Late-Breaking Basic Science Abstracts
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Cardiac Myosin Binding Protein C is an Ultra-early and Cardiac-specific Biomarker of Myocardial Necrosis

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Rationale: We recently demonstrated that proteolytic cleavage fragments of cardiac myosin binding protein C (cMyBP-C) can be detected in the serum after myocardial infarction (MI) in a rat model and in patients with MI. The findings implied that serum cMyBP-C might be useful as a cardiac-specific biomarker for MI. The release kinetics of cMyBP-C in the circulation post-MI remains to be elucidated. Objective: Determine the release kinetics of cMyBP-C as an ultra-early and cardiac-specific biomarker of myocardial necrosis. Method and Results: To determine the exact timing of cMyBP-C release in the bloodstream post-acute MI, left anterior descending (LAD) coronary artery was ligated in adult swine (n=6). ECG showed significant ST elevation. Infarct size represented 12.4 ± 1.9% of total ventricular mass. Blood samples were collected before and at predetermined time points between 30 min and 14 days after LAD ligation. Plasma cMyBP-C level was quantified using a highly sensitive and rapid sandwich enzyme-linked immunosorbent assay. Compared with baseline, cMyBP-C levels were increased in post-MI serum within 45 min (0.64 ± 0.52 ng/ml) after LAD ligation and declined after 16 hrs to the baseline level (0.01 ± 0.00 ng/ml). In contrast, cardiac troponin I (cTnI) level peaked after 6 hrs and returned to baseline after 10 days. To validate these findings in humans, serial blood samples were taken from 5 patients with hypertrophic cardiomyopathy undergoing transcatheter ablation of septal hypertrophy (TAGH). Similar to the swine model, the level of cMyBP-C increased 30 min after TAGH (0.25 ± 0.15 ng/ml) and peaked at 4 hrs (0.56 ± 0.27 ng/ml), confirming that cMyBP-C is a promising ultra-early biomarker of MI. Furthermore, cMyBP-C level was determined in patients with acute coronary syndromes (ACS) from the SYNERGY library population and a healthy control group (n=160 and 61, respectively). Seventy-eight percent (125 out of 160) of patients with ACS had detectable cMyBP-C serum levels (2.9 ± 1.2 ng/ml), implicating serum cMyBP-C as a biomarker for ACS. Conclusion: The rapid appearance of proteolyzed cMyBP-C in the circulation post-acute MI in a swine model and in human patients with ACS and post-TAGH identify serum cMyBP-C as an ultra-early biomarker of myocardial necrosis.


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Replacement Of Mybpc3 Mutation By 5'-trans-splicing In A Knock-out Mouse Model: A Step Towards Gassural Therapy Of Hypertrophic Cardiomyopathy

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Purpose: Hypertrophic cardiomyopathy (HCM) is characterized by asymmetric septal hypertrophy, diastolic dysfunction, myocardial disarray and lacks curative treatment. It is often caused by mutations in MYBPC3 encoding cardiac myosin-binding protein C (cMyBP-C). Most of the mutations alter mRNA splicing and result in aberrant mRNAs and proteins. In the present study we evaluated the feasibility and efficacy of RNA correction using spliceosome-mediated 5'-trans-splicing to remove the mutation in vitro and in vivo in Mybpc3-targeted knock-in (KI) mice. Methods and Results: KI mice carry a G > A transition in exon 6, which results in low levels of mutant Mybpc3 mRNAs and cMyBP-C proteins. We generated a series of FLAG-tagged pre-trans-splicing molecules (PTM) containing wild-type exons 1-6 with binding domains complementary to intron 6 of Mybpc3. The PTMs were packaged in aden-associated virus serotype 6 or 9 (AAV6; AAV9) driven by a cardiomyocyte-specific promoter. Cardiac myocytes isolated from neonatal KI mice (NCMks) were transduced with AAV6 for 7 days and AAV9 was systemically administered in 3-day-old KI mouse for 7 weeks. The efficacy of 5'-trans-splicing was evaluated by RT-PCR, Western Blot and immunofluorescence. The trans-spliced mRNA was amplified only with specific primers only in PTM-transduced samples in vitro and in vivo. The correction of the mutation was confirmed by sequencing. By semi-quantitative PCR trans-spliced mRNA was estimated to represent 33% and 0.14% of total Mybpc3 transcripts in vitro and in vivo, respectively. Whereas the trans-spliced cMyBP-C protein was not revealed by Western blot, up to 9% of FLAG-positive striated cardiac myocytes were detected by immunofluorescence and exhibited correct incorporation of trans-spliced cMyBP-C in doublets in the A-band of the sarcomere. Conclusion: These data provide the first proof-of-concept of 5'-trans-splicing to correct cardiac genetic defects in vitro as well as in vivo. Therefore, spliceosome-mediated 5'-trans-splicing may be a promising approach for the treatment of HCM.


Hematopoietic Id Ablation Contributes To Pathogenesis in the Adult Heart

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The Id inhibitor of DNA binding genes play a crucial role in cardiovascular development. Id double knockout (Id dKO) embryos lacking both Id1 and Id3 develop multiple cardiac defects reminiscent of the “thin myocardial wall” syndrome and are lethal at mid-gestation. While Id genes are only expressed in non-myocardial layers (epicardium, endocardium, endothelium), the myocardium is affected suggesting a paracrine mechanism of action. Maternal injection of Lf1f1 (epicardial Id-dependent signal) failed to rescue inner heart defects seen in Id dKO embryos, suggesting that the endocardium/endothelium play an important role in cardiac development. To circumvent embryonic lethality and study the role of Id in the endocardium/ endothelium in adult mice, we generated a conditional knockout (Id cKO) using the Tie2Cre/loxP system. These mice are Id3 null with endocardial/endothelial-specific Id1 ablation. Surviving Id cKOs develop dilated fibrocardiomyopathy, hematopoietic defects and splenomegaly in adulthood, suggesting that Id loss in Tie2 expressing organs may be responsible. Since Tie2 is also expressed in hematopoietic cells, it is unclear if loss of hematopoietic Id contributes to cardiac pathology in Id cKOs. To address this question, we transplanted WT GFP-labeled bone marrow into lethally irradiated Id cKOs (WT/Id cKOs) to test if Id cKO-deficient hematopoietic system negatively impacts a normal heart (dysregulation). Full bone marrow reconstitution occurred. Within the endocardium, we found marked acellularity and fibrosis in Id cKOs, decreased fibrosis and improved cellularity in WT/Id cKOs, and emerging interstitial fibrosis and mononuclear invasion in id cKOs/WTs. The marked pervasive fibrosis in Id cKO hearts was reduced in WT/Id cKO hearts. Ejection fraction decreased in Id cKOs, improved in WT/Id cKOs and decreased in Id cKOs/WTs (64.4±5.7% n=5 WT/Id cKOs; 58.4±7.97% n=9 Id cKOs/WTs; 56.2±13.3% n=56 id cKOs; 64.3±6.8% n=15 Wts). These results suggest that Id loss in bone marrow cells play an important role in the development of diastolic, fibrotic cardiomyopathy. This research has received full or partial funding support from the American Heart Association.

