed to regulate Ryr2 splicing. *Rbm20*$^{-/-}$ rats present dilated cardiomyopathy (DCM) and sudden death. In *Rbm20*$^{-/-}$ rats, a 24-bp exon in Ryr2 is included. Increase of this exon has been associated with ventricular tachycardia and sudden death. S107 is a stabilizer of RyR multi-protein complex and inhibits abnormal SR Ca^{2+} release. This study is to test whether S107 can protect *Rbm20*$^{-/-}$-induced cardiac dysfunction.

Methods and Results

Cardiomyocytes (CMs) were isolated from 6 month-old *Rbm20*$^{-/-}$ and *Rbm20*$^{+/+}$ rats and incubated with S-107 (10 μM) for 2 hours after isolation. Contractile properties of CMs were evaluated using an IonOptix SoftEdge system within 6 hrs. *Rbm20*$^{-/-}$ deficiency depressed sarcomere peak shortening (PS) and ± dL/dt, the effects of which were significantly alleviated by S-107. Further, sarcomere length exhibited no significant differences between *Rbm20*$^{-/-}$ and *Rbm20*$^{+/+}$ rats, although S-107 prolonged the length of sarcomere. The time-to-50% sarcomere PS (TP_{50}) was significantly shorter in *Rbm20*$^{-/-}$ than that in *Rbm20*$^{+/+}$ rats, while TP_{50} was significantly prolonged in *Rbm20*$^{-/-}$ CMs and S-107 treatment nullified such prolongation. Similar results were also noted in TP_{10} but not in TP_{90}.

Discussion and Conclusion

Our findings reveal that S107 improves contractile function in *in vitro* cultured CMs. Next, we will answer the following questions 1) Does additional 24-bp inclusion in Ryr2 interfere with the RYR2 channel complex? 2) Does S107 improve the contractility of *Rbm20*$^{-/-}$ CMs via stabilizing Ryr2 complex? 3) Does S107 protect *Rbm20*$^{-/-}$ rats from sudden death by regulating Ca^{2+} release? Together, this preliminary data indicate the therapeutic potential for S107 for ventricular tachycardia and sudden death in patients with loss function of RBM20.

059

A High-Content RNAi Screen for Novel Effectors of Cardiac Proteotoxicity

Patrick McLendon, Gregory Davis, James Gulick, Kritton Shay-Winkler, Jeffery Molkentin, Jeffrey Robbins

Cincinnati Childrens Hospital Medical Center, Cincinnati, OH, USA

Proteotoxicity has emerged as an important underlying pathologic mechanism in cardiac disease. Many heart failure phenotypes, including ischemia/reperfusion, pressure overload-induced hypertrophy and desmin-related cardiomyopathy accumulate misfolded protein within cardiomyocytes, potentially leading to cell death and dysfunction. While some success in treating these phenotypes has been achieved by inducing the protein degradation mechanisms mediating protein quality control in cardiomyocytes, we do not know or understand all the players and processes involved in pathologic aggregation. To address this, we developed a cellular model of protein aggregation in primary mouse cardiomyocytes by expressing a fluorescent aggregation-prone protein, a mutated B-crystallin ($CryAB^{R120G}$). The cells developed large intracellular aggregates, consistent with data from mice and humans. We subjected this cell model to a high-content, genome-wide RNAi knockdown screen using a lentiviral shRNA library (15,959 genes). The assay's readout consisted of the quantity of punctate aggregate structures within cardiomyocytes. Using shRNA knockdown, we identified 213 genes in the mouse genome that could substantially reduce aggregate content. The hits span diverse and interesting cellular processes including mitochondrial and apoptotic cell death, calcium signaling, post-translational modifications and metabolism. As a whole, the hits suggest overlapping therapeutic mechanisms between proteotoxicity and other cardiovascular pathologies. Further interrogation of our candidate genes will contribute vital details on the molecular mechanisms of pathologic protein aggregation, and hopefully provide new and interesting therapeutic targets.

060

Genetic Deletion of the γ2-subunit of AMPK Aggravates Cardiac Dysfunction During Pathological Hypertrophy

Maengjo Kim, Lorena Garcia-Menendez, Yong Seon Choi, Richard Palmiter, Rong Tian

University of Washington, Seattle, WA, USA

Mutations in γ2-subunit of AMPK (γ2-AMPK) cause glycogen storage cardiomyopathy. To investigate its biological role(s) in the heart, we generated a cardiac specific knock-out mouse (γ2KO) by crossing transgenic mice (Flox) that flank the exon 6 of γ2-AMPK with loxP sites to βMHC-Cre transgenic mice. In spite of lower glycogen content in the heart, γ2KO mice were born normal and remained indistinguishable from control mice in terms of heart weight and cardiac function for up to 6 months under baseline conditions. When we subjected γ2KO and Flox/Flox control mice to transverse aortic constriction (TAC) and isoproterenol infusion, γ2KO mice developed more severe cardiac dysfunction after cardiac stress compared with control mice. AMPK activity greatly increased after TAC but there was no difference between control and γ2KO hearts. The γ2-AMPK activity was depressed and compensatory increase in the γ1-AMPK activity maintained overall AMPK activity in γ2KO hearts, suggesting that γ2-AMPK activity is critical in the response to chronic stress. Protein expression level of hexokinase II (HKII) was down-regulated both in the whole lysates and the cytosol of γ2KO hearts, which resulted in decline in the pentose phosphate pathway (PPP) flux and increase in oxidative stress. Knock-down of γ2-AMPK subunit also increased oxidative stress in neonatal rat ventricle cardiomyocytes (NRVCMs). HKII overexpression abolished the phenylephrine-induced hypertrophy and hydrogen peroxide-induced cell death in γ2-AMPK knock-down NRVCMs. Taken together, these data indicate that γ2-AMPK regulates pathological hypertrophy via the HKII-PPP axis.

061

An alpha-1A adrenergic receptor agonist to treat heart failure

Megan D. Montgomery, Trevor Chan, Rajesh Dash, Philip M. Swigart, Bat-Erdene Myagmar, Anthony J. Baker, Paul C. Simpson

University of California, San Francisco, San Francisco, CA, USA
San Francisco VA Medical Center, San Francisco, CA, USA

Background: Neurohormonal blockade underlies current heart failure (HF) therapy, but has limits, including adverse effects of alpha-1-adrenergic receptor (α1-AR) blockade in clinical trials such as ALLHAT and V-HeFT. Conversely, α1-agonists protect myocytes and hearts in multiple species and models through pleiotropic mechanisms; however, it is unknown if these findings translate to HF treatment. Therefore, we evaluated α1-AR agonism in HF in vivo.

Methods: We screened α1-AR agonists in neonatal rat and adult mouse ventricular myocytes (NRVM, AMVM), measured blood pressure (BP) by telemetry, and treated mice with HF from cardiotoxin (doxorubicin, DOX) and pressure overload (TAC).

Results: From the 11 α1-AR agonists we tested in NRVMs, we identified A61603 (A6), a selective α1A agonist, as the most efficacious and potent in ERK activation (ratio of phospho- to total-ERK) and protein synthesis, and EC50s matched α1A binding affinity. In AMVMs, A6 activated ERK and protected against DOX, an effect lost with α1A-KO. In isolated hearts, A6 activated ERK target genes. In vivo, A6 10 ng/kg/d 7-day subcutaneous infusion had no effect on BP, but increased cardiac phospho-ERK. A6 10 ng/kg/d started at DOX injection enhanced survival, preserved cardiac function, and reduced cardiac fibrosis and apoptosis; however, A6 had no effect on survival and function after DOX in α1A-KO mice. After TAC, A6 improved cardiac function, reduced cardiac fibrosis and apoptosis, and increased phospho-ERK and bMyHC, without affecting heart or myocyte size.

Conclusions: Overall, the high-affinity α1A-AR agonist A61603, at a low, non-hypertensive dose, prevents cardiac cell death and fibrosis and improves function and mortality in two mouse models of HF. Therefore, α1A-AR agonists have potential as HF therapies.

062

The mitochondrial protein C1qbp binds to cyclophilin D and ATP synthase and regulates the mitochondrial permeability transition in the heart

Manuel Gutierrez-Aguilar, Christopher Baines

University of Missouri-Columbia, Columbia, MO, USA

Opening of the Mitochondrial Permeability Transition (MPT) pore induces necrotic cell death following diverse cardiac insults. Although its biochemical and pharmacological features have been extensively identified, its molecular componentry is still a matter of debate. Models suggest that the pore accessory cyclophilin D (CypD) binds to mitochondrial C1qbp and F_0F_1-ATP synthase to regulate MPT. Here we validate that CypD - through its cyclosporine A-binding domain - can directly interact with C1qbp. Conversely, C1qbp presents multiple potential docking domains for CypD and its N-terminal portion encompassing residues 200 to 279 is required to interact with the α subunit of ATP synthase. We consequently generated mouse strains overexpressing or with decreased levels of mitochondrial C1qbp in the heart to assess the functionality of such interactions. Although C1qbp overexpression resulted in decreased ATP synthase activity, MPT pore readings remained unchanged. Antithetically, partial depletion of C1qbp in *C1qbp*$^{+/-}$ mice resulted in a sensitized MPT pore to Ca^{2+} together with decreased basal mitochondrial respiration. Our results suggest that C1qbp is a novel factor that regulates ATP synthase activity alongside its multiple proposed roles and is required for normal MPT pore regulation.

063

FK 506 Binding Proteins Facilitate the Termination of Spontaneous Ca^{2+} Release in Wild Type Cardiac Ryanodine Receptor (RyR2), but not Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) Associated RyR2 Mutants

Joe Zhang, Helen Waddell, Ella Wu, Jhanvi Dholakia, Janet McLay, Peter Jones

Department of Physiology and HeartOtago, University of Otago, Otago, New Zealand

FK506 binding protein 12.6 (FKBP12.6) is tightly associated with the cardiac ryanodine receptor (RyR2). Early studies indicated that dissociation of FKBP12.6 from RyR2 contributes to abnormal Ca^{2+} release, otherwise known as store overload induced Ca^{2+} release (SOICR), and Ca^{2+} triggered arrhythmias, however these findings remain controversial. In contrast to FKBP12.6 less is known about the role of FKBP12.0, another FKBP family member, in regulating RyR2 function. Here we investigated the impact of FKBP12.6 and FKBP12.0 on SOICR in cells expressing RyR2 wild type (wt), and the arrhythmogenic mutant forms of RyR2 V4653F and R4496C. We found that expression of FKBP12.6 or FKBP12.0 significantly increased the termination threshold of SOICR without changing the activation threshold of SOICR. These changes led to a reduction in the magnitude of Ca^{2+} release per SOICR event. The differences in the properties of individual release events occurred in the absence of changes in the propensity of SOICR. The introduction of arrhythmogenic mutations into RyR2 prevented both FKBP12.6 and FKBP12.0 from regulating the magnitude of SOICR, despite a conserved interaction between both FKBPs and RyR2. Taken together, our results suggest that both FKBP12.6 and FKBP12.0 play critical roles in regulating RyR2 function by facilitating the termination of SOICR. The inability of FKBPs to mediate a similar effect on mutant RyR2 represents a novel mechanism by which mutations within RyR2 lead to arrhythmia.

064

Toll-like receptor 4 knockout alleviates paraquat-induced cardiomyocyte contractile dysfunction through an autophagy-dependent mechanism

Shuyi Wang, Maolong Dong, Jun Ren

Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, WY, USA

Paraquat, a quarternary nitrogen herbicide, is a toxic prooxidant leading to multi-organ failure including the heart although the underlying mechanism remains poorly understood. This study was designed to examine the role of the innate proinflammatory mediator toll-like receptor 4 (TLR4) in paraquat-induced cardiac contractile injury and the underlying mechanisms involved with a focus on autophagy. The autophagy-lysosome pathway is a major pathway governing protein and organelle degradation and recycling to promote cardiac homeostasis.

Wild-type (WT) and TLR4 knockout (TLR4-/-) mice were challenged with paraquat (45 mg/kg) for 48 hrs. Paraquat elicited cardiac mechanical anomalies including compromised cardiomyocyte contractile function, intracellular Ca2 + handling, reduced cell survival, and overt autophagy induction as manifested by increased LC3BII-to-LC3BI ratio and Atg7 levels. In addition, paraquat treatment promoted phosphorylation of AMPK while suppressing the phosphorylation of ULK1 (Ser757) and mTOR. Interestingly, TLR4 knockout significantly attenuated paraquat-induced cardiac contractile and intracellular Ca2 + derangement as well as alterations of the autophagy markers, phosphorylation of AMPK, ULK1 and mTOR. The beneficial effect of TLR4 knockout was associated with inhibition of the AMPK- mTOR-ULK1 signaling cascade. In vitro study revealed that AMPK activation (using AICAR 500 μM) or the mTOR inhibition (using rapamycin 5 μM) negated the beneficial cardiomyocyte mechanical effects of TLR4 inhibition (using CLI-095 400nM) against paraquat (100 μM), supporting a permissive role for AMPK-mTOR in TLR4 inhibition-offered cardioprotection against paraquat.

Our results suggested that TLR4 knockout alleviated paraquat-induced unfavorable changes in cardiac function possibly through attenuation of AMPK-mediated cardiac autophagy.

065

Impair contractility and size-dependent toxicity of silica nanoparticles in adult rat cardiomyocytes

Carlos Enrique Guerrero-Beltrán[a], Jesús Roberto-Garza[a], Alejandra García-García[b], Noemí García[a], Judith Bernal-Ramírez[a], Nancy Ornelas[a], Omar Lozano[c], Elvin Blanco[d], Gerardo García-Rivas[a]

[a]*Tecnológico de Monterrey, Monterrey, Nuevo León, Mexico*
[b]*Centro de Investigación en Materiales Avanzados S.C., Apodaca, Nuevo León, Mexico*
[c]*Université de Namur ASBL, Namur, Belgium*
[d]*The Methodist Hospital Research Institute, Houston, TX, USA*

Nanotechnology, as we know it in the 21th century, is gaining an enormous acceptance in the industry: agriculture, costumer products, additives to food, and more recently to biotechnological and biomedical fields. Nanomaterials [e.g. TiO_2 and SiO_2 (silica) particles] are used to improve or create new functional properties to regular

products, however, recently there has been an enormous evidence showing that nanoparticles might exert potential risks to human health. Several studies have shown that nanoparticles can penetrate to the body by different mechanisms and routes such as the skin, respiratory system and gastrointestinal tract, or by novel clinical diagnostic protocols and therapeutic drugs treatments allowing nanoparticles to a more and extended exposure to biological systems causing inflammatory response, fibrosis or cell death. In this study, we explored for the first time, SiO_2-induced toxicity and impair contractility on isolated adult rat cardiomyocytes exposed to a 24 h incubation with 7 nm or 680 nm SiO_2 particles. Silica was characterized by SEM, DLS and Z potential in H_2O and media culture. IC_{50} was obtained for the 7 nm (99.5 ± 12.4 ug/mL) and 680 nm (>1500 ug/mL), showing a size-dependent toxicity. Both size particles exerted cellular death by necrosis, in a time-dependence, where apoptosis was not evident. We found a SiO_2 membrane association and cellular internalization, analyzed by SEM and confocal microscopy, respectively. Significant glutathione depletion (down to 5 nmol/mg prot *vs.* 15 nmol/mg prot, control group) and reactive oxygen species generation was observed in both treatments at its IC_{50}. Importantly, cardiomyocytes Ca^{2+} transient was affected only by the 7 nm SiO_2 and cellular shortening was reduced significantly by both particles.

In summary, cultured adult rat cardiomyocytes exposed to 7 nm or 680 nm SiO_2 results in a size-dependent cytotoxicity with an impair contractility closely related to increased oxidative stress.

066

G3BP1 differentially regulates two cardiac-enriched, non-coding RNAs and promotes hypertrophy development

Minzhen He, Danish Sayed

Rutgers New Jersey Medical School, Newark, NJ, USA

Posttranscriptional regulation controls transcript processing at multiple levels from translation, stability, to location, hence underscoring its importance in gene regulation. RNA binding proteins (RBP) have been identified as foremost posttranscriptional regulators of coding and non-coding RNA. Ras GTPase-Activating-Protein-SH3-Domain-Binding-Protein-1 (G3BP1) is unique RBP that can function as an endoribonuclease or affect RNA stability and location by binding to its consensus sequence in selective transcripts. Our preliminary data shows that G3BP1 is significantly increased during hypertrophy and assembles in cytoplasmic aggregates called stress granules (SG), which are triage sites for RNA transcripts. RNA immunoprecipitation with G3BP1 followed by bioanalysis showed enrichment of transcripts ranging from 100-2000 bp. Our aim was to identify these transcripts, their fate and role in hypertrophy. A screen of the consensus sequence pulled up two cardiac-enriched non-coding RNAs, pre-miRNA-1 and Growth Arrest Specific 5 (Gas5). We measured primary, pre-miRNA-1, mature miRNA-1 and Gas5 levels in mouse hearts subjected to sham or transverse aortic constriction for 4 days. Interestingly, a ~40% decrease was seen only in pre-miRNA-1-2 and mature miRNA-1 expression, while no change was seen in primary-miRNA-1 or Gas5 transcripts. Similar results were seen in cardiac myocytes expressing exogenous G3BP1. Intriguingly, in-situ hybridization showed translocation of Gas5 to perinuclear foci with hypertrophy or exogenous G3BP1, suggesting sequestration into SGs and hence loss of function. We showed that miRNA-1 targets TFIIB and CDK9, two essential components of transcriptional machinery. On the other hand, Gas5 regulates stability of Pten, and controls PI3K-AKT pathway. In addition, Gas5 has been shown to bind to EIF4E and regulate cap-dependent translation. Inhibition of G3BP1 rescues growth-induced downregulation of miRNA-1 and sequestration of Gas5, hence restoring their downstream targets and function. Thus, we conclude that G3BP1 by regulating processing/function of miR-1 and Gas5, respectively, controls transcriptional and translational increase in gene expression and promote development of hypertrophy.

067

Increasing myocardial fatty acid oxidation protects against pathological hypertrophy and diastolic dysfunction in mice

Stephen Kolwicz, Yong Seon Choi, Ana Mattos, Dan Shao, Tao Li, Eric Smith, Miranda Nabben, Wang Wang, Rong Tian

University of Washington, Seattle, WA, USA

Background: Our previous work demonstrated that increasing myocardial fatty acid oxidation (FAO) by 50%, via deletion of acetyl CoA carboxylase 2 (ACC2), did not cause cardiac dysfunction under non-stressed conditions. Moreover, cardiac dysfunction was preserved and cardiac hypertrophy was attenuated during chronic pressure-overload. Therefore, we further tested the cardioprotective effect of enhanced myocardial FAO using a cardiac-specific inducible deletion of ACC2 (iKO) in mice fed a high fat diet (HFD) or during chronic administration of angiotensin II (AngII).

Methods: Male iKO and control (CON) mice were randomly assigned to HFD (60% kcal/fat) or control diet (CD, 17% kcal/fat) for 12wks. In a separate cohort of iKO and CON mice, AngII or vehicle (saline) was delivered for 4wks by osmotic mini-pumps. Echocardiography and tissue Doppler imaging measured systolic and diastolic function, respectively. Substrate oxidation was assessed in tissue extracts from isolated perfused hearts via ^{13}C NMR spectroscopy.

Results: HFD resulted in obesity and glucose intolerance in both genotypes. HFD and iKO each resulted in a 60% increase in FAO, while FAO was increased 2.5-fold in iKO-HFD vs CON-CD. Systolic and diastolic function was unaltered in iKO and/or HFD hearts. Heart weight to tibia length ratio (HW:TL) increased 20% in CON-HFD but not in iKO-HFD mice ($p < 0.05$). With AngII administration, HW:TL increased 30% and fibrosis increased 3.5-fold in CON ($p < 0.05$ vs CON-vehicle) while both of these parameters were attenuated 50% in iKO ($p < 0.05$ vs CON-AngII). E'/A' and E/E' ratios were significantly altered in CON-AngII while iKO-AngII were similar to CON-vehicle. FAO decreased in CON-AngII but iKO-AngII remained similar to CON-vehicle.

Conclusion: These data suggest that increased cardiac FAO via inducible deletion of ACC2 does not exacerbate cardiac dysfunction during HFD. Furthermore, AngII-induced adverse cardiac remodeling, evidenced by myocardial hypertrophy, fibrosis, and diastolic dysfunction, is attenuated in ACC2-iKO hearts.

068

Pathophysiological impact of a highly prevalent MYBPC3 gene variant causing hypertrophic cardiomyopathy *in vivo*

Diederik Kuster[a,b], David Barefield[a], Suresh Govindan[a], Mayandi Sivaguru[c], Ben August[d], Kyounghwan Lee[e], Rajasekaran Namakkal-Soorappan[f], Roger Craig[e], Sakthivel Sadayappan[a]

[a]*Loyola University Chicago, Maywood, IL, USA*

[b]VU University Medical Center, Amsterdam, The Netherlands
[c]University of Illinois at Urbana Champaign, Urbana, IL, USA
[d]University of Wisconsin-Madison, Madison, WI, USA
[e]University of Massachusetts Medical School, Worcester, MA, USA
[f]University Alabama at Birmingham, Birmingham, AL, USA

Introduction: A polymorphic deletion of 25 base pairs in the cardiac myosin binding protein-C (cMyBP-C) gene (MYBPC3), resulting in a modified C10 domain (cMyBP-C$^{\Delta C10}$), affects ~60 million South Asians and is associated with hypertrophic cardiomyopathy (HCM) and heart failure. We recently showed that cMyBP-C$^{\Delta C10}$ causes contractile dysfunction *in vitro* through a poison polypeptide effect. However, the molecular mechanisms underlying the pathogenicity of cMyBP-C$^{\Delta C10}$ *in vivo* is unknown.

Hypothesis: cMyBP-C$^{\Delta C10}$ expression causes contractile dysfunction and leads to the development of HCM.

Methods and Results: To determine whether cMyBP-C$^{\Delta C10}$ causes HCM and contractile dysfunction *in vivo*, we generated transgenic mice having cardiac-specific expression of cMyBP-C$^{\Delta C10}$ at approximately half the level of endogenous cMyBP-C. At 12-weeks of age, significant hypertrophy was observed in transgenic mice expressing cMyBP-C$^{\Delta C10}$ by calculating the ratio of heart weight to body weight (HW/BW; 4.41 ± 0.09 mg/g nontransgenic [NTG] vs 4.94 ± 0.17 mg/g cMyBP-C$^{\Delta C10}$, $P < 0.05$). Furthermore, hematoxylin and eosin and Masson's Trichrome staining as well as second harmonic generation imaging revealed the presence of significant fibrosis and a greater relative nuclear area in the cMyBP-C$^{\Delta C10}$ hearts compared to NTG controls. Electron microscopic analysis showed normal sarcomere structure in the cMyBP-C$^{\Delta C10}$ hearts compared to the NTG controls. M-mode echocardiography analysis revealed hypercontractile hearts (EF: $53.8\% \pm 4.3\%$ NTG vs $66.4\% \pm 4.7\%$ cMyBP-C$^{\Delta C10}$; $P < 0.001$) and early diastolic dysfunction (E/E': 32.3 ± 5.7 NTG vs 46.3 ± 8.4 cMyBP-C$^{\Delta C10}$; $P < 0.01$) indicating the presence of an HCM phenotype. To assess whether these changes manifest themselves at the myofilament levels, contractile function of single skinned cardiomyocytes was measured. Preserved maximum force generation and an increased Ca^{2+}-sensitivity of force generation was observed in cardiomyocytes from cMyBP-C $^{\Delta C10}$ mice compared to NTG controls (EC_{50}: 3.37 ± 0.01 μM NTG vs 2.90 ± 0.01 μM cMyBP-C$^{\Delta C10}$; $P < 0.001$).

Conclusion: Expression of the HCM-associated cMyBP-C$^{\Delta C10}$ protein is sufficient to cause contractile dysfunction and HCM *in vivo*.

069

Age-dependent cardiac contractile dysfunction is graded by frailty, not age, in senescent C57BL/6 J mice

Hirad Feridooni[a], Arash Boroumandi[b], Nazari Polidovitch[c], Robert Rose[a], Robert Tsushima[b], Susan Howlett[a]
[a]Dalhousie University, Halifax, Canada
[b]York University, Tornoto, Canada
[c]University of Toronto, Toronto, Canada

Frail patients with cardiovascular disease (CVD) experience worse outcomes and higher mortality than non-frail patients, but the links between frailty and myocardial function are unclear. Here we investigated the impact of age and frailty on cardiac hemodynamic function. We quantified frailty in aging mice with a novel frailty index (FI) developed in our laboratory based on the clinical assessment of health deficits. FI scores were measured for middle-aged (205-332 days; $n = 6$) and senescent (783-877 days; $n = 20$) male C57BL/6 J mice. Langendorff-perfused hearts (Tyrode's solution, 1.8 mM Ca^{2+}; 95% O_2/5% CO_2; constant pressure = 80 mmHg) were used to measure heart rate (HR), left ventricular developed pressure (LVDP), rate of pressure development (+dP/dt), and rate of pressure decay (-dP/dt). Spontaneous HR was unaffected by either age or FI scores. By contrast, LVDP declined with age (values were 94 ± 9 vs 66 ± 4 mmHg in middle-aged vs senescent hearts; $P < 0.05$). Age also caused a reduction in +dP/dt (2144 ± 204 mmHg/s vs 1538 ± 98 mmHg/s; $P < 0.05$), and -dP/dt (-2130 ± 164 mmHg/s vs -1477 ± 86 mmHg/s; $P < 0.05$). Interestingly, this age-dependent decline in cardiac contractile function was graded by the FI score. As FI increased, LVDP, +dP/dt, and -dP/dt declined dramatically. Indeed, frailty predicted the reduction in LVDP ($r^2 = 0.36$; $P = 0.005$), +dP/dt ($r^2 = 0.29$; $P = 0.014$), and -dP/dt ($r^2 = 0.30$; $P = 0.012$) in senescent hearts while chronological age did not (r^2 values for LVDP, +dP/dt, and -dP/dt = 0.029, 0.027, and 0.024 respectively; P values = 0.48, 0.49, and 0.51 respectively). Heart weight to tibia length (HW:TL) ratios also increased as frailty increased ($r^2 = 0.18$, $P = 0.01$), whereas chronological age showed no relationship between HW:TL ratio ($r^2 = 0.06$, $P = 0.15$). These results suggest that age-dependent hypertrophy and cardiac contractile dysfunction are more closely linked to frailty than chronological age. This may contribute to adverse outcomes in frail older patients with CVD.

070

Diabetic hyperglycemia acutely affects action potentials and ionic currents through CaMKII activation on murine and rabbit ventricular myocytes

Zhandi Liao, Donald Bers

University of California Davis, Davis, CA, USA

Diabetes mellitus is a complex disease that involves cardiomyopathy and neuropathy. Ca^{2+}-Calmodulin dependent protein kinase II (CaMKII) is a nodal molecule that participates in many physiological and pathological processes in the heart, including modulation of numerous ion channels. Diabetic hyperglycemia has been shown to activate CaMKII, through modification of O-linked N-acetylglucosamine (O-GlcNAc) at S279, leading to cardiac arrhythmias at the whole heart/animal level. However, the effect of acute hyperglycemia on specific ion channels and thus action potentials (APs) through O-GlcNAc activated CaMKII is still unclear. To investigate this question, we measured action potentials (APs) and ionic currents in freshly isolated murine and rabbit ventricular myocytes under acute diabetic hyperglycemia challenge. On rat myocytes, glucose (30mM) perfusion significantly reduced the AP amplitude, depolarized the resting membrane, and prolonged the AP duration. The glucose effects on APs were abolished on myocytes pre-incubated with KN-93 (10□M) or on myocytes isolated from CaMKII□-KO mice, indicating that glucose changes APs in a CaMKII sensitive manner. Acute glucose application significantly reduced $I_{to,fast}$. On rabbit myocytes, however, glucose alone did not alter APs. Only when combined with inhibition of O-GlcNAcase (Thiamet G, 100nM) and low level of oxidative stress (Angiotensin II, 100nM), glucose was able to prolong the AP duration, suggesting a species dependent glucose-CaMKII signaling pathways. Taken together, these data provide evidence for the arrhythmogenesis of acute hyperglycemia at the cellular level and suggest that CaMKII-modulated ionic currents are responsible for the hyperglycemic effects on APs.

