Late-Breaking Basic Science Oral Abstracts I

Inhibition Of Prolyl-tRNA Synthetase As A Novel Mediator Of Cardiac Fibrosis

Joon Seok Park1, Caroline H Lee1, Sungjin Yoon1, Jong Hyun Kim1, Sunghoon Kim2, Bonggyeong Lee2; 1Daejung Pharmaceutical Co., Ltd., Yongin, Korea, Republic of

Introduction: Prolyl-tRNA synthetase (PRS), a member of aminoacyl tRNA synthetases (ARS), is an enzyme that conjugates amino acid proline to its cognate tRNA to generate prolyl-tRNA to be used in protein synthesis. Since ARS are essential for protein synthesis and viability, dysregulation of ARS has been proposed to many human diseases. Cardiac fibrosis is characterized by excess production and deposition of ECM proteins by activated fi broblast. Although proline is a main component of collagen and ECM proteins, the exact mechanism and involvement of PRS in cardiac fibrosis has not yet been elucidated.

Hypothesis: We hypothesized that suppression of PRS would down-regulate collagen synthesis, which could be beneficial in cardiac fibrosis. Results: To validate our hypothesis, we investigated the expression levels of pro-fibrotic markers by overexpression or knockdown of PRS in vitro. We showed that PRS is closely related to the expression of collagen I and α-SMA. Then, we showed that treatment of DWN12088, a novel small molecular selective inhibitor of PRS, decreased expression levels of pro-fibrotic markers in TGFβ1-induced fibrotic environments using various cell-lines and primary fibroblasts. Interestingly, we showed that down-regulation of pro-fibrotic markers by DWN12088 is independent of TGFβ signaling pathway, although TGFβ is required for induction of pro-fibrotic markers. In vivo study, we performed transverse aortic constriction in C57BL/6 mice to evaluate the effect of DWN12088 on cardiac fibrosis.

Conclusions: These results suggest that inhibition of PRS attenuates pressure-overloaded cardiac fibrosis and a selective inhibitor of PRS, DWN12088, could serve as a potent anti-fibrotic agent without affecting critical cellular signaling cascade.


Key Words: Enzyme inhibitors; Fibrosis; Heart failure

Cytosolic RBFox1 in Cardiac Fibrosis Regulation

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RBFox1 is known to be an RNA splicing regulator with enriched expression in cardiac muscle. Loss of RBFox1 expression is a molecular hallmark associated with pathological hypertrophy and heart failure. However, much of our current knowledge about RBFox1 focuses on nuclear RBFox1 with a major impact on global alternative splicing changes in the diseased heart. Yet, RBFox1 gene also generates a cytosolic isoform through alternative splicing (RBFox1c), but the specific function of RBFox1c in heart has not been characterized.

RBFox1c expression is significantly repressed in the mouse failing heart and hypertrophic cardiomyocytes. We performed RNA-seq combined with GO and IPA analysis to determine the impact of RBFox1c expression in culture. Among the genes suppressed specifically by RBFox1c but not the nucleus RBFox1 are groups of pro-infl ammatory genes. Both Motif enrichment analysis and de novo motif discovery identifi ed significant enrichment of RBFox1c binding motif in the 3′UTR of the RBFox1c regulated genes. Using CLIP analysis followed by RT-qPCR, we observed RBFox1c, but not nuclear RBFox1, specifically interacted with targeted inflammatory gene 3′UTR. In the cardiac specific RBFox1 knockout mice, enhanced cardiac fi brosis was observed following TAC, associated with elevated expression of RBFox1c dependent infl ammatory genes. In contrast, cardiac specific expression of RBFox1c significantly reduced cardiac fi brosis and infl ammatory gene expression following TAC, associated with improved ejection fraction and reduced hypertrophic marker gene expression. Further, we tested the effect of RBFox1c expression on cardiac fi brosis response using MVMN conditioned media. We showed the conditioned media from the hypertrophic cardiomyocytes potently reduced fi broblast proliferation. However, RBFox1c expression can suppress phenylephrine and isoproterenol induced fi broblasts proliferation.

RBFox1c regulates cardiac transcriptome reprogramming at two post-transcriptional steps. The RBFox1c nuclear isoform regulates global RNA splicing reprogramming in heart, while the RBFox1c cytosolic isoform regulates infl ammatory gene expression and fi brotic remodeling potentially through potential interaction with their 3′UTR and targeted RNA degradation.

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Key Words: Heart failure; Genomics; Hypertrophy

Optogenetic-induced Mitochondrial Membrane Potential Depolarization and Targeting Cell Death

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Introduction: There is growing evidence that mitochondrial dysfunction is closely associated with the development of cardiovascular diseases, although the precise mechanism is not well deﬁ ned. Normal functioning of mitochondria relies on maintaining the inner membrane potential (a.k.a. ΔΨm) to drive oxidative phosphorylation and redox balance. Thus, developing a tool to induce controlled mitochondrial depolarization and examine the effects on downstream intracellular processes will lead to critical information that helps to reveal the mechanisms underlying mitochondria-induced cellular dysfunction.

Methods and Results: In this study, we developed a new generation optogenetic-based technique for targeting mitochondrial depolarization with light. Particularly, a heterologous light-gated channelrhodopsin protein, named Chr2, was targeted to and expressed on the inner mitochondrial membrane (IMM). We showed that Chr2 formed functional cationic channels on IMM with properties similar to that on the plasma membrane, allowing light-induced targeting ΔΨm depolarization. We also showed that sustained moderate light illumination caused significant cell death in mitochondrial Chr2-expressing cells but not in the mock transfected or mitochondrial YFP-expressing cells. Finally, we provided evidence that the mitochondrial optogenetic-induced cell death is via apoptosis and is independent of the opening of the permeability transition pore.

Conclusion: This new generation optogenetic tool can be used to study the mechanisms how a change of mitochondrial permeability influences cell and organ functions.


Key Words: Cell physiology; Mitochondria; Apoptosis; Cellular Engineering; Cardioprotection

A Peptide Of The Amino-terminus Of Grk2 Induces Hypertrophy And Yet Elicits Cardioprotection After Pressure Overload.

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Heart failure (HF) is a leading cause of death worldwide and a growing burden on public health, and the underlying mechanisms of cardiac remodeling and decomposition to HF remain a focus of research efforts towards therapeutic development. Signaling via G protein-coupled receptors (GPCRs) is critical for normal heart function and is tightly controlled by GPCR kinases (GRKs) with GRK2 (originally a- PK), being intimately involved in HF progression. In addition to its well-characterized role in regulating GPCRs, ongoing research has demonstrated great diversity in the functional roles of GRK2. I have recently investigated GRK2 amino terminal binding interactions through the generation of transgenic (Tg) mice with cardiac-targeted expression of the amino-terminal peptide jARKnt (residues 50–145), in a murine model of trans-aortic constriction (TAC)-induced pressure overload, echocardiography revealed increased left ventricular (LV) posterior wall thickness (1.57 versus 1.37 ± 0.02; n = 10) and LV mass in TgARKnt compared to non-transgenic littermate controls (NLC) 4 weeks after TAC or Sham surgery. Interestingly, despite enhanced hypertrophy at baseline and after acute pressure overload, the progression to HF was paradoxically inhibited in TgjARKnt mice during chronic pressure overload with preserved cardiac function (% Ejection Fraction 57.3 ± 37.3 ± 2.0, n = 11, 10), Further, jARKnt expression limited adverse left ventricular remodeling, with reduced interstitial fibrosis (% area fibrosis 4.1 versus 9.2 ± 0.8; n = 11, 9 hearts) and preserved %a- adrenergic receptor density 4 weeks after surgery. The effect of cardiac jARKnt expression was not consistent with alterations in GRK2 activity at GPCRs as neither GRK2 peptide inhibition (TgjARKct) nor GRK2 overexpression alter hypertrophy, and overexpression hastens HF development. Further, TgjARKnt mice exhibit reduced epididymal white adipose content
and altered mitochondrial respiration, suggesting altered cardiac metabolism. These data support the idea that the [GFP]K peptide embodies a distinct functional interaction and that [GFP]K-mediated regulation of β-adrenergic receptor density may provide a novel means of cardioprotection during pressure-overload induced HF.


