Late-Breaking Basic Science Abstracts
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Late-Breaking Basic Science Oral Abstracts I

Inhibition Of Prolyl-tRNA Synthetase As A Novel Mediator Of Cardiac Fibrosis
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Introduction: Prolyl-tRNA synthetase (PRS), a member of aminocyclotransferase superfamily (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 fibroblast. 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, reduced expression 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 in cardiac fibrosis. TAC results in pressure overload-induced left ventricular hypertrophy and fibrosis, and it is one of the most widely used models to study cardiovascular diseases. 2-week oral treatment of DWN12088 markedly reduced cardiac fibrosis with ED50 of 0.4 mg/kg, based on histological examinations. In addition, we demonstrated that 2-week oral treatment of DWN12088 showed reduced infiltration of inflammatory cells, left ventricle thickness and accumulation of collagen I. Conclusion: These results suggest that inhibition of PRS attenuates pressure-overload-induced cardiac fibrosis and a selective inhibitor of PRS, DWN12088, could serve as a potent anti-fibrotic agent without affecting critical cellular signaling cascades.


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-inflammatory genes. Both Motif enrichment analysis and de novo motif discovery identified significant enrichment of RBFox1c binding motif in the 3′UTRs of the RBFox1c regulated genes. Using CLIP analysis followed by RT-PCR, we observed RBFox1c, but not nuclear RBFox1c significantly interacted with targeted inflammatory gene 3′UTR. In the cardiac specific RBFox1c knock-out mice, enhanced cardiac fibrosis was observed following TAC, associated with elevated expression of RBFox1c dependent inflammatory genes. In contrast, cardiac specific expression of RBFox1c significantly reduced cardiac fibrosis and inflammatory 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 fibrosis response using MVNm conditioned media. We showed the conditioned media from the hypertrophic cardiomyocytes potently induce fibroblast proliferation. However, RBFox1c expression can suppress phenylpyridine and isoproterenol induced fibroblasts 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 inflammatory gene expression and fibrotic remodeling potentially through protein 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 defined. Normal functioning of mitochondria relies on maintaining the inner membrane potential (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 membrane permeability influences cell and organ functions.


Key Words: Heart failure, Genomics; Hypertrophy

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 βARK1), 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 βARKnt (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 = 16) and LV mass in TgβARKnt compared to non-transgenic 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 TgARKnt mice during chronic pressure overload with preserved cardiac function (% Ejection Fraction 57.3 versus 37.3 ± 2.0; n = 11, 10). Further, βARKnt 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 β-adrenergic receptor density 4 weeks after surgery. The effect of cardiac βARKnt expression was not consistent with alterations in GRK2 activity at GPCRs as neither GRK2 peptide inhibition (TgβARKnt) nor GRK2 overexpression altered hypertrophy, and overexpression hastens HF development. Further, TgβARKnt mice exhibited reduced epididymal white adipose content


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

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and altered mitochondrial respiration, suggesting altered cardiac metabolism. These data support the idea that (αβAR) peptide embodies a distinct functional interaction and that βAR-mediated regulation of β-adrenergic receptor density may provide a novel means of cardioprotection during pressure-overload induced HF.

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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 (βARs) 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 explore the role of GRK2 on metabolism and bioenergetics in the adult heart. We hypothesize that desensitization of βARs via an increase in GRK2 will result in decreased fatty acid-fueled respiration and will compromise cardiomyocyte function. Conversely, ablation of GRK2 will result in increased respiration and function, under these conditions. Our results show that basal respiration, maximal respiration, and reserve respiratory capacity (RRC) are highest in the presence of palmate versus glucose (1.6, 3, and 7.2-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) show a 2.2-fold increased respiration (1.5 and 1.45-respectively) and RRC (2.7 and 2.2-fold, respectively) with fatty acids. This correlates with an increase in ATP levels (1.2-1.9-fold). Thus, we propose that increased GRK2, as seen during heart failure, compromises fatty acid-driven mitochondrial respiration, while GRK2 inhibition under these conditions enhances RRC, which is known to improve cellular survival during stress.