071

Role of CaMKII in cardiac frequency adaptation

Tamas Banyasz[a,b], Balazs Horvath[a,b], Bence Hegyi[a,b], Peter P. Nanasi[a], Zhong Jian[b], Rafael Shimkunas[b], Leighton T. Izu[b], Ye Chen-Izu[b]

[a]*Department of Physiology, University of Debrecen, Debrecen, Hungary*
[b]*Department of Pharmacology, University of California Davis, Davis, CA, USA*

Cardiac Ca^{2+}/calmodulin depedent protein kinase II (CaMKII) is known to modulate a regiment of membrane currents and to regulate physiologic and pathologic functions including calcium homeostasis and arrhythmogenesis. The goal of the present study is to explore the role of CaMKII as a "memory molecule" that regulates the gradual adaptation of the action potential (AP) in response to a change of pacing frequency. Using current-clamp technique, we recorded APs when the pacing rate was switched from 1 Hz to 0.1 Hz (at 22°C) in guinea pig ventricular myocytes, and studied the AP duration (APD90) and other parameters during the transient adaptation phase in the control cells and in the cells pre-treated with 1 μM KN-93 to inhibit CaMKII. Our results show that CaMKII inhibition accelerated the APD changes after the frequency switching and significantly abbreviate the adaptation phase, although the steady state APDs at 1 Hz and 0.1 Hz were not significantly affected. Next, we used voltage-clamp to study the effects of CaMKII inhibition on the major K^+ currents involved in AP repolarization. KN-93 did not affect I_{K1}, but completely suppressed the rapid component of I_{Kr}; KN-93 also facilitated the voltage and time dependent activation of I_{Ks} and significantly slowed the deactivation of I_{Ks}. Taken together, our data suggest that CaMKII regulation of I_{Kr} and I_{Ks} plays a key role in the frequency adaptation of cardiac AP. CaMKII serves as a "memory molecule" to effect a gradual adaption of the APD in response to heart rate changes; such adaptation mechanism avoids abruptly changes in APD that may provide a substrate to arrhythmias.

072

Enhanced myocardial repair with CardioClusters

Megan Monsanto, Kristina Fisher, Kevin White, Natalie Gude, Mark A. Sussman

San Diego State University, San Diego, CA, USA

Existing approaches to modify stem cells for myocardial regeneration desperately need an innovative solution that builds upon existing knowledge that will raise efficacy of repair to a new level. Although this deficiency has been attacked through combinatorial stem cell delivery and formation of cardiospheres, there is no evidence that these stem cell injections provide for direct cellular crosstalk to promote stem cell survival and proliferation. Therefore, we created a CardioCluster, a three-dimensional microenvironment consisting of three defined cell populations from the human heart: c-kit^+ cardiac progenitor cells (CPCs), $CD90^+/CD105^+$ mesenchymal stem cells (MSCs) and $CD133^+$ endothelial progenitor cells (EPCs). The size of the CardioCluster can be controlled by the quantity of cells used to create the cluster, allowing them to be infused into the heart without being reduced to single cell suspensions as is the case for cardiosphere-derived cells where the structural and cell-cell contact information is lost when delivered. Unlike cardiospheres, these cardiac cells are combined into a rationally designed cluster with MSCs and CPCs in the central core and EPCs forming the outer layer. EPCs play a vital role in forming neovasculature that will connect the CardioClusters to living heart tissue not damaged by ischemia and allow for revascularization of the damaged myocardium. In vitro we have shown that EPCs are better able to form tubular networks on matrigel-coated plates compared to either CPCs or MSCs. MSCs reinforce the 3D structure by releasing growth factors that attract and maintain cells within the cluster. Upon induction of an oxidative stress by hydrogen peroxide CardioClusters show improved cell survival with a lower percentage of apoptotic and/or necrotic cell populations compared to the three populations individually. Clinically, CardioClusters broaden the application of cell types by creating a single structure to increase engraftment, mitigate inflammation and prevent the progression of heart failure.

073

ALPK2 is a novel regulatory kinase required for heart development

Peter Hofsteen[a], Aaron Robitaille[a], Nathan Palpant[a], Lil Pabon[a], Randall Moon[a,b], Charles Murry[a]

[a]*University of Washington, Seattle, WA, USA*
[b]*Howard Hughes Medical Institute, Seattle, WA, USA*

Understanding the molecular mechanisms of heart development provides clues toward novel therapies for heart disease. Alpha-kinase 2 (ALPK2) is a recently discovered atypical eukaryotic protein kinase with no known function during heart development. Here, we sought to determine the role of ALPK2 by using two models of cardiogenesis: human pluripotent stem cell (hPSC) differentiation towards definitive cardiomyocytes in vitro and zebrafish in vivo. Chip-Seq, quantitative proteomics and RNA analysis over time course cardiomyocyte differentiation revealed ALPK2 is expressed and epigenetically regulated in cardiac progenitor cells (CPCs) and definitive cardiomyocytes. ALPK2 knockdown (KD) results in failure to form CPCs and subsequently contractile cardiomyocytes. Furthermore, cells lacking ALPK2 fail to undergo EMT and show differential expression of proteins involved in cell polarity and microtubule formation. In zebrafish, KD of Alpk2 supports our findings in vitro. Alpk2 KD fish show severe cardiac defects with decreased function and failure to form epicardium. Collectively, ALPK2 is a novel protein kinase required for vertebrate heart development by playing a role in specification and cellular morphogenesis of CPCs.

074

Autophagy Induction using Rapamycin Rescues against Targeted Deletion of PTEN in Cardiomyocytes-Induced Cardiac Contractile Dysfunction

Xiyao Chen[a,b], Xiaoling Zhu[c], Xiaoming Wang[b], Jun Ren[a]

[a]*University of Wyoming College of Health Sciences, laramie, WY, USA*
[b]*Departments of Geriatrics, Xijing Hospital, the Fourth Military Medical University, Xi'an, China*
[c]*Departments of Anesthesiology, Xijing Hospital, the Fourth Military Medical University, Xi'an, China*

Phosphatase and tensin homolog (PTEN) deleted from chromosome 10 has been implicated in the maintenance of cardiac

homeostasis although the underlying mechanism(s) remains elusive. Given the likely role for PTEN in the regulation of autophagy, this study was designed to examine the impact of autophagy induction using rapamycin on PTEN deletion-induced cardiomyocyte contractile dysfunction, if any, and the underlying mechanism involved with a focus on mTORC1 signaling. Adult male C57 wild-type (WT) and cardiomyocyte-specific knockout of PTEN ($PTEN^{-/-}$) mice were treated with intraperitoneal injection of rapamycin (6 mg/kg, i.p.) every other day for one week. Myocardial mechanical and intracellular Ca^{2+} properties were examined in WT and $PTEN^{-/-}$mice using echocardiography and IonOptixSoft-Edge techniques. Protein markers for autophagy and related signaling molecules were evaluated. $PTEN^{-/-}$mice elicited cardiac hypertrophy and contractile function shown as reduced fractional shortening, peak shortening, maximal velocity of shortening/relengthening, prolonged relengthening duration and impaired intracellular Ca^{2+} homeostasis. Interestingly, administration of the mTORC1 inhibitor rapamycin rescued against PTEN deletion-induced geometric and functional defects. In addition, the autophagy protein markers Beclin-1, LC3BII and LC3B-II/I ratio expression were significantly decreased while that of the autophagosome cargo protein P62 was significantly upregulated in hearts from $PTEN^{-/-}$mice, the effects of which were significantly attenuated by rapamycin.In conclusion, our data suggested that the mTORC1 inhibitor (and autophagy inducer) rapamycineffectively rescued against PTEN deletion-induced geometric and functional defects associated with restoration of autophagy.

075

PTP1B knockout rescues against ER stress-induced myocardial contractile dysfunction: Role of autophagy

Xiyao Chen[a,b], Nair Sreejayan[b], Xiaoming Wang[a], Jun Ren[b]

[a]*Departments of Geriatrics, Xijing Hospital, the Fourth Military Medical University, Xi'an, China*
[b]*Center for Cardiovascular Research and Alternative Medicine, University of Wyoming College of Health Sciences, Laramie, laramie, WY, USA*

Endoplasmic reticulum (ER) is an intracellular organelle responsible for protein folding. An accumulation of misfolded/unfolded protein in ER lumen activates unfolded protein response (UPR) (also called ER stress). ER stress increases the overall cardiovascular morbidity and mortality although effective management for ER stress-induced pathology remains elusive. This study was designed to examine the impact of protein tyrosine phosphatase 1B (PTP1B) knockout on ER stress-induced myocardial contractile dysfunction, if any, and the underlying mechanism involved with a focus on autophagy signaling.

Adult male C57 wild-type (WT) and protein tyrosine phosphatase 1B knockout ($PTP1B^{-/-}$) mice were treated with intraperitoneal injection of tunicamycin (1 mg/kg, i.p.) once a day for two days. Myocardial mechanical and intracellular Ca^{2+} properties were examined in WT and $PTP1B^{-/-}$mice using echocardiography and IonOptix Soft-Edge techniques. Protein markers for ER stress and autophagy related signaling molecules were evaluated. The results showed that ER stress led to compromised echocardiographic and cardiomyocyte contractile function, intracellular Ca^{2+} mishandling. Tunicamycin triggered ER stress, increased left ventricular end systolic and diastolic diameter, as well as suppressed fractional shortening and cardiomyocyte contractile capacity. Interestingly, tunicamycin-induced changes in myocardial function were significantly reduced by PTP1B knockout. In addition, the ER stress protein markers GRP78, GADD153,eIF2α, p-eIF2α expression were significantly decreased and autophagy protein markers Atg7, Beclin-1, LC3BII, and LC3B-II/I ratio were significantly increased in hearts from WT mice treated with tunicamycin, the effects of which were significantly attenuated by PTP1B ablation. In conclusion, our data suggested that PTP1B knockout reduced ER stress-induced myocardial contractile dysfunction possibly through regulation of autophagy.

076

Defective branched-chain amino acids catabolism induces metabolic remodeling and exacerbates ischemia/reperfusion injury in heart

Tao Li[a], Zhen Zhang[a], Stephen C. KolwiczJr.[a], Haipeng Sun[b], Yibin Wang[b], Rong Tian[a]

[a]*Mitochondria and Metabolism Center, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA*
[b]*Department of Anesthesiology, UCLA David Geffen School of Medicine, Los Angeles, CA, USA*

The branched-chain amino acids (BCAA), i.e. leucine, isoleucine and valine, are required for protein homeostasis, energy balance, and nutrient signaling. BCAA catabolism in mitochondria is regulated by the branched-chain keto acid dehydrogenase (BCKDH) complex. A mitochondria localized phosphatase 2C (PP2Cm) is a key regulator of BCKDH activity. In our study, the PP2Cm knock-out (KO) mice showed normal cardiac function, assessed by echocardiography at 2 months. Myocardial high energy phosphate content and the isovolumic contractile function in isolated perfused hearts were also normal. However, ^{13}C NMR isotopomer analysis revealed a significant decrease in the relative contribution of glucose to oxidative metabolism (16 ± 3 vs. $26 \pm 2\%$ for KO and WT, respectively, $P = 0.018$) in the KO accompanied by an increase in fatty acid oxidation (51 ± 4 vs. $39 \pm 3\%$ for KO and WT, respectively, $P = 0.020$). Glycogen content in the KO hearts was also reduced by >50% (4.4 ± 0.5 vs. 10.9 ± 1.8 μmol glucose/g, $P = 0.000$). As anticipated, ^{31}P NMR spectroscopy for hearts perfused with non-tracer 2-deoxyglucose indicated that the rate of insulin-stimulated glucose uptake in the KO heart was decreased by >20% (0.47 ± 0.007 vs. 0.60 ± 0.02 2-DG-P/ATP/g/min for KO and WT, respectively, $P = 0.0007$). The pyruvate dehydrogenase (PDH) flux estimated from [4-^{13}C] glutamate/[3-^{13}C] alanine enrichment was also significantly suppressed (8.16 ± 0.96 vs. 12.19 ± 1.15 % for KO and WT, $P = 0.034$). These findings collectively suggest a metabolic remodeling in the KO. When subjected to 25 minutes low-flow ischemia (1% of baseline) and 40 minutes reperfusion, the recovery of cardiac function, PCr, ATP, and Pi during reperfusion in the KO failed to reach the level of WT hearts. Increasing glucose uptake and utilization in the KO by overexpressing insulin-independent glucose transporter GLUT1 rescued the exacerbated I/R injury. In conclusion, our results suggest that defective BCAA catabolism induces cardiac metabolic remodeling by suppression of glucose entry, which contributes to exacerbated I/R injury.

077

Inhibition of late Na^+ current as a therapeutic strategy for treating long QT syndrome

Bence Hegyi[a], Zhong Jian[a], Rafael Shimkunas[a], Leighton T. Izu[a], Sridharan Rajamani[b], Luiz Belardinelli[b], Ye Chen-Izu[a], Tamas Banyasz[a]

[a]Department of Pharmacology, University of California, Davis, Davis, CA, USA
[b]Gilead Sciences Inc., Fremont, CA, USA

Rationale:

Late sodium current (I_{NaL}) persists throughout the action potential (AP) plateau where relatively small changes in the net membrane current can significantly affect AP duration (APD). Increase of I_{NaL} has been reported in several pathological conditions including long QT syndrome 3 (LQT3), hypertrophic cardiomyopathy and heart failure. However, little is known about the role of I_{NaL} in LQT2 caused by reduced I_{Kr}, where APD is prolonged but the Na^+ channel function and the Na^+ homeostasis are maintained. The goal of this study was to test the hypothesis that inhibiting the normal physiological I_{NaL} may counterbalance the reduction of I_{Kr} in LQT2 and normalize the AP duration.

Method:

We used the ^{self}AP-clamp Sequential Dissection (Onion-Peeling) technique to record I_{NaL} and I_{Kr} during the AP under physiological condition in rabbit ventricular myocytes.

Results:

(1) The inward I_{NaL} during the AP can counterbalance the outward I_{Kr} under physiological condition, and the total charge carried by I_{NaL} is comparable to that carried by I_{Kr} during the AP cycle.

(2) When LQT2 was simulated by using E-4031 to block I_{Kr}, using GS-458967, a potent inhibitor of I_{NaL}, was able to counterbalance the reduced I_{Kr} and to restore APD to the normal value.

(3) Pacing the cardiomyocyte at high frequency induced beat-to-beat AP alternans, possibly due to variability in the Na^+ and Ca^{2+} homeostasis. Using GS-458967 to inhibit I_{NaL} effectively reduced the variability seen in the AP dispersion.

Conclusion:

Our data indicate that inhibiting the normal physiological I_{NaL} can counter the reduced I_{Kr} during cardiac AP, and thus provide a novel therapeutic strategy for treating LQT2. Furthermore, inhibiting I_{NaL} is also beneficial for maintaining the Na^+ and Ca^{2+} homeostasis and reducing the beat-to-beat variability caused by tachycardia.

078

Mechano-chemo-transduction in cardiomyocytes contracting under mechanical load

Rafael Shimkunas[a,b], Zhong Jian[b], Bence Hegyi[b], John Shaw[e], Nipavan Chiamvimonvat[d], Kit S. Lam[c], Kenneth Ginsburg[b], Julie Bossuyt[b], Donald M. Bers[b], Leighton T. Izu[b], Ye Chen-Izu[a,b]

[a]Department of Biomedical Engineering, University of California, Davis, Davis, CA, USA
[b]Department of Pharmacology, University of California, Davis, Davis, CA, USA
[c]Department of Biochemistry and Molecular Medicine, University of California, Davis, Sacramento, CA, USA
[d]Department of Internal Medicine/Cardiology, University of California, Davis, Sacramento, CA, USA
[e]Department of Aerospace Engineering, University of Michigan, Ann Arbor, MI, USA

Background: Increased mechanical stress under pathological conditions such as hypertension, infarction and fibrosis can cause arrhythmias and heart failure. However, little is known about the mechano-chemo-transduction mechanisms that underlie heart disease development. We have developed a novel Cell-in-Gel system that can control mechanical loading on cardiomyocytes during excitation-contraction coupling. In this study, we investigate mechanical load effects on modulating cardiomyocytes Ca2+ signaling and contraction dynamics. **Methods:** Freshly isolated adult rabbit ventricular myocytes were embedded in polyvinyl alcohol (10%) and crosslinked with tetravalent boronate-PEG (7.5%) to form a 3D elastic gel. Crosslinked gels containing embedded cells were placed in a chamber perfused with Tyrode's solution and electrically paced at 0.5 Hz. Cell contraction and Ca2+ transients were measured in cardiomyocytes contracting in-gel and compared with load-free cells contracting in solution. **Results:** Cardiomyocytes contracting in-gel showed larger systolic Ca2+ transients than the load-free cells (Fura-2 fluorescence ratio peak height 1.47 ± 0.07 in-gel vs 0.85 ± 0.03 load-free), revealing mechano-chemo-transduction that transduces mechanical stress to intracellular Ca2+ increase. Cells in-gel showed an 8% decrease in the contraction amplitude (fractional shortening 13.5 ± 0.4% in-gel vs 14.7 ± 0.3% load-free) when pulling a mechanical load. Furthermore, 12% of cells in-gel showed mechanical stress-induced alternans in the contraction amplitude and in the Ca2+ transient, which can be arrhythmogenic. Importantly, cardiomyocytes from a heart failure rabbit model showed a marked decrease in the Ca2+ transients caused by mechano-chemo-transduction, compared with the healthy control. **Conclusions:** Our findings demonstrate a compensatory mechano-chemo-transduction mechanism that increases the systolic Ca2+ transient to enhance contractility in response to increased afterload, providing a mechanistic basis for the Anrep effect. Our data also suggest this mechanism is attenuated in heart failure.

079

Fatty acid feeding promotes the maturation of cardiomyocytes-derived from human pluripotent stem cells

Xiulan Yang, Marita Rodriguez, Andrea Leonard, Karin Fischer, Lil Pabon, Hans Reinecke, Nathan Sniadecki, Charles Murry

University of Washington, Seattle WA, USA

One hallmark of postnatal cardiomyocyte development is the switch from glucose to fatty acids for ATP production. Standard cell culture media, however, contain minimal fatty acids. Here we propose to promote the maturation of cardiomyocyte-derived from human pluripotent stem cells (hPSC-CM) by providing fatty acids in the medium. The principal fatty acids in human neonatal serum are palmitic, oleic, and linoleic acids, present at stoichiometries of 2.3/1.8/1 and a total fatty acid concentration of about 300 μM. We developed protocols to feed hPSC-CM with these three fatty acids at the above ratio, loading them onto delipidized albumin as a carrier. We have verified that our lipid-albumin complexes are endotoxin-free and the lipids feeding do not lead to cell toxicity. The lipid-albumin complexes are added to our base medium (RPMI plus B27) and cells are treated for 2 weeks. Fatty acid treatment significantly increases cardiomyocyte force production by 30-50% at the single cell level assessed by using arrays of microposts. The improvement in force generation is accompanied by an increase in the rates of calcium release and reuptake, along with a significant increase in maximum calcium amplitude. We have observed that fatty acids treatment induces a modest but significant increase in cell size, increases expression of KCNJ2, CD36, CPT1b, and PDK4, and enhances mitochondrial maximal respiratory capacity. These pro-maturation effects suggest that fatty acid feeding may

facilitate the utility of hPSC-CMs for cell therapy, disease modeling, and/or drug/toxicity screens.

080

Mechanistic studies of myosin light chain mutations associated with dilated, hypertrophic and restrictive cardiomyopathy

Chen-Ching Yuan, Wenrui Huang, Jingsheng Liang, Katarzyna Kazmierczak, Ana I. Rojas, Rosemeire Kanashiro-Takeuchi, Danuta Szczesna-Cordary

University of Miami, Miller School of Medicine, Miami, FL, USA

In this report we focus on a novel mutation in the ventricular regulatory light chain of myosin identified by exome sequencing in a pedigree with familial Dilated Cardiomyopathy (DCM). The D94A mutation in RLCv was expressed in transgenic mice and the hearts subjected to functional and structural measurements. Echocardiography on DCM hearts confirmed a phenotype resembling DCM in humans. The phenotype observed in DCM mice was significantly different from that monitored in hypertrophic (HCM) or restrictive (RCM) cardiomyopathic hearts in transgenic mice expressing D166V-RLCv and E143K-ELCv mutations, respectively. Left ventricular inner diameter was significantly enlarged in DCM mice but diminished in HCM hearts. In addition, HCM mice showed increased ejection fraction (83%) compared to DCM (35%) and WT (63%) hearts. In addition, HCM mice showed significantly increased posterior wall and interventricular septum thickness compared to WT mice. The RCM and HCM hearts demonstrated significant LV fibrosis in 6 month-old male and female mice. Invasive hemodynamics study showed increased Tau (relaxation time) and decreased maximum speed of relaxation in HCM and RCM mice. Reduced cardiac output and stroke volume were observed in DCM mice, while increased atrial elastance was noted in DCM vs. HCM and WT mice. Studies on skinned papillary muscle fibers showed reduced maximal tension in HCM mice while significantly increased in RCM mice. The myofilament Ca^{2+}-sensitivity was significantly increased in HCM and decreased in DCM mice. Studies of signal transduction pathways showed an upregulation of stress/pathology related genes (ANF, BNP and collagen) in HCM and RCM mice. RCAN1.4, calcineurin regulatory protein was upregulated in HCM but not in DCM mice compared to WT controls. Our data indicate significant molecular and functional differences governing the development of DCM, HCM and RCM in mice expressing disease causing mutations in myosin light chains. Supported by NIH-R01HL108343 (DSC) and AHA 15PRE23020006 (CCY).

081

β1-AR /CaMKII Signaling Cause Cardiac Myocyte Death via Mitochondrial Calcium Overload

Pei Wang, Shengchen Xu, Wang Wang

Mitochondria and Metabolism Center, University of Washington, Seattle, WA, USA

Background: Activation of β1-adrenergic receptors (β1-AR) plays crucial roles in cardiac physiology and pathology. In the short-term, β1-AR activation mediates the fight-or-flight response to boost cardiac performance, whereas chronic β1-AR activation can lead to detrimental outcomes in the heart. It has been proposed that chronic β1-AR stimulation may increase diastolic calcium, which can induce the expression of hypertrophic genes, mitochondrial dysfunction and cell death. However, the specific mechanisms of chronic β1-AR stimulation-induced mitochondrial dysfunction are largely unknown.

Results: In this study, we monitored mitochondrial calcium, membrane potential, reactive oxygen species (ROS) and permeability transition pore (mPTP) openings in cultured adult cardiac myocytes and found that chronic β1-AR stimulation by isoproterenol (ISO, 100 nM for 12-48 hr) induced mitochondrial calcium accumulation before triggering mPTP opening and oxidative stress. Moreover, these effects are mediated by activation of a downstream kinase of β1-AR signaling, the calcium calmodulin kinase II (CaMKII) and depend on mitochondrial calcium uptake via mitochondrial calcium uniportor. Blocking mitochondrial calcium uptake or inhibiting CaMKII activity ameliorated mitochondrial dysfunction as reflected by maintaining of membrane potential and resistant to triggered mPTP opening. These approaches also prevented ISO-induced myocyte death at the later stage (48 hr).

Conclusion: Taken together, we provided direct evidence to show the causal role of mitochondrial calcium overload induced by CaMKII during chronic β1-AR in mitochondrial dysfunction and cardiac myocyte death.

082

Mitochondrial Aldehyde Dehydrogenase 2 (ALDH2) Deficiency Impairs Revascularization in Chronic Ischemia and Contributed to Poor Coronary Artery Collateral Circulation

Xiangwei Liu[a,c], Xiaolei Sun[a,b], Zhen Dong[a], Peng Wang[a], Hong Zhu[a], Aijun Sun[a,b], Jun Ren[a,c]

[a]*Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai 200032, China*
[b]*Institute of Biomedical Science, Fudan University, Shanghai 200032, China*
[c]*Center for Cardiovascular Research and Alternative Medicine, School of Pharmacy, University of Wyoming College of Health Sciences, Laramie, WY, USA*

Objective

Revascularization is an essential process to compensate for cardiac underperfusion. Recent evidence suggested a vital role of aldehyde dehydrogenase 2 (ALDH2) in cardiac protection after ischemia. This study was designed to evaluate whether ALDH2 regulates angiogenesis induced by chronic ischemia, the underlying mechanism involved and the clinical impact of the ALDH2 mutant allele on the coronary collateral circulation.

Approach and Results

Mice limb ischemia was performed. Compared with wild-type group, ALDH2 deficiency significantly reduced perfusion recovery, capillary and small artery density, while increased muscle atrophy. *in vitro,* ALDH2 knockdown reduced proliferation, migration, and tube formation in HUVEC, the effects of which were restored by ALDH2 transfection. Further examination revealed that ALDH2 regulated angiogenesis possibly through the classical HIF-1α / VEGF pathways. To further discern the role of ALDH2 deficiency in the function of stem/progenitor cells, cross bone marrow transplantation was performed between WT and ALDH2-KO mice. Slightly lower perfusion recovery was showed in recipient transplanted with ALDH2-KO bone marrow than that with WT bone marrow both in WT and ALDH2-KO mice. 139 chronic total occlusions patients were

recruited from Zhongshan Hospital. Patients with poor coronary collateral circulation (CCC) (n = 51) exhibited a higher frequency of the AA genotype than those with good CCC (n = 88; 11.76% *vs.* 1.14%, P = 0 .01). On the other hand, the AA group displayed less rich CCC frequency in Logistic regression model compared with the GG group (odds ratio = 0.08 [95% confidence interval, 0.009-0.701]; P = 0 .026).

Conclusion

The current study demonstrated that ALDH2 possesses an intrinsic capacity to regulate angiogenesis of endothelial cells involving HIF-1α, VEGF and stem/progenitor cells. Patients with ALDH2 deficient genotype displayed a higher risk of developing poor coronary collateral circulation. Therapeutic individualization based on ALDH2 allele distribution may improve the therapeutic benefit, especially in the East Asia.

083

Oxidative stress regulates titin elasticity by affecting Ig-domain stability

Nazha Hamdani[a], Jorge Alegre-Cebollada[b], Martin Breitkreuz[a], Lars Leichert[c], Julio M. Fernandez[c], Wolfgang A. Linke[a]

[a]*Dept. of Cardiovascular Physiology, Ruhr University, Bochum, Germany, Bochum, Germany*
[b]*Columbia University, New York, USA*
[c]*Medical Proteome Center, Ruhr University, Bochum, Bochum, Germany*

Background: The elasticity of titin is regulated through several mechanisms, including isoform switching and phosphorylation of unique spring elements (N2-Bus, PEVK). However, the titin springs consist mainly of immunoglobulin-like (Ig) domains, which are centrally involved in the molecular mechanism of titin elasticity. Passive force-regulating mechanisms targeting the Ig-domains of titin have not been described.