Key Words: Heart failure; Ventricular remodeling; Cardiac hypertrophy; Cardiac metabolism

G Protein-coupled Receptor Kinase 2 Negatively Regulates Fatty Acid Utilization and Mitochondrial Bioenergetics

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During cardiac injury or stress, G protein-coupled receptor (GPCR) kinase 2 (GRK2) expression levels and activity are increased, leading to a desensitization of myocardial β-adrenergic receptors (JARs) and contributing to the loss of contractile reserve. Up-regulated GRK2 has been shown to be pathogenic in the post-injured heart and is involved in the promotion of heart failure (HF). There is evidence that GRK2 has other, non-GPCR dependent pathological functions within cardiomyocytes. For example, GRK2 localizes to the mitochondria following oxidative stress, where it acts as a pro-death kinase and decreases fatty acid utilization. As metabolic substrate utilization and bioenergetics are key parameters in the maintenance of cardiomyocyte contractility, our objective is to examine the role of GRK2 on metabolism and bioenergetics in the adult heart. We hypothesize that desensitization of JARs via an increase in GRK2 will result in decreased fatty acid-fueled respiration and will compromise mitochondrial function. Conversely, ablation of GRK2 will result in increased respiration and function, and under these conditions. Our results show that basal respiration, maximal respiration, and reserve capacity (RRC) are highest in the presence of palmitate versus glucose (1.6, 3, and 2-fold, respectively), accompanied by increased (1.3-fold) ATP levels. Moreover, basal and maximal respiration was decreased (1.6 and 1.2-fold, respectively) cardiomyocytes isolated from cardiac-specific GRK2 transgenic mice. This correlates with a decrease in ATP levels and in vivo cardiac fatty acid uptake (1-fold and 1.6-fold, respectively). Conversely, cardiomyocytes isolated from ARKnt, a peptide inhibitor of GRK2, transgenic mice or GRK2 knockout mice, which is known to improve cellular survival during stress. Our data support the idea that the mitochondrial expression of GRK2 is a key factor in the regulation of fatty acid metabolism in the heart.

Author Disclosures: J.M. Pfleger: None. W.J. Koch: None.

Key Words: Adrenergic; Energetics; Metabolism; Mitochondria; Mitochondrial energetics; heart failure; arrhythmias

Heart Failure Induced Upregulation of MicroRNAs in the Human Sinoatrial Node Associated with Pacemaker Dysfunction

Ning Li, Maria Petkova, Brian J Hansen, Prosper S Seseekayombya, Joseph Yannii, Brandon J Biesiadecki, Ahmed Kilic, Peter J Mohler, Paul M Janssen, Jonathan P Davis, Federica Accornero, Mark R Boyett, Halina Dobrzynska, Vladim F Fedorov; The Ohio State University, Columbus, OH, “University of Manchester, Manchester, United Kingdom

Background: Heart failure (HF), a leading cause of morbidity and mortality, involves significant dysfunction of the sinoatrial node (SAN). MicroRNAs (miRNAs) are abundant, non-coding RNAs that ultimately regulate protein expression at the post-transcriptional level and have been implicated in HF. However, nothing is known about the distribution and expression of miRNAs in the human SAN as well as their roles in regulating pacemaker channels and SAN dysfunction (SND) in HF. Methods: The human SAN was isolated and cryopreserved from failing hearts with implantable pacemakers (n=5) or non-diseased donor hearts (n=3) that were cardioplegically arrested in the surgery room. Utilizing immunohistochemistry, the intact 3D intramural SAN structure was precisely identified as the fibrotic region around the SAN artery containing Connexin43-negative pacemaker cardiomyocytes (Figure). Small biopsies from the central SAN compartment were used to characterize the expression pattern of 14 miRs and their associated pacemaker channels. Ingenuity software (Qiagen) was used to predict the relationship between miRs and their targeted mRNA of SAN ion channels Results: Out of 14 studied miRs, 3 miRs (mir-370-3p, mir-133a and mir-1) were significantly upregulated in SAN, but not in atria, during HF. We found that mir-370-3p was predominantly expressed in the human SAN, but not atria. Whereas, both mir-133a and mir-1 were highly expressed in atria vs SAN. All three miRs were predicted to regulate pacemaker HCN1 and/or HCN4 channel expression. RT-PCR showed that HCN1 and HCN4 mRNA were downregulated in the HF SAN. Conclusions: This is the first study to explore the miRs profiles in healthy and diseased human SAN with SND. We demonstrate that miR-370-3p, mir-133a and mir-1, thought to regulate pacemaker HCN1 channel expression, are selectively upregulated in human HF SAN. We propose that understanding the function of miRs in human SAN might lead to novel SND treatments.


Key Words: Sinoatrial node; Heart failure; MicroRNA

Exercise Instigates Apoptosis-inducing Factor Nuclear Translocation and Myocyte Death in Arrhythmogenic Cardiomyopathy

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During cardiac injury or stress, G protein-coupled receptor (GPCR) kinase 2 (GRK2) expression levels and activity are increased, leading to a desensitization of myocardial β-adrenergic receptors (JARs) and contributing to the loss of contractile reserve. Up-regulated GRK2 has been shown to be pathogenic in the post-injured heart and is involved in the promotion of heart failure (HF). There is evidence that GRK2 has other, non-GPCR dependent pathological functions within cardiomyocytes. For example, GRK2 localizes to the mitochondria following oxidative stress, where it acts as a pro-death kinase and decreases fatty acid utilization. As metabolic substrate utilization and bioenergetics are key parameters in the maintenance of cardiomyocyte contractility, our objective is to examine the role of GRK2 on metabolism and bioenergetics in the adult heart. We hypothesize that desensitization of JARs via an increase in GRK2 will result in decreased fatty acid-fueled respiration and will compromise mitochondrial function. Conversely, ablation of GRK2 will result in increased respiration and function, and under these conditions. Our results show that basal respiration, maximal respiration, and reserve capacity (RRC) are highest in the presence of palmitate versus glucose (1.6, 3, and 2-fold, respectively), accompanied by increased (1.3-fold) ATP levels. Moreover, basal and maximal respiration was decreased (1.6 and 1.2-fold, respectively) cardiomyocytes isolated from cardiac-specific GRK2 transgenic mice. This correlates with a decrease in ATP levels and in vivo cardiac fatty acid uptake (1-fold and 1.6-fold, respectively). Conversely, cardiomyocytes isolated from ARKnt, a peptide inhibitor of GRK2, transgenic mice or GRK2 knockout mice, which is known to improve cellular survival during stress. Our data support the idea that the mitochondrial expression of GRK2 is a key factor in the regulation of fatty acid metabolism in the heart.

Author Disclosures: J.M. Pfleger: None. W.J. Koch: None.

Key Words: Adrenergic; Energetics; Metabolism; Mitochondria; Mitochondrial energetics; heart failure; arrhythmias

Late-Breaking Basic Science Oral Abstracts II

Heart Failure Induced Upregulation of MicroRNAs in the Human Sinoatrial Node Associated with Pacemaker Dysfunction

Ning Li, Maria Petkova, Brian J Hansen, Prosper S Seseekayombya, Joseph Yannii, Brandon J Biesiadecki, Ahmed Kilic, Peter J Mohler, Paul M Janssen, Jonathan P Davis, Federica Accornero, Mark R Boyett, Halina Dobrzynska, Vladim F Fedorov; The Ohio State University, Columbus, OH, “University of Manchester, Manchester, United Kingdom

Background: Heart failure (HF), a leading cause of morbidity and mortality, involves significant dysfunction of the sinoatrial node (SAN). MicroRNAs (miRNAs) are abundant, non-coding RNAs that ultimately regulate protein expression at the post-transcriptional level and have been implicated in HF. However, nothing is known about the distribution and expression of miRNAs in the human SAN as well as their roles in regulating pacemaker channels and SAN dysfunction (SND) in HF. Methods: The human SAN was isolated and cryopreserved from failing hearts with implantable pacemakers (n=5) or non-diseased donor hearts (n=3) that were cardioplegically arrested in the surgery room. Utilizing immunohistochemistry, the intact 3D intramural SAN structure was precisely identified as the fibrotic region around the SAN artery containing Connexin43-negative pacemaker cardiomyocytes (Figure). Small biopsies from the central SAN compartment were used to characterize the expression pattern of 14 miRs and their associated pacemaker channels. Ingenuity software (Qiagen) was used to predict the relationship between miRs and their targeted mRNA of SAN ion channels Results: Out of 14 studied miRs, 3 miRs (mir-370-3p, mir-133a and mir-1) were significantly upregulated in SAN, but not in atria, during HF. We found that mir-370-3p was predominantly expressed in the human SAN, but not atria. Whereas, both mir-133a and mir-1 were highly expressed in atria vs SAN. All three miRs were predicted to regulate pacemaker HCN1 and/or HCN4 channel expression. RT-PCR showed that HCN1 and HCN4 mRNA were downregulated in the HF SAN. Conclusions: This is the first study to explore the miRs profiles in healthy and diseased human SAN with SND. We demonstrate that miR-370-3p, mir-133a and mir-1, thought to regulate pacemaker HCN1 channel expression, are selectively upregulated in human HF SAN. We propose that understanding the function of miRs in human SAN might lead to novel SND treatments.