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

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

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Background: Heart failure (HF), a leading cause of morbidity and mortality, involves significant dysfunction of the sinoatrial node (SAN). MicroRNAs (miRs) 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 miRs 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 cardiogenically 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 ion channels and to influence cardiac metabolism. RT-PCR showed that HCN1 and HCN4 channel expression. Furthermore, miR-370-3p downregulated the expression of the HCN1 channel and upregulated the expression of the HCN4 channel. Conclusions: This is the first study to explore the miR 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 and/or HCN4 channel expression, are selectively upregulated in human HF SAN. We propose that understanding the function of miRs in human SAN might be 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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Exercise increases disease penetrance in arrhythmogenic cardiomyopathy (ACM). Yet, how exercise contributes to disease pathogenesis is unclear. Mitochondria potentiate reactive oxygen species (ROS) generation during exercise that are scavenged by antioxidants, such as thioredoxin-2 (Trx2). Here we tested if deficits in Trx2-based ROS buffering act as substrates for exercise-induced cardiac apoptosis in ACM. Homozygote Desmoglein-2-Mutant mice (Dsg2mut/mut) model ACM features, thus WT and Dsg2mut/mut mice were enrolled in a 10 week swimming protocol to evaluate survival and post-effort cardiac function. ROS production by EPR, and mitochondrial ROS-gating protein levels. Survival rate after swimming was ≥84±0.4% in WT, but only 60% (25/22) in Dsg2mut/mut mice (p=0.008). Of the survivors, Dsg2mut/mut mice displayed cardiac dysfunction (Ejection Fraction 57±4 vs 44±0.4% in WT; n=24/cohort, P<0.001) and increased bouts of non-sustained VT. Swimming augmented ROS emission in Dsg2mut/mut right ventricles (RV) than in WT (25±3 vs 15±1 Gasser/total protein [Gtp]; P<0.05) and amplified ROS release from Dsg2mut/mut left ventricles (LV) (39±5 Gtp/ mutant RV; P<0.05). Isolated mutant mitochondria showed reduced ND1410 generation and peroxiredoxin-6, Trx2 and Trx2 reduce (Trx2R) protein levels, with impaired Trx2R function (all P<0.05 vs WT in RV67). When mitochondria bound, Apoptosis-Inducing Factor (AIF) acts as a NADH oxidoreductase, yet upon oxidation, AIF translocates to the nucleus and initiates apoptosis. After exercise, Dsg2mut/mut hearts displayed marked AIF nuclear and chromatin-bound protein levels and increased AIF immunostained nuclei vs WT. Additionally, direct exposure to Trx2 or Trx2 inhibition (by auranofin) in Dsg2mut/mut embryonic stem-derived myocytes elevated AIF-nuclear translocation and apoptosis (via Annexin/PI FACS analysis). Our study reveals a novel causal link between exercise-evoked cardiac redox imbalance and aberrant AIF-Trx2 signaling in ACM, which was associated with increased apoptosis, propensity of arrhythmias and sudden cardiac death. These findings offer a new targetable mechanism for preventing one of the most cited, yet poorly understood, pathological phenotypes (apoptosis) in ACM.


Key Words: Apoptosis; Exercise tests and training; Cardiomyopathy; Redox; Ventricular arrhythmia

Late-Breaking Basic Science Oral Abstracts II

A Cryosection of human SAN central compartment Cx43+ α-actinin immunostaining

B microRNA

mRNA

Relative abundance

370-3p
133a-3p
1-3p
HCN1
HCN4
Control-SAN
Control-RA
HF-SAN
HF-RA
n=3-5 for each group *P<0.05 vs control SAN

Abbreviations: HF - heart failure; IAS - interatrial septum; RA - right atrium; SAN - sinoatrial node.

A) Pure human SAN tissue was collected from the central SAN pacemaker compartment. B) HF induced microRNA and mRNA changes.


Key Words: Sinoatrial node; Heart failure; MicroRNA
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 blood flow patterns.

Hypothesis: Arterial pulsewaves 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 PIONEER HCM (NCT02452242) 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 pulsewave 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 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 oHCM.

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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 quiescent myocardium, overcoming the source-sink mismatch.

Methods: The iPM-spheroids are viable in long-term and exhibit native SAN-like pacemaker cell properties. They also demonstrate small-signal ECG (p=0.0001) and a 2-fold increase in myocardial gap junction, Cx43 (p=0.0030), compared to GFP-spheroids. The iPM-spheroids have superior viability compared to control GFP-spheroids, 87±1% vs 72±5% respectively (p=0.0463). TUNEL staining confirmed apoptotic fibroblast in the periphery. When cultured for ≥2 weeks, iPM-spheroids demonstrated small-α sarcomeric actinin positive cells organized as a mesh in the core, similar to the pacemaker cells in the native SAN. Conclusion: iPM-spheroids can pace and drive the quiescent myocardium, overcoming the source-sink mismatch. The iPM-spheroids are viable in long-term and exhibit native SAN-like pacemaker cell organization. These data provide an in vitro platform on which the design principles of native SAN could be tested.

