Late-Breaking Basic Science Abstracts
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Inhibition Of Prolyl-tRNA Synthetase As A Novel Mediator Of Cardiac Fibrosis

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Introduction: Prolyl-tRNA synthetase (PRS), a member of aminocacyl-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 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 -SMA. Then, we showed that treatment of DWN12088, a novel small molecular selective inhibitor of PRS, reduced expression levels of pro-fibrotic markers in TGF-α 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 TGF- signaling pathway, although TGF-α is required for induction of pro-fibrotic markers. In vivo study, we performed transverse aortic constriction in C57BL/6 mice to evaluate the effect inhibition of pro-fibrotic markers in left ventricle thickness and accumulation of collagen fiber. 2-week oral treatment of DWN12088 showed reduction in infiltration of inflammatory cells, left ventricle thickness and accumulation of collagen fiber. Conclusion: These results suggest that inhibition of PRS attenuates pressure overload 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 cardiomyopathies. 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 nuclear 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′UTR of the RBFox1c regulated genes. Using CLIP analysis followed by RT-PCR, we observed RBFox1c, but not nuclear RBFox1 specifically interacted with targeted inflammatory gene 3′UTR. In the cardiac specific RBFox1c knockout 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 MVMV conditioned media. We showed the conditioned media from the hypertrophic cardiomyocytes potently induce fibroblast proliferation. However, RBFox1c expression can suppress phenylphrine and isoproterenol induced fibroblasts proliferation.

RBFox1c regulates cardiac transcriptome reprogramming at two post-transcriptional steps. The RBFox1c nucleic isoform regulates global RNA splicing reprogramming in heart, while the RBFox1c 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.

Conclusions: 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: 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 decompensation 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.k.a. GRK1), 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 ± 0.02; n = 10) and LV mass in TgARKnt mice (1.37 ± 0.02; n = 10) compared to 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 ± 3.7 vs. 37.3 ± 2.0, n = 11, 10), further, JARKnt expression limited adverse left ventricular remodeling, with reduced interstitial fibrosis (% area fibrosis 4.1 ± 0.9 vs. 9.2 ± 0.8, n = 11, 9 hearts) and preserved β-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 (TgARKni) nor GRK2 overexpression alter hypertrophy, and overexpression hastens HF development. Further, TgJARKnt mice exhibited reduced epididymal white adipose content.


Key Words: Cell physiology; Mitochondria; Apoptosis; Cellular Engineering; Cardioprotection
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. Consequently, 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 palmitate 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). Consequently, cardiomyocytes isolated from (GRK2+/-) a peptide inhibitor of GRK2, transgenic mice or GRK2 knockout mice (1.5-fold) decreased ATP levels (1.5 and 1.45-fold 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.

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

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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None.


Abstracts II

Late-Breaking Basic Science Oral Abstracts II

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

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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 (Tx2). Here we tested if deficits in Tx2-based ROS buffering act as substrates for exercise-induced cardiac apoptosis in ACM. Homozygote Desmoglein-2 (Dsg2) mutant mice (Dsg2mut/mut) model ACM features, thus WT and Dsg2mut/mut mice displayed cardiac dysfunction (Ejection Fraction 57±4 vs 84±0.4% in WT; p=0.008). Of the survivors, Dsg2mut/mut mice displayed cardiac dysfunction (Ejection Fraction 57±4 vs 84±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) (n=20) (25±3 vs 15±1 G mol/s, P<0.001) and isolated mitochondria from mice that emitted the highest ROS showed increased MPT pore formation vs WT (n=11, P<0.001). Mitochondrial ROS-driven MPT pore formation and Ca2+ overload were restored by the ROS scavenger L-arginine vs WT (n=5, P<0.001). Moreover, cardiomyocytes isolated from Dsg2mut/mut mice elevated AIF-nuclear translocation and apoptosis (via AnnexinV/PI FaCS analysis). Our study reveals a novel causal link between exercise-evoked cardiac redox imbalance and aberrant AIF-Tx2 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


Key Words: Sinoatrial node; Heart failure; MicroRNA
Machine Learning Detection of Obstructive Hypertrophic Cardiomyopathy Using a Wearable Biosensor
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Introduction: Obstructive 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 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 wristworn biosensor (Wavehealth, Mtn. View, CA), from oHCM patients enrolled in a digital health substudy of PIONEER HCM (NCT02842242) to unaffected controls from a Wavehealth 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=5 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 wristworn biosensor and machine learning algorithms. These data raise the possibility of a novel approach to the non-invasive detection of oHCM.