Methods and Results: We have elucidated a novel oxidative stress-related mechanism regulating muscle elasticity by altering the stability of titin-Ig domains (Alegre-Cebollada *et al.*, Cell. 2014;156:1235-46). Using single-molecule AFM force spectroscopy, force measurements of isolated skinned human myocytes, and redox proteomics, we show that I-band Ig-domains of titin are weakened by oxidative modification of cryptic cysteines. We demonstrate that mechanical unfolding of these Ig domains exposes hidden cysteines, which now become accessible to disulfide bonding or S-glutathionylation in the presence of millimolar concentrations of oxidized glutathione (GSSG). In the AFM experiments, the cysteines of unfolded titin-Ig domains preferentially formed mixed disulfides with glutathione, which prevented the refolding of these domains. Oxidation by GSSG substantially reduced the passive tension of stretched human myocytes, and the effect was fully reversible with the incubation of reduced glutathione. Exposing perfused mouse hearts to oxidative stress (0.1 mM H_2O_2) revealed that Ig-domains from the distal Ig-region of the titin springs are preferential targets of oxidation, as monitored using ICAT labeling/mass spectrometry.

Conclusions: Titin elasticity in striated muscle is modulated by oxidative stress through reversible weakening of Ig-domain stability via S-glutathionylation of buried cysteines. These titin Ig domains could also represent individual mechanosensors, whose mechanical properties determine mechano-chemical signaling processes in stressed myocytes.

084

Endogenous DRP1 modulates cardiac respiration through mPTP and independent of fission

Huiliang Zhang[a], Sara Bisetto[b], Shey-Shing Sheu[b], Wang Wang[a]

[a]*Mitochondria and Metabolism Center, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA*
[b]*Center for Translational Medicine, Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA*

BACKGROUND: The cardiac mitochondria exhibit a stable morphology with a rather low level of dynamic changes. However, fission and fusion proteins, such as dynamin-related protein 1 (DRP1) are abundant in the heart. Whether these proteins bear other functions in the heart than mitochondrial dynamics regulation are largely unknown. We hypothesize that endogenous DRP1 in the heart regulates mitochondrial respiration independent of fission.

METHODS: Mitochondrial respiration was determined by measuring the OCR with Seahorse assay or Clark type electrode in adult rat cardiomyocytes or mitochondria isolated from adult mouse heart. Confocal imaging was used to quantify mitochondrial morphology in adult cardiomyocytes and H9C2 myoblasts. To evaluate the role of mitochondrial permeability transition pore (mPTP), we monitored superoxide flashes (SOF) and laser-induced mPTP openings, and used cyclophilin D knockout mice (CypD KO). Mitochondrial ROS and Ca^{2+} were also monitored.

RESULTS: Inhibiting the DRP1 GTPase activity by Mdivi-1 or overexpression of the dominant-negative mutant (DRP1-K38A) induced mild mitochondrial morphological changes in adult cardiomyocytes, and inhibited mitochondrial respiration. Modulation of fission/fusion by overexpressing DRP1 or treating cells with S3, a compound facilitates fusion, exhibited significant morphological changes, but failed to influence respiration. Therefore, endogenous DRP1 activity may regulate respiration in the heart and this effect is dissociated with morphological changes. Further, inhibiting DRP1 activity attenuated the frequency of SOF, indicating decreased transient mPTP openings, delayed laser-induced permanent mPTP opening, and increased mitochondrial Ca^{2+}. Inhibiting DRP1 activity decreased mitochondrial ROS levels. The role of DRP1 inhibition on respiration absents in CypD KO myocytes, suggesting the involvement of mPTP in the modulation of respiration by endogenous DRP1.

CONCLUSION: These results suggest that endogenous DRP1 positively regulates respiration in the heart. This effect is likely independent of its role in mitochondrial fission. DRP1 regulation of respiration may involve transient opening of mPTP and contribute to mitochondrial Ca^{2+} and ROS signaling.

085

Vascular stiffening precedes the onset of HFpEF in diabetics with diastolic dysfunction

Ilya Karagodin, Omer Aba-Omer, Rodney Sparapani, Jennifer Strande

Medical College of Wisconsin, Milwaukee, WI, USA

Background

Diabetes mellitus (DM) is frequently associated with both diastolic dysfunction and heart failure with preserved ejection

fraction (HFpEF), but identifying those diabetic patients who progress from asymptomatic diastolic dysfunction to HFpEF remains elusive. DM is also associated with vascular stiffening. The goal of this study is to determine whether vascular stiffening precedes the development of HFpEF in DM patients with diastolic dysfunction and whether this can be identified on 2D transthoracic echocardiography (TTE).

Methods

This study included 64 subjects with DM and diastolic dysfunction. Transthoracic echocardiograms (TTEs) were screened until the earliest documented evidence of diastolic dysfunction was identified. The TTEs were then divided into two groups: Group 1 included TTEs of subjects who were known to progress to HFpEF and Group 2 included TTEs of subjects who remained asymptomatic. Indices of vascular stiffening including aortic distensibility and aortic strain, arterial stiffness, and arterial elastance were recorded and compared between groups.

Results

There were no significant differences between groups in terms of age, gender, race, body surface area, tobacco use, alcohol use, hypertension, or atrial fibrillation. Group 1 ($n = 43$) had significantly less aortic strain than Group 2 ($n = 19$; 6.94% vs. 9.73%, $p = 0.017$). Aortic distensibility was also significantly decreased in Group 1 ($n = 17$) compared to Group 2 ($n = 14$; $1.80*10^{-3}$ vs. $3.45*10^{-3}$ $cm^2dyne^{-1}10^{-6}$, $p = 0.022$). Arterial stiffness and arterial elastance did not significantly differ between groups.

Conclusion

In DM subjects with diastolic dysfunction, reduced aortic strain and distensibility are found on TTEs performed prior to the development of HFpEF. This suggests that reduced aortic strain and distensibility may predict which DM patients develop HFpEF. However, larger prospective studies are needed to further investigate this relationship and to determine whether early interventions to control blood pressure and diabetes can alter the outcome in these patients.

086

Chronic testosterone withdrawal slows calcium transient decay and prolongs contraction in ventricular myocytes isolated from gonadectomised C57BL/6 male mice

Omar Ayaz, Susan Howlett, Robert Rose

Dalhousie University, Nova Scotia, Canada

The extent to which sex differences in cardiac function are influenced by testosterone is unclear. This study determined the impact of chronic testosterone withdrawal on cardiac contraction and Ca2+ homeostasis. Male mice had either a bilateral gonadectomy (GDX) or a sham-operation (at 4-6 wks). Ventricular myocytes were isolated by enzymatic digestion, paced at 2-4 Hz and investigated at 37 °C. In field stimulation experiments, peak Ca2+ transients (fura-2) and contractions were similar in GDX and sham-operated controls, however GDX myocytes had prolonged Ca2+ transients (44 ± 2.3 vs 54 ± 2.7 ms, $P < 0.05$) and contractions (28 ± 1.5 vs 39 ± 3.1 ms, $P < 0.05$). Microelectrode studies showed that action potential duration was prolonged in GDX myocytes compared to sham controls (56 ± 3.0 vs 74 ± 4.6 ms, $P < 0.05$). Voltage clamp studies showed that GDX had no effect on Ca2+ current density or Ca2+ release per unit of Ca2+ current (gain), but supressed peak contractions and transients. Sarcoplasmic reticulum (SR) Ca2+ content (assessed with 10 mM caffeine) was attenuated by GDX, while fractional SR Ca2+ release was unaffected. Subcellular SR Ca2+ release events (Ca2+ sparks, fluo-4) were smaller (0.381 vs 0.373 $\Delta F/F0$, $P < 0.05$), less frequent (7.99 vs 5.84 /100 μm/sec, $P < 0.05$), and decayed more slowly (20.51 vs 22.43 ms, $P < 0.05$) in GDX myocytes than sham controls. Western blots of key Ca2+ handling proteins (Cav1.2, NCX, and SERCA) showed no change in expression in sham vs GDX hearts. These results demonstrate that low testosterone levels disrupt Ca2+ homeostasis and prolong cardiac relaxation. This may promote diastolic dysfunction in older men with very low testosterone levels.

087

Defining the *in vivo* consequences of altered interactions between cMyBP-C and actin on cardiac function in transgenic mice

Sabine van Dijk[a], Kristina Bezold[b], Samantha Harris[a]

[a]*University of Arizona, Tucson, AZ, USA*
[b]*Stanford University, Stanford, CA, USA*

Background: Cardiac myosin binding protein C (cMyBP-C) plays an essential role in proper timing of cardiac contraction, yet the molecular mechanisms are incompletely understood. Until recently most models assumed that cMyBP-C represses cross bridge cycling through interactions with myosin, but evidence for a specific and physiological relevant interaction between cMyBP-C and actin is emerging. We created transgenic mice with altered interactions between cMyBP-C and actin to for the first time study the effects of this interaction on cardiac function.

Methods: To study the functional effects of altered interactions between cMyBP-C and actin, we created two transgenic mouse models with mutations in cMyBP-C that in vitro were previously shown to either increase (L348P) or decrease (E330K) binding affinity of cMyBP-C for actin. We then assessed cardiac function using echocardiography and pressure-volume recordings.

Results: Transgenic mice with the L348P mutation (L348P-Tg) showed cardiac remodelling which was evident primarily as enlargement of the left atria. Echocardiograms in adult L348P-Tg mice demonstrated lower E (674 ± 21 vs 528 ± 25 mm/s for non-Tg vs L348P-Tg) and E' (-24 ± 1 vs -18 ± 1 mm/s), which both suggest that stiffer left ventricles could be the cause of atrial dilation. In addition, isovolumetric relaxation time was prolonged in L348P-Tg (12 ± 0.2 vs18 ± 0.6 s). In contrast, transgenic mice with the E330K mutation showed no signs of diastolic dysfunction. However, preliminary results showed that E330K-Tg mice have a trend toward left ventricle dilation with increased stroke volume (36 ± 2 vs 45 ± 2% in nTg and E330K-Tg) and cardiac output (17 ± 0.6 vs 22 ± 1 mL/min).

Conclusion: These results provide *in vivo* evidence that altered interactions between cMyBP-C and actin affect cardiac performance. More detailed studies, e.g. using pressure-volume recordings and longitudinal characterization of these transgenic mouse models will provide more detailed insight in how an interaction between cMyBP-C and actin influences cardiac contraction.

088

Notch-mediated proliferation of human embryonic stem cell-derived cardiomyocytes

Kaytlyn A. Gerbin, Charles E. Murry

University of Washington, Seattle, WA, USA

Background

Gaining control over cell proliferation is of great interest to the field of cardiac regenerative medicine and hESC-cardiomyocyte cell

therapy. Towards this goal, we have developed a Notch-signaling strategy to enhance the proliferation of hESC-cardiomyocytes both in 2D cell culture and in 3D engineered cardiac tissue.

Methods

Tissue culture polystyrene was pre-coated with anti-IgG, blocked, and subsequently incubated with the Notch ligand Delta-1 or IgG (control) to achieve oriented immobilization. RUES2 hESC-cardiomyocytes were replated on the Delta-1 surfaces and cultured for 2D experiments. For 3D gel experiments, collagen-1 was reacted with EDC/Sulfo-NHS and bound with anti-IgG, which was then incubated with Delta-1 or IgG (control). HESC-cardiomyocytes were resuspended and gelled in PDMS micro-molds. Initial hydrogel optimization was performed using a Notch-luciferase reporter cell line. Cardiomyocyte proliferation was measured following BrdU pulsing and histological analysis for double-labeled BrdU+/βMHC+ cells.

Results

Culturing hESC-cardiomyocytes on Delta-1 surfaces resulted in the induction of Notch signaling by qRT-PCR. HES5 expression increased 2-fold relative to controls, and expression of HES1, HES5, and HEY2 were also upregulated. Statistical analysis showed a significant increase in cardiomyocyte proliferation on 2D surfaces coated with 5, 10, and 20 μg/mL Delta-1 relative to IgG1 controls ($p < 0.05$), with the highest response on 10 μg/mL Delta-1 (22% increase, $p < 0.05$). The Notch-mediated luciferase response was verified using 2D Delta-immobilized surfaces and compared to the optimized 3D gels, which showed significantly higher normalized luciferase expression in Delta-1-collagen than IgG control gels (3-fold increase, $p < 0.001$). Importantly, engineered cardiac constructs formed with Delta-1-collagen demonstrated a significant 10% increase in cardiomyocyte proliferation compared to IgG control gels after 1 week in culture ($p < 0.05$).

Conclusion

Collectively, these studies establish a platform in which Notch signaling can be modulated to enhance cardiomyocyte proliferation in vitro in both 2D tissue culture and in 3D engineered cardiac tissue.

089

Development of an intracellular enzyme replacement therapy for Barth Syndrome

Ana Dinca, Wei Ming Chien, Michael Chin

University of Washington, Seattle, USA

Barth Syndrome (BTHS) is caused by a single gene mutation in the mitochondrial transacylase, tafazzin (TAZ), which results in impaired lipid metabolism leading to dysfunction in highly energetic tissues such as the heart and skeletal muscle. TAZ remodels the signature mitochondrial phospholipid, cardiolipin (CL), which is responsible for providing support to the electron transport chain. BTHS patients suffer from growth deficiencies, cardiomyopathy, hypotonia and neutropenia. Currently, treatment for patients with BTHS is supportive, seeking to ameliorate rather than prevent heart problems, skeletal muscle problems and recurring infections. Protein therapy, on the other hand, might treat and even prevent cardiac, skeletal muscle as well as infection-related morbidities.

We designed a recombinant TAZ protein containing a cell penetrating peptide in its C-terminus, which enables the recombinant TAZ to penetrate cells and then treated TAZ-deficient cells with it. We tested the permeability of the recombinant protein by direct delivery to C2C12 myoblasts and found that the protein is successfully taken up by the cells. We have assessed the enzymatic activity of the delivered protein in primary cardiac fibroblasts by conducting mitochondrial respiration measurements and found that TAZ knockdown cells show a decrease in oxygen consumption as compared to the wild type cells; this is consistent with data from BTHS patient-derived fibroblasts. We also found that treatment with TAZ improves respiration in the knockdown cells. We have acquired a mouse model of BTHS and are testing the recombinant TAZ in vivo.

These results indicate that the protein is able to reach the mitochondria, where it is enzymatically active and able to enhance respiration. As the protein is able to rescue respiration in cells in which tafazzin was absent, this suggests that our approach should not only be able to prevent onset of symptoms, but also rescue the phenotype in already affected tissues.

090

S100A1 DNA-based inotropic therapy protects against pro-arrhythmogenic ryanodine receptor 2 dysfunction

Julia Ritterhoff[a,c], Andreas Seitz[a], Hugo A. Katus[a,b], Patrick Most[a,d]

[a]*University Hopsital Heidelberg, Heidelberg, Germany*
[b]*German Centre for Cardiovascular Research, Heidelberg/Mannheim, Germany*
[c]*Thomas Jefferson University, Philadelphia, PA, USA*
[d]*uniQure GmbH Germany, Heidelberg, Germany*

Background: S100A1 gene therapy presents a promising approach for the treatment of heart failure. S100A1-mediated enhanced calcium (Ca^{2+}) resequestration into the sarcoplasmic reticulum (SR) is critical for its cAMP-independent inotropic effects but raises concerns about potential diastolic SR Ca^{2+} leakage that might trigger fatal arrhythmias. Thus, the goal of this study was to determine the impact of S100A1 on ryanodine receptor 2 (RyR2)-mediated SR Ca^{2+} leakage *in vitro*.

Methods and Results: S100A1 association with the RyR2 was diminished (-50%) in failing cardiomyocytes (CMs) and hearts with S100A1 downregulation. Emplo6ying epifluorescent and confocal Ca^{2+} imaging, adenoviral-mediated S100A1 overexpression (3-4 folds vs. control) in adult rat CMs revealed both decreased SR Ca^{2+}-spark frequency (-50%) and prevented β-adrenergic receptor (β-AR)-triggered Ca^{2+} waves (-62%) despite augmented SR Ca^{2+} load. Equal efficacy was observed in electrically stimulated CMs, where S100A1 overexpression protected against ß-AR triggered diastolic Ca^{2+} waves (occurring in 20% of CMs compared to 80% in controls). In multicellular rat engineered heart tissue (EHT), S100A1-overexpression (6-8 folds vs. control) protected from Ca^{2+}-triggered after-contractions (ACs) (-50%) with preserved enhancement of isometric twitch force (TF, +40%) at 2Hz. S100A1-mediated rescue of contractile failure of endothelin-1-treated EHT (-50% decrease in TF) was associated with protection from Ca^{2+}-triggered ACs. Mechanistically, S100A1-overexpression changed neither PKA/CaMKII-dependent RyR2 phosphorylation nor binding of accessory proteins like FKBP12.6, calmodulin or sorcin to RyR2 but enhanced S100A1/RyR2 stoichiometry. Thiolation of the preserved cysteine of S100A1 (Cys86) has been reported to regulate Ca^{2+} affinity of S100A1. Interestingly, S100A1 mutants lacking Cys86 demonstrated similar effects on calcium transient amplitude and ß-AR triggered diastolic Ca^{2+} waves but lacked protection against pacing-induced SR Ca^{2+} leak.

Conclusion: Our data provide evidence that S100A1 interaction with the RyR2 reverses diastolic RyR2 dysfunction. S100A1 appears to convey a rather unique molecular profile combining cAMP-independent inotropy with protection against Ca^{2+}-triggered arrhythmias.

091

Gender-based differences in myocardial protein *S*-nitrosylation

Qin Shao[a], Elizabeth Murphy[b], Charles Steenbergen[a], Mark Kohr[a,c]

[a]*Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[b]*National Heart, Lung and Blood Institute, Bethesda, MD, USA*
[c]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*

Background: Premenopausal females have been shown to exhibit endogenous cardioprotective signalling mechanisms that are thought to result from the beneficial effects of estrogen, and these effects are reversed following menopause. *S*-nitrosylation is a labile protein modification that increases with cardioprotective interventions, such as ischemic preconditioning.

Objective: To identify a potential role for protein *S*-nitrosylation in gender-dependent cardioprotection.

Methods: We utilized the Langendorff-perfused mouse heart model of ischemia-reperfusion injury with male and female hearts, and *S*-nitrosylation-resin-assisted capture in tandem with LC-MS/MS to identify *S*-nitrosylated proteins and modification sites.

Results: Consistent with previous studies, female hearts exhibited a significant increase in functional recovery compared to male hearts (male: 37.5±5.9% vs. female: 61.4±10.1% of pre-ischemic function, $p<0.05$; n=3 hearts/group). In a separate set of hearts (n=4-8 hearts/group), we identified a total of 177 *S*-nitrosylated proteins in female hearts at baseline compared to 109 *S*-nitrosylated proteins in male hearts (101 common; 76 unique to female; 8 unique to male). Unique *S*-nitrosylated proteins in the female group included the F_1F_0 ATPase subunit O, HSP60, and cyclophilin D. We also utilized label-free peptide analysis to quantify levels of common identifications and noted that SERCA2a *S*-nitrosylation was decreased by nearly 70% in male hearts compared to female; baseline SERCA2a expression was not different. We utilized western blot to assess protein expression differences in the various nitric oxide synthase (NOS) isoforms, as well as *S*-nitrosoglutathione reductase, and found that only endothelial NOS levels were significantly higher in female hearts compared to male (male: 32.1 vs. female: 61.4 arbitrary units normalized to GAPDH expression, $p<0.05$; n=4 hearts/group). This may partly explain the higher levels of *S*-nitrosylation observed in female hearts.

Conclusion: We identified a number of novel *S*-nitrosylated proteins in female hearts that are likely to contribute to gender-specific cardioprotection.

092

The COP9 Signalosome controls the Degradation of cytosolic misfolded Proteins and protects against cardiac Proteotoxicity

Huabo Su[a,b], Jie Li[a,b], Hanming Zhang[a], Ning Wei[c], Xuejun Wang[a]

[a]*University of South Dakota, Vermillion, SD, USA*
[b]*Georgia Regents University, Augusta, GA, USA*
[c]*Yale Univeristy, New Haven, CT, USA*

Background- Cardiac accumulation of misfolded proteins is associated with the progression from a large subset of heart diseases to congestive heart failure. Misfolded proteins are degraded primarily by the ubiquitin-proteasome system (UPS) but the ubiquitin ligases responsible for the degradation remain largely unidentified. The COP9 signalosome (CSN) consisting of 8 unique protein subunits (CSN1 through CSN8) is a deneddylase that removes ubiquitin-like protein NEDD8 from target proteins. CSN is known to regulate cullin-RING ubiquitin ligases (CRLs) and thereby controls ubiquitination of a large family of cellular proteins; however, neither CSN nor CRLs have been demonstrated to regulate the ubiquitination and degradation of cytosolic misfolded proteins. Hence, we sought to investigate the role of CSN in the removal of misfolded proteins and the impact of CSN haploinsufficiency on cardiac proteinopathy.

Methods and Results– Myocardial neddylated forms of cullins and non-cullin proteins were markedly increased in mice with Cops8 haploinsufficiency ($CSN8^{hypo}$). Myocardial performance to degrade a surrogate misfolded protein was significantly decreased by $CSN8^{hypo}$. When crossed with a cardiac proteinopathy mouse model in which a *bona fide* misfolded protein $CryAB^{R120G}$ is overexpressed in the heart, $CSN8^{hypo}$ aggravated the $CryAB^{R120G}$-induced restrictive cardiomyopathy and shortened the lifespan of the mice. The exacerbation of proteinopathy was associated with augmented accumulation of protein aggregates, increased level of NEDD8 conjugates, and reduced levels of total ubiquitin conjugates in the heart. In cultured cardiomyocytes, both CSN8 deficiency and CRL inactivation impaired the ubiquitination and degradation of $CryAB^{R120G}$; CSN8 knockdown resulted in accumulation of protein aggregates and exacerbation of $CryAB^{R120G}$ cytotoxicity.

Conclusions– (1) CSN8/CSN promotes the ubiquitination and degradation of misfolded proteins and protects against cardiac proteotoxicity and (2) CRLs participate in degradation of cytosolic misfolded proteins.

093

Coordinated Protein Turnover of Cardiac Metabolic Clusters in Hypertrophy

Edward Lau, Quan Cao, Dominic Ng, Ding Wang, Maggie P.Y. Lam, Peipei Ping

University of California at Los Angeles, Los Angeles, USA

Protein homeostasis is disrupted in pathological hypertrophy preceding heart failure. How alterations in protein stability and turnover lead to the reorganization of various cardiac pathways is unknown. Regulations of protein expression imposed by turnover are poorly understood due to difficulties of measuring protein half-life in the heart in vivo. Although methods such as inhibition of protein synthesis were used to measure protein stability in vitro, comprehensive understanding of physiological proteome dynamics was limited prior to recent methods suitable for animal model studies.

To investigate the dynamics of proteome remodeling, we used an in-house metabolic stable isotope labeling strategy in conjunction with high-resolution mass spectrometry to measure the abundance and turnover rates of 3,000 + cardiac proteins in six common inbred strains of laboratory mice. Each mouse strain (C57BL/6 J, DBA/2 J, CE/J, A/J, FVB/NJ, BALB/cJ) exhibited contrasting responses to isoproterenol, which permits regression modeling of proteome dynamics over phenotypes.

We completed 1,404 LC-MS/MS experiments to quantify the turnover and abundance of 3,227 proteins from 127,648 peptide time-series. We found that protein interaction partners tended to share similar turnover rates and coordinated changes in protein turnover following isoproterenol, suggesting synchronous renewal of functionally related proteins. To summarize the multi-dimensional data and discover underlying structure, we further performed weighted correlation network analysis, which categorized the proteins into 15 protein modules/eigengenes in an unsupervised manner. Several distinct clusters of coordinated protein turnover

were apparent and corresponded to distinct metabolic processes. Severity of hypertrophy in the mouse strains was positively correlated with the turnover of glycolytic enzymes (r: 0.58 to 0.80). Strikingly, hypertrophy was negatively correlated with the turnover of a module of fatty acid oxidation proteins proteins including ACAT1, ACAD10, HADHA/B, DBT, ACAA2, ETFA, and ACSL1 (r: –0.66 to –0.89). These data provide a first clue on the association between cellular remodeling and protein turnover networks.

094

Data science on proteomics: prioritizing development of high-demand quantitative protein assays for cardiovascular research

Maggie PY Lam[a], Vidya Venkatraman[b,c], T. Umut Dincer[a], Edward Lau[a], Peipei Ping[a], Jennifer Van Eyk[b]

[a]*University of California at Los Angeles, CA, USA*
[b]*Cedars-Sinai Medical Center, CA, USA*
[c]*Johns Hopkins University, MD, USA*

Which proteins are most critical to cardiovascular research? The question is pertinent to the effective dissemination and clinical translation of proteomics technologies to cardiovascular researchers. Although targeted proteomics methods (e.g., SRM/MRM) assays can allow accurate and precise quantification of virtually any proteins, their adoption rates in clinical and basic research settings remain low. Because developing verified MRM assays requires time and labor, it would avail method developers to prioritize protein targets that may attain maximal impact in the cardiovascular research community.

We demonstrate here a new data-driven approach to identify proteins that are most important to research in various systems and disciplines. We performed a large-scale bibliometric analysis of the 24 million research articles curated on PubMed, using the search terms "heart [MeSH term/All fields] or cardiac [All fields]" to retrieve all cardiac-related articles. We then created a custom software tool (BD2KPubMed) to tally the occurrences of each protein being referenced to the retrieved articles, and derived a formula based on normalized compression distance to determine the proteins with the highest semantic similarity to the literature query.

I will describe the identity and functional significance of the 50 most highly investigated in the heart. The majority of cardiovascular research is narrowly focused on only a few distinct proteins. The lists of top-50 proteins illuminate a number of interesting observations on cardiac biology. We observed marked differences in research priorities on human and mouse proteins, which likely represents the divergent focuses between basic and clinical research. In summary, we highlight here a list of high-impact proteins, and describe a software resource to monitor the focal points and trends of biomedical research. We foresee that this resource will avail the development and optimization of high-impact quantitative assays by the proteomics community, and may also be informative for gene and protein annotation projects.

095

The impact of acute exposure of progesterone on mechanisms of cardiac excitation-contraction coupling in isolated murine ventricular myocytes

Jennifer MacDonald[a], Hirad Feridooni[a], Glen Pyle[b], Susan Howlett[a]

[a]*Dalhousie University, Halifax, Canada*
[b]*University of Guelph, Guelph, Canada*

There are receptors for all of the major sex steroid hormones on isolated cardiac myocytes. A number of studies have shown that acute application of estrogen can affect cardiomyocyte calcium homeostasis, but little is known about the acute affects of progesterone. The objective of this study was to determine whether progesterone modified cardiac calcium homeostasis and contractions. Ventricular myocytes were isolated from 6-8 month-old female C57BL/6 mice by enzymatic digestion and acutely exposed to either progesterone (1-10 μM) or vehicle control. In voltage-clamp experiments, calcium currents were recorded with microelectrodes, and calcium transients (fura-2) and cellular contractions were recorded simultaneously in cells paced at 4Hz. Biochemical experiments used ventricular homogenates from hearts retrogradely perfused with either progesterone (1-10 μM) or vehicle control for 35 minutes. Progesterone had a dose-dependent inhibitory effect on peak cardiomyocyte contraction, where the concentration of progesterone causing 50% inhibition (IC50) was 160 ± 50nM ($n = 12$). By contrast, progesterone had no effect on either peak calcium transients or L-type calcium current. To determine whether small contractions were due to changes in myofilament calcium sensitivity, actomyosin MgATPase activity was assayed. While maximal responses to calcium were not affected by progesterone, a higher concentration of calcium was required to achieve 50% myofilament activation in progesterone-treated hearts ($EC50 = 0.94 \pm 0.01$ vs. 1.13 ± 0.05 μM, $p < 0.05$). These data demonstrate that progesterone inhibits cardiac contraction by decreasing myofilament calcium sensitivity. These effects may be important in pregnancy, where serum progesterone levels increase to concentrations above 600nM.

