Intravenous Gene Therapy with Pim-1 via a Cardiotoxic Viral Vector Halts the Progression of Diabetic Cardiomyopathy Through Promotion of Pro-survival Signaling

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Background: Studies in transgenic mice showed the key role of moloney murine leukemia virus 1 (Pim-1) in the control of cardiomyocyte function and viability. Objective: To investigate if Pim-1 is a therapeutic target for the cure of diabetic cardiomyopathy (DCM), a steadily increasing cause of non-ischemic heart failure. Methods and Results: Western blot analysis on hearts of streptozotocin-induced type-1 (T1D) mice showed a time-dependent reduction in Pim-1 (8-fold at 20 wks from T1D induction), a parallel decline in the Pim-1 activator STAT-3 (6-fold) and Akt (7-fold) and an increase of the Pim-1 direct inhibitor miR-11 (6-fold) (P<0.01 vs. age-matched non-diabetic (ND) mice for all comparisons). Moreover, diabetic hearts showed low levels of anti-apoptotic Bcl-2, high levels of pro-apoptotic Bad and increased caspase 3/7 activity (P<0.01 vs. ND for all comparisons). Studies on murine cardiomyocytes challenged with high glucose (HG) confirmed the in vitro expression changes. In in vitro rescue studies, anti-miR-1 boosted Pim-1 and Bcl-2 expression and promoted cardiomyocytes survival under HG. Similarly, transfection with Pim-1 plasmid prevented cardiomyocyte apoptosis. Finally, at 4 wks from T1D induction, mice were randomly assigned to receive an i.v. injection of human (h) Pim-1 via cardiotoxic serotype-9 adenovirus-associated virus (X10101 or X51015 plu, doses decided on pilot titration studies) or empty vector. Expression of hPim-1 was confirmed by Western blot and immunohistochemistry in cardiomyocytes and to a less extent in skeletal muscles (Fig.1a), but not in other organs. Echocardiography showed that hPim-1 gene therapy attenuates diastolic dysfunction and prevents the development of left ventricle dilation and failure in T1D mice (Fig.1b). Conclusion: Down-regulation of pro-survival Pim-1 contributes in the pathogenesis of DCM. Systemic delivery of hPim-1 via cardiotoxic AAV9 represents a novel and effective approach to treat DCM.

Author Disclosures: R. Katare: Employment; Significant; Salary is paid by British Heart Foundation. Research Grant; Significant; British Heart Foundation. A. Caporali: None. L. Zentlin: None. G.S. Newby: None. C. Emanuelli: None. M. Giacca: None. P. Madeddu: British Heart Foundation. Key Words: Cardiomyopathy, Gene therapy, Diastolic function

Reduced Complex I Function of the Mitochondria Accelerates Heart Failure in Mice

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Impaired mitochondrial function is closely associated with heart failure. To define the causal role of mitochondrial dysfunction in the development of heart failure we generated a cardiac-specific model of impaired Complex I function by deleting a subunit encoded by Ndufs4 in the mouse heart (cNdufs4−/−). Complex I activity decreased by ~70% and complex I substrates supported respiration in permeabilized myofibers decreased by ~50% in cNdufs4−/−, whereas complex II activity and complex II substrate supported respiration...
remained unaltered. Mitochondrial volume density and morphology were also similar in cNDf14–/– and control (CON) hearts. Using 31P NMR spectroscopy of isolated perfused hearts, we found that high energy phosphate content and contractile function of the cNDf14–/– hearts were normal suggesting that the deficiency of Complex I was fully compensated. In vivo cardiac function assessed by echocardiography as well as the survival of cNDf14–/– mice was normal in an 8-week period. However, mice were subjected to pressure overload by transverse-aortic constriction (TAC) for 4 weeks the heart weight was increased by 2.5±0.1 folds compared to 1.6±0.1 folds in CON mice (n=5-6 per group; p<0.05). LV fractional shortening (FS) was significantly reduced in TAC-CON (12±2%; n=6) compared to TAC-P (18±2%; n=6; p<0.05). LVFS was 55±4% in sham-operated hearts for both genotypes. LV end-diastolic dimension increased 63% at 4 weeks post TAC in cNDf14–/– (from 3.0±0.01 to 4.9±0.2 mm; p<0.05) while it did not change in TAC-CON (from 3.0±0.01 to 3.5±0.2 mm). Thus, our results show that redox signaling is functionally compensated energetically and functionally under stress conditions but renders the heart highly vulnerable to pathological stimuli. Therefore, despite a large reserve, mitochondrial dysfunction plays a critical role in the pathogenesis of heart failure in response to pathological stress.

Author Disclosures: G. Karamanlidis: None. L. Garcia Menendez: None. S. Kowlcz: None. R. Palazzo: None. R. Tian: None.

Key Words: Heart failure, Mitochondria, Metabolism

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Ryodine Receptor Phosphorylation by CaMKII Promotes Ventricular Arrhythmias and Arrhythmogenic Death in Mice with Heart Failure

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Background: Approximately half of all heart failure patients die as a result of lethal ventricular arrhythmias. In these patients, diastolic calcium release of CaC2 - a sarcoplasmic reticulum (SR) through type 2 ryodine receptors (RyR2) has been linked to sudden arrhythmogenic death. However, the molecular mechanisms behind this release remain unknown. We hypothesized that increased RyR2 phosphorylation by CaC2-calmodulin-dependent protein kinase II (CaMKII) is both necessary and sufficient to promote lethal ventricular arrhythmias. Methods and Results: We generated knock-in mice in which the CaMKII phosphorylation site on RyR2 is constitutively activated (S2814D). In lipid bilayer preparations there was increased RyR2 open probability in S2814D (Po 54.7%±8.0%; n=14) vs. WT mice (Po 13.3%±0.4%; n=14; p<0.001). Also, intact S2814D myocytes had higher diastolic SR CaC2 release events on confocal microscopy as evinced by increased spark frequency (9.8±0.5, n=8) vs. WT mice (6.4±0.3, n=10; p<0.05). At baseline, young S2814D mice had structurally and functionally normal hearts without arrhythmias; however, they developed sustained ventricular tachycardia with increased risk for sudden cardiac death upon catecholaminergic provocation by caffeine/epinephrine (71% vs. 13%; n=8; p<0.05) or programmed electrical stimulation (53% vs. 6%; n=15; p<0.01). Transverse aortic constriction (TAC) surgery in S2814D and WT mice caused a significant decline in survival due to arrhythmogenic death on ECG telemetry (Survival 40% vs. 90%; n=10,11; p<0.05). Conversely, genetic inhibition of the CaMKII site on RyR2 (S2814A) rescues mutant mice from pacing-induced arrhythmias versus WT mice after 10, p<0.01 to 3.5