Key Words: Hypertrophic cardiomyopathy, mHealth, Big Data

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 ventricle (LV) may be followed by adverse remodeling leading to heart failure. The mechanism of adverse remodeling might be linked to the increased wall stress in the dysfunctional myocardium adjacent to the infarct (border zone BZ). We hypothesized 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 regional metabolism. To accurately measure regional metabolism, we developed implantable carbonated-tissue surface-coated 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 metabolic stress and adverse cardiac remodeling following infarct.


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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Introduction: Lymphedema results from decreased lymphatic drainage due to lymph node failure or blockage. To achieve effective lymphatic drainage, continuous lymphatic drainage is required. In this study, we attempted to develop an implantable lymphatic drainage system by using a thin gel that could be easily implanted. Methods: We obtained normal lymphatic endothelial cells (LECs) from a human lymph node sample and induced their differentiation into lymphatic endothelial cells. After the harvesting of the cells, we encapsulated the cells into nanomatrix gel to form a spherical gel. Subsequently, we implanted the gel into the right flank of the New Zealand White rabbit model to test its therapeutic effects on lymphatic drainage. Results: After implantation, the nanomatrix gel encapsulated with lymphatic endothelial cells was extracted from the implanted area at 8 weeks post implantation. Histological examination revealed that the gel contained intact lymphatic vessels and lymphatic endothelial cells, along with extravasated red blood cells and a thin gel nonviolated. Conclusion: The nanomatrix gel encapsulated with lymphatic endothelial cells demonstrated lymphatic drainage ability. In future study, we plan to apply the nanomatrix gel encapsulated with lymphatic endothelial cells to a patient with lymphedema to test its effect on lymphatic drainage.

Author Disclosures: S.I. Grijalva: None. J.H. Sung: None. B.W. Furman: None. P. Han: None. J. Li: None. H.C. Cho: None.

Key Words: Sinoatrial node; Pacing

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Author Disclosures: S.I. Grijalva: None. J.H. Sung: None. B.W. Furman: None. P. Han: None. J. Li: None. H.C. Cho: None.

Key Words: Sinoatrial node; Pacing
Background: Current systems generating lymphatic endothelial cell (LEC) from human induced pluripotent stem cells (iPSCs) have limited value due to low purity, the use of defined components for differentiation, and poor cell survival in vivo. Here, we developed a fully defined system to differentiate iPSCs into LECs and evaluated their therapeutic and engraftment potential when encapsulated in a nanomatrix gel (PA-RGDS). Methods and Results: iPSCs were cultured with cFGSF 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 bFGF for another 6 days and double-sorted by PDPN and FL1A. These iPSC-PDPN-FL14+ cells (iPSC-derived lymphatic endothelial cells, iPSC-LECs) showed highly purified and fully functional LEC characteristics in vitro. These iPSC-LECs express LEC markers such as PDPN, LYVE1, PROX1, and FL13 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 mouse, iPSC-LECs, iPSC-LECs encapsulated with PA-RGDS, human dermal lymphatic endothelial cells (hDLECs), hPSC-LECs, or PBS were injected into the tail of mouse. 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 tail diameter compared to all other groups in which other groups injected with PBS or no treatment. 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 these iPSCs can be differentiated into LECs 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.

Author Disclosures: S. Lee; None. Y. Sohn; None. D. Sohn; None. Y. Yoon; None.

Key Words: Stem cell therapy; Lymphatic disease; Valvular myocardial ischemia, promoting cellular functions such as proliferation, glucose metabo-

24060

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 ischemia, 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 C57/Bl6 mice were studied: one group received a sham procedure (n = 4) and two groups underwent transversa aortic constriction (TAC). Two days after TAC, one group had CGRP-filled osmotic pumps (4 μg kg−1 bw/day) implanted subcutaneously (n = 7) while the second group was TAC alone (n = 7). At day 28, mice from each group were sacrificed. Autopsies were completed and heart tissues were analyzed. Results: Echocardiographic and histological data showed that TAC markedly decreased fractional shortening (FS) and ejection fraction and increased heart, 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 46.2±1.8% vs TAC 25.4±1.1%, p < 0.001; and TAC 25.4±1.1% vs TAC-CGRP 36.6±1.2%, p < 0.001). CGRP significantly reduced apoptotic cell death and lipid peroxidation (an oxidative stress marker measured by malondialdehyde and 4-HNE staining) in the TAC hearts (malondialdehyde immunolaboration protein *SEM: sham 3.5±0.9 vs TAC 14.3±0.57, p< 0.05; and TAC 14.3±0.57 vs TAC-CGRP 5.0±0.12, p < 0.05). TAC alone decreased the level of p-ERK1/2 and increased p-JNK compared to sham. CGRP-TAC hearts had higher p-ERK1/2 levels but equal p-JNK levels compared to the TAC hearts. HIF1α and HIF2 protein levels were not different between experimental groups. Compared to TAC hearts, 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 metabolic, and oxidative stress pathways, decreases myocardy apoptosis and is protective in pressure-induced heart failure. Thus, CGRP is a potential therapeutic agent in preventing the progression of heart failure.