096

Regulation of cell cycle genes in neonatal mouse heart regeneration

HIDEMI KAJIMOTO, Kyohei Oyama, W. Robb MacLellan

University of Washington, Seattle, USA

Background – The mice heart retains a transient capacity for cardiac regeneration within 1 week after birth. Although the exact mechanisms underlying heart regeneration are not fully defined, cardiomyocytes (CMs) proliferation appears to be a critical step. The aim of this study was to examine the regulation of cell cycle genes in CMs in neonatal mouse heart regeneration.

Methods and Results – Cardiac injury model was created by inducing a myocardial infarction (MI) by LAD occlusion in 1-day old (P1) or 7-day old (P7) mice. MI-induced ischemic area was similar between P1 and P7 (P1: 29.6 ± 3.5%, P7: 26.9 ± 0.9%) in TTC staining 3 days after MI. To determine that regeneration was occurring, we measured fibrotic area 21 days after MI using sirius red/fast green staining. Serial sections were cut at 200-μm intervals from the site of the ligature toward the apex. The fibrotic area was significantly larger in P7 (22.3%) than P1 (3.4%) at level 4 and 5. To examine CM proliferation, CM DNA synthesis was quantified by BrdU incorporation after a pulse at postoperative day 0, 7, and 14. BrdU labelled CMs were detected more in P1 (border area: 72.5%, remote area: 50.6%) than P7 (border area: 10.7%, remote area: 10.1%) 21 days after MI. To determine if the enhanced regeneration was related to differences in cell cycle genes, we assessed expression levels of cell cycle genes by qPCR using isolated CMs 7 days after MI. In general, P1-Sham mice showed higher expression levels of all cell cycle genes than P7 hearts. However, of the 16 cell cycle genes examined, only *Plk1* was

significantly induced in P1 hearts after injury. No induction of *Plk1* expression was seen in the hearts of P7 mice.

Conclusions – *Plk1* is a candidate gene that modulates heart regeneration in neonatal mouse CMs.

097

2-Deoxy Adenosine Triphosphate Restores the Contractile Function of Cardiac Myofibril from Adult Dogs with Naturally Occurring Dilated Cardiomyopathy

Yuanhua Cheng[a], Kaley A. Hogarth[b], M. Lynne O'Sullivan[b], Michael Regnier[a], W. Glen Pyle[b]

[a]*University of Washington, Seattle, WA, USA*
[b]*University of Guelph, Guelph, Ontario, Canada*

Background: Dilated cardiomyopathy (DCM) is a major type of heart failure resulting from thinning ventricular walls and loss of systolic function. Canine DCM is a widely-accepted experimental paradigm for studying human DCM. 2-Deoxy-adenosine triphosphate (dATP) can be used by myosin and has been demonstrated as a superior energy-substrate over ATP for cross-bridge formation and increased systolic function. Here we report for the first time the beneficial effect of dATP on cardiac myofibrils from DCM dogs.

Methods: We measured actomyosin NTPase activity, and contractile/relaxation properties of isolated myofibrils obtained from non-failing (NF) and DCM canine hearts.

Results:

Replacement of ATP with dATP significantly increased myofilament activity in both NF and DCM samples. Maximal tension of DCM myofibrils was significantly improved with dATP vs. ATP (64.9 ± 2.0 *vs.* 50.9 ± 1.5 mN/mm^2, $p < 0.005$) and was restored to NF sample level (67.2 ± 2.0 mN/mm^2). dATP also resulted in an increased Ca^{2+} sensitivity of tension. Similarly, dATP increased the kinetics of activation (k_{ACT}) with a step increase in Ca^{2+}, but had little impact on rate of tension redevelopment (k_{TR}) after a release-restretch of myofibrils under steady-state Ca^{2+} conditions. Thus, the ratio of k_{ACT}/k_{TR} was increased for dATP vs. ATP (0.67 ± 0.03 *vs.* 0.50 ± 0.02, $p < 0.005$) to the level seen for NF samples (0.73 ± 0.04), suggesting Ca^{2+} dependent activation kinetics is compromised in DCM myofibrils and can be rescued by dATP. The early slow-phase of relaxation was reduced with dATP vs. ATP ($k_{REL,slow}$, 0.42 ± 0.02 *vs.* 0.56 ± 0.03 s^{-1}, $p < 0.005$) , but its duration was not affected ($t_{REL,slow}$, 154 ± 4 *vs.* 141 ± 4 ms, $p = 0.0625$), nor was fast-phase relaxation ($k_{REL,fast}$, 2.79 ± 0.17 *vs.* 3.01 ± 0.14 s^{-1}, $p = 0.2975$).

Conclusions:

Our findings suggest that myosin utilization of dATP as a contractile substrate improves contractile properties of cardiac myofibrils from naturally occurring DCM canine samples, without compromising relaxation, and elevation of cardiac dATP is a promising approach for treatment of DCM.

098

Investigating the pathogenesis of Δ160E mutation-linked Hypertrophic Cardiomyopathy

Salwa Abdullah, Mark McConnell, Jil Tardiff

University of Arizona, Tucson, AZ, USA

Hypertrophic Cardiomyopathy (HCM) is a primary disease of the myocardium. 10% of HCM is caused by mutations in cardiac troponin T (cTnT) and 65% within the tropomyosin (TM)-binding TNT1 domain. Two of the known mutational hotspots within TNT1 are in the N and C-terminal domains. There is no high-resolution structure for the C-terminal domain limiting both our ability to understand its role in myofilament activation and the molecular mechanism(s) of HCM. TNT1 C-terminal mutations (Δ160E and E163R/K) are located in a putative "hinge region" upstream of the unstructured flexible linker connecting the two main functional domains of cTnT. Our goal is two-fold: to define the spatial relationship of the linker to other thin filament proteins, and to identify the conformational changes induced therein by the Δ160E mutation using Fluorescence Resonance Energy Transfer (FRET) in a fully reconstituted thin filament. To achieve this goal, residues flanking the hinge were sequentially cysteine-substituted (A168C, A177C, A192C and S198C) and labeled with the energy donor IAEDANS. The energy acceptor, DABMI was attached to TM cysteine 190 and FRET measurements were obtained. We found that Δ160E changes the distance between residues 168, 177, 192 and TM C190 in the -Ca^{2+} state, suggesting a significant conformational change of the linker during relaxation. Moreover, Δ160E abolishes the physiological distance change between residue 168 and TM C190 in the + Ca^{2+} state, suggesting a localized conformational change of the linker during activation. These findings are consistent with the pronounced diastolic dysfunction characterizing Δ160E mice. In conclusion, we provided a more precise definition of the linker's boundaries (~aa168-192) in WT thin filament and detected mutation-induced conformational changes during relaxation. We are currently repeating our experiments with myosin subfragment-1. These studies will thus, for the first time, provide information regarding the role of the linker in both myofilament activation and disease.

099

Rise of diastolic Ca^{2+} explains the tachy-brady sinus node arrhythmia of Na^+/Ca^{2+} exchange KO mice

Angelo G. Torrente[a], Audrey Zaini[a], Rui Zhang[a], Jeanney Kang[a], Kenneth D. Philipson[b], Joshua I. Goldhaber[a]

[a]*Heart Institute Cedars Sinai, Los Angeles, CA, USA*
[b]*David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*

Na+/Ca2+ exchange (NCX) is an acknowledged component of sinoatrial node (SAN) pacemaker activity, although its precise role is debated. To explore NCX contribution to SAN activity, we created an atrial-specific NCX KO mouse. These mice live into adulthood with a phenotype (no atrial activity and lack of spontaneous action potentials (APs) in single SAN cells) compatible with impaired depolarization. Using voltage mapping, we recorded depolarizations in the KO SAN, which did not reliably spread to the atria, explaining the absence of P waves on ECG. In KO SAN tissue we also found intermittent bursts of Ca2+ transients instead of the steady periodic pacing observed in WT. To determine the source of burst behavior in KO, we studied intracellular Ca2+ handling in the NCX KO SAN. We found a decrease in Ca2+ current in KO SAN cells, caused in part by elevated subsarcolemmal Ca2+, as well as a further dynamic rise of diastolic Ca2+ during bursts in KO SAN tissue. The rise of Ca2+ correlated with termination of the bursts. Furthermore, increased diastolic Ca2+ was also observed during electrical stimulation of KO SANs. These results suggest that

intracellular Ca2 + accumulation accounts for the tachy-brady burst pacing pattern of NCX KO.

100

Hyperactive mitochondrial dynamics mediates obesity-induced heart dysfunction

Eric Smith, Nicolas Gutierrez Cortes, Huiliang Zhang, Shangcheng Xu, Pei Wang, Wang Wang

University of Washington School of Medicine, Mitochondria and Metabolism Center, Seattle, WA, USA

BACKGROUND: Metabolic syndrome, featured by obesity and diabetes, is an independent risk factor for cardiovascular disease. Hyperlipidemia, an important etiologic facet of metabolic syndrome, is proposed to induce fatty acid oxidation, oxidative stress, and mitochondrial dysfunction; eventually leading to insulin resistance and myocyte death in the heart. However, how obesity induces cardiac mitochondrial dysfunction and whether mitochondrial dysfunction plays any role in the development of heart failure are questions that remain largely unanswered. **METHODS:** We evaluated mitochondrial function in heart tissue isolated from mice fed a high-fat diet (fat calories = 60%) and adult rat cardiac myocytes supplemented with high concentration palmitate (0.3 mM). **RESULTS**: High-fat diet had a significant increase in body weight (145%), blood glucose (129%), glucose intolerance, and heart failure. After only 4 weeks the cardiac NAD^+/NADH ratio decreased 61.2%. High-fat diet also resulted in a 211% increase in transient respiration-coupled ROS-production events localized to individual mitochondria (superoxide flash). Despite increased superoxide flash events, oxidative stress was not detected. Rat cardiomyocytes treated with palmitate displayed a decrease in NAD^+, the NAD^+/NADH ratio, and increased superoxide flash events. Additionally, palmitate treatment increased mPTP opening, cell death, and decreased reactive oxygen species. Inhibition of FA transport to the mitochondria using the carnitine palmitoyltransferase I inhibitor etomoxir (100 μM) attenuated palmitate induced mitochondrial respiration and superoxide flash events. Intriguingly, morphological changes of mitochondria were hyperactive after palmitate treatment, indicating the activation of mitochondrial dynamism. Investigation of proteins that facilitate mitochondrial dynamics revealed that palmitate increased the expression of both DRP1 and OPA1. Finally, inhibiting the activity of a fission protein, DRP1 or increasing NAD^+ levels ameliorated palmitate-induced mPTP opening and myocyte death. These results demonstrate that high fatty acid supply decreases NAD^+ levels, which may lead to mitochondrial dysfunction and myocyte death through mitochondrial dynamics. **DISCUSSION**: Taken together, we have elucidated a novel mechanism in which a high-fat diet may disturb mitochondrial redox and morphology.

101

High saturated fat diets alter endogenous cardiac lipid profiles in mice

Loreta C. Tomasi, Stephen C. Kolwicz

Mitochondria & Metabolism Center, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA

Background: Although reduced consumption of saturated fatty acids (SFA) is recommended for the prevention of cardiovascular disease, the effects of SFA on the myocardial composition of FA, diacylglycerol (DG), and triacylglycerol (TG) is not clear. Therefore, we assessed endogenous cardiac lipid profiles of mice in response to chronic high SFA supply.

Methods: Male mice (10wks old) were fed either a Western diet (WD, n = 4) containing 41% kcal/fat (62% SF) or Surwit diet (SD, n = 4) containing 58% kcal/fat (93% SF) for 12wks. Age-matched controls (CON, n = 5) received a standard chow diet containing 21.6% kcal/fat (32% SF). Composition of FA, DG, and TG in cardiac lipid extracts was assessed by gas chromatography-mass spectrometry.

Results: Body weight increased ~25% and adipose tissue mass increased ~3-fold in both WD and SD mice ($P < 0.05$ vs CON) after high SF feeding. Fasting blood glucose levels were similarly elevated in both WD and SD mice compared to CON. In the endogenous cardiac lipid compartments SFA comprised: 88% of the cytosolic FA pool, 77% of DG and 50% of TG, which was constant in CON, WD, and SD hearts. Further analysis revealed that the abundance of palmitoleate and oleate in TG was increased to a similar degree in both WD and SD ($P < 0.05$ vs CON). In addition, both WD and SD hearts demonstrated decreased linoleate abundance in the FA, DG, and TG pools ($P < 0.05$ vs. CON).

Conclusions: Obesity phenotypes in mice are not drastically changed with different SF diets. Although high SF intake does not affect the relative abundance of SF found in the endogenous cardiac lipid compartments, our data suggest that SFs entering TG storage may be converted to an unsaturated form. Furthermore, the reduction of the unsaturated FA, linoleate, in all lipid compartments may be a consequence of high SF intake.

ACKNOWLEDGEMENTS: This work is supported by funding from the Sao Paulo Research Foundation and the American Heart Association.

102

Postnatal hyperplasia precedes hypertrophy in mice lacking myosin binding protein C (cMyBP-C) – a model of hypertrophic cardiomyopathy (HCM)

Adrian Grimes[a], Emily Farrell[b], Willem de Lange[b], J. Carter Ralphe[b]

[a]*University of Wisconsin School of Medicine and Public Health, Department of Medicine, Madison, WI, USA*
[b]*University of Wisconsin School of Medicine and Public Health, Department of Pediatrics, Madison, WI, USA*

Background: Hypertrophic cardiomyopathy (HCM) is the most prevalent familial heart disease. Some of the most common HCM-causing mutations are in cardiac myosin binding protein C (cMyBP-C). The mechanism(s) through which mutations in cMyBP-C induce hypertrophy remain unclear. Hearts from mice with germline ablation of cMyBP-C are morphologically indistinguishable from wild type (WT) hearts at birth, but develop overt hypertrophy and depressed function by postnatal day (PND)10. The goal of this study was to differentiate signaling pathways between physiologic growth and the pathophysiologic growth that occurs in HCM.

Methods: RNA from left ventricles of WT and cMyBP-$C^{-/-}$ mice were harvested at PND1 and PND10, and hybridized to whole genome microarrays. Genes differentially regulated greater than 1.5-fold between the four groups were identified ($p<0.05$). Immunohistochemistry was used to determine cardiomyocyte cross-sectional area and to quantify proliferating myocytes.

Results: Microarray analysis revealed that the most differentially regulated pathway between cMyBP-C$^{-/-}$ and WT hearts at PND1 was the cell cycle pathway, with higher activation in cMyBP-C$^{-/-}$. However, by PND10, hypertrophic cardiomyopathy became the top scoring pathway in cMyBP-C$^{-/-}$ hearts. Immunohistochemistry confirmed that cMyBP-C$^{-/-}$ hearts contained more proliferative cardiomyocytes at PND1, and had larger cardiomyocyte cross sectional area at PND10, compared to WT. Furthermore, we identified genes differentially regulated between physiologic (WT) and hypertrophic (cMyBP-C$^{-/-}$) growth (ΔPND1-10), including participants in the TGF-ß and extracellular matrix pathways, that have yet to be investigated with respect to their specific involvement in HCM.

Conclusions: This study provides microarray and immunohistochemical evidence for enhanced cardiomyocyte proliferation immediately postnatal in cMyBP-C$^{-/-}$ mouse hearts vs WT. The impact of enhanced or persistent hyperplasia in this model on the development of the hypertrophic phenotype remains to be defined. Furthermore, these data suggest extracellular matrix and TGF-ß signaling pathways as potential targets for therapeutic intervention to ameliorate the development of overt hypertrophy.

103

High glucose Suppresses Branched-chain Amino Acid Catabolism in the heart through downregulation of Kruppel-Like Factor 15

Dan Shao, Zhen Zhang, Sung Won Choi, Haiwei Gu, Danijel Djukovic, Daniel Raftery, Rong Tian

Mitochondria and Metabolism Center, Department of Anesthesiology & Pain Medicine, University of Washington, Seattle, WA, USA

The branched-chain amino acids (BCAAs), including leucine, isoleucine and valine, are essential amino acids for mammals. Emerging evidence suggested BCAAs catabolism was significantly changed during the development of cardiovascular diseases, however the mechanism involved in the regulation of BCAA catabolism is unknown. In mouse hearts with increased glucose uptake and utilization by overexpression of glucose transporter 1 (GLUT1-TG), we observed a 2-fold downregulation of Kruppel-Like Factor 15 (KLF15) and its target genes involved in BCAA catabolism by microarray analysis. Quantitative PCR results confirmed that the mRNA of KLF15 was decreased in GLUT1-TG (-44%, $p<0.05$ vs. WT, $N=6$) as well as its targets encoding BCAA catabolism enzymes e.g. branched chain amino-acid transaminase 2 (BCAT2) (-62%), branched-chain alpha-keto acid dehydrogenase complex (BCKDHC) (-54%), mitochondrial protein phosphatase 2C (PP2Cm) (-63%) ($p<0.05$, $N=6$). Targeted LC-MS analysis of GLUT1-TG hearts found higher intracellular BCAAs levels than WT (by 40%, $p<0.05$) while BCAA metabolites, e.g. α-keto-β-methylvalerate and α-ketoisocaproate were decreased (-37%). These observations led us to hypothesis that high glucose suppressed BCAA catabolism through downregulation of KLF15. To test our hypothesis, neonatal cardiomyocytes (CM) were incubated with normal glucose (NG, 5.5 mM) or high glucose (HG, 25mM) medium. HG treatment significantly decreased the expression of BCAT2 (-58%), BCKDHC (-34%) and PP2Cm (-61%) ($p<0.05$, $N=3$), accompanied by downregulation of KLF15 (-47%, $p<0.05$, $N=3$) compared to NG. In order to examine whether KLF15 was responsible for glucose induced suppression of BCAA catabolism, CM was infected with adenovirus harboring KLF15 to normalize KLF15 expression under HG condition. We found overexpression of KLF15 prevented downregulation of BCAA catabolism genes in response to HG. Altogether, our results suggest that increased intracellular glucose negatively regulates BCAA catabolism through inhibition of KLF15. These findings have important implications for cardiac pathology associated with increased reliance on glucose, such as pathological hypertrophy and heart failure.

104

Altered Ca^{2+} binding properties of Hypertrophic Cardiomyopathy-related cardiac troponin T mutation

Yueh Alison Li[a], Bo Liang[a,b], Kaveh Rayani[a], Glen Tibbits[a,b]

[a]*Simon Fraser University, Burnaby, BC, Canada*
[b]*Child and Family Research Institute, Vancouver, BC, Canada*

Cardiac troponin (cTn) is a three-subunit complex that plays an essential role in cardiac contractility. Each cTn complex is composed of a highly conserved Ca^{2+} binding subunit (cTnC), an inhibitory subunit (cTnI), and a tropomyosin binding subunit (cTnT). Mutations within each subunit may cause changes in both contraction and relaxation of the heart, potentially leading to devastating consequences. Familial hypertrophic cardiomyopathy (FHC) is the most common inherited cardiac muscle disorders. Seventy-one of the FHC mutations discovered thus far involve the cTn complex. This study focuses on the I79N cTnT mutation that has been shown to induce a FHC phenotype that is not detectable clinically, but is associated with a high incident of sudden cardiac death in young patients.

The biophysical properties of the human cTnT mutants were investigated in the reconstituted thin filaments (RTF) containing purified human cTn, rabbit skeletal actin, and human tropomyosin along with WT cTnI, IAANS-labeled control TnC (T53C, C84S, C35S) and WT or I79N cTnT. Fluorometry was used to measure the Ca^{2+} affinity of cTnC within each sample. Functional characterization was also performed in isolated skinned cardiomyocytes, in which the native cTn were exchanged with the recombinant cTn containing I79N cTnT. Changes were observed in the cTnC-Ca^{2+} binding affinity in the RTF as well as altered Ca^{2+} sensitivity in cardiomyocytes in the presence of the cTnT mutation. This suggests that the changes in Ca^{2+} binding kinetics of cTnC due to this mutation may be an underlying mechanism of the pathological remodeling of the myocardium in FHC patients. Elucidation of the molecular and functional properties of this and other mutants will allow us to have a clearer understanding of the mechanism of the pathogenesis of FHC and provide an important basis for rational drug design for patients suffering with FHC.

105

Characterization of Human Induced Pluripotent Stem Cells-derived Cardiomyocytes by simultaneous voltage and calcium optical mapping

Sanam Shafaattalab[a], Eric Lin[a], Christine Genge[a], Glen Tibbits[a,b]

[a]*Simon Fraser University, Burnaby, Canada*
[b]*Child and Family Research Institute, Vancouver, Canada*

Human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) are important models of human cardiac physiology. Despite the number of advantages this model may offer over previous cardiac models, immaturity of these cardiomyocytes can

hamper their adoption as an in-vitro model. The spontaneous and synchronous contraction of hiPSC-CMs is one of the principal indicators of these cells being differentiated into cardiomyocytes. The expression and functioning of several ionic channels, which occur during development, are important for generating action potentials. The *human ether-a-go-go-related* gene (*hERG* or *KCNH2*) encodes the voltage-gated potassium channels, which govern I_{Kr} and participates in the regulation of action-potential duration. Alternate transcripts of *KCNH2* encode 1a and 1b subunits, each with different characteristics. Changes in the expression levels of hERG-1a and hERG-1b during development may also be apparent in pharmacological sensitivities of hiPSC-CMs' action-potential dynamics, as well as their altered rates of spontaneous activity. The hERG current, I_{Kr}, which is active later in the action-potential plateau, has a strong effect on the thermodynamics of calcium efflux via forward-mode sodium-calcium (NCX) activity. Because forward-mode NCX activity is electrogenic, which results in a depolarizing current, changes in the calcium transient can potentially alter membrane-voltage dynamics. Quantitative RT-PCR and digital PCR were used to monitor transcript levels of hERG-1a and hERG-1b up to 150 days of maturation. Simultaneous voltage and calcium recordings, using the potentiometric dye RH-237 and the calcium indicator dye Rhod-2, were used to quantify the rates of spontaneous activity, action-potential profiles, and calcium transient dynamics. The findings show that changes in action-potential duration or increased susceptibility to pharmacological blockage of hERG channels correlate with changes in the expression level of two hERG subunits.

106

Isothermal Titration Calorimetry (ITC) derived thermodynamic analysis of calcium binding to recombinant human Troponin C (TnC) with Familial Hypertrophic Cardiomyopathy (FHC) associated mutations

Kaveh Rayani[a], Charles Stevens[a,b], Alison Li[a], Glen Tibbits[a,b]

[a]*Simon Fraser University, Burnaby, British Columbia, Canada*
[b]*Child and Family Research Institute, Vancouver, British Columbia, Canada*

Interaction of Ca^{2+} with cardiac troponin C (cTnC) is the fundamental process governing regulation of cardiac contractility. The cardiac troponin complex consists of the Ca^{2+} binding cTnC, inhibitory cTnI, and tropomyosin binding cTnT. Mutations in cTnC have been implicated in alteration of heart contraction and relaxation predisposing hypertrophic cardiomyopathy (HCM), the inherited form of which is familial hypertrophic cardiomyopathy (FHC). HCM, with an incidence of 1 in 500, is noted as the main cause of sudden cardiac death (SCD) in young adults.

Increased affinity of the regulatory site II of cTnC for Ca^{2+} elevates sensitivity, observed as an increase in cardiac force production capabilities. Such a change in the affinity of cTnC for Ca^{2+} is characteristic of HCM, a pathological disease state of the heart. Altered Ca^{2+} binding to mutant forms of cTnC affects changes in sarcomeric force production capability. Sequence variation in the form of single residue changes alters tertiary protein structure and Ca^{2+} interaction with site II of TnC. Maintenance of cardiac function in the face of varying temperature is an important property of the heart and sequence based changes in cTnC may alter the ability to produce force with changing temperature.

Isothermal titration calorimetry can be used to study the heat absorption or release upon titration of Ca^{2+} with site II in the N-terminal domain of wild-type cTnC and the protein containing mutations associated with FHC. We found the interaction of Ca^{2+} with each cTnC type to be spontaneous as demonstrated through a favorable Gibbs free energy of interaction. The enthalpic and entropic contributions to the reaction coordinate are significantly different at 25 and 37 °C between the wild-type and mutant TnC. The unique thermodynamic profile of the interaction between FHC mutant cTnC with Ca^{2+} points to structural changes which need to be explored further.

107

Predicted structural and functional effects of cardiac troponin mutations associated with familial hypertrophic cardiomyopathy

Charles Stevens[a,b], Kaveh Rayani[a], Glen Tibbits[a,b]

[a]*Simon Fraser University, Burnaby, BC, Canada*
[b]*Child & Family Research Institute, Vancouver, BC, Canada*

Hypertrophic cardiomyopathy (HCM) affects one in every 500 people. Many HCM cases occur without overt symptoms and patients may be unaware of their condition prior to sudden cardiac death. Familial Hypertrophic Cardiomyopathy (FHC) is a heritable form of HCM caused by mutations in sarcomeric genes, including 70 in the troponin (Tn) complex. The Tn complex contains three proteins:

TnC, a highly conserved protein responsible for initiating cardiac contraction in response to the cytosolic level of Ca^{2+} through a conformational change; TnI, an inhibitory protein; and TnT, a tether to the thin filament.Mutations that alter the ability of cTnC to bind Ca^{2+} have been hypothesized to induce either hypertrophic or dilated cardiomyopathies depending on whether Ca^{2+} affinity is increased or decreased, respectively. We have used molecular dynamics simulation to model the effects of these mutations at the molecular level as an indicator of their potential to induce the FHC phenotype. Six FHC-linked mutations are currently known and have been characterized biochemically.

The FHC-associated mutations A8V, L29Q and C84Y in the N-terminal domain of TnC were used in this study. In each mutant, the modeled structures differed in the degree to which the conformational change resembled the activated form. The change in free energy upon Ca^{2+} was the highest for the A8V mutation, followed by the WT protein, C84Y and finally, L29Q. These results suggest that in addition to direct changes in Ca^{2+} affinity, the energy barrier to transduction of the Ca^{2+}-binding signal may have been altered in the mutated proteins. Our ultimate goal is the development of an *in silico* workflow for the risk-stratification of novel mutations uncovered through next-generation whole genome sequencing.