Conclusions: Abnormal CaMKII phosphorylation of RyR2 CuC2 releases channels plays a critical role in arrhythmogenesis and sudden cardiac death in heart failure. Inhibition of CaMKII phosphorylation of RyR2 may reduce the incidence of death from arrhythmias in patients with heart failure.


Key Words: Heart failure, Remodeling, Signal transduction

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Nrf2 Deficiency Prevents Reductive Stress and Rescues Human Mutant Protein Aggregation Cardiomyopathy (MPAC) in Mice

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Background: We recently discovered that overexpression of the human mutant CuB crystallin (hR120GCrAB) in mouse resulted in protein aggregation cardiomyopathy (PAC) and reductive stress (RS) in the heart tissue (Rajasekaran et al.Cell 2017). Further, we identified that sustained activation of Nrf2 (nuclear erythroid 2 related factor 2) ARE signaling as a causal mechanism for RS in the MPAC mice. We hypothesized that abolishing Nrf2 could prevent RS in the R120GTG mice and thereby rescue them from cardiomyopathy and heart failure. Methods: To elucidate loss-of-function mechanisms for Nrf2 on RS and MPAC, we generated (1) NTG/WT, (2) R120GTG/WT, (3) R120GTG/Nrf2-/-, and (4) R120GTG/Nrf2-/- mice by intercrossing R120GTG mice with Nrf2-/- mice. Primarily, we determined the rate of survival and ECHO-cardiograph analysis to understand the degree of pathogenesis and cardiac function over time (n=6-10). Next, we analyzed the redox state and protein/mRNA expression for major antioxidants including enzymes that involve in glutathione metabolism in the heart. Results: Disruption of Nrf2 prolonged the survival of MPAC mice along with a loss of cardiac dysfunction (by ECHO). The R120GTG mice with Nrf2 deficiency (either Nrf2-/- or NTG-/- ; n=4) had significantly decreased glutathione (but equal to NTG levels when compared with R120GTG/WT cohorts). Such a decline in GSH resulted in quenching of "reducing power" to prevent "reductive stress" and facilitated redox homeostasis in the myocardium. Interestingly, the intercross mice showed no cardiac hypertrophy even at 12 months while the R120GTG exhibited 90% mortality due to PAC/heart failure. Further, protein and mRNA levels for major antioxidants were found to be normal in the R120GTG/Nrf2-/- whereas there was significant down regulation of these parameters in the R120GTG/Nrf2-/- mice compared to NTG/WT suggesting that Nrf2-independent pathways are inadequate to maintain the antioxidant capacity under stressed conditions. Conclusions: These results demonstrate a critical role for Nrf2.
underlying antioxidant potential in the myocardium. Agrostat R S via fer2 might be highly relevant to cure the protein aggregation diseases that are coupled with elevated intracellular reducing potential. This research has received full or partial funding support from the American Heart Association.

Author Disclosures: G. Khandarena: None. M.A. Firpo: None. C.D. Olsen: None. S. Lihwin: None. L.J. Benjamin: None. L. Wang: None. R. Namakkal soorapan: None. Key Words: Cardiac hypertrophy, Oxidative stress, Cardiomyopathy

Coordinated Downregulation of MicroRNA-1 And MicroRNA-133 During Myocyte Hypertrophy Is Required For An Increase In Global Gene Expression and Protein Turnover

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Cardiac hypertrophy is characterized by an increase in global protein and RNA synthesis that is accompanied by an increase in proteasomal-dependent protein turnover. However, the regulation and coordination of these two functions has not been elucidated. The objective of our study was to investigate the role of microRNA (miR) in this process. MiR-1 and miR-133, which are coexpressed in muscle tissue, are downregulated during skeletal and cardiac muscle hypertrophic growth. Our data reveal a role for miR-1 in regulating global mRNA and protein synthesis by suppressing key molecules that include TATA-binding protein (TBP), general transcription factor II B (GTFII), cyclin-dependent kinase 9 (cdk9), and eukaryotic initiation factor 4e (eif4e), through highly conserved targeting sites. Indeed, the levels of miR-1 inversely correlate with the expression of these molecules during postnatal cardiac growth and myocyte hypertrophy. Accordingly, supplementing cardiac myocytes undergoing hypertrophic growth with exogenous miR-1 inhibits total mRNA and protein synthesis by 40% and 52%, respectively, preceded by downregulation of miR-1 targets, and vice versa. TBP binds to the TATA box and recruits RNA polymerase II through binding to TIFII. Thus, via inhibiting the expression of these target proteins, miR-1 should inhibit the association of RNA polymerase II to the genomic DNA. To confirm this possibility, we supplied cardiac myocytes undergoing hypertrophy with exogenous miR-1 or a control adenovirus and performed chromatin immunoprecipitation using RNA polymerase II, followed by library construction and sequencing, and microarray analysis (ChIP-on-ChIP). The results confirmed that miR-1 inhibits the association of RNA polymerase II with a broad spectrum of genes, including housekeeping genes by −80%. On the other hand, increasing miR-133 levels inhibited proteasomal-mediated protein degradation and increased ubiquitinated proteins in cardiac myocytes equivalent to the effect of the proteasomal inhibitor epoxomicin. Conversely, knockdown of miR-133 reversed the effect epoxomicin. Thus, the coordinated downregulation of miR-1 and miR-133 during hypertrophic muscle growth results in enhancing global gene expression and protein turnover.