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Detection And Therapy Of Ischemia-reperfusion Injury Using Hydrogen Peroxide-responsive Molecularly Engineered Polymer Nanoparticles

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The main culprit in the pathogenesis of ischemia/reperfusion injury (IR) is the overproduction of hydrogen peroxide (H2O2), a causal agent for apoptosis and inflammation that lead to cellular damage and organ dysfunction. In this study, we generated and characterized novel H2O2-responsive nanoparticles formulated from polylactide co-polymer containing vanillyl alcohol (PVAX) during IR injury. PVAX, in the presence of H2O2, generates completely into three safe compounds, cyclohexanedimethanol, VA and CO, PVAX efficiently scavenges H2O2 in a dose-dependent manner, with the intrinsic anti-oxidant and anti-inflammatory properties in vitro. Using In Vivo Imaging System, we demonstrated that PVAX loaded with rubrene as a fluorophore robustly imaged H2O2 generated during IR in the hind-limb IR injury in vivo, demonstrating their potential for bioimaging of H2O2. PVAX nanoparticles injected into the site of hind-limb IR injury also exerted highly potent anti-inflammatory and anti-apoptotic activities resulting in a significantly less cardiac damage compared to the vehicle treated group. We then examined the effect of PVAX in doxorubicin (DOX)-mediated cardiomyopathy since a major mechanism of DOX-mediated toxicity has been shown to involve increased oxidative stress. We found that i.p. injection of 100 μg of PVAX daily resulted in significant decrease in apoptosis as well as PARP-1 and caspase activation in both heart and liver compared to vehicle treated groups.

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Furthermore, 10 days after DOX administration PVAX treated animals, compared to vehicle treated animals, demonstrated 31% less decrease in body weight, 28% improvement in functional capacity (as indicated by significantly improved maximal running time (WT= 75% ± 10%; PVAX=71%; p<0.05%). Moreover, PVAX administered daily for 7 days demonstrated no histological or functional abnormalities in various organs. Thus, we conclude that PVAX is a novel multifunctional nanoparticle that possesses intrinsic anti-oxidant and anti-inflammatory properties, and has a tremendous potential to be used as a theranostic agent for IR injury in heart and other organs.


Key Words: Stem cells; Aging; Cardiac regeneration

22954

Communication with Cardiomyocytes in Contact Co-culture Repprograms Mesenchymal Stem Cells for Improved Cardiomyogenesis

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Background: Bone marrow mesenchymal stem cells (MSC) can differentiate to morpho-functional cardiomyocytes (CM) when maintained in conducive culture conditions in vitro and post-transplantation in the ischemic heart albeit with an as yet undefined mechanism. We hypothesized that propensity of MSC for reprogramming to adopt cardiac phenotype and their myocardial reparability could be enhanced if the cells were co-cultured with CM in vitro prior to transplantation. Methods and results: Bone marrow cells were isolated from GFP expressing transgenic adult rats and analyzed for MSC specific surface markers by flow cytometry. The cells were co-cultured with PKH-26 labeled rat neonatal CM at 1:3 ratios in a dual chamber dish separated by a semi-permeable membrane or by direct contact co-culture for 0-4 days. Conventional fluorescence microscopy revealed formation of nano-tubular structures between nano-tubular structures in contact co-culture with CM. Live cell imaging showed transient exchange of cytosolic contents between the two juxtaposed cell types which was indicated by PKH26 red fluorescence dye transfer. Flow cytometry revealed increased percentage of double positive GFP+MSC in time-dependent manner (up to 81.98 ± 5.91% of GFP+MSC received the red dye transferred from CM at day 3 of co-culture). Cardiac specific gene expression including GATA4, Nkx2.5 and MEF2c was upregulated in vitro and post-transplantation in the ischemic heart albeit with an as yet undefined mechanism. Our findings provide an insight into the mechanisms which underlie the morpho-functional cardiogenic differentiation of MSC post-transplantation in an infarcted heart. Author Disclosures: V. Lai: None. S. Jiang: None. K.S. Prindle: None. K.H. Haider: None.

Key Words: Myocardial infarction; Regenerative medicine stem cells; Transplantation; Molecular biology

22894

Telomeric Shortening Induces a Senescent Cardiomyopathy that is Independent from Chronological age

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Telomeric shortening is viewed as the hallmark of organism, organ, and cellular senescence. It remains, however, to be documented whether loss of telomeric DNA in cardiac stem cells (CSCs) and/or cardiomyocytes is causally involved in the manifestations of the aging cardiomyopathy. To address this issue, the heart of mice carrying a deletion of the RNA component of telomerase (Terc) was studied at 3-7 months of age. This allowed us to define whether telomere shortening in stem cells and their progeny promotes ventricular dysfunction independently from chronological age. The structural and functional characteristics of the aging cardiomyopathy were defined initially in senescent 30-month-old wild-type mice (WT). By echo-Doppler, MRI, and invasive hemodynamics, deterioration in systolic and diastolic indices of myocardial contractility were detected in these senescent mice. Quantitative parameters obtained by MRI documented the presence of chamber dilation and attenuated ventricular torsion capacity and diastolic strain rate. With respect to young mice, a 50% reduction in telomere length was detected in CSCs and cardiomyocytes isolated from 30 month-old mice. Importantly, 7-month-old Terc−/− mice showed severe ventricular dysfunction comparable to that seen in 30 month-old WT. Telomere length in Terc−/− mice CSCs and myocytes was ~50-70% shorter than in age-matched WT cells but was comparable to that detected in 30 month-old WT cells. The number of CSCs was 60% lower in Terc−/− than age-matched WT cells, and the fraction of BrdU-positive CSCs decreased 1.4-fold, from 25% to 14%. The absence of Terc led to a 50% reduction in myocyte turnover, which was coupled with myocyte hypertrophy and significant myocyte loss. BrdU and KI67 labeling were reduced, respectively, by 65% and 60% in Terc−/− myocytes. Old CSCs formed a senescent progeny composed of cardiomyocytes, which carried markedly shortened telomeres, were consistently larger in volume, and showed a severe depression in cell shortening and re-lengthening. Our findings document that telomeric shortening is the central determinant of CSC aging and reduced myocyte turnover. The accumulation of senescent CSCs and cardiomyocytes leads to pathological cardiac remodeling and heart failure (HF= 75% ± 10%; PVAX=71%; p<0.05%). Moreover, PVAX administered daily for 7 days demonstrated no histological or functional abnormalities in various organs. Thus, we conclude that PVAX is a novel multifunctional nanoparticle that possesses intrinsic anti-oxidant and anti-inflammatory properties, and has a tremendous potential to be used as a theranostic agent for IR injury in heart and other organs.