108

Post-Surgical Atrioventricular Nodal Arrhythmogenesis In The Neonate Heart

Cici Chenliu[a,b], Pauline Dan[a,b], Yang Qu[a,b], Eric Lin[a,b], Shubhayan Sanatani[b], Xiao Ye Sheng[a,b], Glen Tibbits[a,b]

[a]*Simon Fraser University, Burnaby, BC, Canada*
[b]*Child and Family Research Institute, Vancouver, BC, Canada*

The atrioventricular node and junctional region (AVN, AVJ, respectively) exhibit tremendous heterogeneities between cell types,

function and conduction properties. For these reasons, the AVJ remains poorly understood, particularly in the neonate heart. In neonates with congenital heart disease corrective surgeries are often necessary to decrease morbidity and mortality. However, serious rhythmic disorders such as Junctional Ectopic Tachycardia (JET) and Atrial Ventricular (AV) conduction blocks often occur in the neonate heart after cardiopulmonary bypass (CPB) surgery. Recent clinical reviews suggest that the relative risks for JET significantly correlates with increased CPB duration, aortic cross clamp time, young age (< 1 year), as well as, the use of β adrenergic agonists. We hypothesize that ischemic reperfusion (I/R) injury to the neonate heart during CPB surgery can selectively disrupt AVJ function and promote these arrhythmias. We used patch clamping of rabbit AVN cells and optical mapping of the 10-d and 56-d old rabbit epicardium to observe the electrical events stimulated by ischemia and subsequently reperfusion. We found in the ex-vivo heart that 4 out 20 (20%) hearts developed a JET like arrhythmia while 12 of 20 (60%) exhibited AV blocks. All 10 of the 56 day old heart remained in sinus rhythm after these perturbations. Our results suggest that I/R injury in the neonate AVJ is a critical mediator in the etiology of these rhythmic disorders in the neonate heart.

109

Comparison of the development and progression of cardiac dysfunction in various mouse models of metabolic stress

Vongai Nyamandi, Marysol Garcia-Patino, Julia Nogueira Varela, Kathleen MacLeod

University of British Columbia, Vancouver, BC, Canada

Background: Obesity-induced metabolic stress is a risk factor for the development of cardiac dysfunction or obesity cardiomyopathy. The reasons for this have been investigated in various animal models of diet-induced obesity. However, although a number of these models exhibit biochemical signs of cardiac dysfunction, overt cardiac contractile impairment is not always apparent. The aim of the current study was to compare mouse models of metabolic stress in relation to the development and progression of cardiac dysfunction and identify the factors contributing to the differences in the severity, if any, of cardiac dysfunction in each model.

Methods and results: Six-week old male C57Bl/6 mice were placed on a chow (CH), high fat (HF) or high fat-high sucrose (HFHS) diet. After 2 weeks, half of the HF mice were injected with 40mg/kg streptozotocin (HF-STZ) for 3 days; the remaining animals were injected with vehicle. Weight gain, monitored weekly for 26 weeks, was similar in HF, HFHS and HF-STZ groups. Cardiac function, measured by echocardiography, was unchanged in all groups after 12 weeks of high fat feeding. At weeks 18 and 24, HFHS showed signs of both diastolic and systolic dysfunction, while HF-STZ exhibited only diastolic dysfunction. In contrast, cardiac function was largely preserved in HF at both time points. All 3 groups had comparable decreases in glucose tolerance and insulin sensitivity as measured by intraperitoneal glucose and insulin tolerance tests, respectively. Changes in serum triglycerides and cholesterol were also comparable, while no differences in free fatty acids compared to CH were detected.

Conclusion: The HFHS diet, which resembles a 'Western' diet high in fat and sugar, induced the most severe cardiac dysfunction. This is not due to differences in weight gain or in glucose or insulin tolerance or circulating lipids, suggesting that other mechanisms contribute to the deleterious cardiac effects of HFHS.

110

Mice with heterozygous deletion of ROCK2 are protected against myocardial ischemia-reperfusion injury

Marysol Garcia-Patino[a], Vongai Nyamandi[a], Julia Nogueira Varela[a], Zhengping Jia[b], Kathleen MacLeod[a]

[a]*Molecular and Cellular Pharmacology Research Group, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada*
[b]*Neurosciences & Mental Health, the Hospital for Sick Children, and Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, ON, Canada*

Over-activation of Rho kinase (ROCK) has been reported to contribute to ischemia-reperfusion (I/R) injury, based on the cardioprotective effect of pharmacological inhibition of ROCK before or after ischemia. However, since ROCK inhibitors are non-isoform selective, the relative contribution of the two ROCK isoforms, ROCK1 and ROCK2, to this effect is not clear. In the present study we investigated the contribution of ROCK2 using mice with heterozygous deletion of ROCK2 (ROCK2+//0). At 11 weeks of age, basal systolic cardiac function and cardiac dimensions of male ROCK2+//0 mice and their wild type (WT) littermates, evaluated using echocardiography, were not significantly different. Myocardial ischemia was induced in vivo by occlusion of the left anterior descending coronary artery for 40 minutes. After 24 hours of reperfusion, fractional shortening and ejection fraction were significantly decreased and left ventricular internal diameter significantly increased in WT hearts, consistent with the development of I/R injury. However, cardiac function and dimensions in ROCK2+//0 mice were unchanged compared to baseline. ROCK2 expression was lower, while changes in ROCK1 were not detected in ROCK2+//0 hearts. Total ROCK activity, assessed by measuring the phosphorylation of its targets MYPT and ezrin/radixin/moesin, was not changed in either WT or ROCK2+//0 hearts after 24 hours of reperfusion. Phosphorylation of Akt, a component of the reperfusion injury salvage kinase (RISK) signalling pathway, was significantly higher in post-I/R ROCK2+//0 than WT hearts. In contrast, the mRNA expression of inflammatory mediators, measured by RT-PCR, was not significantly different between post-I/R WT and ROCK2+//0 hearts. These results suggest that ROCK2 contributes to the development of cardiac dysfunction after I/R injury, and that its partial deletion reduces myocardial I/R injury by activating the RISK pathway rather than by reducing cardiac inflammation. Isoform-specific inhibition of ROCK2 may represent a novel form of treatment to reduce I/R injury.

111

2-deoxy-ADP as a substrate for oxidative phosphorylation and creatine kinase

Jason Murray, Farid Moussavi-Harami, David Marcinek, Michael Regnier

University of Washington, Seattle, WA, USA

2-deoxy-ATP (dATP) is a nucleotide used in DNA synthesis but present in extremely low levels in post-mitotic cells. Elevation of dATP content in cardiomyocytes increases both the magnitude and rate of contraction and is a potential therapy for cardiomyopathies. In cardiomyocytes that have been virally transfected to overexpress the enzyme ribonucleotide reductase (RR), elevation of dATP to as little as 1% of the total adenine nucleotide pool improves

contractility. Ribonucleotide reductase synthesizes deoxy-ADP from cytosolic ADP, but the primary pathway by which dATP is produced from dADP in the cardiomyocyte is unknown. The goal of this study is to understand the metabolic processes involved in dATP synthesis.

Using preparations of enriched mitochondria from mouse ventricular myocardium, we have shown that dADP is a possible substrate for oxidative phosphorylation in mitochondria. Mitochondrial respiration was measured using high resolution respirometry in the presence of substrates for the electron transport chain using either ADP or dADP to stimulate respiration. Maximum respiratory capacity using dADP was approximately half that of ADP-dependent respiration, and higher concentrations were required to activate mitochondrial respiration. Use of an uncoupling agent to separate oxidation from phosphorylation shows that these differences are not due to inhibition of the electron transport system. However, at ratios physiologically relevant to RR-overexpression (2% of the total adenine nucleotide pool), the kinetics and maximum respiratory capacity of mitochondria are unchanged.

We also found that dADP is phosphorylated to dATP via the enzyme creatine kinase. We are currently in the process of making kinetic measurements to determine the affinity of creatine kinase for dADP compared to ADP.

These data suggest that cardiomyocytes can generate dATP from dADP through the same metabolic pathways as ATP, and that the levels of dADP associated with upregulation of RR do not alter the cell's ability to synthesize ATP.

112

Rapamycin induces mitochondrial remodeling to rejuvenate energy metabolism and energetics in old hearts

Ying Ann Chiao, Stephen Kolwicz, Nathan Basisty, Michael MacCoss, Rong Tian, Peter Rabinovitch

University of Washington, Seattle, WA, USA

Recently, we showed that rapamycin (a caloric restriction mimetic) can reverse age-related cardiac dysfunction in 10 weeks, highlighting its therapeutic potential for cardiac aging. At this timepoint, rapamycin reversed the age-related decrease in levels of mitochondrial proteins, without elevations of mitochondrial number or biogenesis.

Longitudinal echocardiographic analysis revealed that while diastolic function of old mice began to improve 2-4 weeks following treatment, this progressed over the course of 10 weeks. We studied whether mitochondrial remodeling had a similar time course.

Rapamycin treatment reduced phosphorylation of TORC1 target S6 and TORC2 targets AKT and PKCα in old hearts by 1 week. The mRNA expression of PCG-1α, a mitochondrial biogenesis marker, increased in the first 2 weeks of rapamycin treatment but subsequently returned to control. Autophagy (LC3 II/LC3 I ratio and ATG5 levels) increased at 1 week. Concordantly, proteomics analysis showed a mixture of increased and reduced levels of mitochondrial proteins at 1 week but an overall increase at 2 weeks of rapamycin treatment. These findings suggest that protein turnover in the first 2 weeks of rapamycin treatment rapidly remodels the cardiac mitochondrial proteome.

^{13}C NMR spectroscopy of isolated perfused heart extracts revealed that fatty acid oxidation was reduced by 30% in old control hearts, consistent with the proteomic data. Strikingly, 1 to 2 weeks of rapamycin treatment reversed the age-related decrease in fatty acid oxidation. This reversal was also accompanied by increased PCr/ATP ratio in old hearts treated with rapamycin as early as 2 weeks after treatment.

Overall, our results suggest that rapamycin induces mitochondrial remodeling in the first 2 weeks of treatment to rejuvenate energy metabolism and energetics. However, the slower rate of improvement in cardiac performance indicates that there are additional, later steps required to fully translate mitochondrial enhancement into improved cardiac function in old hearts.

113

CD29/Beta-1 integrin identifies and contributes to pathologic cardiac fibrosis

Stephen Farris, Deri Helterline, April Stempien-Otero

University of Washington, Seattle, WA, USA

Background: Activated fibroblasts are critical to cardiac fibrosis (CF); however, fibroblast markers PDGFRa, CD90 and DDR2 are neither sensitive nor specific. We identify CD 29/Beta-1 Integrin as a unique marker of activated cardiac fibroblasts.

Methods: Fresh ventricular mononuclear cells were studied in mice [wild-type (WT) and fibrotic (SR-uPA)] and human subjects receiving a ventricular assist device or transplant. Mice carry a Green Fluorescent Protein (GFP)-labeled collagen1 1 reporter gene. Flow cytometry, cell culture and qRT-PCR were used.

Results: SR-uPA Coll1a1-GFP mice develop plasmin dependent, cardiac specific fibrosis beginning after 5 weeks and plateauing at 12-15 weeks. GFP+ cells have >20-fold collagen expression compared to GFP- cells ($N = 4$ mice. $P<0.05$). Active CD29 was present on 96±1.5% of GFP+ cells (WT and fibrotic mice including all time points. N=11-13 per genotype). However, PDGFRa, CD90 and DDR2 were present on only 61±4, 82±2 and 13±3.5%, respectively ($N \geq 3$ per marker) of GFP+ cells.

Compared to 4 weeks of age, activated fibroblasts (GFP+) were decreased at 8 weeks in all WT CD29+ (22.6±2% to 8.8±4%), PDGFRa+ (11.5±1.8 to 3.4±1.3%) and CD90+ cells (30.2±4.6 to 14.8±15.4%). Conversely, GFP expression remained elevated in SR-uPA CD29+ (23.7±2% to 16.4±9%), PDGFRa+ (9.8±1.9 to 8.7±5%) and CD90+ cells (33±1.7% and 24.5±11%). $P \leq 0.03$ for WT and $P \geq 0.3$ for fibrotic hearts, all markers. $N = 6$-8.

Freshly isolated human CD29+ cells have 3.3±0.3 fold increased collagen expression vs. CD29- cells ($P \leq 0.002$, $N = 4$ subjects). CD29+ cultured human cardiac fibroblasts had 2.2 and 1.8-fold increased collagen expression with and without TGF stimulation vs. CD29- cells (both $P < .05$). Presence of CD90 had no effect.

Conclusions: CD29/β1 Integrin identifies essentially all activated fibroblasts in murine hearts and may contribute towards progression of human cardiac fibrosis. This implicates potentially critical protease – matrix – integrin signaling in CF.

114

Sinoatrial node dysfunction in aged and frail mice assessed using high resolution optical mapping

Motahareh Moghtadaei, Sara Rafferty, Susan Howlett, Robert Rose

Dalhousie University, Halifax, Nova Scotia, Canada

The normal heartbeat is initiated in the specialized pacemaker cells of the sinoatrial node (SAN), which generates spontaneous action

potentials (APs) that then propagate to the rest of the atrial myocardium. The aging process is associated with impairments in SAN function; however, not all individuals age at the same rate and the health status of elderly adults varies from fit to frail. We have used a non-invasive 31 item clinical frailty index (FI) to quantify frailty in young (11.6±0.5 weeks; $n=9$) and aged (110.9±2.6 weeks; $n=9$) male wildtype C57BL/6 mice. Frailty was higher in aged mice compared to young mice; FI ranged from 0.032-0.097 in young mice and 0.161-0.363 in old mice. Patterns of electrical conduction within the SAN of intact atrial preparations isolated from these mice were investigated using high resolution optical mapping. Using custom MATLAB-based software we assessed SAN optical APs (OAPs), cycle length (CL) and SAN conduction velocity (CV) in atrial preparations in sinus rhythm. OAP measurements from the leading pacemaker site demonstrated diastolic depolarizations confirming that mapping was done in the SAN region of the right atrial posterior wall. We found that that CL was longer ($P<0.05$) in old mice (209.5±13.0 ms) compared to young mice (171.42±11.7 ms) while SAN CV was lower ($P<0.05$) in old mice (5.69±0.01 cm/s) vs. young mice (6.57±0.12 cm/s). Interestingly, changes in CL and SAN CV were with frailty. Specifically, CL increased as FI score increased (range: 137.0-282.3 ms; $r^2=0.33$; $P<0.05$) while SAN CV decreased as FI score increased (range: 7.1-5.4 cm/s; $r^2=0.77$; $P<0.001$). Overall, mice with highest FIs had the longest cycle lengths and lowest SAN CVs indicating that frailty is a strong predictor of SAN dysfunction. Our results show that frailty may be a better determinant of SAN dysfunction than chronological age.

115
Assessment of sinoatrial node activity and atrial conduction as a function of age and frailty in mice

Hailey Jansen, Sara Rafferty, Susan Howlett, Robert Rose

Dalhousie University, Halifax, Nova Scotia, Canada

Heart rate (HR), a major determinant of cardiac output, is determined by the intrinsic activity of the sinoatrial node (SAN). Impairments in SAN function and atrial conduction increase as a function of age, which can result in conditions such as sick sinus syndrome and atrial fibrillation. However, chronological age does not necessarily reflect biological age because individuals age at different rates such that the health status of older adults varies from fit to frail. Here, we have quantified frailty in young mice (~14 weeks; $n=27$) and aged mice (~2 years; $n=33$) using a non-invasive clinical frailty index (FI) and studied SAN activity and atrial conduction as a function of age and frailty. HR, SAN function, and atrial conduction were assessed in anesthetized mice by recording ECGs in conjunction with intracardiac programmed stimulation. Baseline HR was lower ($P<0.05$) while corrected sinus node recovery time (cSNRT) was prolonged ($P<0.05$) in aged vs. young mice. Similarly, P wave duration (a measure of atrial conduction time) was prolonged ($P<0.05$) in aged mice. Importantly, these parameters were graded by FI score such that as FI increased measures of SAN function and atrial conduction progressively declined. Specifically, FI score was a strong predictor of the increases in cSNRT ($r^2=0.20$; $P<0.001$) and P wave duration ($r^2=0.36$; $P<0.001$). Intrinsic SAN function was also studied *in vivo* by blocking the autonomic nervous system with propranolol and atropine (10mg/kg each). Aged mice showed a greater response to autonomic blockade as indicated by a larger drop ($P<0.05$) in HR. This reduction in HR was also correlated with FI ($r^2=0.13$; $P<0.05$) indicating that the autonomic nervous system compensates for the reduction in SAN function with age and frailty. These data demonstrate that SAN function and atrial conduction decline with age and that frailty may be a better predictor of these changes.

116
Distinct effects of wildtype and mutant forms of atrial natriuretic peptide on atrial electrophysiology in mice and humans

Iuliia Polina, Rui Hua, Sarah MacLeod, Motahareh Moghtadaei, John Sapp, Jean Francois Legare, Stacy O'Blenes, Robert Rose

Dalhousie University, Halifax, Nova Scotia, Canada

Atrial natriuretic peptide (ANP) is a hormone with numerous beneficial effects in the cardiovascular system. ANP can elicit physiological effects via two receptors denoted NPR-A (coupled to guanylyl cyclase signalling) and NPR-C (coupled to inhibitory G-proteins). Recently, an ANP frameshift mutation that results in the generation of a mutant ANP (mANP) was shown to cause atrial fibrillation (AF) in people. Using optical mapping we found that wildtype ANP sped conduction throughout the atria and was not associated with atrial arrhythmias during burst pacing whereas mANP slowed atrial conduction and profoundly increased susceptibility to atrial arrhythmias. The aim of this study was to determine the mechanisms by which mANP causes AF. We performed patch-clamp experiments in right atrial myocytes from wildtype and NPR-C knockout (NPR-$C^{-/-}$) mice as well as from human cardiac surgery patients to determine the signalling pathways mediating the effects of ANP and mANP. ANP and mANP had no electrophysiological effects in baseline conditions in mice; however, in the presence of isoproterenol (ISO; 10 nM), both peptides had potent effects. Specifically, after application of ISO, mANP (10-100 nM) decreased ($P<0.05$) action potential (AP) duration and upstroke velocity (V_{max}), whereas ANP (10-100 nM) did the opposite and increased ($P<0.05$) these AP parameters. Surprisingly, mANP and ANP had no effects on sodium current (I_{Na}); however, mANP potently decreased ($P<0.05$) L-type Ca^{2+} current ($I_{Ca,L}$) while ANP increased ($P<0.05$) $I_{Ca,L}$. These opposing effects of ANP and mANP on $I_{Ca,L}$ were very similar in human right atrial myocytes. The inhibitory effects of mANP on $I_{Ca,L}$ were completely absent in myocytes from NPR-$C^{-/-}$ mice whereas the stimulatory effects of ANP were maintained in NPR-$C^{-/-}$ myocytes. This study demonstrates that ANP and mANP have opposing effects on atrial electrophysiology associated with the activation of different NPRs, which may explain the mechanisms by which mANP causes AF.

117
Muscle Ring Finger-1 (MuRF1) Enhances Autophagic Flux In vivo

Traci Parry[a], Megan Quintana[a], Joseph Hill[b], Monte Willis[a]

[a]*University of North Carolina, Chapel Hill, NC, USA*
[b]*University of Texas Southwestern, Dallas, TX, USA*

The ubiquitin-proteasome and autophagy systems are complementary protein degradation pathways that play a critical role in protein quality control in cardiomyocytes. Moreover, ubiquitin ligases themselves have been implicated in the regulation of autophagy at multiple key points, including the ubiquitin ligases TRAF6 (substrate Beclin1), TRIM13 (substrate p62), and RNF184 (substrate BNIP1) in non-

cardiovascular-related cells. Our laboratory studies the role the muscle specific ubiquitin ligase MuRF1 (Muscle Ring Finger-1) and have identified that increased MuRF1 (in MuRF1 Tg+ mice) is cardioprotective in cardiac I/R injury, while also more susceptible to heart failure when challenged with pressure overload-induced cardiac hypertrophy. Since these phenotypes are consistent with increased autophagic flux, we tested the hypothesis that MuRF1 positively regulated cardiac autophagy in vivo. Healthy MuRF1-/- hearts had decreased autophagic flux, demonstrated by significantly decreased LC3II (after Bafilomycin A1 treatment), decreased p62, and decreased Vps-34 by immunoblot. Conversely, dual cardiac MuRF1/GFP-RFP-LC3 Tg+ hearts demonstrated significantly increased red and green puncta, consistent with increased autophagic flux, along with significant increases in p62 and Vps-34 protein by immunoblot. No changes in mitochondrial fission (Mfn1, Opa1) or fission (Fis1, Drp1) genes were detected in MuRF1 Tg+ and MuRF1-/- hearts by RT qPCR analysis, with the exception of MuRF1-/- Fis1 (significantly elevated). These findings demonstrate the first ubiquitin ligase in a non-cancer cell that regulates autophagy, linking the UPS and autophagy systems in a more direct manner. This mechanism, regulated by a yet-to-be-identified substrate, may explain in part MuRF1's cardioprotective effects in cardiac I/R injury, which predisposes the heart to heart failure in chronic pressure overload-induced cardiac hypertrophy. Understanding MuRF1's regulation of autophagy provides useful insight for the development of therapies targeting autophagy in heart disease.

118

Calcineurin and protein phosphatase 2A modulate cardiac gap junction conductance in guinea-pig left atrium: Role of Cx43 and Cx40 phosphorylation state

Fiona Hatch[a], Samantha Salvage[a], Abdul Waheed[a], Chris Fry[b], Rita Jabr[a]

[a]*University of Surrey, Guildford, UK*
[b]*University of Bristol, Bristol, UK*

Atrial arrhythmias mainly occur due to abnormal action potential (AP) propagation. Myocardial AP conduction velocity is a function of gap junction (GJ) electrical conductance (G_j) which in turn depends on the phosphorylation state of GJ proteins, connexins (Cx). We have shown in guinea-pig left ventricle that the Ca^{2+}-dependent serine/threonine (Ser/Thr) protein phosphatase (PP) calcineurin A (Cn) targets Cx43-pSer365 leading to PKC-mediated Cx43-Ser368 phosphorylation causing reduced G_j, however, the involvement of PPs in the left atrium (LA) remains unknown. In this study we investigated the role of Cn and Ca^{2+}-independent PP1 and PP2A in modulating G_j and Cx43 and Cx40 phosphorylation.

LA strips from Dunkin-Hartley guinea-pigs were mounted in an oil-gap chamber and G_j measured in control (Na=149.4mM), or low-Na solution (Na=29.4mM to raise $[Ca^{2+}]_i$ ($\uparrow[Ca^{2+}]_i$). Inhibitors were: for Cn, cyclosporine-A (CysA;5μM;$n=6$) or Cn auto-inhibitory peptide (CAIP;50μM;$n=3$); for PP1, okadaic acid (OA;100nM;$n=4$) or tautomycin (TTM;5nM;$n=7$); for PP2A, OA (2nM;$n=4$) or fostriecin (FST;100nM;$n=6$). Western blots measured total-Cx43 (T-Cx43) and Cx43-pSer368 in inhibitor-perfused tissues. Immunoprecipitated T-Cx40 was probed for Ser/Thr phosphorylation levels ($n=4$). Values are mean±SEM, G_j normalised to control (=100%). Differences were tested by ANOVA, with post hoc parametric tests; significance was at $p<0.05$.

Raised $[Ca^{2+}]_i$ significantly and reversibly decreased G_j. This decrease was attenuated by CysA and CAIP ($\uparrow[Ca^{2+}]_i$ 55±5.8%; $\uparrow[Ca^{2+}]_i$+CysA 85±10%; $\uparrow[Ca^{2+}]_i$+CAIP 104±10%); and 2nM OA or FST $\uparrow[Ca^{2+}]_i$ 54±3%; $\uparrow[Ca^{2+}]_i$+OA 78±8%; $\uparrow Ca^{2+}$+FST: 77±6%) but not TTM ($\uparrow[Ca^{2+}]_i$ 65±5%; $\uparrow[Ca^{2+}]_i$+TTM 66±4%). Protein expression levels of Cx43-pSer368 increased in $\uparrow[Ca^{2+}]_i$ (control 0.05±0.1; $\uparrow[Ca^{2+}]_i$ 1.09±0.04) which was significantly diminished by CysA (0.55±0.1), CAIP (0.03±0.01) and FST (0.58±0.05) but not TTM (1.01±0.04). Also, immunoprecipitated T-Cx40 Ser/Thr phosphorylation significantly increased in $\uparrow[Ca^{2+}]_i$ which was reduced by CysA, CAIP and FST but not TTM.

Calcineurin and PP2A but not PP1 alter the phosphorylation of Cx43 and Cx40 to mediate a decrease in G_j during $\uparrow[Ca^{2+}]_i$.

119

An acute bout of exercise impacts cardiac CapZ regulation

Glen Pyle

Cardiovascular Research Group, Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada

Cardiac CapZ (cCapZ) binds to the barbed ends of filamentous actin and regulates the addition of actin monomers to the sarcomere. We have shown that reduced cCapZ impacts the ability of the heart to respond to acute pathological stress, making it a potential therapeutic target in ischaemia-reperfusion injury. Others have shown that acute, non-pathological stressors designed to mimic exercise can alter cCapZ levels and actin dynamics. However, it is not known if these changes in cCapZ occur in vivo during acute exercise, or how cCapZ is controlled under physiological stress. To determine how acute exercise impacts cCapZ regulation we subjected mice to a single bout of swimming (20 min, 32°C) and measured changes in cCapZ protein levels, along with key regulatory elements. Acute exercise increased myofilament CapZIP protein levels by 51% in association with a 23% reduction in CapZIP S179 phosphorylation, a combination that stabilizes CapZ binding to actin filaments. Neither myofilament-associated Hsc70 nor Bag3 protein levels were significantly impacted by exercise. To determine the importance of cCapZ in the response to acute exercise we subjected cCapZ-deficient transgenic (TG) mice to the same swimming protocol. CapZ TG mice exhibited no significant changes in myofilament cCapZ, CapZIP, Hsc70, or Bag3 protein levels, or CapZIP phosphorylation. Interestingly telethonin levels decreased by 45% in CapZ TG myofilaments with exercise, suggesting an overall instability in the myofilaments. In a separate series of experiments mice swam until exhaustion. Wildtype mice were exhausted by 643 ± 68s, whereas cCapZ TG mice swam for only 407 ± 42s before reaching exhaustion. Together these data underscore the importance of cCapZ in the response to acute exercise, and show that CapZIP is a key element in the regulation of CapZ binding to actin during a bout of exercise.

120

Gender-dependent depression of myocardial contractility with activation of the estrogen receptor GPR30

Kaley Hogarth, Glen Pyle

Cardiovascular Research Group, Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada

Estrogen receptors are powerful players in the regulation of myocardial function and the pathogenesis of cardiovascular disease. Historically estrogen receptors have been thought to mediate their

effects through genomic mechanisms. We and others have challenged this dogmatic paradigm by showing rapid, nongenomic effects on the heart with estrogen receptor activation. The most recently discovered estrogen receptor, GPR30, protects hearts against ischaemia-reperfusion injury following acute activation, but the physiological effects of acute GPR30 agonism and the underlying intracellular mechanisms of action are poorly understood. We perfused isolated mouse hearts of both genders with the GPR30 agonist G1 (100 nM) for up to 10 min. In female mice left ventricular developed pressure (LVDP) declined significantly from 81 ± 2 mmHg to 74 ± 2 mmHg following G1 exposure, and +dP/dt also decreased significantly from 3602 ± 146 mmHg/s to 3366 ± 135 mmHg/s. The decrease in LVDP was significant by 3 min perfusion, and was driven by reduced systolic pressure. By contrast, hearts from male mice were unresponsive to GPR30 activation. To determine if the observed myocardial depression was due to changes in cardiac myofilaments we measured actomyosin MgATPase activity and myofilament protein phosphorylation. Again, male myocardium was unresponsive with no significant changes in actomyosin MgATPase activity or myofilament protein phosphorylation. By contrast samples from female hearts exhibited a 35% decrease in desmin phosphorylation, and a 30% decline in the phosphorylation of both troponin T and tropomyosin. Interestingly there was no change in actomyosin MgATPase activity following GPR30 activation in female mouse hearts. These data are the first to show that murine hearts have rapid and gender-dependent physiological responses to acute GPR30 activation, despite similar protein levels of the receptor in males and females, and that the cardiodepressant response of hearts from female mice cannot be explained by changes in myofilament activation.