Key Words: Sinoatrial node; Heart failure; MicroRNA

Cryosection of human SAN central compartment


Key Words: Sinoatrial node; Heart failure; MicroRNA
Machine Learning Detection of Obstructive Hypertrophic Cardiomyopathy Using a Wearable Biosensor

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Introduction: Hypertrophic cardiomyopathy (HCM) is a heart muscle disease characterized by left ventricular (LV) hypertrophy without a systemic etiology and is associated with heart failure, stroke and sudden death. Disease prevalence is estimated at 1:500, but ~84% remain undiagnosed. Patients with obstructive HCM (oHCM) have dynamic obstruction of the LV outflow tract and characteristic abnormalities in arterial bloodflow patterns.

Hypothesis: Arterial pulsuswaves recorded with a wearable biosensor and analyzed with machine learning algorithms could identify a signature of oHCM when compared to unaffected controls.

Methods: We compared baseline arterial pulse wave morphology, obtained by photoplethysmography using an investigational wristband biosensor (Wavelet Health, Mtn, View, CA), from oHCM patients enrolled in a digital health substudy of PIONER HCM (NCT02452424) to unaffected controls from a Wavelet Health database. Five minute recordings were obtained at rest, and data sets were divided into training and validation cohorts. A beat-by-beat machine learning model was developed using a predefined feature set to calculate an HCM probability score, and an optimal threshold score was determined. The model was evaluated using summary statistics and an ROC area-under-curve metric.

Results: Arterial pulsuswave recordings were obtained from 14 patients with oHCM at rest and 81 unaffected controls. An oHCM machine learning classifier was developed based on 42 calculated metrics. After training and cross-validation (n=9-oHCM, n=48 control), the model achieved 98% accuracy. Application of this model to a validation cohort (n=6-oHCM, n=33 control) confirmed an increased probability in oHCM patients compared to unaffected controls (0.40 ± 0.13 vs. 0.18 ± 0.10; p=0.006). Analysis of the ROC curve in the pooled cohort shows an area under the curve of 0.98. Conclusion: This first-of-its-kind study suggests that a signature of arterial bloodflow in oHCM when compared to unaffected controls can be identified with the combination of a wristband biosensor and machine learning algorithms. These data raise the possibility of a novel approach to the non-invasive detection of HCM.

Key Words: Hypertrophic cardiomyopathy; mHealth; Big Data

Induced Pacemaker Spheroids As A Model To Reverse-Engineer The Native Sinoatrial Node

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Background: The sinoatrial node (SAN) has intricate architecture, which facilitates the spontaneous action potentials generated from the SAN to pace and drive the neighboring myocardium. We sought to create an engineered SAN that recapitulates the native SAN’s ability to overcome source-sink mismatch. We hypothesized spheroids consisting of induced pacemaker cells (iPM) can pace and drive surrounding quiescent myocardium.

Methods: We compared baseline arterial pulse wave morphology, obtained by photoplethysmography using an investigational wristband biosensor (Wavelet Health, Mtn, View, CA), from oHCM patients enrolled in a digital health substudy of PIONER HCM (NCT02452424) to unaffected controls from a Wavelet Health database. Five minute recordings were obtained at rest, and data sets were divided into training and validation cohorts. A beat-by-beat machine learning model was developed using a predefined feature set to calculate an HCM probability score, and an optimal threshold score was determined. The model was evaluated using summary statistics and an ROC area-under-curve metric.

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Author Disclosures: E.M. Green; Employment: Significant; Myokardia. Ownership Interest: Significant; Myokardia. R. van Mourik; Employment: Significant; Wavelet Health. Ownership Interest: Significant; Wavelet Health. C. Woltus; Employment: Significant; Myokardia. Ownership Interest: Significant; Myokardia. S.B. Heitner; None. O. Dur; Employment: Significant; Wavelet Health. Ownership Interest: Significant; Myokardia. H.C. Cho; None. Key Words: Hypertrophic cardiomyopathy; mHealth; Big Data

Regional Assessment of Pyruvate Metabolism in the Remodeled Heart Using Dynamic Nuclear Polarization Carbon13 Magnetic Resonance

James J Pilla1, Terence Gade1, Gabor Mizsei2, Norman Butler3, Yoshiaki Saito1, Akito Imai1, Keitaro Okamoto1, Christopher L Gade2, Gabrielle Pilla1, Jerry Zsido1, II1, Joseph H Gorman1, III1, Robert C Gorman1; 1Univ of Pennsylvania, Philadelphia, PA, 2Weill Med College of Cornell Univ, New York, NY

Introduction: After infarction compensated remodeling of the left ventricular (LV) may be followed by adverse remodeling leading to heart failure. The mechanism of adverse remodeling may be linked to the elevated wall stress in the dysfunctional myocardium adjacent to the infarct (border zone BZ). We hypothesize that increased BZ stress results in altered metabolism which could drive the transition from compensated to adverse remodeling. To evaluate BZ and remote metabolism we compared the regional uptake and intracellular conversion of 1-13C-pyruvate using hyperpolarized (HP) 13C MR.

Methods: An established pre-clinical posterolateral infarct model of LV remodeling was used to investigate region metabolism. To accurately measure regional metabolism, we developed implantable carbon-tuned surface coils placed on the epicardium over the BZ and remote regions (Fig. Top). A coronary catheter was placed for direct injection of the HP substrate to maximize deliver and eliminate cavity blood pool signal. MR was performed at 6-weeks post infarct with a spectra acquired every 1.5s for each region simultaneously during HP infusion under physiologic and DOB stress conditions. The resulting spectra from each coil were analyzed to measure lactate, alanine, bicarb, and total flux.

Results: Under physiologic (Pre-DOB) conditions the percent difference between remote and BZ lactate, alanine, and total flux was only slightly elevated in the remote region whereas bicarb flux was greater in BZ compared to remote (Fig. Bottom). DOB stress produced an increase in remote metabolite flux compared to BZ with lactate, alanine and total flux reaching significance and bicarb flux shifting from greater in BZ Pre-DOB to greater in remote. Conclusion: These findings demonstrate an impaired metabolic response to pharmacologic stress in BZ myocardium which may provide a mechanism for the established association of mechanical stress and adverse cardiac remodeling following infarct.

Author Disclosures: J.J. Pilla; None. T. Gade; None. G. Mizsei; None. N. Butler; None. Y. Saito; None. A. Imai; None. K. Okamoto; None. C.L. Gade; None. G. Pilla; None. J. Zsido; None. J.H. Gorman; None. R.C. Gorman; None. Key Words: Ventricular remodeling; Cardiac metabolism; Cardiac MRI; Imaging agents; Ischemic heart disease

Late-Breaking Basic Science Posters

Therapeutic Effects of Human Pluripotent Stem Cell-derived Lymphatic Endothelial Cells Encapsulated With Nanomatrix Gel on Experimental Lymphedema

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Author Disclosures: S.I. Grijalva; None. J.H. Sung; None. B.W. Furman; None. F. Han; None. N. Li; None. N.C. Cho; None. Key Words: Sinoatrial node; Pacing

Late-Breaking Basic Science Posters

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Background: Current systems generating lymph endothelial cell (LEC) from human induced pluripotent stem cells (iPSCs) have limited value due to low purity, the use of undefined conditions for differentiation, and poor cell survival in vivo. Here, we developed a fully defined system to differentiate hPSCs into LECs and evaluated their therapeutic and engraftment potential when encapsulated in a nanomatric gel (PA-RGDS). Methods and Results: hPSCs were cultured with GSK3α inhibitor on collagen-coated plates for 2–3 days to induce differentiation into the mesodermal lineage. The mesodermally differentiated cells were then cultured with VEGF, VEGFR, EGF, and SDFγ for another 6 days and double-sorted by PDPP and FL14. These hPSC-PDPP+FL14+ cells (hPSC-derived lymphatic endothelial cells, hPSC-LECs) showed highly purified and fully functional LEC characteristics in vitro. These hPSC-LECs express LEC markers such as PDPP, LYVE1, PROX1, and FL35 and at the mRNA and protein level, and formed tube-like structures in Matrigel. We next determined the lymphatic vascular reparative effects of engineered hPSC-LECs. After inducing lymphedema in the tail of mouse, hPSC-LECs were encapsulated with PA-RGDS and transplanted with or without PA-RGDS compared to the other groups at day 28. At day 45, mice injected with PA-RGDS encapsulated hPSC-LECs showed significant decrease in the tail diameter compared to all other groups including those injected with hPSC-LECs. Histological examination demonstrated that the skin thickness was significantly reduced and the density of lymphatic vessels was markedly increased when the hPSC-LECs were encapsulated with PA-RGDS compared to others. Conclusion: This study demonstrated for the first time that hPSC can be differentiated into LECs in a clinically compatible manner with a high yield. Furthermore, nanomatrix encapsulated hPSC-LECs can substantially improve lymphedema in mouse tail through enhancement of cell survival and lymphatic neovascularization. This engineered hPSC-LEC therapy represents a novel option for treating lymphedema.