Author Disclosures: D. Sayed: None. M. He: None. Z. Yang: None. M. Abbadellatif: None. Key Words: Cardiac hypertrophy, Gene expression, Proteasome

PDE3A: A Component of a Molecular Scaffold That May Integrate Cyclic AMP and SERCA2 Transduction Pathways in Cardiac Muscle

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Many cardiac functions are regulated by multiple spatially and functionally distinct pools of cAMP. By hydrolyzing cAMP, cyclic nucleotide phosphodiesterases (PDEs) regulate the amplitude, duration, and compartmentation of cAMP-mediated signaling. Although PDE3A inhibitors raise cAMP and produce acute inotropic effects, the detailed mechanisms are unclear. Immunohistochemical (IHC) staining of human myocardium indicates that PDE3A colocalized with desmin, APLP1, and SERCA2 to sarcomere 2-Bands, while PDE3B colocalized with sarcomeric proteins (DIA, MYH7, ACTA1) and ATP synthase, Cytochrome C. During sucrose gradient centrifugation of mouse cardiac membranes, PDE3A cofractionates with sarcoplasmic reticulum (SR) Ca2+ 2-ATPase (SERCA2) and phospholamban (PLB), and IHC staining indicates that PDE3A colocalized with SERCA2 and desmin in mouse heart. In addition, Western blots and LC-MS/MS analysis of PDE3A immunoprecipitates indicates that murine PDE3A immunoprecipitates (CD-IP) with SERCA2. Similarly, in solubilized cardiac microsomes, PDE3A CD-IP with SERCA2 and other signaling molecules thought to be components of an AKAP/SERCA2 macromolecular regulatory complex, including PLB, PKAII, P2P2A, and APLP1, but not AKAP LBC. In human myocardial microsomes, the PKA catalytic subunit (PKAc) phosphorylates PDE3A1 and A2 isoforms, but not PDE3A3, and significantly increases PDE3A catalytic activity. cAMP or PKAc significantly increase Ca2+ uptake into human SR vesicles, and cilostamide, a SERCA3-selective inhibitor, potentiates the effect of cAMP on Ca2+ uptake into mice and human SR vesicles. SERCA2 Ca2+ ATPase activity and Ca2+ uptake were increased in SR vesicles from PDE3A-Knockout (KO) mice, compared to WT. In lysates from KO hearts, SERCA2 expression was increased and that of PLB decreased, and phosphorylation of PLB at Ser-16 (pPLB/PLB ratio) was increased. In KO lysates, due to the loss of PDE3A activity, PKA was activated, as evidenced by increased phosphorylation of PKA substrates and PLB. Taken together, these data suggest that, as a component of SERCA2-containing macromolecular complexes in murine and human myocardium, PDE3A regulates a discrete cAMP pool important in regulating contractility by modulating Ca2+ uptake into the SR.
S1P Lysase: A Novel Therapeutic Target for Ischemia/Reperfusion Injury of the Heart


Aims: Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid that promotes cardiomyocyte development in bone marrow or in spleen between SR-BI null and wild type littermates. To assess the functionality of this putative IL-1 receptor form, we treated both SR-BI null mice and SR-BI+/– mice with a nontoxic SPL inhibitor THI raised S1P levels and reduced SPL activity (n=8). These results reveal for the first time that SPL is an ischemia-induced enzyme that is inhibited by a common food additive, and thus is a novel target for preventing cardiac I/R injury.

Opposite Effects of Genetic Deletion of the IL-1 Receptor and IL-1 Receptor Antagonist in an Experimental Mouse Model of Pulmonary Hypertension

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Pulmonary arterial hypertension (PAH) is a life threatening condition with high morbidity and mortality. Inflammatory mechanisms are proposed to play a significant role in disease progression, particularly PAH associated with other diseases e.g. systemic sclerosis. Previous studies have described PAH in fat fed Apolipoprotein E (apoE) null mice (apoE-/–). Our group has previously reported that ApoE-/– mice with interleukin-1 receptor deletion (IL-1R1–/–) have reduced diet-induced atherosclerosis, and lower systemic blood pressure compared to ApoE+/– mice on the same diet. We subsequently hypothesized that ApoE+/– IL-1R1–/– double null mice would exhibit a reduced PAH phenotype compared to ApoE+/–. We reported at the AHA Scientific Session last year that, surprisingly, after 8 weeks on a high fat (Pig) diet ApoE+/– IL-1R1–/– mice exhibited significantly higher RSPV (mean 70 mmHg vs 50 mmHg, n=7 p<0.01) and more severe pulmonary vascular muscularization than ApoE+/– mice. Further investigation has revealed expression of a putative alternatively primed IL-1R1–/– transcript expressed predominantly in lungs (but not heart) of the ApoE+/– IL-1R1–/– double null mice. To assess the functionality of this putative IL-1 receptor form, we treated both ApoE+/– and ApoE+/– IL-1R1–/– mice with Interleukin 1 receptor antagonist (IL-1Ra) or placebo for 4 weeks via a subcutaneous osmotic mini pump, following an initial 4 weeks on diet. Echocardiography and cardiac catheterization was performed at 8 weeks prior to collecting lung tissue and serum. ApoE+/– and ApoE+/– IL-1R1–/– mice treated with IL-1Ra had significantly reduced RSPV (23 and 33 mmHg respectively) and significantly reduced pulmonary vascular remodeling (55% and 66% respectively). These data suggest that IL-1Ra may have beneficial effects in treating PAH and that alternative IL-1 receptor signaling in the lung may be important in driving PAH progression.