121

Prevascularized cardiac constructs to promote tissue survival and rapid host integration *in vivo*

Meredith Roberts[a], Dominic Tran[a], Kareen Coulombe[a,b], Nathan Palpant[a], Maria Razumova[a], Kaytlyn Gerbin[a], Jill Weyers[a], Sarah Dupras[a], Michael Regnier[a], Charles Murry[a], Ying Zheng[a]

[a]*University of Washington, Seattle, WA, USA*
[b]*Brown University, Providence, RI, USA*

Cardiac tissue engineering is a strategy to restore contractile muscle lost during a myocardial infarction and to model cardiac diseases for new therapeutic discovery. Although promising, many approaches are unable to recapitulate native myocardial structure (i.e. myocyte alignment and perfusable vasculature). Systemic integration with host circulation is essential for the survival and therapeutic benefit of engineered cardiac grafts, yet the fabrication of interconnected vasculature within electrically synchronized contractile tissues has not yet been achieved. We have engineered perfusable vascular networks that are compatible with cardiac construct formation, structure, and function. Our vascular networks are patterned using a soft lithographic injection molding technique to create endothelialized microchannels within native collagen matrix under uniaxial strain. We show that matrix-remodelling stromal cells are required for hESC-derived cardiomyocytes to structurally and functionally integrate within the dense collagen matrices that are required for microvessel patterning. The constructs generate length dependent contractions at the order of 0.1 mN/mm^2 and can be paced up to 2 Hz. Co-culture with stromal cells additionally results in cardiomyocyte organization and maturation as seen by increased sarcomere length (from 1.2 to 1.7μm) and alignment. We further demonstrate that stem cell derived endothelial cells of an endocardial-like origin are an ideal cell source for generating vascular constructs with high vessel density. These hESC-ECs undergo robust tube formation and demonstrate high angiogenic activity, and preliminary *in vivo* studies suggest hESC-EC seeded vascular constructs are able to integrate with host systemic perfusion in a rat myocardial infarction model. Our study provides insights on the cellular, mechanical, and chemical conditions required to produce engineered heart tissues that are both functional and vascularized. Future *in vivo* evaluation of the functional benefit and systemic incorporation of vascularized heart grafts will move us closer to clinical applications of engineered tissue for heart regeneration.

122

Modulation of Cardiac Stem Cell Growth by Oxygen Tension and Sirtuin 1

Michael A. Bellio, Claudia O. Rodrigues, Victoria Florea, Aisha Khan, Joshua M. Hare, Ivonne H. Schulman

University of Miami, Miami, FL, USA

Background: Myocardial infarction produces severe hypoxia within regions of the myocardium. Adult hearts contain endogenous cardiac stem cells (CSCs) that contribute to cardiac repair but presumably lose proliferative abilities in the setting of hypoxia. However, no molecular mechanism has yet been established to account for insufficient regeneration. We propose that the histone deacetylase Sirtuin 1 (SIRT1) is a mediator of this hypoxia-induced decrease in regeneration due to the protein's well-established roles in cell cycle progression and conferring protection from senescence and oxidative damage.

Hypothesis: Hypoxic oxygen levels impair CSC proliferation through reduced SIRT1 expression.

Methods and Results: First, human CSCs (hCSCs) were isolated based on CD117+ expression and subsequently grown at room air (21% O_2), physiologic (5% O_2), and ischemic hypoxic (0.5% O_2) conditions for 96 hours. Physiologic oxygen significantly enhanced hCSC growth rate compared to both room air and ischemic hypoxia groups (33% and 46% respectively, N=3, $p<0.05$). This effect was accompanied by a significant increase in SIRT1 protein expression in physiological growth conditions. Similar results have been found with mouse CSCs (mCSCs), where ischemic hypoxia reduced CSC proliferation and DNA synthesis to 25±2.0% (N=3; $p<0.05$) and 54 ±7.0% (N=7; $p<0.05$), respectively, relative to cells grown in room air. SIRT1 protein expression was decreased by 58±10.0% (N=4; $p<0.05$). Interestingly, there was no difference in growth rate when mCSCs were cultured at room air versus physiological oxygen. Furthermore, SIRT1 protein was 59±6.0% less stable (N=4; $p<0.05$) following 8 hours of cyclohexamide treatment in mCSCs exposed to ischemic hypoxia for 72 hours compared to room air. SIRT1 knockdown by RNA interference confirms the proteins role in CSC proliferation by significantly reducing growth in both hCSCs and mCSC grown in room air (N=3 and, $p<0.05$).

Conclusion: These results suggest that SIRT1 expression is regulated by oxygen tension and is one contributing mechanism that regulates CSC proliferation in hypoxia. Manipulation of SIRT1 may serve as one avenue for the enhancement of CSC self-renewal under hypoxic conditions.

123

Covalent Modification of Cardiac Troponin C Alters Myocardial Ca^{2+} Sensitivity

Ian Robertson[a,b], Sandra Pineda-Sanabria[a], Yin-Biao Sun[b], Malcolm Irving[b], Brian Sykes[a]

[a]*University of Alberta, Edmonton, Alberta, Canada*
[b]*King's College London, London, UK*

Heart failure is characterized by the inability of the heart to supply the body with a sufficient supply of blood. One way to treat the reduced cardiac contractility typical of heart failure is to directly enhance the muscle's response to Ca^{2+}. During systole, cystolic Ca^{2+} levels spike and bind to the regulatory domain of the sarcomere protein troponin C (cTnC). Binding of Ca^{2+} to cTnC triggers a conformational change in cTnC that culminates in its association with troponin I, and the initiation of myocardial contraction. Compounds that sensitize cardiac muscle to Ca^{2+} by binding to cTnC are termed Ca^{2+}-sensitizers, and although they hold promise, their potential has yet to be realized. A limitation in their development has been their multiple pharmacological effects - many Ca^{2+} sensitizers also inhibit phosphodiesterase 3 (PDE3), which leads to an increase in cytosol Ca^{2+} levels and the development of arrhythmias. Levosimendan is the most widely prescribed "Ca^{2+}-sensitizer", but there is still debate about its biological activity; therefore, design of a more specific Ca^{2+}-sensitizer is needed. Using Mass Spectrometry and NMR spectroscopy, we show that levosimendan forms a reversible covalent bond with the thiol group of Cys^{84} of cTnC located in the hydrophobic cleft. To study the covalent interaction, we characterized the structure and function of cTnC labeled at Cys^{84} with an analog of levosimendan (dfbpma). The structure indicates that dfbpma binds in the hydrophobic cleft of cTnC. Functional studies indicate that when cTnC-dfbpma is exchanged into cardiac muscle trabeculae, Ca^{2+} sensitivity is dramatically enhanced. The results indicate that not only is cTnC a bonafide druggable target, but also that more specific drugs for cTnC could be designed taking into consideration the chemical reactivity of its hydrophobic cleft.

124

The Role of a Novel AMPK Activator in the Protection Against Angiotensin II-Induced Myocardial Hypertrophy

Ian Robertson, Nobutoshi Matsumura, Shereen Hamza, Carrie Soltys, Miranda Sung, Grant Masson, Donna Beker, Jason Dyck

University of Alberta, Edmonton, Alberta, Canada

The WHO estimates that approximately 25% of adults worldwide have hypertension. Because hypertension is linked to severe morbidities such as arteriosclerosis, myocardial hypertrophy and eventually heart failure, the discovery of new therapeutics that not only treat hypertension, but also its complications, such as hypertrophy, is needed. We tested the novel complex I inhibitor and indirect AMP-activated kinase (AMPK) activator, R118 (Rigel Pharmaceuticals, Inc.), to examine whether or not AMPK activation alone is sufficient to prevent the development of angiotensin II (Ang II)-induced hypertension and cardiac hypertrophy. Male C57BL/6 mice were administered control diet or diet with R118 (200 mg/kg) for two weeks followed by an additional two weeks of Ang II (1.4 mg/kg/day) or saline infusions. Blood pressures were recorded using telemetry and tail-cuff to assess the vascular effects of R118. Echocardiography was employed to assess in vivo cardiac function and morphology at baseline and following the four-week study. We found that while R118 did not prevent the Ang II-induced increase in blood pressure, it decreased several parameters linked to cardiac hypertrophy, most notably left ventricular posterior wall thickness. To evaluate the molecular mechanisms involved in the anti-hypertrophic effects of R118, R118 was tested in rat neonatal cardiomyocytes. Cells were pretreated with R118 2 hours prior to a 24-hour treatment with phenylephrine to induce cardiomyocyte hypertrophy. R118 treatment resulted in an increase in AMPK activation and a decrease in protein expression via a decrease in p70S6 kinase and eukaryotic elongation factor-2 activation. Taken together, these data suggest that although R118 fails to prevent Ang II-induced hypertension, it is able to prevent myocardial hypertrophy through inhibition of protein synthesis. Whether or not this anti-hypertrophic effect is beneficial or detrimental in the setting of hypertension is yet to be established.

125

Cardiac evaluation in canine model of X-linked myotubular myopathy after correction of muscle pathology with AAV8-MTM1

Jennifer Strande[a,h], Michael Lawlor[a], David Mack[b], Karine Poulard[c], Melissa Goddard[d], Jessica Snyder[b], Robert Grange[e], Jon Doering[e], Virginie Latournerie[f], Philippe Veron[f], Hui Meng[a], Lin Yang[c], Fujun Liu[c], Larine Buscara[f], Samia Martin[f], Michael O'Callaghan[g], Federico Mingozzi[f], Alan Beggs[i], Anna Buj-Bello[f], Martin Childers[b]

[a]*Medical College of Wisconsin, Milwaukee, WI, USA*
[b]*University of Washington, Seattle, WA, USA*
[c]*University of Florida, Gainesville, FL, USA*
[d]*Wake Forest University, Winston Salem, NC, USA*
[e]*Virginia Polytechnic and State University, Blacksburg, VA, USA*
[f]*Généthon, Evry, France*
[g]*Audentes Therapeutics, San Franscisco, CA, USA*
[h]*University Pierre and Marie Curie, Paris, France*
[i]*Boston Children's Hospital, Boston, MA, USA*

X-linked myotubular myopathy (XLMTM) is a congenital muscle disorder caused by deficiency of the lipid phosphatase, myotubularin. Patients typically present with severe hypotonia and respiratory failure which leads to early mortality. Myotubularin is expressed in the heart, although the heart is not known to be affected in XLMTM. Gene replacement therapy with the adeno-associated virus serotype 8 vector (AAV8) expressing myotubularin under the muscle-specific desmin promoter delivered intravenously in Mtm1-KO mice restored skeletal muscle function but resulted in cardiotoxicity; specifically the development of cardiac fibrosis. The goal of this study was to explore potential cardiotoxicity during a dose escalation study, testing the efficacy of AAV8-MTM1 in XLMTM dogs. Five groups were studied: Wild-type or female carrier controls (N=5); affected dogs given saline only (N=4) or affected dogs given AAV8-MTM1 at the following dosages: (5E12 vg/kg, N=3), (2.5E13 vg/kg, N=3) or (8E13 vg/kg, N=3). AAV8-MTM1 was administered at 10 weeks of age. Comprehensive 2D and Doppler echocardiograms were performed serially between 17 and 52 weeks of age and cardiac pathology was conducted at the terminal end-point. No evidence of gross or histological cardiac pathology was observed in any of the groups. Echocardiogram analysis revealed mild variations of cardiac structure (left ventricular (LV) wall thickness and LV chamber size), systolic function (ejection fraction and fractional shortening) and diastolic function (mitral inflow velocities and mitral annular tissue velocities). There were neither severe abnormalities nor specific

abnormal patterns observed between groups or within any specific group. Therefore, these small changes of heart structure and function most likely represents normal variation found within growing dogs and may be specific to this breed/mix. It would be unlikely that these changes are related to the XLMTM disease process or the AAV8-MTM1 treatment; however, these data must be interpreted cautiously due to small sample size and inter-subject variability.

126

A Novel Gene Therapy of Ribonucleotide Reductase for a Large Animal Heart Failure Model

Shin Kadota[a], Sam Teichman[b], Michael Laflamme[a], Charles Murry[a], Michael Regnier[a], Gregory Mahairas[b]

[a]*University of Washington, Seattle, WA, USA*
[b]*BEAT Biotherapeutics Corp, Seattle, WA, USA*

Background: Deoxy-adenosine triphosphate (dATP) has been demonstrated to be a superior substrate over ATP for myosin cross-bridge formation. dATP is normally produced along with other deoxyribonucleotide triphosphates to supply substrate for DNA synthesis, and this process is severely down-regulated in non-replicating cardiomyocytes. Previously we have shown that the expression of ribonucleotide reductase (RNR) increased intracellular dATP, cardiomyocyte contraction and cardiac performance in rodents.

Methods and Results: We have developed an adeno-associated virus serotype 6 (AAV6) vector to deliver the entire human RNR enzyme complex under the control of a cardiac-troponin T promoter. Incorporation of a 2A sequence results in the independent translation of two subunits of RNR. We induced myocardial infarction (MI) in Yucatan minipigs by 75-min balloon occlusion of the left anterior descending artery. Two weeks after MI (Day0), AAV6-RNR (1×10^{13}, 5×10^{12}, and 1×10^{12} viral genomes), or saline, were delivered via antegrade coronary infusion. Left ventricular ejection fraction (LVEF) by echocardiography improved in the high- and medium-dose groups (*$p<0.05$, **$p<0.01$ vs sham).

LVEF	Sham (n=4)	High-dose (n=5)	Medium-dose (n=4)	Low-dose (n=4)
Day -14	58.3±1.7%	62.4±3.4%	54.0±2.5%	56.8±0.8%
Day 0	46.3±1.8%	44.0±3.3%	44.3±4.8%	48.5±2.2%
Day 28	38.5±3.4%	50.0±1.7%*	37.8±5.1%	45.8±4.1%
Day 56	34.8±4.1%	51.2±2.0%**	47.8±3.3%*	44.5±5.7%

LV end-systolic dimension at day 56 was shorter in the high-dose group (33.4±1.5 mm) than in the sham (46.3±1.8 mm, P=0.01). Hemodynamic parameters such as +dP/dt, -dP/dt and LV end-diastolic pressure also improved in the high-dose group. There was neither difference in histopathologic appearance of heart nor other organs in treated animal compared to control, without any safety or tolerability concerns.

Conclusions: Targeted gene therapy using AAV6-RNR, which is independent of calcium and adrenergic signalling, resulted in reversal of cardiac dysfunction in a large-animal heart failure model with no safety concerns. These results support the therapeutic potential of AAV6-RNR for patients with heart failure.

127

Cardioprotective role of miR-181c in obesity

Brittany Dunkerly[a,b], Junaid Afzal[c], Igal Madar[d], Djahida Bedja[c], Jorge Henao-Mejia[e], Richard Flavell[e], Roopa Biswas[f], Charles Steenbergen[a], Samarjit Das[a]

[a]*Department of Pathology, Johns Hopkins University, Baltimore, MD, USA*
[b]*Notre Dame of Maryland University, Baltimore, MD, USA*
[c]*Department of Cardiology, Johns Hopkins University, Baltimore, MD, USA*
[d]*Division of Nuclear Medicine, Russell H. Morgan Department of Radiology, Johns Hopkins University, Baltimore, MD, USA*
[e]*Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA*
[f]*Department of Anatomy, Physiology and Genetics, School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA*

Recent reports suggest that mi(cro)RNAs, non-coding RNAs, can regulate many genes. We identified a nuclear encoded miRNA (miR-181c) that translocates into mitochondria to regulate a mitochondrial gene, and ultimately affects mitochondrial function. In an obesity model, we found up-regulation of miR-181c in the heart. To investigate the significance of this increase in miR-181c, we used an antagomir against miR-181c. Consistent with our previous findings that miR-181c overexpression decreases mt-COX1 protein, treating H9c2 cells with antagomir-181c significantly increases mt-COX1 protein expression compared to scramble treatment. Previously, we showed that cardiac dysfunction with miR-181c overexpression is associated with excessive production of ROS, and consistent with this, ROS decreased with antagomir-181c treatment. We subjected H9c2 cells to H_2O_2, 200 μM for 8 hrs, and antagomir-181c treatment showed significant cytoprotection compared to H9c2 cells exposed to scramble. To study the effect of lipid, we incubated H9c2 cells with oleate (100 μM) bound to 99% pure BSA in 0.1% DMSO for 48 hr. As reported by others, we found that lipid-load significantly reduced cellular viability compared to BSA-control, using an MTT assay. Treatment with antagomir-181c can rescue the phenotype. To further investigate, we used a more physiological model, miR-181c/$d^{-/-}$ mice. *In vivo* imaging (PET-CT) showed higher uptake of (18)F-fluorobenzyl triphenyl phosphonium into the mitochondria of the heart/kidney/liver tissue of miR-181c/$d^{-/-}$ mice, suggesting higher mitochondrial membrane potential or greater mitochondrial volume in these tissues. Using both Electron Microscopy and light-scattering at 540 nm by isolated heart mitochondria, we found that the mitochondria are smaller in the miR-181c/$d^{-/-}$ cardiac myocytes, and genomic DNA-qPCR showed the number of mitochondria was markedly higher in the miR-181c/$d^{-/-}$ heart compared to the WT group. Taken together, the data indicate that the obesity-induced increase in miR-181c in the heart contributes to myocardial injury, and that lowering miR-181c expression is protective.

128

Understanding the physiological role of γ2-AMPK

Naveen Bojjireddy, Rong Tian

University of Washington, Seattle, WA, USA

AMP activated protein kinase (AMPK) is a heterotrimeric complex consisting of catalytic α subunit and regulatory β and γ subunits. AMPK α and β subunits have two isoforms (α1, α2 and β1, β2) and γ subunit has three isoforms (γ1, γ2 and γ3). Point mutations in the γ2

subunit cause glycogen storage cardiomyopathy associated with cardiac hypertrophy and arrhythmias. Molecular mechanisms contributing to this phenotype is elusive. To understand the physiology role of γ2-AMPK we have adopted two strategies. 1. To identify novel interacting partners we generated HEK293 cell line stably expressing flag-tagged human γ2-subunit and performed mass-spectrometry of proteins pulled down by anti-flag antibody. 2. To understand the subcellular localization of γ subunits we have generated GFP- γ1, GFP- γ2 and GFP- γ3 constructs; expressed them in COS7 cells and performed confocal microscopy. GFP-tagged- γ subunits were uniformly distributed between nucleus and cytosol. Since AMPK is stress responsive protein kinase we evaluated whether GFP-tagged γ subunits have altered cellular distribution during stress. Either by activation with AMPK activator (A769662) or under metabolic stress condition only GFP- γ2 accumulated in the nucleus with no effect on cellular distribution of GFP- γ1 of GFP- γ3. To further strengthen this observation we have performed nuclear-cytoplasmic fractionation studies using Flag- γ2 overexpressing stable cell line. We observed that under metabolic stress conditions α2, β1 and γ2 subunits accumulate in the nucleus and we have also demonstrated that the nuclear accumulation of α2β1γ2 complex is AMPK activity dependent. The nuclear translocation of γ2-AMPK complex was associated down-regulation of the pre-rRNA transcription. We have identified several novel γ2 interacting proteins that are involved in the transcriptional regulation of rRNA from our proteomic studies of flag-tagged γ2-AMPK expressing cells; we are currently validating these candidates to understand the novel role of γ2-AMPK in stress response.

129

Pharmacological inhibition of soluble epoxide hydrolase preserves mitochondrial efficiency and cardiac function post-MI in aged mice

Kristi L. Jamieson, Victor Samokhvalov, Maria Akhnokh, Xiuhua Wang, Zamaneh Kassiri, John M. Seubert

University of Alberta, Edmonton, Alberta, Canada

Background: Cardioprotective effects of epoxyeicosatrienoic acids (EETs) toward acute myocardial ischemia-reperfusion injury have been recognized; however, it remains unclear whether EET-mediated cardioprotection is sustained in the aged population. Our study investigates the protective effects of EETs by inhibiting soluble epoxide hydrolase (sEH), the enzyme responsible for EET metabolism, following surgical occlusion of left anterior descending artery (LAD) in aged animals.

Methods: Age matched 18 month old sEH null (KO) and littermate wild-type (WT) mice were subjected to LAD-ligation to induce myocardial infarction (MI). In parallel, aged C57Bl/6 mice received sEH inhibitor, trans-4-[4-(3-adamantan-1-y1-ureido)-cyclohexyloxy]-benzoic acid (*t*AUCB; 10mg/L) or vehicle in drinking water for 4 days prior and 7 days post-surgery. Cardiac structure and function was assessed by echocardiography prior to and 7 days post-surgery. Mitochondrial enzymatic activities of respiratory complexes I, II, IV, and citrate synthase were assessed. Respiratory control ratios were determined using a Clark-type electrode.

Results: Hearts from *t*AUCB-treated mice showed preserved ejection fraction and percent fractional area change compared to WT counterparts. However, no preservation of cardiac function was observed in sEH KO groups. Mitochondrial functions were better preserved following myocardial infraction in hearts from *t*AUCB-treated and sEH KO mice based on higher respiratory control ratios compared to WT controls. *t*AUCB treatment increased post-MI enzymatic activity of complex I and II.

Conclusion: Our data suggest that while genetic deletion of sEH showed minor protective effects post-MI, pharmacological inhibition of sEH resulted in sustained mitochondrial bioenergetic efficiency and improved cardiac function.

130

Organ-Specific VWF Promoter Activity in Response to Hypoxia and Microthrombotic Consequences

Anahita Mojiri, Maryam Nakhaei-Nejad, Nadia Jahroudi

University of Alberta, Edmonton, Alberta, Canada

Background: Von Willebrand factor (VWF) is an endothelial specific pro-coagulant protein that has a major role in initiation and growth of thrombosis. It exhibits a heterogeneous expression pattern in ECs of different organs. In vivo VWF expression is significantly increased in response to hypoxia in ECs of lung, heart, liver and brain, but not Kidney. We explored the differential response of VWF promoter to hypoxia in heart and lung compared to kidney EC and determined the functional consequences with regard to microthrombosis in vivo.

Method: Adenoviral vectors containing VWF promoter-LacZ transgene were used in transduction of mice and cultured human ECs of lung, heart and kidney. LacZ expression patterns were determined by immunofluorescent staining (IF). VWF transcriptional response to hypoxia was analysed in cultured ECs by determining the endogenous VWF and transgene LacZ mRNA levels. Major organs from hypoxic mice were analysed by IF to detect platelets, VWF and fibrinogen colocalization representing microthrombosis.

Results: Mice transduced with Adenoviral vectors demonstrated LacZ expression in ECs of organs in a pattern consistent with previously reported organ-specific activities of the VWF promoter fragments in transgenic mice. In cultured ECs, hypoxia resulted in increased levels of endogenous VWF and exogenous VWF-LacZ transgenes in heart and lung ECs but not in that of kidney. Colocalization of platelets, VWF and fibrinogen, which together are strong evidence of microthrombosis, were observed in heart and lung vasculature but not kidney of hypoxic mice.

Conclusion: ECs of various organs exhibit differential response to hypoxia with regard to VWF transcriptional activity in vivo and in vitro. Specifically kidney ECs do not up-regulate VWF transcription in response to hypoxia while lung and heart ECs do. Consistent with VWF up-regulation, evidence of microthrombosis is observed in vasculature of heart and lung but not kidneys of mice exposed to hypoxia.

131

Resveratrol improves exercise capacity in mice with heart failure by enhancing skeletal muscle oxidative capacity and vascular function

Miranda Sung, Nikole Byrne, Ian Robertson, Victor Samokhvalov, Jody Levasseur, Kelvin Jones, John Seubert, Jason Dyck

Cardiovascular Research Centre, University of Alberta, Edmonton, AB, Canada

Exercise intolerance is a hallmark symptom of heart failure (HF) that results from a combination of cardiopulmonary, vascular and

skeletal muscle specific effects. Recently, we have shown that resveratrol increases survival and activity of mice with HF, which occurs in the absence of an improvement in systolic cardiac function, suggesting non-cardiac beneficial effects. Since resveratrol is known to be an exercise mimetic, we hypothesized that resveratrol may improve exercise capacity in mice with HF via direct effects on the skeletal muscle and vasculature. To investigate this, 8-week old male C57/BL6 mice were subjected to sham or transverse aortic constriction (TAC) surgery to induce pressure overload-induced HF. Three weeks post-surgery when mice were in HF (EF<40%), a cohort of mice from both sham and TAC groups were administered resveratrol (14 mg/d) in their diet. HF mice treated with resveratrol for 2 weeks had increased baseline physical activity levels and improved exercise capacity compared to vehicle treated HF mice (Treadmill duration (min): Sham, 36.64±6.88; TAC, 10.14±1.69 and TAC+resveratrol, 28.30±5.32, $p<0.05$, n=7-15/group). Consistent with HF inducing direct skeletal muscle dysfunction, ADP-stimulated O2 consumption of isolated EDL and soleus muscle was reduced by ~50% in mice with HF. However, resveratrol restored O2 consumption rates of skeletal muscle fibers similar to that observed in sham mice ($p<0.05$, n=5/group). Furthermore, HF-induced skeletal muscle insulin resistance and vascular dysfunction was restored by resveratrol treatment. Taken together, these data suggest that resveratrol may have direct actions on skeletal muscle and vasculature to increase exercise tolerance by restoring skeletal muscle mitochondrial oxidative capacity, insulin sensitivity and vascular function. Our data suggest that resveratrol supplementation may be an effective adjunct therapy for the treatment of HF and has the potential to improve the quality of life HF patients.

132

Sorcin interacts with the mitochondrial calcium uniporter and inhibits calcium transport in mitochondria

Christian Silva-Platas, Gerardo Garcia-Rivas

Tecnologico de Monterrey, Monterrey, Nuevo Leon, Mexico

Sorcin is a cytosolic protein with EF calcium binding domains relevant as an accessory element during inactivation of L type calcium channels and ryanodine receptors, key elements in calcium signaling. Intramitochondrial calcium is a key factor in the myocyte physiology. In energy demanding conditions is crucial as it can activate dehydrogenases in Krebs cycle but also in organelle dysfunction during calcium overload and apoptosis. Mitochondria calcium transport is mediated by the calcium uniporter (MCU) but little is known about its regulation. Using protein sequence analysis we found three segments in the N terminus region of MCU that are significantly similar to those segments in L type channel and RYR2 which interact with sorcin during calcium transients. Thus here is evaluated if sorcin interacts with the MCU and the effect on calcium transport. The purified recombinant sorcin showed the characteristic property of calcium induced conformational change (EC_{50} 4,4 M Ca^{+2}). Using SDS-PAGE and western blot techniques, the incubation of sorcin with rat heart mitoplasts resulted in its translocation to membranes in dependence of free Ca^{+2}. The immunoprecipitation of sorcin, followed by SDS-PAGE and western blot analysis, revealed that the MCU is coprecipitated indicating a Ca^{+2} dependent interaction between these two entities. In isolated rat heart mitochondria, the dynamic of Ca^{+2} was determined using the metalochromic indicator arsenazo III. Here, sorcin inhibited the uptake of Ca^{+2} in the nanomolar range. In conclusion, a direct interaction of sorcin with MCU can modulate the calcium dynamic in mitochondria. This encourages for more investigation as sorcin could emerge as a new regulator protein of MCU.