Author Disclosures: S. Lee: None. Y. Sohn: None. D. Sohn: None. Y. Yoon: None.
Key Words: Stem cell therapy; Lymphedemic disease; Valvular myocardial ischemia, promoting cellular functions such as proliferation, glucose metabo-
lism and angiogenesis. Therefore, we investigated the role of Zmynd8 in the pathobi-
logy of cardiac ischemia in a model of HIF-1 activation.

Hypothesis: Zmynd8 modulates the HIF-1 response in ischemic heart disease by inhibiting enhancer activity of HIF-1 target genes.

Methods & Results: We expressed an inducible cardiac-specific, oxygen-stable form of HIF-1α in a mixed strain of mice that genetically express increased Zmynd8 (Z+). Compared to HIF-induced wild-type (WT) mice, these mice did not exhibit the expected HIF-1 phenotype of increased heart weight to body weight (6.4±0.3 vs. 4.4±0.2 vs. 5.1±0.3 mg/g WT vs. Z+ vs. uninduced; p<0.05) with reduced ventricular function and as-

associated chamber dilation. Semi-quantitative qPCR analysis of HL-1 and H9C2 cardiac cell lines transduced with CMV-Zmynd8 expression plasmids for Zmynd8 and oxygen-stable HIF-1α resulted in striking reduction of multiple HIF-1 target genes such as PDK1 (45% reduction) compared to HIF-1α plasmid alone. RNA mediated knockdown of Zmynd8 alleviated this negative regulation (65% increase). Bioinformatic analysis of human Zmynd8 and HIF-

1α ChIP-seq data indicates that Zmynd8 binds to the enhancer of 78% of HIF-1 regulated genes. This further supports our observation that Zmynd8 modulates HIF-1 activity in the heart.

Conclusion: We have discovered a new regulator of HIF-1 action that modifies the hypoxic response, likely through chromatin remodeling. We suggest that this new form of regulation can modify the pathophysiology of ischemia and potentially provide new targets for therapy.

Key Words: Hypoxia; Epigenetics

Differential, Sex Related, Response To High-fat Diet In Mice Impairs Cardiac Autophagy And Mitophagy

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Introduction: Premenopausal women, as well as females in animal studies, have a reduced risk of cardiovascular diseases and a reduced myocardial susceptibility to ischemia/reperfusion (I/R) injury. However, with constantly increasing prevalence of obesity, the impact of obesity-induced female on cardiac autotophagy remains unclear.

Methods & Results: We determined autophagy and mitophagy pathways in both female cardiac protection and deleterious effect of obesity. Methods: Male and female C57BL/6J mice (n =20 per group) of 8 weeks old were fed a low-fat (LFD, 10% fat) or high-fat (HFD, 60% fat) diet for 12 weeks. At 12 weeks, hearts from mice were studied under basal conditions and after global no-flow ischemia and reperfusion on a Langendorff perfusion system. Results: In both males and females, HFD significantly increases body weight (31.4 vs 42.3g and 22.1g vs 39.9g respectively), fat mass (+200% in males vs +100% in females) and glucose (+75mg/dl and +54mg/dl, respectively) (p<0.01). HFD tends to decrease flow reperfusion after global no-flow ischemia and to increase infarct size (p=0.04) in both sexes. In males and females, HFD increases pS6K75-UK1 and decreases Parkin levels under basal conditions (all p<0.006). Moreover, HFD tends to decrease pThr172-AMPK only in females. After I/R, Parkin levels remain lower in HFD groups without sex-difference (p<0.004) but a drastic increase of LC3-II occurs only in females under HFD (p<0.047). Conclusion: All together, these results suggest an impairment of autophagy and mitophagy pathways under HFD. A more drastic change occurs in females as HFD results in a more pronounced cardiac autophagy.

Key Words: Ischemia reperfusion; Obesity; Sex differences; Autophagy; Mitochondria

ZMYND8 Regulates the Hypoxic Response in Cardiomyocytes

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Introduction: The Zmynd8 gene contains a variety of recognized motifs including a bro-
madomain and PHD finger, which are implicated in cooperative binding to acetylated nucleosomes. It was recently identified as a key regulator of cancer progression and tran-
scriptional repression by directing modification of histone methylation in the enhancer regions of genes. Mutations and dysregulation of Zmynd8 have been found in multiple cancer types, however its role in the cardiac myocardium is unclear. Hypoxia-inducible factor 1α (HIF-1α) regulation and stabilization is a common feature of both cancer and 2017 AHA Late-Breaking Basic Science Abstracts

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20460 Alpha Calcitonin Gene-Related Peptide (CGRP) Protects Against Pressure-induced Heart Failure

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Introduction: Calcitonin gene-related peptide (CGRP), a 37 amino acid neuropeptide, is a potent vasodilator, and plays a critical role in the efferent functions of the sensory nervous system. A protective role for CGRP in cardiovascular diseases (hypertension, cardiac isch-

emia, and failure) has been well established by our laboratory and others. In the present study we determined whether long-term exogenous administration of α-CGRP protects against pressure-induced heart failure.

Method: Three groups of nine-week-old C57BL/6J mice were studied: one group received a sham procedure (n =4) and two groups underwent transverse aortic constriction (TAC). Two days after TAC, one group had CGRP–fitted osmotic pumps (4 mg/kg bw/day) implanted subcutaneously (n=7) while the second group was TAC only (n=7). At day 28, mice were evaluated for echocardiographic and hemodynamic parameters. Results: Echocardiographic and histological data showed that TAC markedly decreased fractional shortening (FS) and ejection fraction and increased heart and lung weight, cardiac hypertrophy, and fibrosis compared to sham. However, the TAC-CGRP mice had preserved cardiac function and less cardiac fibrosis (FS ±SEM: sham 36.6±1.2% vs TAC 25.4±1.1%, p<0.001; and TAC 24.5±1.1% vs TAC-CGRP 36.6±1.2%, p<0.001). CGRP significantly reduced apoptotic cell death and lipid peroxidation (an oxida-
tive stress marker measured by malondialdehyde and 4-HNE staining) in the TAC hearts (p=0.007 vs sham). Western blot analysis showed that TAC-CGRP hearts had lower p-AMPK and nuclear Sirt1 level, regulatory proteins of energy metabolism. Conclusion: Our results suggest that CGRP mediated through energy metabolism, and oxida-
tive stress pathways, decreases myocyte apoptosis and is protective in pressure-induced heart failure. Thus, CGRP is a potential therapeutic agent in preventing the progression of heart failure.

Key Words: Heart failure; Cardioprotection; Cardiac hypertrophy; Cardioprotective drugs

Targeting Trpv4 Channels Protects Heart From Pathological Remodeling Following Myocardial Infarction

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Heart failure is one of the leading causes of death which is often characterized by patho-
lological fibrosis. Cardiac remodeling following myocardial infarction is a multiphase repara-
tive process which involves replacement of damaged tissue with physiological (reparative) fibrosis to form scar that limit the expansion of the infarct. Myofibroblasts are critical mediators of this process, however, their function and role in pathological fibrosis leading to heart failure. We have previously demonstrated that the mechanosensitive ion channel TRPV4 (transient receptor potential vanilloid channel 4) regulates cardiac fibroblast differentiation into myofibroblasts. However, the physiological or translational significance of TRPV4 in cardiac remodeling following MI is unknown. To further explore this, we have induced MI (permanent LAD ligation) in WT and TRPV4–/– mice and measured cardiac function for 8 weeks. Separately, WT mice were given an orally active..
TRPV4 antagonist GSK1939374, immediately after MI surgery and followed for 5 weeks. 2D-echocardiographic revealed that the cardiac function (ejection fraction and fractional shortening) is preserved post-MI in both TRPV4KO and GSK1939374-treated WT mice compared to either WT or vehicle treated mice. Further, we found reduced cardiac fibrosis at infarcted and remote zones in TRPV4KO and GSK1939374-treated WT mice compared to their MI control parts. Furthermore, TRPV4KO hearts exhibited decreased cardiac apoptosis (TUNEL assay) and increased capillary density (CD31 staining) post-MI compared to WT hearts. Our results thus suggest that targeting TRPV4 protects heart from myocardial infarction-induced damage by preserving cardiac structure and function via reduced myocyte apoptosis, diminished fibrosis and increased revascularization, and identifies TRPV4 as a novel therapeutic target for heart failure.

This research has received full or partial funding support from the American Heart Association.