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Key Words: Ischemia reperfusion; Apoptosis Inflammation; New technology; Cardiomyopathy

22950

Identification of Novel Non-steroidal Modulators of Vitamin D Receptor with Cardioprotective Property without Hypercalcemic Effect

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Vitamin D is a multifunctional, steroid hormone responsible for regulating various biological processes. Vitamin D therapy has been shown to prevent cardiac hypertrophy and improve cardiac dysfunction. However, its clinical utility has been limited by hypercalcemia. In this study, we report on a novel vitamin D agonist tool compound discovered using computer aided drug discovery (CADD) that possesses significant anti-hypertrophic activity without hypercalcemia. Using this CADD approach, we screened approximately 4 million unique compounds virtually using chemical features/ descriptors from known vitamin D receptor (VDR) agonists that had minimal demonstrated hypercalcemic activity. These hit compounds were vetted using an ensemble structure-based screen of the VDR. The top 174 CADD molecules were evaluated using the GeneBlazer® Cell-Based VDR Assay to identify those compounds that significantly regulated the transcriptional profile of VDR; we identified 5 compounds that showed considerable activation at 100 μM or lower. The compound possessing the greatest VDR binding activity (known as VDR136) showed a significant concentration-dependent suppression of phenylephrine (PE)-induced cardiac hypertrophy in adult cardiomyocyte culture in vitro and mouse infused with PE via osmotic pump in vivo. In addition, VDR136 significantly suppressed cardiac hypertrophy and progression to heart failure induced by transverse aortic constriction (TAC) as compared to the vehicle treated group as determined by 17% decrease in HW/BW ratio and 41% improvement in fractional shortening. VDR 136 also demonstrated effective suppression secondary hyperparathyroidism in transgenic knockout mice, a model of vitamin D deficiency. Most importantly, we observed no significant hypercalcemia even at supra-physiological concentrations of VDR136. In contrast, calcitriol, a naturally occurring, commercially available vitamin D hormone, caused significant hypercalcemia and hyperphosphatemia. Thus, VDR136 represents a novel VDR agonist with significant cardioprotective properties that lack the hypercalcemic effect otherwise common with vitamin D analogs, and may provide a novel therapeutic option for the treatment of cardiac hypertrophy and heart failure.


Key Words: Heart failure; Hyperphosphor; Vitamins; Calcium; Drugs

22966

Sorafenib-induced Cardiotoxicity is Mediated by Inhibition of c-kit+ Cardiac Stem Cells

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Introduction: Tyrosine kinase receptor (RTK) inhibitors have been previously shown to be cardiotoxic in cancer patients with comorbidities, but the mechanism of this cardiotoxicity has not been elucidated. Hypothesis: The multi-kinase inhibitor Sorafenib is known to inhibit several RTKs including c-kit, a receptor found on cardiac progenitor cells in the heart and bone marrow. Patients with comorbidities have pre-existing cardiac damage, and administration of Sorafenib is risky in the cardiac donor POP. We hypothesize that the donor myocyte turnover and exacerbating cardiac dysfunction. Methods and Results: Male 12 week old C57BL/6 mice were pretreated with 40 mg/kg/d intraperitoneal Sorafenib (n=20) or vehicle (n=20) for 3 weeks. Sorafenib treatment dramatically exacerbated LV dysfunction measured by echocardiography and may provide a novel therapeutic option for the treatment of cardiac hypertrophy and heart failure.


Key Words: Heart failure; Hyperphosphor; Vitamins; Calcium; Drugs
cardiovascular events shows diurnal variation with a peak in the morning. The circadian change in vascular contractility is one of the mechanisms for this circadian variation. We hypothesize that the vascular intrinsic clock contributes to generating the circadian rhythm of the contractility. However, its underlying mechanism still needs to be clarified. The circadian change in vascular contractility is one of the mechanisms for this circadian variation.
myocardial remodeling and dysfunction. Thus cardiac fibroblast GSX3 and GS3-3 induces opposite effects on MI-induced cardiac remodeling and dysfunction. Furthermore, these data are, to our knowledge, the first to establish the driving role of cardiac fibroblasts in MI-induced remodeling.


Key Words: Myocardial infarction; Fibrosis; Heart failure; Remodeling

 Modeling Cardiovascular Disease in Supravalvular Aortic Stenosis and Williams-Beuren Syndrome Using Human Induced Pluripotent Stem Cells

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Supravalvular aortic stenosis (SVAS) and Williams-Beuren syndrome (WBS) are genetic disorders of elastin (ELN) and are characterized by hyper-proliferation of vascular smooth muscle cells (SMCs) that can lead to blockage of the arterial vessels and sudden cardiac death. While SVAS is typically caused by heterozygous loss-of-function mutations in the ELN gene, WBS is a microdeletion disorder resulting from heterozygous loss of 1.5-1.8 Mb pairs of DNA from chromosome 7 including the gene, WBS is a microdeletion disorder resulting from heterozygous loss of 1.5-1.8 Mb pairs of DNA from chromosome 7 including the ELN gene. Although WBS patients display a complex phenotype including neurobehavioral defects, the cardiovascular lesions in patients with SVAS and WBS are virtually identical and manifested as hyper-proliferation of vascular SMCs. The use of animal models and primary cell culture to study SVAS and WBS has been very informative. However, Eln±/- and WBS haploinsufficiency mice do not develop human-like aortic occlusive defects, and human tissue accessibility is limited. It is therefore of great importance to establish a self-renewable human model for studying SVAS and WBS. Human induced pluripotent stem cells (hiPSCs) can be derived from a person’s own somatic cells by forced gene expression and self perpetuation. We recently established hiPSC lines from SVAS and WBS patients. SVAS and WBS iPSC-derived SMCs (iPSC-SMCs) had reduced ELN expression, decreased smooth muscle alpha actin (SM α-actin) filament bundles, and increased proliferation and migration, compared to control iPSC-SMCs. Recombinant ELN or enhancement of small GTPase RhoA signaling rescued actin filament bundle formation and inhibited hyper-proliferation. Several candidate small molecules including vinblastine have been tested to see whether rescue of actin filament bundle formation in SVAS or WBS iPSC-SMCs leads to inhibition of hyper-proliferation. Our results showed that low dose of vinblastine rescued actin filament bundle formation, inhibited hyper-proliferation, and decreased extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation in SVAS and WBS patients. SVAS and WBS iPSC-derived SMCs (iPSC-SMCs) recapitulate key cardiovascular pathological features of patients and may provide a promising strategy to study disease mechanisms and to develop novel therapies.