133

19,20-EDP protects HL-1 cardiac cells against LPS-induced cytotoxicity through activation of mitochondrial function and biogenesis

Victor Samokhvalov, Kristi L. Jamieson, John M. Seubert

University of Alberta, Edmonton, Alberta, Canada

BACKGROUND: Cellular and molecular mechanisms through which epoxy metabolites of long-chain omega-3 polyunsaturated fatty acids (*"fish oil"*) regulate mitochondrial quality control in cardiac cells is unknown. We investigated the role cytochrome P450 epoxygenase metabolites of docosahexaenoic acid, epoxydocosapentaenoic acids (EDPs) in regulation and protection of mitochondria.

METHODS: HL-1 cardiac cells were exposed to LPS (1μg/ml) for 24 hrs, treated with vehicle, 19,20-epoxydocosapentaenoic acid (19,20-EDP, 1 μM) and/or a soluble epoxide hydrolase (sEH) inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*AUCB, 10 μM), to prevent metabolism. Cell viability, injury, mitochondrial function and biogenesis were assessed.

RESULTS: LPS treatment decreased HL-1 cell viability, contractility, mitochondrial oxidative activity, respiratory control ratios and ATP levels which were attenuated by co-treatment with 19,20-EDP. Examination of cellular ultrastructure via electron microscopy revealed extensive damage and accumulation of aberrant mitochondria in HL-1 cells following LPS treatment. We further explored LPS-triggered mitochondrial damage showing that LPS decreased activities of key regulators of mitobiogenesis, notably pCREB (Ser133), NRF1/ NRF2 DNA binding activities and SIRT1 enzymatic activity. Interestingly, our data demonstrate that 19,20-EDP alone increased the activity of these regulators thereby initiating mitochondrial biogenesis. Furthermore, our results indicate that 19,20-EDP effectively prevented LPS-induced decline in mitochondrial biogenesis. 19,20-EDP also limited LPS-promoted accumulation of aberrant mitochondria. Combining 19,20-EDP with *t*AUCB potentiated the protective effects against LPS-induced cytotoxicity suggesting that inactivation of she, which effectively prevents degradation of EDPs plays crucial role in producing biological effects.

CONCLUSION: In summary, our data demonstrated a critical role of DHA metabolites, EDPs, acting as intracellular lipid mediators protecting cardiac cells against LPS-induced injury by regulating mitochondrial function and biogenesis.

134

19,20-EDP targets mitochondrial pathways to produce protection of HL-1 cells against hypoxia-reoxygenation injury

Victor Samokhvalov, Kristi L. Jamieson, John M. Seubert

University of Alberta, Edmonton, Alberta, Canada

BACKGROUND: Hypoxia-reoxygenation (H/R) injury promotes extensive damage to cardiomyocyte mitochondria triggering cell death. Recently, we demonstrated that epoxy metabolites of long-chain omega-3 polyunsaturated fatty acids, specifically docosahexaenoic acid (DHA), found in *"fish oil"* produce potent cardioprotective effects. In this study, we investigated whether the

cardioprotective effects of cytochrome P450 epoxygenase metabolites of DHA, epoxydocosapentaenoic acids (EDPs), involve regulating and protecting mitochondria following H/R injury.

METHODS: HL-1 cardiac cells were subjected to hypoxia (<1%O2) for 24 hrs followed by 6 hrs of reoxygenation. Cells were treated with vehicle, 19,20-epoxydocosapentaenoic acid (19,20-EDP, 1 μM) and/or a soluble epoxide hydrolase (sEH) inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*AUCB, 10 μM), to inhibit enzymatic degradation. Cell viability, contractility, mitochondrial function and biogenesis were assessed.

RESULTS: H/R exposure caused a dramatic decrease in contractility and overall cell viability. Furthermore, H/R insult induced a pronounced decline in mitochondrial bioenergetic function such as total mitochondrial oxidative activity, respiratory control ratios and depletion in the levels of ATP. H/R resulted in almost complete downregulation of key regulatory factors required for mitobiogenesis such as NRF1/2, pCREB (Ser133) and SIRT1. The most important finding of this study is that co-treatment with 19,20-EDP resulted in a robust preservation of cell viability, contractility and remarkably, protection of mitochondrial function and biogenesis. We also demonstrated that addition of 19,20-EDP promoted mitobiogenesis under normal conditions and prevented its decline in HL-1 cells after H/R. Addition of 19,20-EDP together with *t*AUCB greatly potentiated protective effects highlighting an essential physiological relevance of sEH inhibition in EDPs-produced protection.

CONCLUSION: Our data provide convincing evidence that protective effects of DHA (*"fish oil"*) may be attributed to the CYP epoxygenase metabolites, EDPs, which regulate mitochondria function and biogenesis.

135

Identification of Pathologic Circulating Factors in Children with Dilated Cardiomyopathy

Xuan Jiang[a], Brian Reid[a], Brian Stauffer[a,c], Shelly Miyamoto[b], Carmen Sucharov[a]

[a]*University of Colorado School of Medicine, Aurora, CO, USA*
[b]*Children's Hospital Colorado, Aurora, CO, USA*
[c]*Denver Health and Hospital Authority, Aurora, CO, USA*

Introduction: Heart failure (HF) is a major public health issue that is associated with increasing prevalence, high mortality, frequent hospitalization, and imposes a significant societal economic burden. Despite the demonstrated benefits of current medical and device therapy in adult HF patients, no substantial improvement in survival has been observed in children with HF, suggesting the underlying cellular mechanisms are uniquely regulated in children with HF. The myocardium of children with idiopathic dilated cardiomyopathy (IDC) demonstrates a gene expression pattern consistent with myocardial remodeling: increased atrial natiuretic factor (ANF) and β-myosin heavy chain (MHC) and decreased sarcoplasmic reticulum calcium ATPase (SERCA) and α-MHC. We have shown that the serum from pediatric IDC patients would recapitulate this pathologic gene expression pattern in neonatal rat ventricular myocytes (NRVMs). The objective of this study was to characterize the serum circulating factors that cause pathologic response in NRVMs with the ultimate goal of identifying new drug therapy candidates for pediatric and adult patients.

Methods: Exosomes were isolated from the serum of 23 pediatric IDC patients and 3 non-failing (NF) control children with normal heart function. Exosome quantitation was measured by the AchE activity known to be enriched within exosomes. To determine if exosome from pediatric IDC patients would have an effect on FGP expression, neonatal rat ventricular myocytes (NRVMs) were treated with exosome from IDC patients and NF controls. RNA was isolated from the cells after 72 hour treatment, followed by RTqPCR for the above described genes. Exosomal RNA sequencing was performed from serum of 2 pediatric IDC patients and 2 NF controls to identify the candidate serum-circulating factor(s) that cause the pathologic response.

Results: The number of exosomes extracted from the serum of pediatric IDC patients was 2 to 10 fold less than the one from NF controls, suggesting the existence of protective factors in the NF exosome. Treatment of NRVMs with exosomes from IDC patients induced hypertrophy of NRVMs similar to exposure to phenylephrine (PE) after 72 hour incubation. Besides, treatment with exosome from IDC patients also resulted in up-regulation of βMyHC, ANF and BNP, suggesting that most the serum effect in NRVMs is mediated by exosome. Ongoing work is to identify the potential candidate factors responsible for the pathological response in the NRVM model by performing exosomal RNA-seq from serum of IDC patients and NF controls.

Conclusions: Exosomes of pediatric IDC patients can induce a pathological response that is similar to the response induced by serum, indicating most of the serum effect is mediated by the factors in the exosome. Serum fractionation shows that these factors are present in both low and high molecular weight fractions, suggesting the observed effect is medicated by miRNAs which can be associated with vesicles and proteins. High-throughput screening will be used to identify new drug therapy candidates for pediatric IDC patients.

136

The mitochondrial calcium uniporter balances energetic supply with cardiac workload during sympathetic stress and modulates mitochondrial permeability transition in ischemia-reperfusion injury

Timothy S. Luongo, Jonathan P. Lambert, Ancai Yuan, Xueqian Zhang, Santhanam Shanmughapriya, Erhe Gao, Polina Gross, Steven R. Houser, Muniswamy Madesh, John W. Elrod

Temple University, Philadelphia, PA, USA

Background: The high metabolic demand of the heart makes it essential that a tightly controlled system be in place to regulate energy production. Contractility is mediated by a variable flux in intracellular calcium ($_{i}Ca^{2+}$), which is theorized to be integrated into mitochondria to regulate oxidative phosphorylation and meet energetic demand. In addition, Ca^{2+}-overload of mitochondria during stress is known to trigger mitochondrial permeability transition pore (MPTP) opening and cell death. Recent studies have reported that the *Mcu* gene encodes the channel-forming portion of the mitochondrial calcium uniporter (MCU) and is required for $_{m}Ca^{2+}$ uptake. **Objective:** To examine the role of $_{m}Ca^{2+}$ signaling in the heart, we generated a conditional, cardiomyocyte-specific knockout model ($Mcu^{fl/fl}$ x αMHC-MerCreMer), and deleted *Mcu* in adult mice (*Mcu-cKO*). **Results:** Loss of *Mcu* completely ablated acute $_{m}Ca^{2+}$ uptake and MCU channel activity in isolated adult cardiomyoctes. *Mcu-cKO* mice were protected against *in vivo* myocardial ischemia-reperfusion (IR) injury by preventing the activation of the MPTP, reducing necrotic cell death, and preserving LV function. In

addition, while we found no baseline cardiac phenotype, *Mcu*-cKO mice lacked contractile responsiveness to β-adrenergic receptor stimulation, examined by LV pressure monitoring during isoproterenol infusion, and in parallel were unable to activate Ca^{2+}-dependent mitochondrial dehydrogenases. Further experimental analyses in isolated adult cardiomyocytes confirmed a lack of energetic responsiveness to acute sympathetic stress. **Conclusion:** These results support the hypothesis that MCU-dependent $_{m}Ca^{2+}$ uptake is necessary to modulate mitochondrial metabolism during the 'fight or flight' response to support cardiac contractility.

137

Age-specific differences in ryanodine 2 receptor phosphorylation contribute to age-specific responses to phosphodiesterase 3 inhibition in heart failure therapy

Kathleen Woulfe

University of Colorado, Aurora, CO, USA

Background: Recent studies suggest that the pathophysiology of pediatric heart failure is distinct from adult heart failure; however, currently the same therapies are used to treat both patient populations (β-blockers and renin-angiotensin-system antagonism). Not surprisingly, these treatments do not confer improved outcomes in children with idiopathic cardiomyopathy (IDC). Conversely, long-term phosphodiesterase 3 inhibition (PDE3i) in adult heart failure patients is associated with an increased risk of sudden death and arrhythmias, whereas pediatric patients respond well to prolonged PDE3i. Based on the increased potential for arrhythmias in adult IDC patients treated with PDE3i, we propose PDE3i affects phosphorylation of the ryanodine2 (RyR2) receptor. RyR2 regulates Ca^{2+}-induced Ca^{2+} -release from the sarcoplasmic reticulum (SR) leading to contraction. When RyR2 is dysregulated, Ca^{2+} can "leak" and lead to inappropriate contractility, thus arrhythmias. There are three known phosphorylation sites that affect RyR2 activity: serine-2030 (Ser2030, phosphorylated by protein kinase A, PKA), serine-2808 (Ser2808, phosphorylated by PKA and Ca^{2+} -calmodulin kinase II, CaMKII), and serine-2814 (Ser2814, phosphorylated by CaMKII). We hypothesize that differential phosphorylation of the three sites on the RyR2 receptor between adult and pediatric patients is responsible for the increase in arrhythmias seen in adult HF patients treated with PDE3i. **Methods**: RyR was isolated from left ventricular tissue explanted from pediatric and adult patients with IDC (treated with standard medical therapy with or without PDE3i) and compared to non-failing donor control samples. Phosphorylation of RyR at Ser2030, Ser2808 and Ser2814 was identified by Western blot with phospho-specific antibodies. **Results**: RyR2 phosphorylation differed between pediatric and adult IDC patients treated with PDE3i. Importantly, PDE3i treatment returned phosphorylation to non-failing levels in pediatric patients. **Conclusions**: The difference in RyR2 phosphorylation between pediatric and adult patients treated with PDE3i could contribute to the increase in arrhythmias in adult patients.

138

Selective blockade of β1 and β2 adrenergic receptors in a mouse model of pediatric hypertrophy

Kathleen Woulfe

University of Colorado, Aurora, CO, USA

Background: The randomized control trial of carvedilol (non-selective adrenergic receptor (AR) antagonist) in pediatric HF did not show the significant reduction in morbidity and mortality seen in adult HF. We propose that the age-related differences in response to carvedilol results from differences in β-receptor expression in pediatric and adult patients. While β1-AR are down-regulated in all HF patients, pediatric failing hearts have a 25% decrease in β2-AR whereas β2-AR expression is not altered in adult HF patients. This could explain the lack of positive response to non-selective β-blockers in pediatric HF patients. We hypothesize that specific β1-AR antagonism, combined with partial β2-AR blockade, will show improvement in an adult mouse model of pathologic hypertrophy but not in the pediatric mouse model. **Methods:** Young and old mice were implanted with mini-osmotic pumps eluting isoproterenol with CGP (β1-AR selective antagonist) alone and in combination with either low or high dose ICI 118551 to provide increasing degrees of β2-AR blockade. We measured morphometric endpoints such as heart weight to body weight ratio and studied the treatment effect on cAMP production and phospholamban (PLB) phosphorylation in the mice. **Results:** β1-AR blockade improved morphometric measures of hypertrophy in both young and old mice. In young mice, the combination of β1-AR-blockade and β2-AR blockade did not decrease hypertrophy. In contrast, the combination of β1-AR blockade and partial β2-AR blockade (low dose ICI) decreased hypertrophy in adult mice, however β1-AR blockade and high dose β2-AR blockade increased hypertrophy. **Conclusions:** These results suggest current non-selective β-blockers used to treat HF may not benefit pediatric HF patients due to the blockade of the β2-ARs which are already down-regulated. In fact, treatment with a selective β1-AR antagonist improved morphometric measures in our pediatric mouse model reiterating β1-AR-specific blockers are a better therapeutic choice in pediatric HF patients.

139

The histone methyltransferase Smyd5 regulates cardiac hypertrophy

Mickey R. Miller[a], Caiyi C. Li[b], Alexa Anderson[a], Li Wang[a], Stephen T. Smale[b], Sarah Franklin[a]

[a]*Nora Eccles Harrison Cardiovascular Research & Training Institute, University of Utah, Salt Lake City' UT' USA*
[b]*Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA*

During stress, cardiomyocytes adapt by undergoing hypertrophic growth and altering their gene expression profile. This selective regulation of transcription represents the molecular underpinnings driving the morphological and physiological remodeling in heart disease and likely includes both maladaptive and compensatory mechanisms aimed at mitigating disease-induced remodeling. However, the proteins responsible for modifying chromatin to accomplish these gene expression changes in the heart during hypertrophy and failure are largely unknown. Overcoming this knowledge gap by understanding the mechanistic basis for genomic regulation in cardiac disease could identify new therapeutic targets for human intervention. The Smyd family of histone methyltransferases regulates gene expression through the post-translational modification of histone proteins, a core component of the chromatin structure. While Smyd1, 2, and 3 have been studied for their involvement in cell growth and differentiation during development and disease, the remaining two family members, Smyd4 and 5, have remained virtually uncharacterized. Recently we discovered that Smyd5 expression is differentially

regulated in the myocardium in a mouse model of pressure-overload hypertrophy; however, its role in the heart is completely unknown. To determine the function of Smyd5, we generated constitutive and inducible, cardiac-specific Smyd5 knockout mice and have characterized their cardiac phenotype under basal conditions and after stress (pressure-overload hypertrophy and isoproterenol administration). When compared to wild type mice, these animals display elevated heart weight/body ratios and left ventricular wall thickening (observed in histological tissue sections) concomitant with increased fibrosis and ANF expression. Our results show that while Smyd5 is dispensable for cardiac development, it is a critical regulator of hypertrophic cell growth and pathologic gene expression in the cardiomyocyte and highlight a novel role for this histone methyltransferase in the myocardium.

140

Increased c-Myc protein levels are detrimental to cardiac function during pressure overload hypertrophy

Margaret Bruce[b], Dolena Ledee[b], Lincoln Smith[a], Danny El-Nachef[a], Michael Portman[a,b], Aaron Olson[a,b]

[a]*University of Washington, Seattle, WA, USA*
[b]*Seattle Children's Research Institute, Seattle, WA, USA*

Background: The proto-oncogene c-Myc (Myc) regulates cardiac hypertrophy; however many of Myc's myocardial effects are incompletely understood. Critically, Myc's effect on cardiac function is unclear. Additionally, Myc causes oncologic transformation in cancer, but the molecular mechanisms affecting this transformation have not been extensively evaluated during cardiac hypertrophy. We tested whether augmenting Myc protein levels during established pressure overload hypertrophy a) affects cardiac function and b) activates molecular mechanisms present during oncologic transformation. Further, we determined whether the molecular changes from Myc augmentation are also present in failing hearts.

Methods: Pressure overload hypertrophy was created by transverse aortic constriction (TAC) in cardiac specific Myc-inducible mice (Myc-TAC, $n = 6$) and non-transgenics (Cont-TAC, $n = 6$). Cardiac function was followed with serial echocardiograms. Three weeks after TAC, Myc was induced for seven days. After that time, tissue was collected for analysis. A separate group of failing hearts (Failure, $n = 5$) had decreased function (shortening fraction $11 \pm 1\%$) throughout serial evaluations.

Results: Although function was similar prior to Myc induction; Myc-TAC had significantly reduced fractional shortening (FS) compared to Cont-TAC (Myc-TAC $13 \pm 2\%$ versus Cont-TAC $20 \pm 2\%$). Cardiac mass increased by 67% in Myc-TAC versus Cont-TAC. Lung wet weight was also significantly greater in Myc-TAC indicating pulmonary edema (12.5 ± 1.2 mg/mm versus 8.6 ± 0.4 mg/mm, respectively). Myc-induction increased protein post-translational modifications by O-GlcNAcylation, pyruvate kinase M2 isoform (PKM2, a glycolytic enzyme) protein levels and glutaminase protein levels (glutamine metabolism), which are all mechanisms in oncologic transformation. Compared to Cont-TAC, Failure hearts had increased Myc mRNA, O-GlcNAcylation and PKM2.

Conclusion: Myc is detrimental to cardiac function during pressure overload hypertrophy. Thus, Myc and/or its molecular mechanisms represent therapeutic targets for heart failure. Failing hearts also exhibited some similar molecular changes as occur during oncologic transformation, which require further evaluation.

141

The effects of beta-adrenergic drugs on embryonic ventricular cell proliferation and differentiation and their impact on donor cell transplantation

Tiam Feridooni, Adam Hotchkiss, Mark Baguma-Nibasheka, Feixiong Zhang, Kishore Pasumarthi

Dalhousie University, Halifax, Nova Scotia, Canada

The beta-adrenergic receptors (β_1 and β_2-AR) and catecholamine ligands are present in the heart as early as embryonic day 8 (E8). Targeted disruption of genes coding for enzymes involved in catecholamine biosynthesis resulted in embryonic lethality between E11.5 and E15.5 possibly due to cardiac structural defects. Several studies also suggested that the adrenergic system is critical for contractility of embryonic heart. However, it is not known whether β-AR signaling plays any role in the proliferation and differentiation of ventricular cells in the embryonic heart. In the present study, we investigated the effects of a nonselective beta AR agonist isoproterenol (ISO) on cell cycle activity and differentiation of cultured cardiac progenitor cells (CPC) and developmentally advanced cardiomyocytes (CM) derived from E11.5 mouse ventricles. Tritiated thymidine incorporation and cell proliferation rates were significantly reduced in both CPC and CM in cultures treated with 1 μM ISO. The ISO mediated effects on cell cycle activity could be completely abolished by co-treatment of E11.5 cultures with ICI 118, 551 (β_2 AR antagonist). In contrast, co-treatment of ventricular cells with ISO and metoprolol (β_1 AR antagonist) significantly increased the cell cycle activity of CPC compared to that in control or ISO treated cultures. Furthermore, ISO treatment significantly increased the percentage of differentiated CM compared to that in control cultures. Subsequent analysis of downstream signaling events revealed that β-AR stimulation of E11.5 ventricular cells can lead to downregulation of Erk and Akt phosphorylation levels followed by significant decreases in cyclin D1 and CDK4 levels. Consistent with the *in vitro* results, we found that chronic stimulation of recipient mice with ISO after intracardiac cell transplantation significantly decreased the graft size while metoprolol (β_1-AR antagonist) protected the grafts from the inhibitory effects of systemic catecholamines. Collectively, these results underscore the importance of β-AR signaling in the developing heart.

142

Revealing cardiac roles of the obscurin protein family

Stephan Lange, Matthew Klos, Stephanie Myers, Nancy Dalton, Eric Devaney, Kirk Peterson, Ju Chen

UC San Diego, La Jolla, CA, USA

The muscle protein obscurin was shown to play structural and signaling roles for sarcomere organization, and in the architecture of the sarcoplasmic reticulum. Despite indications for its importance in muscle formation, function and signaling, obscurin knockout mice develop normally and display no changes to cardiac development or physiology. Therefore, we hypothesized that the lack of a cardiac phenotype in the obscurin knockout mice may be explained by redundant functions between obscurin and other members of the obscurin protein family. Indeed, it was demonstrated that the N-terminus of obscurin and its

close homologue obscurin-like 1 (Obsl1) interact both with myomesin and the C-terminal Ig-domain in titin (Ig-M10).

To reveal essential roles for cardiac development and function that are shared between proteins of the obscurin family, we generated mice that lack Obsl1. Cardiac specific knockouts of Obsl1 were then crossed with obscurin knockouts to generate double knockout mice (dKO). Analysis of single and dKO mice for obscurin and/or Obsl1 revealed no changes to myofibrillar organization and sarcomeric structure on the microscopy level. However, we noticed dramatic changes to calcium-handling proteins and calcium-dependent signaling pathways in single knockout mice, which became more pronounced in hearts of dKO mice.

On the physiological level we found that mice lacking Obsl1 display no changes to heart development or physiology, similar to obscurin knockouts. Only dKO mice for obscurin and Obsl1 develop a late-onset cardiomyopathy after 9 months of age, which results in heart failure and sudden cardiac death in a majority of the mice at 14 months of age.

Taken together, our data indicate that sarcomeric interactions of obscurin and/or Obsl1 with titin and myomesin are not essential for sarcomerogenesis and cardiac development. However, both proteins are crucial for calcium homeostasis as well as calcium-dependent signaling pathways, and ultimately long-term survival of mice.

143

Histone Deacetylase Inhibition Improves Cardiac Function and Attenuates Adverse Tissue Remodeling Post Myocardial Infarction with Upregulation of Wisp-1.

Lillianne Harris[a], Daniel Herr[a], Harinath Kasiganesan[a], Sabina Wang[a], Donald Menick[a,b]

[a]*Medical University of South Carolina, Charleston, SC, USA*
[b]*Ralph Johnson VA Medical Center, Charleston, SC, USA*

Introduction: Myocardial infarctions (MI), or heart attacks, are one of the most prevalent and damaging cardiovascular maladies worldwide. Adverse tissue remodeling, after an infarction, results in necrotic and fibrotic tissue which dampen cardiac function over time. WNT-Inducible Secreted Protein-1 (Wisp-1) is upregulated in response to an MI; while implicated in supporting cell survival and beneficial matrix deposition, its regulation post-MI is unclear. Histone deacetylase (HDAC) expression and activity are increased post-MI and are implicated in tissue remodeling by epigenetic regulation and deacetylation of histone and non-histone proteins. Wisp-1 is transcriptionally regulated by activators that are subject to acetylation. Given the pro-survival/angiogenic/fibrotic functionality of Wisp-1, we hypothesized that broad HDAC inhibition improves cardiac function post myocardial infarction by specifically upregulating Wisp-1.

Materials and methods: Male, 10-12 week old CD1 mice were subjected to left anterior descending coronary artery ligation to model an MI. Mice were injected (intraperitoneal) daily (7 days) with either vehicle or HDAC inhibitor, SAHA. Sham-operated mice served as surgical controls for our experiments. Each experimental cohort consisted of 12 mice per group. 7 and 14 days post-MI, we obtained echocardiograms to assess cardiac function of end-diastolic volume and ejection fraction. Left ventricles were excised and assayed for Wisp-1 expression (rt-q-pcr, and western blot) and DNA-protein interactions (chromatin immunoprecipitation). Each *in vitro* assay was done in triplicate and repeated three times. Statistical analysis of variance (ANOVA) or Student's T-test, determined significance between experimental groups ($p < 0.05$).

Results: Mice injected with SAHA showed improved cardiac function post-MI compared to mice treated with vehicle control ($p < 0.05$). Rt-q-pcr revealed that Wisp-1 is upregulated fifteen fold in ventricles from vehicle mice compared to sham-operated ($p < 0.05$). However, left ventricles from mice treated with SAHA show an almost forty fold induction of Wisp-1 ($p < 0.05$) over sham. This trend is also observed at the protein level as determined by western blot. Furthermore, we observed that transcriptional activators of WISP-1, (p300, ß-catenin andTCF47) are recruited to the proximal promoter of Wisp-1 at a greater abundance in mice treated with SAHA, than vehicle treated mice.

Conclusion: Our data suggests that HDAC inhibition post-MI, may support improved cardiac function by regulating the expression of Wisp-1. Additional studies need to address which HDACs might be involved in regulating Wisp-1 and to determine whether class specific HDAC inhibitors are more efficacious in promoting upregulation of this gene. We also need to address if acetylation of upstream signaling molecules in the WNT/Wisp-1 pathway might also be involved in promoting an increase in Wisp-1 expression post-MI that also supports improved cardiac function and tissue remodeling.

144

Biochemical and biophysical properties of the dilated cardiomyopathy associated cardiac troponin I mutation - P16T

Jordan Klaiman, Joseph Powers, Robert Langan, Maria Razumova, Michael Regnier

University of Washington, Seattle, WA, USA

Dilated cardiomyopathy (DCM) is characterized by progressive dilation and impaired systolic function of the left or of both ventricles. DCM is the most common cardiomyopathy and can result from a variety of factors, including genetic mutations. Interestingly, mutations found on the thin filament regulatory proteins have been associated with some of the most severe phenotypes of DCM and so it is important to understand how these mutations result in loss of contractile function. Here we investigate the cardiac troponin I (cTnI) P16T mutation, which has been associated with DCM. This mutation is located on the N-terminal extension of cTnI and is in close proximity to two important protein kinase A (PKA) targets ($Ser^{23/24}$). In addition, threonine is an amino acid that is able to be phosphorylated by various kinases and so this substitution may affect the overall phosphorylation of the N-terminal extension and thus, contractile function. In this study, we test how the P16T mutation affects biochemical properties of cTnI and its interaction with cTnC, and whether it alters contractile function and its regulation following PKA treatment. Preliminary experiments have demonstrated that residues $Ser^{23/24}$ on cTnI-P16T can be phosphorylated by PKA treatment. Using a fluorescent probe coupled to cTnC the C-I interaction was monitored. Preliminary data indicates that in the presence of Ca^{2+} cTnI-P16T does not affect the C-I interaction compared to WT cTnI. In ongoing experiments we will determine if PKA phosphorylates the threonine substituted at residue 16 and will characterize how the cTnI-P16T mutation affects the Ca^{2+} binding affinity of the cardiac troponin complex and the contractile properties of demembranated trabeculae. We will also characterize these biochemical and contractile properties following treatment

with PKA. In conclusion, this mutant may have milder effects on these steady state measures than some of the other thin filament mutations associated with DCM.