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Key Words: Heart failure; Cardioprotection; Cardiac hypertrophy; Cardioprotective drugs
TRPV4 antagonist GS2193874, 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 GS2193874-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 GSK2193874-treated WT mice compared to their MI 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.

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

23094

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

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Introduction: There is an urgent need for better arrhythmic risk stratification in non-ischae-emic 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 these patients. Hypothesis: We hypothesize that TTNV status can predict potentially life threatening ventricular tachycardia (VT) or fibrillation (VF) and development of new persist-ent 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 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 pre-dicted the risk of receiving an inappropriate ICD therapy for VT (HR: 2.36, 95% confidence interval [CI]=1.11–5.02, P=0.026). TTNV status was also a risk factor for developing new persistent AF (HR = 4.4, 95% CI = 1.45–13.1, 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 powerfully in combination with fibrillating injury by MRI, may provide an efficacious target for future treatment 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 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 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-1/-arrestin-2 heterotetramer, carboxylate that β1-adrenergic receptor-mediated cardioprotective signal- ing through β-arrestin1 stimulates processing of miR-125b-5p in the mouse heart (Figure A-C). We hypothesize that β1-adrenergic receptor/-arrestin1-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 sensitiv-ity 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

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 yrs) under light anesthesia with ketamine. Monkeys with LV hypertrophy, a ≥5 cm2 and e’/e’<10 were defined as DD. Ten monkeys with DD were enrolled in a 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 mon-keys (16.46%) 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 (systolic 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 Estrotesto 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 6.1±1.7 cm/s, a decrease of E/e’ (12.74±2.23 to 10.53±3.29) and a decrease of SBP (129±5 to 115±16 mm Hg). These parameters remained stable and unchanged in the ve-hicle group. Conclusions: The incidence of naturally occurring hypertension in adult 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)

24056

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-
nally studied nonhuman primates (n=162; 164 males) maintained for their lifetimes under
controlled 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 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 HTG
(100–200, 200–500, 500–1000 and >1000mg/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 as-
 sociations for break points in the intersections of these features.

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Key Words: Triglycerides; Hyperlipidemia; Hypertension

23931

Selective Inhibition Of Hdac3 Prevents Diabetic Cardiomyopathy
In Ove66 Mice Via Mir-200a-mediated Mir7 Activation
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Although histone deacetylases (HDACs) was the argaim target for the initiation and
development of diabetic cardiomyopathy (DCM), which isofem of plays their 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
Ove66 mice with age-matched wild-type mice were given the selective HDAC3 inhibitor (HDAC3i)
RGFP106 or vehicle for 3 months before the cardiac function was examined with EchO. Results
indicated that HDAC3 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 HDAC3i. Oxidative stress as a major cause of DCM is also inhibited by HDAC3i.

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Key Words: Cardioprotect; Diabetes (Type I); 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 treatments. OVE66, the no. 1 cause of death globally. One of such path-
ways is CAM-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 CAM levels. Furthermore, published literature
reveals a strong correlation between single nucleotide polymorphisms (SNPs) in proteins
and risk of developing CVDs by varying CAM/PKA signaling. This aspect of AKAPs has
been largely explored. Hence, we hypothesized that mAKAP SNPs alter CAM/PKA sign-
aling in muscles individuals susceptible to CVDs. We analyzed selected mAKAP SNPs found
in human patients with CVDs through multiple online tools that predict functional
effects of SNPs to finally make 20 SNPs, Ser(216)Asp(R) in PDE4D binding domain and
Glu(212)4Gly(214) 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,
S165SR mutant showed increased binding to PDE4D at baseline but significantly reduced
binding after stimulation with 1 Mμm isoproterenol as compared to WT. Similarly, E2124G
mutant exhibited significantly lower PKA binding at baseline and higher binding after stimu-
lum. CAM levels and PKA activity were significantly lower at baseline but higher after stimulation
in S165SR mutant cells. Also, E2124G expressed cells showed no significant
change in CAM levels when compared to WT but PKA activity was significantly lower at
basal levels followed by abrupt increase after stimulation. PDE activity assay was in con
grunt with CAM changes in S165SR mutant cells. Fluorometric assay showed higher intracellular
calcium in E2124G mutant cells after stimulation. Lastly, immunoblotting data showed al-
tered phosphorylation of hypertrophic markers in both mutants. To conclude, human mAKAP
SNPs may pre-dispose humans to the risk of developing CVDs by affecting CAM/PKA sig-
aling and thus confirming the clinical significance of PKA-mAKAP/PDE4D 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 car-
diomyocytes will contribute to the development of better disease models, therapies, and
regenerative medicine approaches for cardiac disease. Using the WTC human CPC line
and the CRISPR/Cas9 system, we have fluorescence tagged ~20 target genes represent-
ing key cellular organelles including a few cardiac-specific genes. Edited iPSC lines are
differentiated 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 organelle 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 in the
differentiated cells using image-based assays. In experiments initiated to date we have successfully differentiated multiple gene edited iPSC lines representing major cardiac structures (1) which are specific to cardiomyocytes (α-skeletal and ACTN2). Some of the gene edited hiPSC lines are fluorescence-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.