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Key Words: Stem cell biology; Smooth muscle regulation

MiR-24 Up-regulation Underlies Excitation-contraction Uncoupling in Heart Failure

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Chronic heart failure is a complex clinical syndrome with impaired myocardial contractility. Failing cardiomyocytes exhibit decreased efficiency of Excitation-contraction (E-C) coupling. The down-regulation of junctophilin-2 (JP2), a structural protein anchoring the sarcoplasmic reticulum (SR) to T-tubules (TTs), has been identified as a major mechanism underlying the defective E-C coupling. However, the regulatory mechanism of JP2 remains unknown. Here we report that miR-24, a microRNA up-regulated in heart failure, is an important upstream regulator of cardiac JP2. Bioinformatic analysis predicted two potential binding sites of miR-24 in the 3′-untranslated regions of JP2 mRNA. Luciferase assays confirmed that miR-24 suppressed JP2 expression by binding to either of these sites. In the aortic stenosis model, miR-24 was up-regulated in failing cardiomyocytes. Adenovirus-directed over-expression of miR-24 in cardiomyocytes decreased JP2 expression and reduced Ca2+ transient amplitude and E-C coupling gain. In vivo silencing of miR-24 by a specific anti- target in an aorta-constricted mouse model effectively protected cardiomyocytes from structural and functional E-C uncoupling, and prevented the mouse from developing heart failure.


Key Words: Heart failure; Excitation-contraction coupling (ECC); Calcium; Micromora

Antifibrotic Effect Of Transplanted Cardiomyocytes Mediated By Dynamic Activation Of The Matrix Proteolytic Cascade In Acute Myocardial Infarction

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Background: Intramyocardial cardiomyocyte transplantation in the infarcted myocardium enhances tissue viability and improves left ventricular (L/V) function in both small and large animal models. However, the underlying biological mechanisms by which cardiomyocytes exert their beneficial effects have not yet been fully elucidated. Objective: We tested the hypothesis that injected cardiomyocytes would alter the proteolytic pathway post myocardial infarction (MI) and thereby attenuate adverse remodeling. Methods: MI was induced in Wistar Kyoto rats, followed by peri-infarct injections of 2X106 syngeneic cardiomyocytes (n=25). Animals injected with vehicle only served as controls (n=20). Collagen synthesis were performed to test the direct interaction between fibroblasts and cardiomyocytes. Results: In vitro, cardiomyocytes stimulated fibroblasts’ excitation of gelatinases MMP2 and MMP9 as early as 24 hours in coculture. In vivo, a differential MMP/TIMP profile was evident in the peri-infarct region 7 days post MI and treatment: MMP2 and MMP9 were upregulated in the cardiomyosphere-treated myocardium compared to control (p<0.05 treated vs control), whereas increased MMP9 was detected. Plasma levels of ICTP, an index of collagen degradation, were increased by day 7 post cardiomyosphere transplantation (p<0.01 treated vs control). In addition, collagen synthesis, evaluated by hydroxyproline assay, was decreased (p<0.01 treated vs control). Picocircus red tissue staining (for regional collagen deposition) was decreased compared to the control group at day 21. These changes in the cardiomyosphere-transplanted post-MI myocardium was associated with significant improvements in LV morphological and functional parameters and increased capillary and vessel formation. Conclusion: Cardiomyocytes intramyocardially-injected in the infarcted myocardium alter the MMP pathway to favor a collagenolytic and antifibrotic profile. These findings begin to reveal how cardiomyocytes attenuate post MI adverse remodeling, a principle central to their beneficial actions in ischemic heart disease.

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Key Words: Stem cell therapy; Stem cell biology; Myocardial infarction; Remodeling; Extracellular matrix

Global RNA Splicing and Regulation in Fetal and Diseased Hearts

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Background: The complexity of transcriptome and proteome is contributed by alternative splicing of mRNA. Altered mRNA splicing is also implicated in many human diseases including cancer. However, little knowledge is available about the scope of alternative splicing at whole genome level during cardiac development and diseases and even less about the mechanisms underlying the regulation of mRNA splicing in response to pathological injury in heart. Methods and Results: In our current study, we have identified global alternative splicing changes associated with both development and pathological remodeling in mouse heart using deep RNA-sequencing. The alternative RNA splicing events observed in failing hearts resemble the profile observed in fetal hearts, suggesting a fetal-like RNA splicing program induced in diseased hearts. We subsequently examined the expression profiles of RNA splicing regulators in neonatal, normal adult, and failing adult mouse hearts, and identified Fox1 as a significantly induced regulator during cardiac development in both mouse and zebrafish, and down-regulated in both mouse and human failing hearts. Fox1 knock-down mediated by morpholino in zebrafish embryos led to lethal phenotype associated with impaired cardiomyocytes maturation and differentiation. Interestingly, this phenotype could be rescued by re-expressing both zebrafish and mouse Fox1 gene. Finally, using bioinformatic analysis, a significant number of the alternatively spliced exons identified in the failing heart harbor a conserved Fox1 binding motif in flanking introns, suggesting that Fox1 may serve as a conserved and major regulator for alternative RNA splicing during cardiac development and diseases. Conclusion: Our study provided the first comprehensive analysis of mRNA splicing regulation in heart during post-natal development and heart failure, and identified Fox1 as a potential key regulator for RNA splicing in heart. This study expands our current understanding to the complexity of cardiac transcriptome in both development and diseases, and reveals the functional importance of RNA-splicing regulation in heart.