Key Words: Ion channels; Fibrosis; Myocardial infarction; Cardioprotection

Titin Truncating Variants Predict Life-threatening Arrhythmias in Patients With Dilated Cardiomyopathy


Introduction: There is an urgent need for better arrhythmic risk stratification in non-ischemic dilated cardiomyopathy (DCM), where the benefit of ICD implantation is unclear. Titin truncating variants (TTNtv) are the commonest genetic cause of DCM and are associated with early-onset non-sustained ventricular tachycardia and atrial fibrillation (AF) in these patients. Hypothesis: We hypothesize that TTNtv status can predict potentially life threatening ventricular tachycardia (VT) or fibrillation (VF) and development of new persistent AF in DCM patients with CRT-D or ICD devices. Methods: We studied 117 DCM patients with an ICD or CRT-D and documented device-recorded arrhythmia over a median period of 4.2 years. Patients were stratified by TTN genotype (28 positive for a TTNtv, 89 negative). The primary outcome was time to first device-treated VT >200bpm or VF. Secondary outcome measures included time to first development of persistent AF. Results: TTNtv predicted the risk of receiving an appropriate ICD therapy for VT/VF hazard ratio (HR) = 4.9, 95% confidence interval [CI]=2.5–9.7, P<0.0001]. TTNtv were also a risk factor for developing new persistent AF (HR = 4.4, 95% CI = 1.45–14.3, P=0.006). Conclusion: TTN tv status is an important risk factor for clinically significant arrhythmia in patients with DCM and CRT-D or ICD devices. TTN tv status alone, or more powerfully in combination with fibrosis imaging by MRI, may provide an effective approach for risk stratifying the need for ICD therapy in DCM patients.


Key Words: Genetics; Ventricular arrhythmia; Cardiomyopathy; Heart failure; adult; Ventricular tachycardia

Microna-125b-5p Protects The Heart From Acute Myocardial Infarction By Repressing Pro-apoptotic Bak1 And Klf13 In Cardiomyocytes

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Cardiac injury is accompanied by dynamic changes in the expression of microRNAs (miRs), small non-coding RNAs that post-transcriptionally regulate target genes. MiR-125b-5p is down-regulated in patients with end-stage dilated and ischemic cardiomyopathy and has been proposed as a biomarker of heart failure. We previously showed using the [β-areinstin-β arrested-2 (βAR2)]-cardiomyocyte-mediated cardioprotective signal- ing through β-areinstin1 stimulates processing of miR-125b-5p in the mouse heart (Figure A-C). We hypothesize that [β-areinstin receptor]-β-areinstin1-responsive miR-125b-5p confers cardioprotection against acute myocardial infarction. Using cultured cardiomyocyte (CM) and in vivo approaches, we show that miR-125b-5p is an ischemic stress-responsive protector against CM apoptosis. CMs lacking miR-125b-5p exhibit an increased sensitivity to stress-induced apoptosis, while CMs overexpressing miR-125b-5p have increased phospho-AKT pro-survival signaling. Moreover, we demonstrate that loss-of-function of miR-125b-5p in the mouse heart causes abnormalities in cardiac structure and function after acute myocardial infarction. Mechanistically, cardioprotection elicited by miR-125b-5p is in part attributed to repression of the pro-apoptotic genes Bak1 and Klf13 in CMs (Figure D). In conclusion, these findings reveal a pivotal role for miR-125b-5p in regulating CM survival during acute myocardial infarction.

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Key Words: MicroRNA; Cardioprotection; Receptor-mediated signaling; Apoptosis

Naturally Occurring Hypertension is Related to Cardiac Diastolic Dysfunction in Rhesus Monkeys

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Introduction: The lack of effective treatment for Diastolic Dysfunction (DD) is partially due to the differences between widely used preclinical rodent models and humans in the physiology and function of the heart. In previous studies, we have demonstrated that rhesus monkeys with naturally occurring adult onset Type 2 Diabetes (T2DM) frequently have DD that is similar in characteristics to DD in diabetic patients. To further characterize DD in rhesus monkeys, we studied the relationship between hypertension and DD, and evaluated the response of rhesus monkeys with DD to Entresto (sacubitril/valsartan). Methods: Blood pressure, fasting plasma glucose and cardiac function were measured in 322 adult rhesus monkeys (Macaca mulatta, 7–22 yrs) under light anesthesia with ketamine. Monkeys with LV hypertrophy, e<5cm/s and E/e'<10 were defined as DD. Ten monkeys with DD were enrolled in the validation study and divided into the Entresto group (n=5) and the vehicle group (n=5). Cardiac function and blood pressure were measured before and at the end of 13 weeks of treatment. Results: Among the 322 adult rhesus monkeys studied, 53 monkeys (16.4%) had SBP>140 mm Hg or DBP>90 mm Hg. Among the 174 monkeys with fasting glucose >80 mg/dl, 67 monkeys had isolated DD, and 8 had DD+ SO (diastolic dysfunction). The incidence of isolated DD was 31% in monkeys with SBP<140 mm Hg and 74% in monkeys with SBP>140 mm Hg. Following Entresto administration (1.66 to 3.33mg/kg) for 13 weeks, DD, and BP evaluation showed an increase of e'(5.27±0.51) to 6.41±1.7 (cm/s), a decrease of E/e' (12.74±2.23 to 10.53±2.9) and a decrease of SBP (128±5 to 113±16 mm Hg). These parameters remained stable and unchanged in the vehicle group. Conclusions: The incidence of naturally occurring hypertension in adult rhesus monkeys was similar to that in adult humans. Entresto reduced blood pressure, but led to no significant improvement of DD in monkeys. The extent of change in rhesus monkeys was similar to that observed in clinical trials. In rhesus monkeys, as in patients, hypertension is significantly related to cardiac diastolic dysfunction. These monkeys, therefore, provide important new opportunities to understand the pathogenesis of DD, as well as to predict the human response to new therapeutic agents.


Key Words: Diastolic function; Hypertension; Drugs; Diabetes (Type II)

Longitudinal Evaluation of the Associations between Severe Hypertriglyceridemia and Cardiovascular Features

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Introduction: For at least 35 years the associations between insulin resistance, hyperin- sulinemia, hypertriglyceridemia and hypertension have been described. Nevertheless, the existence of an independent association between hypertriglyceridemia (HTG) and cardio- vascular diseases (CVD) remains controversial. This may be due to the insufficiency of lon- gitudinal study data that include severe elevations of TG. The purpose of this study was to characterize the associations between systolic blood pressure and various cardiovascular features under different degrees of naturally-occurring HTG severity and to determine the
independent effect of severe HTG. Methods: We characterized TG in a large cohort of longitudi-

dually studied nonhuman primates (n=227; 164 males) maintained for their lifetimes under

different conditions of energy intake and dietary choices. The TG ranged from normal TG levels <100

mg/dl, to mild HTG 100–200 mg/dl, high HTG 200–500 mg/dl, very high 500–1000 mg/dl and severe

>1000 mg/dl. The Kruskal Wallis H test was applied, as the data samples were not normally distributed. Results: Severe hypertriglyceridemia was statistically significantly related to systolic blood pressure and was significantly higher (p<0.0001) at the severe HTG levels of 500–1000 mg/dl and >1000 mg/dl compared to all other TG levels. Diastolic blood pressure and mean arterial blood pressure were not significantly different among the normal to severe HTG groups. HDL cholesterol was significantly lower (p<0.0001) at all levels of TG (100–200, 200–500, 500–1000 and >1000 mg/dl) compared to normal TG <100 mg/dl, and was not related to the severity of the HTG, a finding similar to the relationship with body weight. By contrast, LDL cholesterol was significantly higher (p<0.05) in monkeys with severe HTG >500–1000 mg/dl, as was systolic blood pressure. Conclusions: In conclusion, much of the association between triglyceride levels and cardiovascular features, including blood pressure, may be principally determined by the severely elevated triglyceride levels, possibly highlighting the importance of longitudinal within subject evaluation of such as-

sociations for break points in the intersections of these features.

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Mhaskar: None. B.C. Hansen: None.