145
Cardiac myocyte growth defect in lamin A/C deficient mice

Kyohei Oyama, W. Robb MacLellan

University of Washington, Seattle, WA, USA

Lamin A/C, encoded by *Lmna* gene, is nuclear intermediate filament protein ubiquitously expressed in differentiated cells. Mutation of the *Lmna* gene causes multiple tissue disorders, collectively termed laminopathies, which are commonly associated with dilated cardiomyopathy (DCM). However the exact role of lamin A/C in cardiac myocytes (CMs) and the mechanism whereby loss of lamin A/C causes DCM are poorly understood. We examined the effect of lamin A/C loss on cardiac growth and development of DCM using the lamin A/C mutant mice model (LmnaD8-11). LmnaD8-11 mice develop DCM at 4-6 weeks of age with upregulation of fetal gene expression (beta-MHC and ANP). Despite the development of DCM, CMs from 4-6 week LmnaD8-11 mice were dramatically smaller than wild type (WT) CMs. This reduction in CM size is first apparent 2 weeks after birth ($1400\,\mu m^2$ vs $1100\,\mu m^2$; $p < 0.01$). LmnaD8-11 CMs also demonstrated reduced cycling postnatally, which was secondary to reduced cell cycle gene expression including E2F1 and upregulation of cell cycle inhibitor, p21. Surprising, this reduced cell cycle activity postnatally did not affect CM number but resulted in decreased ploidy in LmnaD8-11 CMs. LmnaD8-11 hearts at 2 weeks had 16 % less CMs with greater than three nuclei compared to WT hearts. These data demonstrate that loss of lamin A/C results in defects in both CM cell cycle and hypertrophic growth, which may underlie the development of DCM in these mice.

146
A high incidence of a MYBPC3 gene variant (25 bp deletion) of South Asian descendents in the United States (DOSA study)

Aravindakshan Jagadeesan[a], Nalla Banu Durai[b], Robert Molokie[b], Suresh Govindan[a], Stephanie Kliethermes[a], Thriveni Sanagala[a], Sakthivel Sadayappan[a]

[a]*Loyola University Chicago, Maywood, IL, USA*
[b]*University of Illinois - Chicago, Chicago, IL, USA*

Background: Approximately 40% of cases of hypertrophic cardiomyopathy (HCM) are caused by mutations in the *MYBPC3* gene, which encodes the contractile regulatory protein cardiac myosin binding protein-C (cMyBP-C). Previously, we identified a novel 25-base-pair deletion mutation in *MYBPC3* that leads to the replacement of 65 wild-type amino acids with 58 novel amino acids in the C'-region (cMyBP-$C^{\Delta C10}$). This variant affects ~60 million people, primarily of South Asian descent (4% of population), and has been significantly associated with HCM and heart failure (HF) among carriers. However, the prevalence of this mutation among South Asians in the United States is unknown.

Hypothesis: The presence of cMyBP-$C^{\Delta C10}$ is highly prevalent among South Asians living in the United States.

Methods and Results: The objective of this study was to identify the presence of the cMyBP-$C^{\Delta C10}$ mutation within South Asians living in the United States. An Institutional Review Board protocol was approved both at Loyola University Chicago and University of Illinois at Chicago. After receiving consent, 2 cc blood samples were collected from 294 individuals of South Asian ancestry who were 18 years or older. To determine the presence of cMyBP-$C^{\Delta C10}$ variant, genomic DNA was extracted from deidentified blood samples and used to perform PCR followed by agarose gel electrophoresis. PCR-based genetic screening showed that 33 individuals were positive for the cMyBP-$C^{\Delta C10}$ variant. This data indicated a mutation frequency of 11.22% in the population, and an allele frequency of 5.61%, a higher prevalence than was expected.

Conclusion: These data demonstrate that the cMyBP-$C^{\Delta C10}$ variant is highly prevalent among South Asians living in the United States. Ongoing studies are focused on clinically evaluating these individuals for an HCM phenotype and associated risk factors. The present study is a crucial step to support genetic testing for South Asians who are at a high risk for heart disease.

147
β-adrenergic signaling activates glycogen autophagy in the heart

Kimberley Mellor[a], Ellie Stevens[a], Upasna Varma[b], Lea Delbridge[b]

[a]*University of Auckland, Auckland, New Zealand*
[b]*University of Melbourne, Melbourne, Victoria, Australia*

Autophagy disturbance and glycogen mishandling in the heart play a role in many cardiac pathologies but the mechanisms are not well understood. We have recently demonstrated that an autophagy process specific for glycogen ('glycophagy'), which is distinct from macro-autophagy, is modulated by metabolic stress and is an important regulator of glycogen content in the heart.[1, 2] The aim of this study was to elucidate β-adrenergic signaling regulation of glycogen handling and autophagy in the heart.

Excised hearts (male Sprague Dawley 8 weeks) were perfused with 10^{-6}M isoproterenol (β-agonist) or control buffer for 5 or 60 minutes, snap frozen, homogenized and protein extracted. Cultured neonatal rat ventricular myocytes (NRVMs) were exposed to isoproterenol for 30 minutes, lysed and frozen. Glycophagy and macro-autophagy markers, signaling intermediates and glycogen handling proteins were analyzed by immunoblot. Glycogen content was measured by amyloglucosidase assay.

Isoproterenol-induced β-adrenergic activation was evidenced by an increased phospho(Ser14)- to total glycogen phosphorylase ratio after 5min of isoproterenol perfusion (62% increase $p<0.05$). Glycophagy markers GABARAPL1 and acid α-glucosidase were markedly increased by β-adrenergic signaling activation (3-fold and 1.5-fold increase respectively, $p<0.05$) consistent with depletion of glycogen content (71% lower after 5min and fully depleted after 60min Isoproterenol perfusion, $p<0.05$). Similarly, isoproterenol induced glycogen depletion in NRVMs (64% decrease, $p<0.05$). Macro-autophagy markers, LC3BII:I ratio and p62 protein expression

were not changed in the isoproterenol perfusion groups. Inactivation of glycogen synthase (phosphorylation-Ser641) was only evident after 60min isoproterenol perfusion.

This is the first study to show that β-adrenergic signaling positively regulates glycophagy. These findings suggest that β-adrenergic regulation of glycophagy is distinct from macro-autophagy, demonstrating that autophagy sub-types are influenced differentially by signaling modalities. Further studies of *in vivo* β-adrenergic regulation of glycophagy in disease states are warranted.

1. Delbridge et al. Am J Physiol Heart In Press, 2015.
2. Mellor et al. Am J Physiol Heart 306(8):H1240-5, 2014.

148

Developmental differences in neonate and adult cardiac extracellular matrix modulate cellular recellularization

C.W. Jenny Chang[a], Janhavi Sharma[a], Andrew Wong[a], Jayne Squirrell[b], Kevin Eliceiri[b], Javier López[a], Leigh Griffiths[a]

[a]*University of California, Davis, Davis, USA*
[b]*University of Wisconsin, Madison, Madison, USA*

Background: The limited regenerative capacity of cardiac tissues has motivated various approaches to engineer a functional myocardial tissue patch. Here, we aim to develop an unfixed xenogeneic extracellular matrix (ECM) biomaterial with preserved native structure and function properties for recellularization. We hypothesize that a sequential antigen-removal (AR) paradigm, utilizing differential protein solubility combined with targeted depolymerization and solubilization of sarcomeric components, will preserve native ECM structure and function properties and enhance recellularization capacity. We further hypothesize that ECM niche of varying post-natal developmental ages will modulate recellularization capacity and cellular differentiation.

Methods: We generated ECM scaffolds from neonatal (3 d) and adult (135 d) rat ventricular myocardium using our developed AR approach (AR-ECM) or sodium dedocyl sulfate (SDS) decellularization. All ECM scaffolds were then subjected to recellularization with rat fetal cardiomyocytes. Collagen fibers within AR and SDS scaffolds were evaluated using picosirius red staining, second harmonic generation and scanning electron microscopy to evaluate collagen fiber integrity. Recellularization capacity of resultant ECM scaffolds was assessed via histology and flow cytometry.

Results: Histology images revealed that AR-ECM was clear of sarcomeric components, nuclear and cytoplasmic content while residual nuclear and cytoplasmic debris remained in SDS scaffolds. Collagen fibers and their birefrigent properties were preserved in AR-ECM, but disrupted in SDS-decellularized samples. Flow cytometry data showed that significantly more viable cells repopulated AR-ECM than SDS decellularized scaffolds. Critically, recellularization of neonatal ECM was significantly greater than for adult ECM. Additionally, beta and total myosin heavy chain expressions were higher in neonatal than adult ECM scaffolds. Lastly, recellularized AR-ECM cultured in serum exhibited spontaneous beating activity and intact response to isoproterenol, indicating maintained beta-receptor function. **Conclusions:** Collectively, these findings demonstrate that our AR method produces an ECM biomaterial with preserved structure and function properties with enhanced recellularization capacity for cardiac tissue engineering.

149

Acute AMPK activation attenuates cardiomyocyte glycogen accumulation induced by high glucose

Upasna Varma[a], Kimberley Mellor[b], Lea Delbridge[a]

[a]*The University of Melbourne, Melbourne, Australia*
[b]*The University of Auckland, Auckland, New Zealand*

Background: Cardiac glycogen accumulation has been observed in humans and in diabetic *in vivo* and *in vitro* models.[1] We have shown that extracellular glucose and insulin status influences intracellular glycogen content and activation of 'glycophagy'- a glycogen specific autophagy pathway distinguished from protein macro-autophagy.[2] AMPK signaling is deregulated in diabetes and modulates mTOR, a key component of autophagy regulation. This study examined the effects of acute AMPK activation on the modulation of cellular glycogen content under simulated diabetic conditions *in vitro*.

Methods: Neonatal rat ventricular myocytes were cultured in 5 mM or 30 mM glucose for 24 hours. AMPK agonist (AICAR, 1 mM) was administered for the final 30 min. Immunoblot was used to measure expression and activation of key signaling markers. Glycogen content was assessed by amyloglucosidase assay.

Results: Glycogen levels were increased in cells exposed to high glucose (3.9-fold, $p < 0.05$). This was abrogated by AICAR, associated with an increase in phosphorylated AMPK (Thr172, 2.9-fold, $p < 0.05$). Downstream mTOR phosphorylation (Ser2448) was surprisingly increased with AICAR in high glucose conditions (2.2-fold, $p < 0.05$), concomitant with a decrease in the macro-autophagy marker LC3BII:I (0.67-fold, $p < 0.05$). High glucose induced a reduction in phosphorylated Akt (Ser473, 0.67-fold, $p < 0.05$). Total expression levels of glycophagy markers, GABARAPL1 and STBD1, was stable.

Discussion: This study provides further evidence that glycogen accumulation is a pathology associated with diabetic conditions. It is the first to indicate that acute AMPK activation attenuates glycogen accumulation in cardiomyocytes exposed to high glucose. Further investigation is necessary to elucidate the effects of AMPK on the enzymatic regulation of glycogen. These findings are of particular importance in considering the cardiac implications of current AMPK-related therapeutic interventions in patients with diabetes.

1. Delbridge LM, Mellor KM, Taylor DJ, Gottlieb RA. *Am J Physiol Heart Circ Physiol* In Press, 2015.
2. Mellor KM, Varma U, Stapleton DI, Delbridge LM. *Am J Physiol Heart Circ Physiol* 306:H1240-5, 2014.

150

Association between Serum Visfatin level and Coronary Artery Disease: A Meta-Analysis

Pradyumna Agasthi[a], Sivakanth Aloor[b], Kalaivani Sivakumar[a], Anekwe Onwuanyi[a]

[a]*Morehouse School of Medicine, Atlanta, GA, USA*
[b]*University of Miami, Miller School of Medicine, Miami, FL, USA*

Background: Visfatin is a novel adipokine shown to be associated with systemic inflammation, obesity, metabolic syndrome, and carotid atherosclerosis. It is hypothesized that visfatin plays a role in monocyte activation, plaque destabilization, the promotion of

angiogenesis, and endothelial dysfunction leading to development of atherosclerotic lesions. The association between visfatin and coronary artery disease (CAD) is obscure.

Purpose: The aim of the present study is to conduct a meta-analysis to evaluate the relationship between circulating visfatin levels and CAD.

Methods: We searched MEDLINE, CIINHAL and COCHRANE databases for studies reporting serum visfatin levels in the patients with CAD and healthy controls. We included case controls, cohort and cross-sectional studies. We calculated the weighted standardized mean difference (SMD) in serum visfatin levels between the CAD and control groups.

Results: Our search strategy yielded 107 articles and we included 6 studies (case-control) enrolling 742 participants. The median age of the CAD group was 62.33 yrs. (IQR 60.8 – 66.8) compared to 60.7 (IQR 58.5 – 64) in the control group. The median body mass index in the CAD group was 26.05 kg/m^2 (IQR 24.7- 27) compared to 25.9 kg/m^2 (IQR 24.7-27) in the control group. The unweighted median serum visfatin levels in the CAD group were 19.5 ng/ml (IQR 14.9 - 35.6) compared to 13.8 ng/ml (IQR 11.25 – 14.35) in the control group.

The SMD of serum visfatin level was 0.66 (95% CI 0.34 - 0.97) $p < 0.001$ comparing those in the CAD group and control group.

Conclusion: An elevated serum visfatin level is significantly associated with the presence of CAD. Current findings demonstrate the need to further clarify the role of visfatin in the development of CAD and its value as a therapeutical target in the cardiovascular disease.

151

Relationship between Serum YKL-40 levels and Coronary Artery Disease: A Meta Analysis

Pradyumna Agasthi[a], Sivakanth Aloor[b], Maihemuti Axiyan[a], Anekwe Onwuanyi[a]

[a]*Morehouse School of Medicine, Atlanta, GA, USA*
[b]*University of Miami, Miller School of Medicine, Miami, FL, USA*

Background: YKL-40 is a novel inflammatory marker secreted by vascular smooth muscle cells (VSMCs) and activated lipid-laden macrophages in atherosclerotic plaques. YKL-40 modulates chemotaxis, cell attachment and migration of VSMCs and the formation of branching tubules suggesting its role in angiogenesis and in the process of atherosclerotic plaque formation. The relationship between YKL-40 and coronary artery disease (CAD) is unclear. We conducted a meta-analysis to investigate the association between serum YKL-40 levels and presence of CAD.

Methods: We searched MEDLINE, CIINHAL and COCHRANE databases for studies reporting serum YKL-40 levels in the patients with CAD and healthy controls. We included case controls, cohort and cross-sectional studies. We calculated the weighted standardized mean difference (SMD) in serum YKL-40 levels between the CAD and control groups.

Results: Our search strategy yielded 51 articles and we included 11 studies enrolling 3289 participants. The median age of the CAD group was 61 yrs. (IQR 60 - 63) compared to 58 yrs. (IQR 56 - 59) in the control group. The median body mass index in the CAD group was 26.2 kg/m2 (IQR 25.8 – 27.4) compared to 25.89 kg/m2 (IQR 25.4 – 26.4) in the control group. The unweighted median serum YKL-40 levels in the CAD group were 88 ng/ml (IQR 70.5 - 141) compared to 54.66 ng/ml (IQR 40.6 – 90.4) in the control group. The SMD of YKL-40 level was 1.607 (95% CI 0.708 - 2.505) $p < 0.001$ comparing those in the CAD group and control group.

Conclusion: An elevated serum YKL-40 level is significantly associated with the presence of CAD. Further studies are needed to confirm this association by addressing potential confounding factors.

152

Saturated high-fat diet-induced obesity increases adenylate cyclase of myocardial beta-adrenergic system and does not compromise cardiac function

Daniele Vileigas, Adriana Deus, Danielle Silva, Loreta Tomasi, Dijon Campos, Katashi Okoshi, Carlos Padovani, Antonio Cicogna

Sao Paulo State University, Botucatu, SP, Brazil

Background: Cardiac function is closely associated with the sympathetic nervous system that is hyperactive in obesity. Although myocardial beta-adrenergic pathway and heart function were assessed in obese animals induced by unsaturated high-fat diet, there are limited studies that analysed these two parameters using saturated fatty acids. This study aimed to evaluate cardiac function and the components of myocardial β-adrenergic system in obese rats induced by saturated high-fat diet.

Methods: Male *Wistar* rats were separated into two groups: control ($n = 18$; standard diet) and obese ($n = 19$; saturated high-fat diet) fed for 30 weeks. Nutritional profile and obesity-related comorbidities were evaluated. Cardiac structure and function were assessed by echocardiography. Myocardial protein expression of β_1 and β_2 receptors, adenylate cyclase (AC), Gαs protein and protein kinase A (PKA) was performed by Western Blotting. Cardiac cyclic adenosine monophosphate (cAMP) levels and PKA activity were assessed by ELISA.

Results: Obese rats showed increased final body weight, total body fat and adiposity index ($p < 0.001$) and various comorbidities such as hypertension ($p = 0.013$), glucose intolerance ($p < 0.001$), insulin resistance ($p = 0.013$) and dyslipidemia, characterized by hypertriglyceridemia ($p = 0.007$) and hypercholesterolemia ($p < 0.05$). There was no difference in the heart rate. The ratio between left atrium and aorta diameters was higher in obese group ($p = 0.033$) and the ratio between posterior wall thickness and left ventricular diastolic diameter was similar in both groups. Systolic and diastolic function assessed by endocardial fractional shortening, ejection fraction and E/A ratio were similar between groups. Protein expression of myocardial AC was significantly elevated in obese group compared to control ($p = 0.025$); there was no change in the other β-adrenergic components evaluated.

Conclusion: Obesity induced by saturated high-fat diet increased AC protein levels of myocardial β-adrenergic pathway and did not cause cardiac dysfunction.

Financial support: Sao Paulo Research Foundation

153

High unsaturated fatty acid diet does not rescue cardiac dysfunction or lipid energy metabolism in rats with aortic stenosis

Loreta C. Tomasi[a,d], Dijon H.S. Campos[a], Danielle F. Vileigas[a], Katashi Okoshi[a], Carlos H. Padovani[b], Gilson M. Murata[c], Stephen C. Kolwicz[d], Antonio C. Cicogna[a]

[a]*Department of Internal Medicine, Botucatu School of Medicine - Sao Paulo State University, Botucatu, SP, Brazil*
[b]*Department of Biostatistics, Botucatu Institute of Biosciences - Sao Paulo State University, Botucatu, SP, Brazil*
[c]*Department of Biochemistry, Institute of Chemistry - University of Sao*

Paulo, Sao Paulo, SP, Brazil
[d]*Mitochondria & Metabolism Center, Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA*

Introduction: Pathological hypertrophy is associated with decreased fatty acid oxidation (FAO). Although high-fat diets (HFD) have been show to have positive effects, limited studies have utilized high unsaturated fatty acid (UFA) diets after the establishment of left ventricular hypertrophy (LVH). Therefore, we hypothesized that increased UFA supply would prevent the downregulation of FAO and attenuate cardiac dysfunction in a rat model of LVH.

Methods: Male *Wistar* rats (80 g) underwent aortic stenosis (AS) or Sham surgery. After 6 weeks, rats received either normolipid diet (N, 17% calories from fat) or high UFA diet (H, 40% calories from fat) for 12 weeks yielding 4 groups: Sham-N ($n = 13$), AS-N ($n = 11$), Sham-H ($n = 12$), AS-H ($n = 14$). Cardiac function was assessed by echocardiography at 6 and 18 weeks after surgery. Metabolic enzymatic activity, FAO-related gene expression and myocardial triacylglycerol (TAG) and free-fatty acid (FFA) composition were measured in hearts at the end of 18 weeks.

Results: Prior to dietary treatment, AS rats showed evidence of LVH and diastolic dysfunction with elevated systolic function. Cardiac function and morphometry remained stable in all groups over the final 12 weeks and HFD had no effect in Sham or AS groups. AS hearts had decreased expression of FAO-related genes (CD36, CPT1β and MCAD) and was unchanged with HFD. Phosphofructokinase activity was increased in both AS groups compared to Sham. Myocardial TAG content was ~25% lower in AS-N and AS-H (vs. respective Sham). Decreased abundance of oleate and linoleate was observed in the FFA and TAG pools in AS hearts, which was not prevented with HFD.

Conclusion: High UFA supply did not improve diastolic dysfunction or the downregulation of FAO in a rat model of established LVH. A decreased amount of the UFAs, oleate and linoleate, in myocardial FFA and TAG pools is associated with pathological hypertrophy.

Financial support: Sao Paulo Research Foundation and the American Heart Association.

154

Determining the Role of Branched Chain Amino Acid Utilization in Cardiac Substrate Utilization

Lauren Abell, Stephen Kolwicz, Tao Li, Daniel Raftery, Rong Tian

University of Washington, Seattle, USA

The metabolism of three essential branched-chain amino acids (BCAA) leucine, isoleucine and valine is significantly altered during the development of cardiovascular and metabolic disease. Impairment of BCAA catabolism due to the deletion of mitochondrial localized protein phosphatase 2C (PP2Cm), a key enzyme in activating BCAA catabolism, increases levels of BCAAs within the body. The PP2Cm-knock out (KO) mouse, which has elevated BCAA levels in the heart, shows a significant decrease in the relative contribution of glucose to oxidative metabolism and an increase in fatty acid oxidation compared to control mice. At six months of age PP2Cm-KO mice develop hyperglycemia and hyperinsulinemia, have a higher susceptibility to stress-induced heart failure and demonstrate decreased ability to recover after ischemia reperfusion (I/R) injury. Interestingly, increasing glucose uptake in PP2Cm-KO mice through overexpression of an insulin independent glucose transporter GLUT1 rescues and improves the cardiac response to IR injury, suggesting that enhancing glucose utilization can compensate for defective BCAA catabolism.

We hypothesize that increased BCAA levels within the heart lead to decreased glucose uptake and utilization. To determine the interplay between glucose and BCAAs in the PP2Cm-KO heart, we utilize a cardiac tissue-specific stable isotope tracer (SIT)-based metabolomics platform to identify regulatory mechanisms of cardiac metabolism *in vivo*. PP2Cm-KO and WT control hearts are perfused with mixed substrate buffer containing either uniformly labelled ^{13}C-glucose or ^{13}C-BCAAs. Metabolite content of heart tissue is extracted and analysed using gas chromatography mass spectrometry (GCMS) or liquid chromatography mass spectrometry (LCMS).

Preliminary findings in hearts perfused with 13C-glucose have shown a decrease in mean ^{13}C enrichment in PP2Cm-KO mice in the presence and absence of BCAAs. TCA cycle and glycolytic intermediates showed decreased levels of ^{13}C incorporation on GC/MS and LC/MS. We predict that labelling experiments involving ^{13}C labelled BCAAs will show interaction and utilization in noncannonical pathways.

155

Right ventricular energy metabolism in a porcine model of acute pressure overload

Masaki Kajimoto[a], Muhammad Nuri[a,b], Dolena Ledee[a], Aaron Olson[a,b], Nancy Isern[c], Michael Portman[a,b]

[a]*Seattle Children's Research Institute, Seattle, WA, USA*
[b]*University of Washington, Seattle, WA, USA*
[c]*Pacific Northwest National Laboratories, Richland, WA, USA*

Background: Substrate metabolism is greatly influenced by various stresses including pressure load. Acute right ventricular (RV) pressure-overload is one of the common reasons for RV dysfunction in children. The potential causes including pulmonary arterial (PA) hypertension and RV outflow tract obstruction. However, RV substrate metabolism is poorly understood under these conditions. We assessed RV substrate metabolism during exposure to acute pressure-overload induced by PA banding (PAB).

Methods: Fourteen infant male mixed breed Yorkshire piglets were randomized to undergo median-sternotomy (Control group) and median-sternotomy and PAB (PAB group). The PABwas tightened to increase the RV systolic pressure 2-fold above the baseline. 13-Carbon (13C)-labeled lactate, medium-chain fatty acid (FA) and mixed long-chain FAs were infused as oxidative substrates for citric acid cycle (CAC) for 60 minutes, and then RV tissue was extracted for metabolic analysis.

Results: RV systolic pressure in PAB group increased by 2-fold of baseline after PAB (24 ± 1 and 51 ± 3 mmHg, $P < 0.01$). In addition, RV end-diastolic pressure in PAB group was increased by around 2-fold of baseline after PAB (6 ± 1 and 11 ± 2 mmHg at baseline and endpoint respectively, $P < 0.01$). LV systolic blood pressure and cardiac output maintained similar levels during experiments in both groups. Proton nuclear magnetic resonance (1H-NMR) showed 81% lower [Phosphocreatine]/[ATP] in PAB group. Fractional contributions of FAs to CAC were significantly lower in PAB group than the Control group (medium-chain FA; 14.5 ± 1.6 vs. 8.2 ± 1.0 %, long-chain FAs; 9.3 ± 1.5 vs. 5.1 ± 1.1 %, Control vs. PAB respectively) by 13C-NMR. Lactate oxidation seemed to be slightly inhibited by PAB, but it did not reach statistical difference between 2 groups.

Conclusions: Acute RV pressure-overload affected the contribution of FA substrates to CAC, and resulted in low energy status.

156
Filling the HFpEF Gap to Guide New Therapies - The Development of HFpEF Animal Models

Kersten Small, Huawei Zhao, Chin-Hu Huang, Shu-Yu Sun, Valdeci DaCunha, Jeffrey Madwed

Merck Research Labs, Kenilworth, NJ, USA

Heart failure with preserved ejection fraction (HFpEF) lacks clinically validated evidence-based mechanisms for therapeutic intervention. The absence of animal models displaying HFpEF or HFpEF phenotypes (e.g., diastolic dysfunction with impaired ventricular relaxation and reduced ventricular compliance) represents a challenge to the pharmaceutical industry in the identification of beneficial treatments for this syndrome. We, at Merck, have set out to develop rodent and non-rodent animal models of HFpEF. Model criteria for HFpEF have been defined and include (a) LVEDP > 16 mmHg, (b) slow LV relaxation, (c) decreased LV compliance, and (d) preserved ejection fraction. To date, multiple rodent models with well-defined risk factors have been assessed to determine if any meet these criteria. Models that failed to meet HFpEF criteria include diabetic ZSF1 rats fed high fat diet for 50 weeks, aged (45 week old) db/db mice, hypertensive Dahl SS rats (varying in salt diet, age and vendor) as well as SHR (varying in age and diet). In each case, either EF was preserved without an increase in LVEDP and diastolic dysfunction, or LVEDP and diastolic dysfunction increased, but EF was reduced. More recently, encouraging data has been observed in three models: (1) Supra-aortic banding in rats, (2) SHR plus L-NAME and ISO, and (3) SHR plus DOCA. In each, mean LVEDP exceeded 16 mmHg; left ventricle relaxation time was increased nearly 2-fold as compared to naïve rats, LV compliance was decreased, while ejection fraction was preserved. Details of these models will be presented with the hope that we, and others, can build upon them to create models suitable for identification of novel therapeutic treatments.