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Key Words: Heart failure; Genomics; Cardiac development; Molecular biology

Treatment with Cell Lysate from Genetically Modified Stem Cells Containing Pre-formed Trophic Factors Effectively Rescues the Infarcted Heart

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Overview: A plethora of bioactive molecules released from mesenchymal stem cells (MSC) has shown their paracrine activity and contributed to preserved infarcted heart function.
Here, we demonstrated the therapeutic benefits of cell lystate containing pre-formed bioactive molecules in MSC genetically modulated to co-overexpress Akt and angiopoietin-1 (Ang-1). Methods and Results: Rat MSC were genetically modified for simultaneous overexpression of Akt and Ang-1 genes (pAkt&Ang-1) using our optimized protocol. MSC transduced with adenoviral vector without therapeutic gene (pAkt&Ang-1) were used as controls. After 48 hours of respective treatments, protein lysates were isolated by freeze-thaw cycles. Real-time PCR based array showed that beside Akt and angiopoietin-1, pAkt&Ang-1 had 25 growth factor genes including VEGF, BMP, interleukin, HGF and epiregulin with 2-fold upregulation as compared to pAkt&Ang-1. Lysate was cytoprotective for cardiomyocytes and endothelial cells and promoted endothelial cell migration and tube formation in vitro. Treatment of endothelial cells with pAkt&Ang-1 enhanced their proliferation (22.7 ± 1.8% vs 9.4 ± 1.3% for pAkt vs pAkt&Ang-1). Treatment of the infarcted heart with pAkt&Ang-1 (n=12 animals/group) significantly reduced cardiomyocyte apoptosis, increased cardiomyocyte DNA synthesis (Ki67+ cardiomyocytes/histological section 2.1 ± 0.4 vs 0.7 ± 0.2 for pAkt vs pAkt&Ang-1) and increased blood vessel density (29.4 ± 0.5 vs 16.1 ± 0.3 for pAkt vs pAkt&Ang-1). Lysate treated infarcted hearts also showed enhanced mobilization of bone marrow stem cells into the infarcted heart. These molecular and cellular events led to attenuated infarct size (27.8 ± 4.0% vs 37.2 ± 1.1% for pAkt vs pAkt&Ang-1) and preserved left ventricular function in pAkt&Ang-1 treated animal hearts. Conclusion: Treatment with cell lystate containing pre-formed trophic factors effectively rescued the infarcted heart by intricate mechanisms involving reduced myocardial apoptosis, enhanced cardiomyocyte proliferation, higher mobilization and homing of endogenous stem cells and improved angiogenesis. Our noninvasive approach will facilitate future stem cell therapy for heart failure in the clinical perspective.


Key Words: Stem cells; Cardiac regeneration; Angiogenesis; Proteins; Apoptosis

A Novel Paradigm of MicroRNA Regulated B Cell Functions On Insulin Resistance: Mir-150 Regulates Insulin Sensitivity Through Controlling Antibody Production

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Low degree adipose tissue inflammation induced by chronic nutrient excess is a causal factor for insulin resistance, which is the hallmark of type II diabetes and a major contributor to cardiovascular diseases associated with obesity. Extensive efforts have been contributed to define the roles of myeloid cells and T cells in this picture. However, bone cells are involved in this complex and how their function is regulated, especially by microRNAs, are poorly understood. Using a unique knockout mouse model, we discovered a novel mechanism that mir-150 exerts profound effects on chronic nutrient overload induced systemic insulin resistance by controlling B cell functions, specifically antibody production. Our previous study demonstrated that mir-150 is a key regulator for hematopoietic lineage development, especially for the formation for B lymphocytes. High-fat diet (HFD) feeding exacerbated the development of systemic insulin resistance and glucose intolerance in mir-150 KO mice compared to wild type control mice. No significant differences were observed between mir-150 and control groups in the aspects of HWI induced body weight gain, food intake, and adiposity, mir-150 deficiency did not affect macrophage activation, which has been previously demonstrated as the major contributor to diet induced adipose tissue inflammation and insulin resistance. Interestingly, mir-150 KO mice exhibited an increased adipose tissue B cell infiltration, adipose tissue inflammatory cytokine production, and liver steatosis, which is accompanied by dramatically enhanced circulating antibody production that can directly contribute to the development of insulin resistance. Four genes are identified as bona fide mir-150 target genes, including ELKS, CBL, EGRs, and EEF1, which are important components of multiple metabolic related signaling pathways. In summary, our study demonstrated that mir-150 is a novel and potent regulator for obesity induced systemic insulin resistance through controlling antibody productive B cells. Thus, it provides a novel paradigm of microRNA regulation in the context of insulin resistance, and opens a new pathway for insulin resistance and glucose intolerance in mir-150 KO mice compared to wild type control mice. B cell functions, specifically antibody production. Our previous study demonstrated that mir-150 is a key regulator for hematopoietic lineage development, especially for the formation for B lymphocytes. High-fat diet (HFD) feeding exacerbated the development of systemic insulin resistance and glucose intolerance in mir-150 KO mice compared to wild type control mice. No significant differences were observed between mir-150 and control groups in the aspects of HWI induced body weight gain, food intake, and adiposity, mir-150 deficiency did not affect macrophage activation, which has been previously demonstrated as the major contributor to diet induced adipose tissue inflammation and insulin resistance. Interestingly, mir-150 KO mice exhibited an increased adipose tissue B cell infiltration, adipose tissue inflammatory cytokine production, and liver steatosis, which is accompanied by dramatically enhanced circulating antibody production that can directly contribute to the development of insulin resistance. Four genes are identified as bona fide mir-150 target genes, including ELKS, CBL, EGRs, and EEF1, which are important components of multiple metabolic related signaling pathways. In summary, our study demonstrated that mir-150 is a novel and potent regulator for obesity induced systemic insulin resistance through controlling antibody productive B cells. Thus, it provides a novel paradigm of microRNA regulation in the context of insulin resistance, and opens a new direction for treating insulin-resistance related diseases by generating specific antibodies. This research has received full or partial funding support from the American Heart Association.


Key Words: Insulin resistance; Micromammal system; Obesity; Cardiovascular disease

Newly Identified Pro-inflammatory Mechanism of Calcification via Formation of a S100A9-Annexin-5 Membrane Complex

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Atherosclerotic plaques containing “spotty” calcifications consisting of calcified vesicles precursors to microcalcification...have increased local stress, which may promote microfractures and fatal rupture. Chronic renal disease (CRD) and mineral imbalance accelerates calcification and the release of matrix vesicles (MV). Using molecular imaging, we previously linked early calcification with inflammation determined as macrophage accumulation. Here, we hypothesized an inflammation-dependent mechanism of calcification via macrophage-released microcalcification-generating MV...in addition to the commonly accepted pathway of osteogenic differentiation of vascular smooth muscle cells. In human carotid plaques, macrophages associated with calcified vascular structures (n=127; p<0.0001), while a hydroyapatite-binding molecular imaging agent visualized calcifying MV. Immunogold labeling showed release of CD68-positive MV containing S100A9 and Annexin-5 (Annx5; Fig A and B). Under CRD conditions, including levels of calcium and phosphate (CaP) comparable to those observed in CRD patients, macrophages released MV with high calcification (p<0.01) and aggregation potential (n=3; Fig. C), which expressed exosomal markers (TS101 and CD9). CaP-stimulated mouse macrophages (RAW 264.7) have increased pro-inflammatory (M1) markers (n=3; p<0.01, Fig. D). Silencing S100A9 in vitro and genetic deficiency in S100A9/− mice reduced MV calcification, while stimulation with S100A9 increased their calcification potential. Externalization of phosphatidylserine (PS; Fig. E, green), after CaP stimulation and interaction of S100A9 and Annx5, indicated that an Annx5-S100A9 membrane complex facilitates hydroxypatite nucleation within the macrophage-derived MV membrane. Our results support the novel concept that macrophages release calcifying MV, enriched in S100A9-Annx5 complex, which accelerates microcalcification formation in CRD.