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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 dif-
derent 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 iPSC-derived cardiac organoids from wild-type and several
biological and genetic backgrounds and generated fluorescent protein (GFP and RFP) reporters under the control of the FHF marker Hcn4 and the SHF marker Tbx1, 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 in vivo process. Consistently, these populations exhibit a high degree of similarities with FHF/SHF cells isolated from early embryos, determined by RNA-sequence 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 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.

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Key Words: Stem cell biology; Cardiac development; Stem cells; Progenitor cell

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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 E46K 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 WT subjects and ALDH2*2 carriers. 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 TNF-α and IL-1β, as evidenced by up-regulation of cell adhesion molecules and augmented adhesion 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 and atherosclerosis

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 (~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 (0.1 mg/kg/dy) 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). 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 LV function in a rabbit model of ischemic HFrEF.


Key Words: Heart failure; Ischemia reperfusion; Natriuretic peptide; Ejection fraction; Fibrosis

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
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 control littermate controls (Ctrl) (2-6 week-old, n=10-11 each) were anesthetized with isoflurane and cardiac contractility was assessed by echocardiography at baseline. Isoprenaline (0.1μg/kg/min) 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. Atropine injection found a significant effect of atropine on the responses of Cav3 OE versus Ctrl genotypes at 2 min; however, in Cav3 OE mice cardiac contractility recovered to 83±3 %EF by ~68±8 %EF. After Iso injection cardiac contractility was increased to ~91±3 %EF in both groups and the atropine injection did not show significant differences. Conclusion: A substantial part of patients with CHF share 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.

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 outcomes 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 PCIs. 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 activating primary PCI. Results: A total of 257 LATIN centers (Brazil 95, Colombia 113, Mexico 49) 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 (TTD) 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 therapies. Telemedicine has important public health implications as a global approach to urgent AMI care in developing countries.

Author Disclosures: Y. Wang: None.

Key Words: Aging; Arteries; Cardiovascular disease.

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, immunohistochemistry 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 100mM) to primary cultured VSMCs isolated from young (8 mo) and old (30 mo) FKB1 rat aorta 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, DAMGO1. 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 PDGF-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.
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 identify vasodilator non-responders. All cases were genotyped using HumanOmni5 with single nucleotide polymorphism (SNP) call rate >99%, minor allele frequency (MAF)>3%, and Hardy-Weinberg equilibrium. 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 49 less severe PAH cases. QQ-plots showed no evidence of genomic inflation (lambda=1.00). Association of the intronic SNP rs8057488 (MAF=0.27) 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). 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: Myocardial infarction; Telemedicine; STEMI; Systems of care

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

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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 AAAs. Using a pharmacological inhibitor and genetically deficient mice, we identified that calpain-2 (a major ubiquitous isoform) 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 floxied 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 of 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 (Cra-: 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 AAAs in mice (Cra-: 75%; Cre+: 7%; P< 0.001). These findings suggest that calpain-2 plays a critical role in AngII-induced AAA development in diet-induced obese mice.

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

Key Words: Abdominal aortic aneurysm; Angiotsensin II

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

Aida Jawaid, Weihua Jiang, Jessica J. Moorleighen, Venkateswaran Subramanian; University of Kentucky, Lexington, KY

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 AAAs. Using a pharmacological inhibitor and genetically deficient mice, we identified that calpain-2 (a major ubiquitous isoform) 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 floxied 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 of 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 (Cra-: 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 AAAs in mice (Cra-: 75%; Cre+: 7%; P< 0.001). These findings suggest that calpain-2 plays a critical role in AngII-induced AAA development in diet-induced obese mice.

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

Key Words: Abdominal aortic aneurysm; Angiotsensin II

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