Key Words: Triglycerides; Hyperlipidemia; Hypertension

23931

Selective Inhibition Of Hdac3 Prevents Diabetic Cardiomyopathy

In Ove66 Mice Via Mir-200a-mediated Mrz Activation

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Although histone deacetylases (HDACs) were an important target for the initiation and development of diabetic cardiomyopathy (DCM), which isomorphs of them plays the key role remains unclear. The present study was designed to determine whether DCM could be pre-

vented by selective inhibition of HDAC3 and the underlying mechanism. Male type 1 diabetic OVE26 and age-matched wild-type mice were given the selective HDAC3 inhibitor (HDAC3i)

RGFP966 or vehicle for 3 months before the cardiac function was examined with Echo. Results

indicated that HDAC3i treatment improved cardiac function in the diabetes group. HDAC3 activity was significantly increased in the heart of diabetic mice, which was blocked by the treatment of HDAC3. Oxidative stress as a major cause of DCM is also inhibited by HDAC3. Mechanistically cardiac mr-200a, which targets and destabilizes kuch-kuch like E3-associated protein (KEAP1), was significantly up-regulated and KEAP1 expression was markedly inhibited by HDAC3 in the diabetic mouse. Immunoprecipitation shows that the binding of KEAP1 in the diabetic heart was decreased by the treatment of HDAC3 when we pull down nuclear factor-E2-related factor 2 (NRF2). Meanwhile, the nuclear localization of NRF2 and its downstream anti-oxidative stress genes, NADPH quinine oxidoreductase 1 (NQO1), heme oxy-
genase-1 (HO-1), were markedly up-regulated in the HDAC3i treated diabetic mouse group.

Author Disclosures: Z. xu; None. Y. zheng; None. Z. zhang; None. J. sun; None. L. cai; None.

Key Words: Cardioprotection; Diabetes (Type II); Antioxidant

24010

Muscle-specific A-Kinase Anchoring Protein

Polymorphisms Pre-dispose Humans to Cardiovascular Diseases by Affecting Cyclic AMP/PKA Signaling

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In-depth understanding of key cardiac signaling pathways is crucial in finding new targets for cardiovascular disease (CVDs). The no 1 cause of death globally. One of such path-

ways is cAMP-dependent PKA signaling which is modulated by scaffold proteins, A-kinase anchoring proteins (AKAPs). Muscle-specific AKAP (mAKAP) regulates expression of hyper-

trophic factors partly by controlling cardiac cAMP levels. Furthermore, published literature revealed a strong correlation between single nucleotide polymorphisms (SNPs) in proteins and risk of developing CVDs by varying cAMP/PKA signaling. Here, we have successfully differentiated multiple genetic effects to finalize two SNPs, Ser(S)1653Arg(R) in PDE4D3 binding domain and Glu(E)2124Gly(G) in 3`-PKA binding domain of mAKAP. After making both mAKAP mutant plasmids from WT using site-directed mutagenesis, we studied them in HEK 293T cells. Four separate samples were used for each experiment. In immunoprecipitation studies, S1653R mutant showed increased binding to PDE4D3 at baseline but significantly reduced binding after stimulation with 1 μM isoprostane as compared to WT. Similarly, E2124G mutant exhibited significantly lower PKA binding at baseline and higher binding after stimu-

lation. cAMP levels and PKA activity were significantly lower at baseline but higher after stimulation in S1653R mutant cells. Also, E2124G expressed cells showed no significant change in cAMP levels when compared to WT but PKA activity was significantly lower at baseline. Also we found that mAKAP SNPs may predispose humans to the risk of developing CVDs by affecting cAMP/PKA sig-

naling and thus confirming the clinical significance of PKA-mAKAP-PDE4D3 interaction.

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Key Words: Cardiovascular disease; Cell signaling; Cardiovascular health; Molecular biology; Gene mutations

24068

Developing a Cardiomyocyte Pipeline for Gene Edited hiPSCs

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The Allen Institute for Cell Science (AICS) is creating an open source collection of fluores-

cently tagged human induced pluripotent stem cell (hiPSC) lines to model cell organization and dynamics of stem cells and differentiated hiPSC-cardiomyocytes. Understanding the sub-cellular organization and the structure/function relationships of organelles within cardiomyocytes will contribute to the development of better disease models, therapies, and regenerative medicine approaches for cardiac disease. Using the WTC human iPSC line and the CRISPR/Cas9 system, we have fluorescently tagged ~20 target genes represent-

ing key cellular organelles including a few cardiac-specific genes. Edited iPS lines are differentiating into cardiomyocytes using established protocols with either small molecules or a combination of cytokines and small molecules, which produce high-purity monolayers of beating cardiomyocytes within 1–2 weeks. Differentiation into cardiomyocytes serves as an important quality control criterion for our gene editing efforts, but also comprises an important aspect of our predictive cell modeling efforts. We plan to study the changes in localization and organization of these tagged organelles as the stem cells differentiate into cardiomyocytes using live fluorescent cell imaging. Here, we present our cardiac differen-

tiation methods for multiple edited hiPSC lines and the quantitative and qualitative assays used to determine the efficacy of differentiation, including myofibril contraction, cardiac protein expression, and transcriptome profiling by bulk and single cell RNAseq. Additionally, we confirm the localization of cardiac proteins such as troponin T and alpha-actinin to the myofibrils in differentiated cells using image-based assays. In experiments initiated to date we have successfully differentiated multiple gene edited iPSC lines representing major cellular Structures, 2 of which are specific to cardiomyocytes (sNK1V and ACTN2). Some of the gene edited hiPSC lines are fluorescently-tagged for structures including focal adhe-

sions, actin and microtubule cytoskeleton, mitochondria, nuclear envelope, desmosomes, and endoplasmic reticulum, which are all publically available to the community.


Key Words: Stem cells; Cellular Engineering; Cardiac development; Stem cell biology

24063

Heart Fields Are Induced by Coordinated Activity of Wnt and Bmp Signaling and Identified by Cd184 and EphA2 in Psc-Derived Organoids

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Over the past few decades, major advances have been made in identifying the origins of cardiac cells from developing embryos. In particular, the discovery of the first heart field (HF) and the second heart field (SHF), led us to understand how diverse lineages and dif-

ferent anatomical structures of the heart arise during cardiogenesis. However, it remains unknown how the two heart fields are specified and segregated, a fundamental step toward understanding heart formation and developing pluripotent stem cell (PSC)-based therapeu-

tic strategies. Here, we generated PSC lines from two different human tissues, the somites, the heart anlagen, and generated both non-fluorescent protein (GFP and RFP) reporters under the control of the FHF marker Hcn4 and the SHF marker Tbx5, respectively. We demonstrate how GFP+ cells and RFP+ cells appear from two distinct areas of mesodermal cells and develop in a complementary fashion, simi-

lar to the two heart fields process. Consistently, these populations exhibit a high degree of similarities with FHF/SHF SHF cells isolated from early embryos, determined by RNA-sequencing analysis. Through a series of bioinformatics approaches, we found that Bmp and Wnt are among the most differentially regulated pathways in the two populations. Importantly, an increased activity of Bmp or Wnt signaling resulted in selective induction of PSC+ or PEP+ cells within mesodermal cells, enabling us to generate heart field-specific cells from PSCs. We further found that FHF/SHF cells can be distinguished and isolated by the surface proteins CD184 and EphA2. This study provides fundamental insights into understanding the specification of two cardiac origins that enable generation of chamber-specific populations for studying heart field/chamber-specific heart disease in cell culture.


Key Words: Stem cell biology; Cardiac development; Stem cells; Progenitor cell
A Combined Basic Science and Population Science Approach Demonstrating the Potential for Simvastatin to Mitigate Cardiomyocyte Disease after Lower Hemi Body Radiotherapy

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Radiation is a cornerstone of successful cancer treatment, with one-half to two-thirds of all patients receiving radiation therapy. Survivors of cancer treated with radiation are at increased risk for cardiovascular disease (CVD). Understanding how radiation causes CVD will allow development of novel therapies. Irradiation of the lower hemi body, but not the upper hemi body, with 10 Gy in rats increases risk factors for CVD and results in cardiac fibrosis qualitatively similar to total body irradiation, suggesting radiation injury to the heart can be indirect. Simvastatin, an inhibitor of liver cholesterol synthesis administered continuously to rats (10 mg/kg/day) after 10 Gy lower hemi body irradiation mitigated against increased blood cholesterol and cardiac fibrosis. These findings indicate simvastatin limits transmission of a signal from the lower hemi body that decreases risk for and occurrence of CVD independent of any direct exposure of the heart to radiation. Bivariate examination of 3,607 patients following therapeutic lower hemi body irradiation using Chi-square, Wilcoxon rank-sum and t-tests was used to examine risk factors for CVD in patients diagnosed with congestive heart failure, myocardial infarction, atrial fibrillation, and cardiomyopathy before 80 years of age. We found that 47.4% of patients age 70–80 developed CVD compared to 29.7% who received simvastatin (p = 0.001, n = 293 and 361, respectively). Patients who were male, overweight, smokers, and had hypertension were not indicative of increased risk for CVD. Race and hypertension were not indicative of increased risk for CVD. These clinical findings, taken together with the results from our animal studies, support a new research paradigm where radiation-induced heart disease can be indirect, with abdominal organs exporting factors that cause CVD. Simvastatin can be developed to mitigate and treat CVD after therapeutic radiation.