Key Words: Calcification; Inflammation; Arteriosclerosis; Vascular

Inhibition of PAR-4 Prevents Myocyte Apoptosis but Impairs Cardiac Healing and Function after Myocardial Ischemia

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Background: Protease-activated receptors-4 (PAR-4) is a low affinity thrombin receptor with an under-studied function. PAR-4 is involved in platelet activation, but its role in inflammation is controversial. Specific functions of PAR-4 in myocyte growth and cardiac function have not yet been described. In this study, we elucidated the contribution of PAR-4 as a potential mediator of remodeling in human ischemic cardiomyopathy compared to non-failure heart controls and in mice after myocardial ischemia. Immunofluorescence microscopy showed increased PAR-4 expression in cardiomyocytes and coronary smooth muscle cells, but not in fibroblasts or endothelial cells. PAR-4 knockout (KO) mice showed greater left ventricle dilatation and decreased contractility compared to WT mice at 7 and 30 days post infarct. Conclusions: These studies show that PAR-4 is a positive regulator of myocyte apoptosis. However, PAR-4 deletion impaired myocardial healing and results in adverse cardiac remodeling and function after chronic myocardial infarction. These results indicate that the use of PAR-4 antagonists should be limited to acute phases after ischemic insults to preserve myocardial loss and should be avoided as long-term therapy against the progression to heart failure.


Key Words: Proteolytic enzymes; Ischemic heart disease; Thrombin; Inflammation; Ventricular remodeling
Endogenous Myocyte Regeneration and Diabetic Cardiomyopathy

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The recognition that the mammalian heart contains a pool of resident c-kit-positive cardiac stem cells (CSCs) that regulate cell turnover and tissue repair raises questions concerning their role in the etiology of the diabetic myopathy. The objective of the current study was to determine whether the negative effects of diabetes on the adult heart are dictated by defects in CSC growth and lineage commitment. Type I insulin-dependent diabetes mellitus was induced in mice by streptozotocin administration. The kinetics of CSCs and cardiomyocytes was measured 3, 7, 10, 20, and 30 days after the onset of diabetes, by implementing a hierarchically structured cell system, which allows the quantitative analysis of the rate of cell turnover. The multiple variables required to apply this mathematical model were measured. They included first the number of CSCs, LCOs (lineage committed cells: myocyte progenitors/transplants), telomere lengths, and post-mitotic cardiomyocytes in the left ventricular (LV) myocardium. Additionally, the fraction of cycling CSCs and the length of their cell cycle were measured, together with the number of cardiomyocytes dying by apoptosis and necrosis. The diabetic heart was characterized by a severe time-dependent loss in LV post-mitotic cardiomyocytes, dictated primarily by a defect in cell renewal. Physiologically, 20% of myocytes were replaced per month in the adult mouse heart through activation, cell cycle reentry, and differentiation of CSCs. Surprisingly, a value of 6%, 1.4%, 0.5%, 0.07%, and 0.05% was found at 3, 7, 10, 20 and 30 days after the induction of diabetes, respectively. Typically, the myocardium showed a progressive increase in old cardiomyocytes expressing the senescence-associated protein p16INK4a and p53. Importantly, the severe impairment in myocyte regeneration was coupled with an increased in LV end-diastolic pressure, and a decrease in LV end-systolic pressure, LV developed pressure, positive and negative dP/dt. Collectively, our data indicate that the diabetic myopathy has to be viewed as a stem cell disease in which the alterations in the other cardiac cell classes are all secondary events, resulting from defects in replication and lineage specification of the controlling cell, i.e., the CSC.


Key Words: Stem cells

Cardiotrophin-1: A New Key Molecule In Vascular Fibrosis, Arterial Stiffness And Senescence

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Background: Cardiotrophin-1 (CT-1), a cytokine belonging to the interleukin-6 family, is increased in hypertension and in heart failure. We hypothesized that CT-1 excess promotes vascular fibrosis and dysfunction, whereas CT-1 absence may reduce the negative effects of diabetes on the vasculature. In this study, we evaluated whether CT-1 affects vascular fibrosis, arterial stiffness, and telomere length in diabetic mice.

Methods: CT-1 (20 µg/Kg/day) or vehicle were administrated to Wistar rats for 6 weeks, and 29 month-old WT and CT-1-null mice were used. Vascular media thickness, collagen and fibronectin were increased in hypertension and in heart failure. We hypothesized that CT-1 excess promotes vascular fibrosis and dysfunction, whereas CT-1 absence reduces the negative effects of diabetes on arterial stiffness. In vivo measurements and histological and immunohistochemical analyses were performed in mice treated for 6 weeks with CT-1 or vehicle in order to evaluate the influence of CT-1 on vascular fibrosis, and to compare the findings in control to those in diabetic animals.

Results: CT-1 treatment did not modify blood pressure levels. In vivo measurements and histological and immunohistochemical analyses in diabetic mice showed that vascular media thickness, collagen and fibronectin were increased in CT-1-null mice compared with WT. Furthermore, we also found that CT-1-null mice exhibited decreased arterial stiffness and increased telomere length in the aorta compared with WT mice. Collectively, our data indicate that CT-1 has a pro-fibrotic and pro-senescent role in the vasculature. These findings support the view that CT-1 is a new key molecule in vascular fibrosis, arterial stiffness and senescence.

Conclusion: This study provides new insights into the role of CT-1 in vascular fibrosis and arterial stiffness and suggests a potential therapeutic role for CT-1 in the treatment of vascular disease.