Key Words: Pulmonary hypertension, Transgenic models, Inflammation
Epigenetic Control of Cardiac Growth by the Histone Methyltransferase Ezh2

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Heart homeostasis is controlled by cardiac-specific gene transcription. Much less is known on the simultaneous gene repression that must occur to allow execution of specialized functions during the heart’s lifetime. Therefore, stable repression of non-cardiac transcriptional programs is likely essential for cardiac maintenance. Stable gene repression is established by tri-methylation of the lysine 27 of histone H3 (H3K27me3) via Enhancement of zeste homolog 2 (Ezh2). However, whether Ezh2 functions in the mammalian heart or in heart disease is not known. Methods: Ezh2 was inactivated in the mouse second heart field by Met2Cre-driven recombination. In vivo, cellular and molecular approaches were used to identify key Ezh2 targets whose epigenetic repression is essential for cardiac maintenance. Results: Ezh2 inactivation led to cardiac hypertrophy, fibrosis and fetal gene reactivation in adult mice. Ezh2-deficient hearts developed hypertrophy upon hypotrophic stimuli, suggesting that Ezh2 represses a pro-fibrosis program in the heart. Indeed, adult Ezh2-deficient hearts over-expressed fibrosis markers (Aimentin, collagens) and the pro-fibrosis factors peroxisrin (Paxrin), connective tissue growth factor (Ctgf) and Tgfβ3. Mutations upregulating Tgfβ3 are present in arrhythmogenic right ventricular dysplasia (ARVD), characterized by adherens junction remodeling. Indeed, Ezh2-deficient cardiomyocytes remodeled adherens junctions, as shown by induction of E-cadherin and redistribution of β-catenin, modeling some aspects of ARVD. We uncovered key Ezh2 targets acting upstream Tgfβ3 in the heart. The homeodomain transcription factor Six1, which activates Tgfβ3 signaling, and its coactivator Eya1, were upregulated in Ezh2-deficient cardiomyocytes. Indeed, the Six1 promoter had decreased H3K27me3 and increased PolII and H3 acetylation in Ezh2-deficient hearts. Furthermore, Six1 and Eya1 induced cardiac hypertrophy in cardiomyocytes and coactivated Nppa expression. Conclusion: Ezh2 is essential for heart maintenance, as it epigenetically represses key transcription factors like Six1 and Eya1, which, upon derepression, activate Tgfβ3 signaling and pro-fibrosis signaling, contributing to a pro-fibrotic phenotype.

Author Disclosures: P. Delgado-Olguin: None. A. Tarakshovsky: None. B.G. Bruneau: None.

Key Words: Gene expression, Cardiac hypertrophy, Fibrosis

MicroRNA-21 Mediates Cardioprotection with PKGI-α Over-expression through Upregulation of Hydrogen Sulfide

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Background: Activation of cyclic GMP-dependent protein kinase (PKG) with pharmacologic agents or its overexpression protect against ischemia/reperfusion injury in the heart and in cardiomyocytes. Moreover, Akt phosphorylation and hydrogen sulfide (H2S) generation were involved in the protective effect. Since Akt positively regulates microRNA (miR-21), we hypothesized that PKG-driven H2S generation is mediated by miR-21. Methods: Stable gene repression is established by tri-methylation of the lysine 27 of histone H3 (H3K27me3) via Enhancement of zeste homolog 2 (Ezh2). However, whether Ezh2 functions in the mammalian heart or in heart disease is not known. Methods: Ezh2 was inactivated in the mouse second heart field by Met2Cre-driven recombination. In vivo, cellular and molecular approaches were used to identify key Ezh2 targets whose epigenetic repression is essential for cardiac maintenance. Results: Ezh2 inactivation led to cardiac hypertrophy, fibrosis and fetal gene reactivation in adult mice. Ezh2-deficient hearts developed hypertrophy upon hypotrophic stimuli, suggesting that Ezh2 represses a pro-fibrosis program in the heart. Indeed, adult Ezh2-deficient hearts over-expressed fibrosis markers (Aimentin, collagens) and the pro-fibrosis factors peroxisrin (Paxrin), connective tissue growth factor (Ctgf) and Tgfβ3. Mutations upregulating Tgfβ3 are present in arrhythmogenic right ventricular dysplasia (ARVD), characterized by adherens junction remodeling. Indeed, Ezh2-deficient cardiomyocytes remodeled adherens junctions, as shown by induction of E-cadherin and redistribution of β-catenin, modeling some aspects of ARVD. We uncovered key Ezh2 targets acting upstream Tgfβ3 in the heart. The homeodomain transcription factor Six1, which activates Tgfβ3 signaling, and its coactivator Eya1, were upregulated in Ezh2-deficient cardiomyocytes. Indeed, the Six1 promoter had decreased H3K27me3 and increased PolII and H3 acetylation in Ezh2-deficient hearts. Furthermore, Six1 and Eya1 induced cardiac hypertrophy in cardiomyocytes and coactivated Nppa expression. Conclusion: Ezh2 is essential for heart maintenance, as it epigenetically represses key transcription factors like Six1 and Eya1, which, upon derepression, activate Tgfβ3 signaling and pro-fibrosis signaling, contributing to a pro-fibrotic phenotype.

Author Disclosures: P. Delgado-Olguin: None. A. Tarakshovsky: None. B.G. Bruneau: None.

Key Words: Gene expression, Cardiac hypertrophy, Fibrosis

Antibody-Sortagging: A Universal Approach Towards Targeted Molecular Imaging and Cell Homing in Cardiovascular Disease

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Targeting of contrast agents to unstable atherosclerotic plaques offers the potential to identify such plaques before rupture, allowing suitable interventions and thus avoiding myocardial infarction and death. Similarly, homing of stem cells to disease sites increases the efficacy of regenerative cell therapy while reducing the number of cells required. Currently, targeting can be achieved via chemical conjugation to specific antibodies, which typically results in the loss of antibody functionality and in severe cell damage. An ideal conjugation technique should...
ensure retention of antigen binding activity and functionality of the targeted biological component (e.g. stem cells). Here we report a novel, gentle, robust, highly reproducible, and site-specific coupling method utilizing the Staphylococcus aureus sortase A enzyme to conjugate a single-chain antibody (scFv), anti-GPib/IIIa-scFv, to nanoparticles and cells for molecular imaging and stem cell homing in cardiovascular disease. This scFv specifically binds to activated platelets, which play a pivotal role in atherosclerosis, thrombosis and inflammation. The conjugation procedure involves chemical and enzyme-mediated coupling steps. The scFv was successfully conjugated to magnetic particles of iron oxide (powerful contrast agents for magnetic resonance imaging), and to model CHO cells. The conjugation efficiency was between 50-70% and the bioactivity of the scFv after coupling was preserved. The targeting of scFv-coupled CHO cells and nanoparticles to activated platelets was strong and specific as demonstrated in in-vitro static adhesion assays, in a flow chamber system under shear stress and in mouse intravital microscopy. In conclusion, this unique biotechnological approach provides a versatile and broadly applicable tool for procuring targeted regenerative cell therapy as well as targeted molecular imaging in cardiovascular, inflammatory diseases and beyond.