Human iPSC-Derived Endothelial Cells Predict Predilection to Atherogenesis by Endothelial Proinflammatory Activation

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Introduction: Coronary artery disease (CAD), the direct outcome of atherosclerosis, is the leading cause of death in the United States. Previous studies demonstrated that impaired function of aldehyde dehydrogenase 2 (ALDH2), a key enzyme for alcohol metabolism, is linked to increased susceptibility to CAD. A single-nucleotide polymorphism that generates E487K mutation (ALDH2*2) reduces enzymatic activity of ALDH2 to less than 40% of the wild type (WT) and is present in ~560 million people. However, it remains unclear how ALDH2 regulates atherosclerotic progression. Hypothesis: Recent studies suggest a critical role of ALDH2 in plaque development and endothelial activation. Therefore, we hypothesize that endothelial cells of ALDH2*2 carriers possess greater susceptibility to proinflammatory activation, whereby endothelial cells recruit immune cells, leading to increased risk of atherogenesis. Methods: To study the patient-specific effects of ALDH2*2 mutation on endothelial proinflammatory activation, we generated and characterized iPSC-derived endothelial cells (iPSC-ECs) from 5 WT subjects and 5 ALDH2*2 subjects. We exposed the iPSC-ECs to pro-inflammatory conditions and assessed the level of endothelial proinflammatory activation by gene expression analysis and monocyte adhesion assay. Results: Our preliminary data show ALDH2*2-iPSC-ECs exhibit impaired ALDH2 function resulting in metabolic dysregulation compared to WT. Presence of ALDH2*2 mutation resulted in enhanced inflammatory response in the iPSC-ECs when treated with proinflammatory cytokines such as TNFa and IL-1β, as evidenced by up-regulation of cell adhesion molecules and increased adher- ence to monocytes. The ALDH2*2-iPSC-ECs also exhibited an increased basal expression of vascular endothelial growth factor receptor 1 (FLTR1) gene, which was further augmented upon inflammatory stimulation. FLTR1 is a receptor for vascular endothelial growth factor ligands, playing a critical role in endothelial homeostasis and biology. Conclusion: Taken together, we elucidate the effects of impaired ALDH2 function on increased susceptibility to atherogenesis by endothelial proinflammatory activation using patient-derived iPSC-ECs.

Sacubitril/Valsartan Attenuates Fibrosis and Improves Left Ventricular Function in a Rabbit Model of HFrEF

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Background: Sacubitril/Valasrtan (SAC/VAL), a drug combining a nephrilysin inhibitor and an angiotensin receptor blocker, was shown to reduce myocardial infarct size and left ventricular (LV) dysfunction in preclinical models of myocardial infarction (MI). In the PARADIGM-HF trial, SAC/VAL prevented the clinical progression of patients with heart failure (HF) more effectively than enalapril. Whether SAC/VAL attenuates cardiac fibrosis and improves LV function in a rabbit model of MI-induced HF with reduced ejection fraction (HFrEF) is unknown. Methods: Anesthetized adult male NZW rabbits (~2.5kg) underwent left thoracotomy and the left anterior descending (LAD) coronary artery was identified and occluded for 45 min followed by reperfusion. Weekly echocardiography was performed to confirm reduced EF (~40%), which was uniformly achieved at 5 weeks post MI. Subsequently, rabbits were randomized to orally receive placebo (volume-matched water, BID), SAC/VAL (10 mg/kg, BID) or VAL (0.1mg/kg/day) starting on week 6. At 10 weeks post MI, rabbits were sacrificed and hearts were harvested, fixed with 10% formalin and embedded in paraffin to assess myocardial fibrosis (Picosiris red staining). Operators performing echocardiography, Picosiris red staining and analysis were blinded to treatment allocation. Results: Two weeks after treatment initiation, a significant improvement in LVEF was observed in the SAC/VAL group compared to both placebo and VAL, a benefit that lasted throughout the entire study (Fig A). The functional improvement observed was associated with a significant reduction in LV scar size compared to placebo at week 10 (Fig B). However, when compared to VAL, the decrease in scar size did not reach statistical significance despite a clear trend. Conclusion: Our results suggest that SAC/VAL may offer superior benefits compared to equivalent dose of stand-alone VAL, in attenuating LV scar size and improving LVEF in a rabbit model of ischemic HFrEF.

Need of Treating Residual Inflammatory Activity in Coronary Heart Disease: The Value of High Sensitive CRP and LDL in a Real World Cohort

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Introduction: Inflammation drives atherosclerosis and its complications. Thus, CANTOS as the first anti-inflammatory outcome trial in this population produced positive results. However, it is unclear how many patients qualify for an anti-inflammatory therapy in everyday practice. Hypothesis: This study analyzes how many patients with coronary heart disease (CHD) on
guideline conformance therapy show an increased residual risk in order to define the need for an anti-inflammatory treatment in a real world setting. Methods: High sensitive C-reactive protein (hsCRP) and low density lipo-protein (LDL) levels were determined in 700 all comer patients between June 2016 and June 2017 in our center. Patients lacking CHD, such with chronic-inflammatory diseases, acute inflammation, and on immunosuppressive medication were excluded. Patients were divided in the following groups: elevated hsCRP (≥2mg/dl), normal hsCRP (≥2mg/dl), off target LDL-cholesterol (≥70mg/dl), on target LDL-cholesterol (<70mg/dl), Univariate logistic regression and backward selection was performed in order to define factors influencing hsCRP. Results: From 700 patients 221 fulfilled the inclusion and exclusion criteria. HsCRP was increased in 45% of these patients. Patients with on target LDL Levels showed lower hsCRP concentrations than those with off target values of LDL confirming a positive association between both, (1.92mg/dl vs. 3.15mg/dl, p=0.005). However, despite guideline-conform LDL control 34% of patients with a LDL-cholesterol <70mg/dl had elevated levels of hsCRP (≥2mg/dl) suggestive of residual inflammation. After logistic univariate regression LDL-cholesterol ≥70mg/dl (OR 2.15, p=0.014), heart failure (OR 3.07, p<0.001) and diabetes mellitus (OR 2.22, p=0.021) independently predicted elevated levels of hsCRP. Heart failure (OR 4.56, p<0.001) and diabetes together (OR 3.04, p=0.012) identified as co-precursors increased hsCRP following the backward selection. Conclusions: A substantial part of patients with CHD shares a residual inflammatory risk defining a need for an anti-inflammatory therapy. Residual inflammation is particularly prevalent in patients with heart failure and diabetes.


Key Words: Arteriosclerosis; Inflammation and inflammatory markers; LDL; Cardiovascular disease; Cardiovascular therapeutics

Cardiac-Specific Overexpression Of Caveolin-3 Expedites Cardiac Relaxation After Adrenergic Stimulation

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Introduction: Caveolae are membrane localized signaling platforms that compartmentalize signal transduction proteins such as GPCRs. Previous studies from our laboratory have demonstrated that overexpression of caveolin-3 in cardiac myocytes (Cav3 OE) protects against pressure-overload induced heart failure. Additionally, Cav3 OE mice present increased heart rate variability with lower nocturnal heart rates, but the mechanisms behind these phenotypes remain unclear. Hypothesis: Since Cav3 OE mice are protected from stressful cardiac stimuli, we tested the rationale that Cav3 OE hearts may show altered parasympathetic control of cardiac responses to adrenergic stimulation. Methods: Cav3 OE mice and transgenic negative littermate controls (Cht) (12-16-week-old, n=10–11 each) were anesthetized with isoflurane and cardiac contractility was assessed by echocardiography at baseline. Isoprenaline (Isuprel USP [Iso], 300 mg/l p.m.) was administered –10 min after baseline recordings and cardiac function recorded at 2 min, 5 min, and 10–15 min after Iso. The same animal cohort received a single atroion injection (2 mg/kg) i.p 20 min before isoflurane anesthetization and iso challenge echocardiography was performed as described above. Results: At baseline, no differences in cardiac contractility (% ejection fraction, %EF±SD) were detected between the two groups –68±8 %EF. After isoproterenol injection cardiac contractility was increased to ~91±3 %EF in both genotypes at 2 min; however, in Cav3 OE mice cardiac contractility recovered to 83±3 %EF by ~68±8 %EF. After iso challenge whereas Cht animals maintained increased contractility of 92±3 %EF (p = 0.001). Importantly, Cav3 OE mice pre-treated with atropine no longer showed increased recovery at 5 min or 10–15 post iso (p = 0.007). A three-way ANOVA of the time-course after iso injection found a significant effect of atropine on the responses of Cav3 OE versus Cht hearts to iso (p = 0.007). Conclusion: We show for the first time that Cav3 OE mice show a faster recovery from hypercontractility after isoproterenol stimulation. Since atropine abrogated this recovery, these data suggest that Cav3 OE mice exhibit increased parasympathetic response to isoproterenol stimulation. Since atropine abrogated this recovery, these data suggest that Cav3 OE mice exhibit increased parasympathetic response to isoproterenol stimulation.