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Key Words: Sinoatrial node; Pacing

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

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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 link to the elevated wall stress in the dysfunctional myocardium adjacent to the infarict (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 MRI. Methods: An established pre-clinical postero lateral infarct model of LV remodeling was used to investigate region metabolism. To accurately measure regional metabolism, we developed implantable carbon-labeled 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, bicap, 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 bicap flux was greater in BZ compared to remote (Fig. Bottom). BZ DOB stress produced an increase in remote metabolite flux compared to BZ with lactate, alanine and total flux reaching significance and bicap 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 metabolic stress and adverse cardiac remodeling following infarct.

Late-Breaking Basic Science Posters

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

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Key Words: Ventricular remodeling; Cardiac metabolism; Cardiac MRI; Imaging agents; Ischemic heart disease
Background: Current systems generating lymphatic endothelial cell (LEC) from human inducible pluripotent stem cells (iPSCs) have limited value due to low purity, the use of undefined components for differentiation, and poor cell survival in vivo. Here, we developed a fully defined system to differentiate iPSCs into iLECs and evaluated their therapeutic and engraftment potential when encapsulated in a nanomatrix gel (PA-RGDS). Methods and Results: iPSCs were cultured with SGK3i inhibitor on collagen-coated plates for 2–3 days to induce differentiation into the mesodermal lineage. The mesodermally differentiated cells were then cultured with VEGFC, VEGFA, EGF, and SFRF for another 6 days and double-sorted by PDPN and FLT4. These iPSC-PDPN+FLT4+ cells (iPSC-derived lymphatic endothelial cells, iPSC-LECs) showed highly purified and fully functional LEC characteristics in vitro. These iPSC-LEC express LEC markers such as PDPN, LYVE1, PROX1, and FLT4 at the mRNA level and the protein level, and formed tube-like structures in Matrigel. We next determined the lymphatic vascular reparative effects of engineered iPSC-LECs. After inducing lymphedema in the tail of mice, iPSC-LECs, iPSC-LECs encapsulated with PA-RGDS, human malignant lymphatic endothelial cells (hMECs), PA-RGDS, or PBS were injected into the tail of mice. Tail thickness significantly decreased in the groups injected with iPSC-LECs with or without PA-RGDS compared to the other groups at day 28. At day 45, mice injected with PA-RGDS encapsulated iPSC-LECs showed significant decrease in the perimeter of inferior epigastric and all other groups injected with PBS (n=8 per group). Histological examination demonstrated that the skin thickness was significantly reduced and the density of lymphatic vessels was markedly increased when the iPSC-LECs were encapsulated with PA-RGDS compared to others. Conclusion: This study demonstrated for the first time that iPSC can be differentiated into iLECs in a clinically compatible manner with a high yield. Furthermore, nanomatrix encapsulated iPSC-LECs can substantially improve lymphema in mouse tail through enhancement of cell survival and lymphatic neovascularization. This engineered iPSC-LEC therapy represents a novel option for treating lymphedema.

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Key Words: Stem cell therapy; Lymphatic disease; Valvular
TRPV4 antagonist GSK1923934, immediately after MI surgery and followed for 5 weeks. 2D-echocardiography revealed that the cardiac function (ejection fraction and fractional shortening) is preserved post-MI in both TRPV4KO and GSK1923934-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 GSK1923934-treated WT mice compared to their WT counter parts. Furthermore, TRPV4KO hearts exhibited decreased cardiomyocyte 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