Identification of a Novel Loss-of-Function Calcium Channel Gene Mutation in Short QT Syndrome

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Background: Short QT Syndrome (SQTS) is a genetically determined ion-channel disorder, which may cause malignant tachyarrhythmias and sudden cardiac death (SCD). Thus far, mutations in 5 different genes encoding potassium and calcium channel subunits have been reported. We present, for the first time, a novel loss-of-function mutation coding for a L-type calcium channel subunit in a 17-year old female patient who survived an episode of SCD.

Methods and Results: The ECG revealed a QT interval of 317 ms (95% CI: 329 ms) with tall, narrow, and symmetrical T-waves. Invasive electrophysiological testing showed short ventricular refractory periods and increased vulnerability to induce ventricular fibrillation. DNA screening of the patient identified no mutation in previously known SQTS genes, however a new variant was found at a heterozygous state was identified in the CACNA2D1 gene (nucleotide c.2264G>A). The patient identified no mutation in previously known SQTS genes, however a new variant was found at a heterozygous state was identified in the CACNA2D1 gene (nucleotide c.2264G>A).

Conclusions: This variant did not modify the expression of the co-expressed of the two other L-type calcium channel subunits, Cav1.2, suggesting a deficient trafficking of the L-type calcium channel towards the cell membrane. Therefore, knockdown of Synv1 in NRVCMs using Synv1-targeted siRNA decreases activation of the ERSR, as measured by the expression of nodal transcription factors ATF6 and XBP1, and downstream targets, indicating that Synv1 is a global regulator of the ERSR. Furthermore, knockdown of Synv1 increases cell death during ER stress, demonstrating critical role of Synv1 in cardiomyocyte. Finally, it is not yet known whether protein quality control machinery in the ER responds to protein misfolding in other cellular compartments. However, since in addition to degrading misfolded proteins in the ER, as an E3 ligase, Syvn1 is involved in posttranslational, proteasome-mediated degradation of misfolded proteins, it is possible that Synv1 is a cornerstone of protein quality control in both ER and cytosolic compartments.

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

Author Disclosures: S. Doroudgar: None. C.C. Glimbskoti: None.

Key Words: Cardioprotection, Stress, Ischemic heart disease

Synoviolin is a Novel Central Regulator of Endoplasmic Stress Response and Protein Quality Control in the Heart

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Recently the endoplasmic reticulum stress response (ERSR) has been shown to be critical for minimizing ischemic damage in the heart. We now identify synoviol1 (Synv1; Hrd1) as a novel global regulator of the ERSR. Accordingly, Synv1 may be an important modulator of ischemic damage in the heart. Isoxyl results in misfolding of proteins synthesized in the ER, activating the ERSR, which can be cytoprotective or death-promoting. In the heart, ERSR-mediated protection is afforded through the activation of ATFS, a nodal transcription factor of the ERSR that we have shown to be activated by ischemia. Activated ATFS up-regulates proteins that protect from cell death during ischemia. Here we show that the E3 ubiquitin ligase, Synv1, is up-regulated by ATFS in the adult mouse heart, in vivo, and is a central regulator of ER stress that has never been studied in the heart. Using neonatal rat ventricular cardiomyocytes (NRVCMs), we demonstrate, for the first time, that Synv1 is expressed in cardiomyocytes, and that it is up-regulated by ER stress. Knockdown of Synv1 in NRVCMs using Synv1-targeted siRNA decreases activation of the ERSR, as measured by the expression of nodal transcription factors ATF6 and XBP1, and downstream targets, indicating that Synv1 is a global regulator of the ERSR. Furthermore, knockdown of Synv1 increases cell death during ER stress, demonstrating critical role of Synv1 in cardiomyocyte. Finally, it is not yet known whether protein quality control machinery in the ER responds to protein misfolding in other cellular compartments. However, since in addition to degrading misfolded proteins in the ER, as an E3 ligase, Synv1 is involved in posttranslational, proteasome-mediated degradation of misfolded proteins, it is possible that Synv1 is a cornerstone of protein quality control in both ER and cytosolic compartments.

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

Author Disclosures: S. Doroudgar: None. C.C. Glimbskoti: None.

Key Words: Cardioprotection, Stress, Ischemic heart disease

In vivo Differentiation of Epigenetically Reprogrammed Endothelial Progenitor Cells into Cardiomyocytes Enhances Functional and Anatomical Post-infarct Myocardial Repair

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Background: Currently, bone marrow derived endothelial progenitor cells (BM-EPCs) are being used clinically to improve vascularization in patients with ischemic heart disease. While it is generally accepted that EPCs participate in vascular repair of the ischemic myocardium, there exists no evidence that these cells are capable of trans-differentiating into functional cardiomyocytes. Therefore, gene therapy approaches to replenish adult cardiac regenerative potential fail.

Methods and Results: The ECG revealed a QT interval of 317 ms (95% CI: 329 ms) with tall, narrow, and symmetrical T-waves. Invasive electrophysiological testing showed short ventricular refractory periods and increased vulnerability to induce ventricular fibrillation. DNA screening of the patient identified no mutation in previously known SQTS genes, however a new variant was found at a heterozygous state was identified in the CACNA2D1 gene (nucleotide c.2264G>A). The patient identified no mutation in previously known SQTS genes, however a new variant was found at a heterozygous state was identified in the CACNA2D1 gene (nucleotide c.2264G>A).