Key Words: Microrna; Signal transmission; Beta-blocker; Cardioprotective drugs; Heart failure

22919 β-arrestin1 Stimulates the Processing of a Subset of MicroRNAs

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MicroRNAs (miRs) are small, non-coding RNAs that modulate diverse biological functions through the repression of target genes. While several signaling pathways have been shown to regulate miR biogenesis, the seven transmembrane receptor (7TMR; also known as G protein-coupled receptor)-mediated signaling pathways that control miR biogenesis are only beginning to be unveiled. Originally identified as mediators of 7TMR desensitization, β-arrestins are now recognized as adaptor proteins that transduce signals to multiple effector pathways, resulting in unique downstream responses such as promotion of cardiomyocyte survival. Based on a published β-arrestin interaction map, we tested the hypothesis that β-arrestins play a role in the regulation of miR biogenesis. Using both HEK 293 cells overexpressing β-arrestin receptors (β1/2/5A) and in vivo mouse hearts, we examined whether the β-arrestin carvedilol (Carv), which activates β-arrestin-mediated signaling, can regulate miR expression. Up-regulation of human miR-190 was induced by Carv and was prevented by using siRNA directed against β-arrestin1. The Carv effect was also blocked by the β1/2/3A antagonist metoprolol, indicating a receptor-mediated mechanism of action. Interestingly, while Carv did not increase the expression of human primary miR-190 transcript, it did increase the expression of the precursor form of miR-190. Similarly, Carv activated the expression of five mature or mature miRs from mouse hearts in a β-arrestin1-dependent manner. Mechanistically, we demonstrated that β-arrestin1 controls miR processing by forming a nuclear complex with HnRNPA1 and Drosha, which are two components of the Drosha microprocessor complex. Lastly, a cardiac myocyte transduction assay indicated that β-arrestin1 regulated the expression of cardioprotective miRs by repressing apoptotic or anti-proliferative genes. In conclusion, we identify a novel mechanism by which β-arrestin1 signaling pathways regulate miR processing and discover new and potentially important miR-target pairs that are required for cardioprotective signaling.

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Author Disclosures: M. Charles; DiaMedica, Winnipeg, Canada

Background: The kallikrein-kinin system (KKS) has been previously linked to glucose homeostasis via increasing insulin sensitivity, vasodilation and more recently decreasing hepatic glucoseogenesis. The Bradykinin 2 receptor (BK2R) is a widely expressed G protein-coupled receptor associated with the beneficial effects of the KKS. In this study, we report a novel agonist monoclonal antibody (mAb) to the BK2R with beneficial anti-diabetic and cardiovascular properties.

Methods: In vivo – male ZDF fa/fa rats (11 weeks of age, n=8 per arm) were treated twice weekly SC with the BK2R antibody (0.2, 0.4, 0.04 mg/kg) for 4 weeks. In vitro – an insulin-stimulated in vitro radio-labeled glucose uptake assay using isolated rat adipocytes was used to analyze insulin sensitization activity of the antibody (1 mg/mL) after a 1 hour incubation with a humanized mAb. Results: In vivo – Overall there dose dependent reductions in various markers including HbA1c (6.2 vs. 8.8% control, p=0.0103), a reduced increase in FBG (33.1 vs. 110 mg/dl, p=0.0058), and a reduction in total cholesterol (-12 vs. +9 mg/dl, p=0.0156) after 21 days. Treatment also prevented the development of hypertension (unchanged vs. control where BP increased 25 mm Hg, p=0.0004) in addition to a reduction in heart rate (~60 bpm, p=0.001). The mAb treatment saw no effect on weight gain or food intake compared to controls. The BK2R antibody outperformed Sitagliptin (10 mg/kg po daily) and Exenatide (1 ug/rat BID) controls on all measures except lipid reduction, where the BK2R mAb had no discernible effect. In an OGTT at day 21 the mAb reduced glucose iAUC compared to the negative control (2495 vs. 3773, p<0.0001) and exhibited a clear secretagogue effect. In vivo – A glucose uptake assay demonstrated insulin stimulated glucose uptake activity (up to 57%, p=0.01) following incubation with a humanized version of the mAb suggesting an additional insulin sensitization M0A. Conclusion: A novel agonist GPCR antibody to the BK2R with insulin secretagogue and insulin-sensitizing activity has been reported with beneficial anti-diabetic and CV effects.

Author Disclosures: M.S. Williams; Employment; Significant; DiaMedica Inc. M. Charles; Employment; Significant; DiaMedica Inc.

Key Words: Monoclonal antibodies; Type 2 Diabetes; Insulin resistance; Cardiovascular disease

22898 A Novel Protein Phosphatase Exacerbates Myocytes Death and Represses Cardiac Contractility under Stress

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Regulation of sarcoplasmic reticulum (SR) calcium-ATPase (SERCA) activity is necessary for calcium homeostasis in cardiomyocytes, has a major impact on cellular viability and cardiac contractility. The key regulators for SERCA activity include protein kinases like CAM- dependent protein kinase A and calcium/calmodulin dependent protein kinase II, and protein phosphatase like protein phosphatase 1. In this report, we have discovered that protein phosphatase 2C (PP2Cε) is a novel serine/threonine protein phosphatase specifically targeted to SR membrane in cardiomyocytes. Here we assessed the hypothesis that PP2Ce is an important player in calcium cycling and cardiac remodeling in heart. PP2Ce was detected...
to interact with phospholamban in heart. Expression of PP2Ce blunted β-adrenergic stimu-
lated increase of phospholamban phosphorylation without affecting phosphorylation of ry-
anoctin. Neptunylated cardiac myosin showed reduced CaMKII stimulation of cardiac myosin
phosphorylation. By contrast, the expression of PP2Ce in cardiac myosin increased CaMKII stim-
ulation of cardiac myosin phosphorylation. Therefore, PP2Ce may negatively regulate cardiac
myosin phosphorylation.

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Key Words: Phosphatases; Phospholamban; Calcium

22891

Is Myocardin a limiting factor for cardiac programming?