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

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Introduction: There is an urgent need for better arrhythmia risk stratification in non-ischemic aetiology dilated cardiomyopathy (DCM), where the benefit of ICD implantation is unclear. Titin truncating variants (TTNV) are the commonest genetic cause of DCM and are associated with early onset non-sustained ventricular tachycardia and atrial fibrillation in this patient group. We examined these patients. Hypothesis: We hypothesize that TTNV 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 TTNV genotype (28 positive for a TTNV, 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: TTNV predicted the risk of receiving inappropriate ICD therapy for VT (HR=3.73, 95% confidence interval [CI]=1.87–7.49, P=9.9×10−4) and persistent AF (HR=2.5–5.6, 95% CI=2.8–8.0, P=0.001). TTNV loci was independent of all covariates, including replacement fibrosis measured by late-gadolinium enhancement (LGE), adjusted HR = 8.2, 95% CI 1–9–83.6, P=0.005. Individuals with both a TTNV and fibrosis had a markedly greater risk for appropriate device therapy than those with neither (HR =16.1, 95% CI 3.5–79.3, P=0.001). TTNV were also a risk factor for developing new persistent AF (HR = 4.4, 95% CI = 1.4–15.1, P=0.006). Conclusion: TTNV status is an important risk factor for clinically significant arrhythmia in patients with DCM or CRT-D or ICD devices. TTNV status alone or more specifically in combination with fibrosis assessed by MRI, may provide an effective stratification tool for predicting the need for ICD therapy in DCM patients.

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

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Microrna-125b-5p Protects The Heart From Acute Myocardial Infarction By Repressing Pro-apoptotic Bak1 And Kif13 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-translationally regulate target genes. MiR-125b-5p is downregulated 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-arrestin-β1–arrestin-β2/ockr receptor, canonical β1-arrestin receptor-mediated cardioprotective signaling through β-arrestin-1 stimulates processing of miR-125b-5p in the mouse heart (Figure A-C). We hypothesize that β1-arrestin receptor-β-arrestin-1-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 protective receptor 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 Kif13 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

24055

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 genetic 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 years) under light anesthesia with ketamine. Monkeys with LV hypertrophy, a ≥5 cm and e′≤10 were defined as DD. Ten monkeys with DD were enrolled in thevalidation 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 (16.8%) 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+ SD (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 13.33 mg/kg) for 13 weeks, DD and BP evaluation showed an increase of e′ (5.27±0.31 to 6.4±1.27 cm/s), a decrease of Ei (12.7±4.23 to 10.5±3.29) and a decrease of SBP (128±15 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.

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

24056

Longitudinal Evaluation of the Associations between Severe Hypertiglyceridemia and Cardiovascular Features

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Introduction: For at least 35 years the associations between insulin resistance, hypertriglyceridemia, hyperglycemia and hypertension have been described. Nevertheless, the existence of an independent association between hypertriglyceridemia (HTG) and cardiovascular diseases (CVD) remains controversial. This may be due to the insufficiency of longitudinal 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 longitudinally studied normonoblem patients (n=227; 164 males) maintained for their lifetimes under constant environmental and dietary conditions. 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 all severe HTG levels compared to normal Tg. Mean arterial blood pressure was not significantly different among the normal to severe HTG groups. HDL cholesterol was significantly lower (p<0.0001) at all levels of HTG (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: 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 associations for break points in the interactions of these features.


Key Words: Triglycerides; Hyperlipidemia; Hypertension

23831

Selective Inhibition Of Hdac3 Prevents Diabetic Cardiomyopathy In Ove26 Mice Via Mir-200a-mediated Nrf2 Activation

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Although histone deacetylases (HDACs) was are the target protein for the initiation and development of diabetic cardiomyopathy (DCM), which isofroms of which plays the key role remains unclear. The present study was designed to determine whether DCM or DCM could be prevent 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) RPP666 or vehicle for 3 months before the cardiac function was examined with Echo. Results indicated that HDAC3 treatment suppressed cardiac function in the diabetes group, HDAC3i activity significantly increased in the heart tissue, which was blocked by the treatment of HDAC3i. Oxidative stress as a major cause of DCM is also inhibited by HDAC3i. Mechanistically cardiac miR-200a, which targets and destroys Akt-like p600A-associated protein (1) (KEAP1) mRNA, was significantly up-regulated and KEAP1 expression was markedly inhibited by HDAC3i in the diabetic mouse. Immunoprecipitation shows that the binding of KEAP1 in the diabetic heart was decreased by the treatment of HDAC3i when we pull down nuclear factor-ε-related factor 2 (NRF2). Meanwhile, the nuclear localization of NRF2 and its downstream anti-oxidative stress genes NADPH oxidoreductase (NQO1), heme oxygenase-1 (HO-1), were markedly up-regulated in the HDAC3i treated diabetic OVE26 mice. These results suggest that HDAC3 prevents DCM likely via miR-200a-mediated degradation of KEAP1 and consequently activation of the NRF2-regulated antioxidant pathway.