Conclusions: Taken together, our results suggest that epigenetically reprogrammed EPCs display a more plastic phenotype and improve post-infarct cardiac repair by both neo-cardiogenesis and neurovascularization.


Cardiac regeneration, Endothelial progenitor cell, Stem/progenitor cells, None.

M. Thal, P. Krishnamurthy, E. Lambers, E. Hoxha, S. Verma, D. Losordo, R. Kishore

Cardiac regeneration, Endothelial progenitor cell, Stem/progenitor cells, None.

Increases in Sodium Permeability Trigger the Activation of ME2/NFAT and BNP/MHC Gene Expression in Cardiac Myocytes via the Salt-Inducible Kinase 1 Network

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Cardiac hypertrophy (CH) generally occurs as the result of a sustained mechanical stress, due to elevated systemic arterial blood pressure (BP). In animal models however, elevated salt
intake associate with CH without significant increases in BP. Thus, we hypothesize that CH may not be entirely related to a mechanical stress but also to other factors associated with elevated BP such as abnormal sodium homeostasis. To test this hypothesis, we examined the effect of increases intracellular in sodium (Na[i]i) (5-10 mM) on transcription factors (TF) and genes associated with CH by incubating HL-1/cells (a line of cardiac origin) with 3 mM NaCl. Elevations in [Na[i]i]i induced a large increase in brain nitric peptide gene expression (11 fold, n=6 at 12 hs), and a moderate increase in structural genes such as myosin heavy chain (% increase 160±20, n=5 at 12 hs) (mRNA expression levels by Taqman and also luciferase-tagged reporters). Furthermore, the TF ME2F (ANOVa P<0.008) and NFAT (ANOVa P<0.003) also increased in a time dependent manner. Elevation of [Na[i]i]i is associated with activation of salt-inducible kinase 1 (SIK1, or snrk-t), an essential kinase for cardiac development. In HL-1 cells, elevations in [Na[i]i]i increased SIK1 activity, revealed by an intramolecular Förster resonance energy transfer (FRET)-based sensor showing the structural alteration of SIK-dependent phosphorylation of T182 residue, ([Na[i]i]i) not capable to increase ME2F/NFAT activity or gene expression in cells expressing two SIK2 mutants (lacking SIK1 phosphorylation motif) when compared to cells expressing the wild type SIK1. SIK1-dependent activation of ME2F involved phosphorylation of HDAC5, because its activation was abolished in cells expressing HDAC5 lacking the SIK1 phosphorylation domain (S255A). The mechanisms by which increases in [Na[i]i]i activate SIK and TF are initiated by a parallel increase in intracellular calcium via the reverse Na[i]/Ca[i] exchanger, activation of CaMKI and phosphorylation of SIK1 (S252), in conclusion, sustained and moderate increases in myocyte sodium permeability may directly influence myocardial growth by controlling transcription activation and gene expression throughout the activation of the SIK network.

Author Disclosures: P. Sergej: None. K. Venetsanou: None. P.J. Chedrese: None. H. Takemori: None. M. Mikolla: / Flt4 muscle cells expressing alpha smooth muscle actin and myosin, h-caldesmon, and endothelial and exposed to cardiac, smooth muscle and endothelial-specific differentiation media, /H11001 /H11001 /H11001 /H11001 embryonic stem cells and identified eight potential candidate cell surface markers. We utilized /H11001 markers we performed microarray analysis of Isl-1 expression (t12 mice per group) to receive human NGF via intra-myocardial AAV-2-Gal and intra-venous AAV-NFG-Gal to serve as non-diabetic controls. In separate mice, successful LV GT at 2 weeks after AAV-2-Gal and AAV-NFG-Gal was confirmed by X-Gal staining and β-gal activity assay, respectively. In comparison with non-injected mice, intra-venous AAV-NFG-Gal enhanced β-gal activity in lmb muscles, but not in other studied organs, including liver and kidneys. High concentrations of human NFG were detected (ELISA) in plasma at 2 and 12 weeks after NGF, but not of β-gal. As presented in Table 1, echocardiography showed a progressive deterioration of cardiac function and LV contractile dysfunction in β-gal-treated diabetic mice. By contrast, β-gal-treated diabetic mice were protected. Moreover, diabetes caused microvascular rarefaction in the LV myocardium, which was prevented by NGF. Our results provide the first evidence that AAV-mediated intra-myocardial or systemic NGF can prevent diabetes-induced cardiac dysfunction and suggest the therapeutic potential of this gene therapy approach.

Author Disclosures: A. Aaslan: None. K. Schenke-Layland: None. B. Van Handel: None. H. Mikola: None. R. MacLellan: None. Key Words: Beta-adrenergic receptor agonists, Myocardial contraction, Pharmacology

23352 Novel Cell Surface Markers to Identify and Isolate Endogenous or Induced Pluripotent Stem Cell-Derived Human Cardiovascular Progenitor Cells

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Introduction: Given their robust capacity for cardiovascular differentiation, the recently described Isl-1 positive cardiovascular progenitor cell (CPC) would be of great benefit for cardiac cell therapy. However, at present, surface markers that characterize early human CPCs are unknown. Surface markers that identify mouse CPCs (Kdr, c-Kit) are less specific for identifying human CPCs and intracardiac markers (Isl-1, Nkx2.5) would require genetic modification of the cells and complicate their clinical use. Purpose of study: We sought to develop markers capable of identifying endogenous human CPCs and determine culture conditions that support their expansion in vivo. A secondary goal was to utilize these markers to isolate and characterize multipotent CPCs from human induced pluripotent stem cells (iPSCs) for potential use in regenerative therapies. Results: To determine more specific surface markers we performed microarray analysis of Isl-1 /Kdr+ CPCs isolated from mouse embryonic stem cells and identified eight potential candidate cell surface markers. We utilized FACS and immunofluorescence on fetal human hearts to determine the sensitivity and specificity of these markers alone and in combination to identify Isl-1 + CPCs. The combination of FRT1 and FRT4 best identified the Isl-1 + CPCs in human hearts. Isolated endogenous and human iPSC-derived FRT1 /FRT4 + cells expressed CPC-specific genes including Nkx2.5, Sox18, Meis2 and c-Myc, but no markers of differentiated cardiac cells. When endogenous and human iPSC-derived FRT1 /FRT4 + cells were plated on matrigel-coated plates and exposed to cardiac, smooth muscle and endothelial-specific differentiation media, spontaneously beating colonies were seen after 21 days. Immunocytochemical staining of these colonies identified cardiomyocytes expressing Troponin C and sarcomeric actin, smooth muscle cells expressing alpha smooth muscle actin and myosin, h-caldesmon, and endothelial cells expressing CD31, WVF and elNOS. Conclusion: Cell surface markers FRT1 /FRT4 + can be used to reliably identify and isolate CPCs. FRT1 /FRT4 + CPCs from human iPSC differentiate into all three cell types of the cardiovascular lineage making them potential candidates for clinical cell therapy.