Key Words: Heart function tests; Adrenergic; Pharmacology; Heart rate; Heart rate variability; Cardioprotection

MFG-E8 Fragment Medin and Arterial Aging

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Background: Aging increases milk fat globule EGF-VIII (MFG-E8) expression in the rat, nonhuman primate and human aortic walls, facilitating the invasion, proliferation, and pro-inflammation of vascular smooth muscle cells (VSMCs). The MFG-E8 C-terminal fragment medin has been reported to be involved in the necrosis of VSMCs at the inflammatory sites of aortic dissection, however, its cleavage processes, aortic wall levels with aging and bio-role in VSMCs remains to be determined. Material and Methods: In this study, immunofluorescence, immunohistochernistry and western blot analysis demonstrate that MFG-E8 and its fragment medin (Figure), activated matrix metalloproteinase type II (MMP-2) and platelet derived growth factor receptor type-beta (PDGFR-β) protein levels were markedly increased in human grossly normal aortic walls from old (n = 10, >50 yrs) vs. younger donors (n = 10, <25 yrs). Importantly, exposure of medin peptide (20 to 100 nM) to primary cultured VSMCs isolated from young (8 mo) and old (40 mo) FBSK rat aortae significantly increased MMP-2 activation, PDGFR-β expression, and migratory capacity measured by a modified Boyden chamber in a dose-dependent manner in both also. Separate exposure of activated MMP-2 to recombinant human MFG-E8 protein and also to MFG-E8 enriched old human aortic protein markedly increased the cleavage product medin in both, which was substantially inhibited by an MMP inhibitor, DAS0101. Exposure of medin to VSMCs did not significantly affect the expression of cell cycle related proteins. In addition, PDGF-BB treatment markedly activated MMP-2 in both young and old VSMCs, which was substantially reduced by a PDGFR-β inhibitor, RTK. Conclusion: Taken together, targeting MFG-E8 or its cleavage product medin is a novel approach to the prevention or treatment of large arterial aging or age-associated disease.

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Key Words: Aging; Arteries; Cardiovascular disease

Utilizing Telemedicine to Construct Population-Based STEMI Systems of Care in Developing Countries

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Background: Major disparities exist between developed and developing countries in the outcomes of patients presenting with Acute Myocardial Infarction (AMI). Telemedicine has emerged as a powerful, cost-efficient, and scalable tool. Whether telemedicine can improve AMI results remains unclear. Methods: We incorporated a hub and spoke strategy for Latin America Telemedicine Infarct Network (LATIN) to expand access to care in regions in Brazil, Colombia, and Mexico that lacked AMI care. Small clinics and primary care health centers in remote areas (spokes) were strategically connected to hubs that could perform 24/7 primary PCI. Experts at 3 central sites in Uberlandia, Sao Paulo, and Bogota, provided urgent EKG diagnosis and tele-consultation for the entire LATIN network by triggering ambulance dispatch and implementing standardized AMI protocols. Results: A total of 257 LATIN centers (Brazil 95, Colombia 113, Mexico 48) were networked using similar telemedicine protocols. In Colombia, LATIN coverage was established to cover 31% of the nation’s 48 million population. With this expanded geographic reach, 4,894 (1.2%) of the 401,095 screened patients were diagnosed as having STEMI. A total of 2,041 (43.5%) STEMI were urgently reperfused. Primary PCI was performed in the majority of patients - 1,578 (77.3%) that were referred for urgent perfusion. The major reasons for non-treatment included insurance denials, lack of ICU beds and chest pain >12 hours. Time to Telemedicine Diagnosis (TDD) was 5.1 minutes, and tele-ECG accuracy was 98%. D2B time for the cohort was 53 minutes, but chest pain to treatment time was >6 hours. Overall, in-hospital mortality was 5.8%. Conclusion: LATIN demonstrates the feasibility of creating a population based and telemedicine-guided AMI management strategy that can hugely expand access to reperfusion strategies. Telemedicine has important public health implications as a global approach to urgent AMI care in developing countries.

Genome-Wide Association Study of Vasodilator Response in Pulmonary Arterial Hypertension

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Introduction: Pulmonary arterial hypertension (PAH) is a rare and fatal disease associated with variable therapeutic response, suggesting a genetic contribution. Vasodilator-responsive PAH accounts for a minority of PAH cases and is associated with dramatically improved survival over vasodilator-nonresponsive PAH. The objective of our study was to identify genetic influences on vasodilator drug response in PAH. Methods: Two cohorts of patients with Group I PAH confirmed by right heart catheterization were derived from the PAH Biobank (NHBLI R24HL105333), representing over 40 US institutions. Differences between hemodynamics at rest (baseline mean pulmonary arterial pressure, mPAP) and after vasodilator (nitric oxide, prostacyclin) administration were determined to derive from the PAH Biobank (NHBLI R24HL105333), representing over 40 US institutions. differences between hemodynamics at rest (baseline mean pulmonary arterial pressure, mPAP) and after vasodilator (nitric oxide, prostacyclin) administration were determined to define acute vasodilator drug response (with unchanged cardiac index) as a continuous measure. All cases were genotyped using HumanOmni5 with single nucleotide polymorphism (SNP) call rate >0.99%, minor allele frequency (MAF)>3%, and Hardy-Weinberg p-value>0.01. Analysis was restricted to cases with European ancestry. We performed linear regressions in an additive model for acute vasodilator drug response with adjustment for baseline mPAP, age, gender, and first 3 principal components. A Bonferroni-corrected alpha=3x10^-4 was used in the discovery cohort and alpha=0.05 was used in the replication cohort. Results: The discovery cohort included 434 PAH cases and the replication cohort included 49 less severe PAH cases. QQ-plots showed no evidence of genomic inflation (lambda=1.00). Association of the intronic SNP rs8057488 (MAF 0.05) in the sorting nexin 29 gene (SNX29) with acute vasodilator drug response reached genome-wide significance in the discovery cohort (beta= -7.03 mmHg, p=3.39x10^-8). A significant association between rs8057488 and acute vasodilator drug response was also observed in the replication cohort (beta = -6.48 mmHg, p=0.03). Conclusion: These findings implicate a novel association between SNX29 variation and differential responses to vasodilator treatment in Group I PAH. While requiring further replication in a larger independent cohort, these observations advance our understanding of the molecular underpinnings in PAH.


Key Words: Abdominal aortic aneurysm; Angiotensin II

Inducible Depletion of Calpain-2 Attenuates Obesity-accelerated Abdominal Aortic Aneurysms in Mice

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Background and Objective: Recent clinical studies demonstrated that abdominal adiposity is associated with increased risk of abdominal aortic aneurysm (AAA) development. Calpains are non-lysosomal calcium dependent cysteine proteases that are highly expressed in human and experimental AAs. Using a pharmacological inhibitor and genetically deficient mice, we identified that calpain-2 (a major ubiquitously expressed) plays a critical role in Angiotsensin II (AngII)-induced AAA formation in mice. In addition, calpain inhibition strongly suppressed adipose tissue inflammation in obese mice. The purpose of this study was to determine the functional contribution of calpain-2 in obesity-accelerated AAA.

Methods and Results: Calpain-2 floxed mice that were hemizygous for β-actin Cre-ERT2 were produced by breeding male Cre-ERT2 to female calpain-2 floxed mice. At 8 weeks of age, male non-Cre littermates (Cre-) and Calp-2 x Cre-ERT2 (Cre+) mice were injected with tamoxifen (25 mg/kg, i.p.) daily for 5 consecutive days. After 2 weeks, Western blot analyses showed a complete depletion of calpain-2 protein in the aorta and periaortic adipose tissue from Cre+ mice compared to non-Cre littermates. Mice were fed a high fat diet (60% Kcal) for 20 weeks. After 16 weeks of diet feeding, mice were infused with AngII (1,000 ng/kg/min) by osmotic minipumps for 4 weeks. Depletion of calpain-2 had no effect on high fat diet-induced body weight gain, fat mass, glucose and insulin tolerance. Interestingly, calpain-2 depletion significantly attenuated AngII-induced expansion of ex-vivo maximal diameter of abdominal aortas in obese mice (Cre-: 1.4 ± 0.14; Cre+: 0.9 ± 0.04 mm; P<0.001). In addition, calpain-2 depletion significantly reduced the incidence of AngII-induced AAs in mice (Cre-: 75%, Cre+: 7%; P< 0.001). Conclusion: These findings suggest that calpain-2 plays a critical role in AngII-induced AAA development in diet-induced obese mice.

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Key Words: Pulmonary circulation; Pulmonary hypertension; Genome-wide association studies (GWAS)
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