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Key Words: Cardioprotect; Diabetes (Type 2); 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 pathways is cAMP-dependent PKA signaling which is modulated by scaffold proteins, A-kinase anchoring proteins (AKAPs). Muscle-specific AKAP (mAKAP) regulates expression of hypertrophic markers in cardiac myocytes using live fluorescent cell imaging. Here, we present our cardiac differentiation 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 in the myofibrillar cells using image-based assays. In experiments initiated to date we have successfully differentiated multiple gene edited iPSC lines representing major cellular subunits (2) of which are specific to cardiovascular disease (aSN61 and ACTN2). Some of the gene edited hiPSC lines are fluorescently-tagged for structures including focal adhesions, 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 (FHF) and the second heart field (SHF), led us to understand how diverse lineages and different 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 therapeutic strategies. Here, we generated a simple system with a single cardiac progenitor and a 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, similar to the in vivo process. Consistently, these populations exhibit a high degree of similarities with HFC/SHF cells isolated from early embryos, determined by RNA-sequenceing 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 GFP+ or RFP+ cells from mesodermal cells, enabling us to generate heart field-specific cells from PSCs. We further found that GFP/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 organs 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 Cardiovascular 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 radiotherapy. 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, and with 10 Gy in rats increases risk factors for CVD and results in cardiac fibrosis quantitatively 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 CVD independent of any direct exposure of the heart to radiation. Bivariate examination of 3,687 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 a diagnosis of chronic kidney disease and diabetes also had significantly higher risk of CVD. Race and hypertension were not indicative of increased risk for CVD. These clinic findings, taken together with the results from our animal studies, support a new research paradigm where radiation-induced heart disease can be indirect, with organ systems exporting factors that cause CVD. Simvastatin can be developed to mitigate and treat CVD after therapeutic radiation.

Conclusion: After therapeutic radiation, simvastatin can be developed to mitigate and treat CVD exporting factors that cause CVD. Simvastatin can be developed to mitigate and treat CVD after therapeutic radiation.

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

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Background: Sacubitril/valsartan (SAC/VAL), a drug combining a neprilysin 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 (3–2.5 kg) 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 (9.1 mg/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 (Picosirisus red staining). Operators performed electrocardiography, Picosirisus 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.

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 E40K 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 atherosclerosis. 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 carriers. We exposed the iPSC-ECs to an inflammatory condition 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 TNF-α and IL-1β, as evidenced by up-regulation of cell adhesion molecules and augmented adherence to monocytes. The ALDH2*2-iPSC-ECs also exhibited an increased basal expression of vascular endothelial growth factor receptor 1 (FLT1) gene, which was further augmented upon inflammatory stimulation. FLT1 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.

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Key Words: Endothelial function; Inflammation and inflammatory markers; Stem cell biology; Stem cells; Cell aging and senescence

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 conformation therapy show an increased residual inflammatory as opposed to an increased residual lipid 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 lipoprotein (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). Uni-locus regressive 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) suggesting 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 levels of hsCRP. Heart failure (OR 4.56, p<0.001) and diabetes (OR 3.04, p=0.012) identified as co-precipitators increased hsCRP follow- ing 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.

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

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

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**Introduction:** Caveolae are membrane localized signaling platforms that compartimentalize 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 adaptability with lower resting heart rate compared to control mice, but the mechanistic basis remains 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 control litterate controls (Ctrl) (12-16 week-old, n=10–11 each) were anesthetized with isoflurane in a recirculating system. Cardiac contractility was assessed by echocardiography at baseline. Isoprenaline (Isuprel USP, 300 nM i.p.) 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 atropine injection (2 mg/kg i.p.) 20 min before isoflurane anesthesia 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 iso injection cardiac contractility was increased to ~91±3 %EF in both groups at 2 min; however, in Cav3 OE mice cardiac contractility recovered to 83.3±6 %EF by 5 min post iso challenge whereas Ctrl animals maintained increased contractility of 92.3±6 %EF (p<0.001). Importantly, Cav3 OE mice pre-treated with atropine no longer showed increased recovery in 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 Ctrl 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 abro-...
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 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 >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=5x10^-8 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 485 cases. QQ-plots showed no evidence of genomic inflation (lambda=1.00). Association of the intronic SNP rs8057488 (MAF=0.05) 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: Genome-wide association study; Pulmonary hypertension; Genome-wide association studies (GWAS)
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