Author Disclosures: A. Aaslan; None. K. Schenke-Layland; None. B. Van Handel; None. H. Mikola; None. R. MacLellan; None. Key Words: Cardiac regeneration, Stem cell therapy, Progenitor cell

23690 Specific Interaction with Tyrosine 308 of β2-Adrenoceptor Promotes Preferential Gβγ Coupling

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There are two clinically important subtypes of β2-adrenergic receptors (β2ARs), β2AR and β3AR. We have previously proposed a novel therapeutic strategy for heart failure by combination of β2AR blocker and β2AR agonists. Unlike the Gαi-coupled β2AR, β3AR couples dually to Gαi and Gαq proteins. Our previous studies in cardiacomyocytes have demonstrated that while β2AR agonists promote dual Gαi and Gαq coupling, fenoterol and methoxalenfetol stereoisomers induce differential Gαq/12 coupling with the RAR-isomers being Gαq-selective and the S2-isomers dually Gαq/12-activating. The data demonstrate that the two stereoisomers stabilize distinct active conformations of β2AR. As revealed by comparative molecular field analysis of fenoterol derivatives, hydrogen bond formation with tyrosine residue Y308 confers the agonist high potency. PTX-sensitive responses were also observed for the RAR-1/2-phenyl (EC50 = 16 ± 3 nM, p<0.05) and RAR-phenyl (EC50 = 80 ± 22 nM, p<0.05) derivatives. In contrast, the positive inotropic effect of RAR-fenoterol was PTX-insensitive (EC50 = 176 ± 78 nM, EC50PTX = 145 ± 64 nM; p<0.64), akin to the case of RAR-fenoterol. Also, RAR-fenoterol-stimulated activation of extracellular signal-regulated kinase 1/2 in PTX-resistant in HEK293 cells stably expressing wild-type β2AR but PTX-sensitive in HEK293 cells expressing the β2AR-Y308A mutant. Thus we conclude that specific interaction between certain RAR-isomers and Y308 stabilizes the β2AR in a conformation favoring selective Gαi coupling, a criterion in the identification of novel therapeutic agents for heart failure.

Author Disclosures: A. Woon; None. K. Józwiak; None. A. Pizilnaka; None. M. Kolinski; None. J.A. Kozocas; None. L. Toll; None. M.J. Tanga; None. V. Zernetkina; None. R. Paul; None. M. Bernier; None. L.W. Wainer; None. R. Xiao; None. Key Words: Beta-adrenergic receptor agonists, Myocardial contraction, Pharmacology

23711 Nerve Growth Factor Gene Therapy via Either Intra-Mycardial or Systemic Delivery of Adeno-associated Viral Vectors Prevents Diabetic Cardiomyopathy in Mice

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Diabetes mellitus causes cardiac dysfunction and heart failure, Nerve growth factor (NGF), which exerts cardiovascular protective and regenerative effects, is downregulated in the diabetic heart. Gene transfer (GT) with adeno-associated viruses (AIVs) is capable of long lasting transgene expression. Moreover, ANV-α-isoforms (2 ANV/9) shows a preferential tropism for myocardies. Here, we investigated whether intramyocardial or systemic ANV-mediated GT could prevent cardiomyopathy in a mouse model of diabetes. Two weeks after type-1 diabetes induction by streptozotocin (40 mg/Kg/day IP for 5 days), diabetic CD1 male mice were randomized (n=12 mice per group) to receive human NFG or β-Gal control via either 4 injections of an ANV-2 vector (total dose: 1X107 viral particles) in the left ventricle (LV) wall or a single injection of an ANV-2 vector (1.5X1010 viral particles) in the tail vein. Age-matched CD1 male mice received intra-myocardial ANV-2-β-Gal or intra-venous ANV-NFG-Gal to serve as non-diabetic controls. In separate mice, successful LV GT at 2 weeks after ANV-2-βGal and ANV-NFG-Gal was confirmed by X-Gal staining and β-gal activity assay, respectively. In comparison with non-injected mice, intra-venous ANV-NFG-Gal enhanced β-gal activity in limb muscles, but not in other studied organs, including liver and kidneys. High concentrations of human NFG were detected (ELISA) in plasma at 2 and 12 weeks after GT of NFG, but not of β-Gal. As presented in Table 1, echocardiography showed a progressive deterioration of cardiac function and LV contractile dysfunction in β-Gal-treated diabetic mice. By contrast, β-Gal-treated diabetic mice were protected. Moreover, diabetes caused microvascular rarefaction in the LV myocardium, which was prevented by NFG. Our results provide the first evidence that ANV-mediated intra-myocardial or systemic NFG can prevent diabetes-induced cardiac dysfunction and suggest the therapeutic potential of this gene therapy approach.
Identification of Nitric Oxide-dependent miRNAs Determining Chromatin Changes During Mouse Embryonic Stem Cells Differentiation

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Introduction: MicroRNAs (miRNA) are a class of small, non-coding RNA molecules recently emerged as important regulators of gene function through their effects at post-transcriptional level. No or little information, however, is available about the effect of miRNAs on chromatin structure during ES differentiation. Methods & Results: A miRNA profiling was performed in mouse ES (mES) cultured with or without leukemia inhibitory factor (LIF) and in the presence or absence of the NO donor DETANO which elicits a early expression of mesoendodermic/vascular differentiation markers. In this condition, the presence of NO determined a rapid expression of miRNA-200 family members. Real-time PCR analysis confirmed, in fact, that miRNA-200a, -200c, -200d, -202, -429 were up-regulated about 5 fold on average above control level. Direct over-expression of miRNA-200a, -200c, -200d and -429 alone or in combination had an important effect on chromatin structure. Specifically, the global acetylation of histone H3 lysine K9 (H3K9Ac) was significantly increased in mES as well as NIH-3T3 cells paralleled by a significant upregulation of global histone acetylase activity (HAT). The expression of miRNA-200 family members also had a positive effect on histone H4 Lysine 20 tri-methylation (H4K20me3) which is a modification known to be associated to differentiation, aging and repressed chromatin formation. Mechanistically, the miRNA-200 family is known to repress the Smad interaction protein Sip1/2EB2. Consistently, the direct knockdown of Sip1/2EB2 by shRNA interference elicited a similar effect on chromatin structure thus suggesting this factor as a negative effector of the NO- and miRNA-200-dependent chromatin remodeling. Conclusion: Taken altogether our data identifies for the first time the miRNA-200 family as responsible of adaptive chromatin changes during the NO-dependent process of mES differentiation and suggest Sip1/2EB2 as an important effector of this process.

Author Disclosures: J. Rosati: None. F. Spallotta: None. B. Illi: None. M.C. Capogrossi: None. C. Gaetano: None.

Key Words: Stem cells, Nicotin oxide, MicroRNA

Density of Collagen in the Infarcted Myocardium Determines Engraftment and Angiogenesis by Induced Pluripotent Stem Cells (iPSC)

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We hypothesized that amount of collagen deposition determines the efficiency of iPSC from a patch applied to infarcted area after myocardial infarction (MI). Transgenic mouse with overexpression of adenyl cyclase VI (AC6) in which collagen synthesis is decreased upon ischemia was used. In vitro: iPS were transfected with plv-RES-ZsGreen-first Luciferase lentivirus vectors bearing a cardiac-sodium calcium exchange promoter (PRSGC) for generation of cardiac progenitors in the patch. Expression of collagen I and collagen III was measured in heart tissues of both AC6 and wild-type (WT) mice. In vivo: iPSC (1x10^5 cells) was placed over the entire infarcted area at 7 days after MI in AC6 and WT mice. Engraftment efficiency of progenitor cells was assessed using a combination of in vivo bioluminescent imaging (BLI) and postmortem ex vivo analysis. Echocardiography was performed and hearts were harvested for analysis at 4 weeks after cell patch, iPSC+patch hearts showed significantly higher BLI compared to differentiation of cardiomyocytes (31%±1.68) from baseline (7%±0.08). Heart function was progressively improved from week 2 to week 4 and was associated with reduced left ventricular fibrosis, collagen I, and collagen III in AC6 mice as compared to WT mice (Fig1). Myocyte proliferators derived from iPSC showed significantly higher engraftment and angiogenesis in AC6 mice with sparsely distributed connective tissue suggesting that density of collagen determines the penetration and engraftment of iPSC in the infarcted myocardium.


Key Words: Stem/progenitor cells, Myocardial infarction, Gene expression

Inhibition of Plasminogen Activator Inhibitor-1 (PAI-1) Corrects Diabetic Endothelial Progenitor Cell (EPC) Dysfunction in vitro and in vivo


The dysfunction of diabetic CD34+ cells limits their utility in autologous cell therapy for vascular complications. Previously, we showed that transient inhibition of transforming growth factor-beta 1 (TGF-β1) enhances vascular reparative function of diabetic CD34+ cells. Expression of PAI-1, the major gene product of TGF-β1 activation, is increased by high glucose and insulin exposure in endothelial cells and serum of diabetics. We asked whether the beneficial effects of TGF-β1 blockade on CD34+ cells function are mediated by inhibition of PAI-1 and whether blocking of PAI-1 could correct diabetes associated dysfunction. Plasma determinations of PAI-1 and TGF-β1 were compared in type 2 diabetic (n=17) and type 1 (n=7) diabetic patients with micro- and macro-vascular disease. CD34+ cells from these individuals were analyzed for cell survival, proliferation, cell cycle analysis and migration. The effect of TGF-β1 phosphorodiamidate morpholino oligomers (PMO) treatment on PAI-1 levels was also determined. In CD34+ cells, PAI-1 was blocked using either lentivirus expressing PAI-1 shRNA or PAI-1 siRNA. In vivo homing ability of PAI-1 inhibited CD34+ cells was assessed using ischemia/reperfusion (I/R) injury model. Plasma PAI-1 level was markedly increased in type 2 diabetic patients compared to type 1 (<0.05) and directly correlated with TGF-β1 plasma levels (r=-0.44). TGF-β1 PMO treatment resulted in a reduction of PAI-1 mRNA expression (p=0.0018 in diabetic, p=0.05 in non-diabetic), PAI-1 blockade promoted CD34+ cell proliferation and cell survival in the absence of growth factors (p=0.05). PAI-1 blockade enhanced the migration of the cells in response to SDF-1α (p=0.01) and improved the in vivo re-endothelialization of CD34+ cells in the I/R model. Our results show that the cytotactic activity of TGF-β1 in CD34+ cells is mediated through PAI-1. Blocking PAI-1 corrects key functional defects in diabetic CD34+ cells. This approach will offer a promising novel therapeutic strategy to allow autologous cells therapy in diabetics with severe vascular dysfunction.


Key Words: Endothelial progenitor cell, Type 2 Diabetes, Angiogenesis
Late-Breaking Basic Science Abstracts From the American Heart Association's Scientific Sessions 2010, Chicago, Illinois, November 13–17, 2010

Circ Res. 2010;107:e32-e40; originally published online November 12, 2010; doi: 10.1161/RES.0b013e3182014899

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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