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Recent reports have shown that non-myocytes may be reprogrammed into cardiomyocytes
using three cardiac transcription factors (cTFs), Gata4 (G), Mef2c (M) and Tbx5 (T). Here,
we sought to identify potentially sensitized substrates for reprogramming strategies and
test the effect of the co-activator Myocardin. Adult mouse Sce-1+/+ cardiac progenitor cells
(CPCs) that express many endogenous cTFs, were used in parallel with adult mouse tail tip
fibroblasts (TTFs). Exogenous cTFs were expressed via doxycycline-inducible lentiviral vec-
tors in various combinations. High throughput qRT-PCR was used to monitor 29 cardiovas-
cular lineage markers two weeks post-induction. Co-expressing GMP increased more than
half the analysed cardiac transcripts in both CPCs and TTFs, with no significant difference
between the cell types under the conditions tested. However, in neither cell background
was protein detection for the induced sarcomeric genes (Actc1, Myh6, Myl2) or calcium
regulators (Ryr2, Pin) detected. Myocardin, which is a transcriptional co-activator for Sce1, Gata4 and
Tbx5, was not expressed in cardiac progenitor cells. We therefore speculated that Myocardin
was a limiting factor in reprogramming. Adding the cardiac-enriched Myocardin isoform
B (Myo) to GMP activated an increased number of genes in CPCs and TTFs with 60% and
94% overlap, respectively, with the genes induced by GMP alone. Importantly, MyoGMP
triggered detectable protein expression for the targets examined (Actc1, Myh6, Nopx) in
CPCs and to a lesser extent in TTFs. Alone, Myocardin was sufficient to induce 70% of the
cardiac markers obtained with MyoGMP in CPCs but only 27% of those in TTFs. In sum-
mary: (1) GMP induced cardiac gene expression in CPCs and TTFs, but no cardiac protein
expression was observed under the conditions tested. (2) Complementing CPCs with Myocardin
induced additional cardiac transcripts and also cardiac protein expression, indicating a more com-
plete cardiac differentiation program by this combination. (3) Myocardin alone induces a
more efficient cardiac program in CPCs than in fibroblasts, suggesting that the presence of
endogenous cTFs in CPCs predisposes them to cardiac differentiation and confirming the
role of Myocardin as a critical cofactor.

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M.D. Schneider: None.

Key Words: Stem/progenitor cells; Cardiac regeneration; Gene expression; Myocardin; Stem
cell therapy

22787

Integrin β1 Signals Through Extracellular Signal-regulated Kinase To
Promote The Survival Of Adipose Tissue-derived Stem Cells In The
Infarcted Hearts

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Adipose tissue-derived stem cells (ASCs) are a promising cell source for myocardial re-
generation. However, reported improvement of cardiac function has been modest, partly
due to low survival rate of injected ASCs in hostile ischemic hearts, with the underlying
mechanisms remaining largely unknown. Integrin β1 belongs to a family of receptors for
many extracellular matrix proteins. This study was to investigate the roles of integrin β1
in mouse ASC survival using both a murine model of myocardial infarction and an in vitro
cell culture. Integrin β1 expression in ASCs was either eliminated via lentivirus carrying
arrNA (ASC[β1−/−]) or overexpressed by lentiviral infection (ASC[β1+/+]). For in vitro study,
BALB/c mice (n = 5/group) were subjected to a permanent occlusion of left anterior descend-
ing coronary artery. Luciferase-labeled control ASC (ASC[β1+/+]), ASC[β1−/−] or ASC[β1+/+]
were injected into the border zone of hearts. Injected ASCs in live mice was imaged using
Bioluminescent Imaging System. For in vitro study, ASCs were treated with 10 mM
H2O2 or with intracellular signal-regulated kinase (ERK) inhibitor PD98059 for 2 h. Cell vi-
ability was measured by lactate dehydrogenase (LDH) release from cells with damaged
membrane. The in vivo results showed that injected ASCs survived in infarcted hearts for
7 days. There were more ASCs detected in ASC[β1−/−] injected hearts compared to ASC[β1+/+]
injected hearts, indicating that inte-
grin β1 is involved in survival of injected ASCs in ischemic hearts, and overexpression of
integrin β1 enhances ASCs to resist hostile myocardial environment. The following in vivo
results confirmed the in vivo findings. Upon H2O2 exposure, overexpression of integrin β1
in ASCs significantly decreased LDH release compared with other groups, while integrin β1
cut down expression of ASCs in ischemic hearts, and overexpression of integrin β1 enhances
ASCs to resist hostile myocardial environment. The following in vivo
results confirmed the in vivo findings. Upon H2O2 exposure, overexpression of integrin β1
in ASCs significantly decreased LDH release compared with other groups, while integrin β1
cut down expression of ASCs in ischemic hearts, and overexpression of integrin β1 enhances
ASCs to resist hostile myocardial environment. The following in vivo
results confirmed the in vivo findings. Upon H2O2 exposure, overexpression of integrin β1
in ASCs significantly decreased LDH release compared with other groups, while integrin β1

LDH release from ASCs was not different from ASCs (p<0.01), suggesting that ERK pathway participates in integrin (β1)-mediated ASC survival. This finding may help to guide the design of novel therapies for improving ASC-mediated repair capacity by increasing the ability of ASCs to persist in ischemic hearts.

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Key Words: Stem/progenitor cells

22834 Sarcolemmal K<sub>ATP</sub> Channelopathy in Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells of Diabetic Patients

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Background: Cardiovascular complications are one of the major causes of death in patients with metabolic disorders such as diabetes mellitus. Cardiac sarcolemmal K<sub>ATP</sub> channels function as metabolic sensors that link energy metabolism to membrane excitability and are required for adaptation of the myocardium to physiological and pathological stress. To investigate whether the functional sarcolemmal K<sub>ATP</sub> channel is expressed in patient-specific cells, we derived cardiomyocytes from induced pluripotent stem cells reprogrammed from non-diabetic (N-iPSC-CMs), type 1 (T1DM-iPSC-CMs), and type 2 diabetic (T2DM-iPSC-CMs) patients.

Methods and Results: Efficient cardiac differentiation was confirmed with cardiac-specific immunostaining and live-labeling of cardiomyocytes with GFP under transcrinal control of cardiac promoter myosin light-chain-2. Immunohistochemistry showed that expression and co-localization of the cardiac sarcolemmal K<sub>ATP</sub> channel subunits Kir6.2 and SUR2A were higher in N-iPSC-CMs compared to T1DM-iPSC-CMs and T2DM-iPSC-CMs. Single channel recordings in the inside-out patch clamp configuration demonstrated the presence of functional sarcolemmal K<sub>ATP</sub> channels in all groups. These channels were reversibly blocked by 10 μM glibenclamide and 2 mM ATP. Single sarcolemmal K<sub>ATP</sub> channel kinetics were similar in N-iPSC-CMs and T1DM-iPSC-CMs with a typical single channel current amplitude of 2.17 ± 0.03 pA and unitary conductance 54 ± 0.68 pS. However, T2DM-iPSC-CMs channels exhibited fast flickering between typical single channel current amplitude of 2.17 ± 0.03 pA and unitary conductance 54 ± 0.68 pS.

Conclusion: These findings indicate that expression and co-localization of cardiac sarcolemmal K<sub>ATP</sub> channel subunits Kir6.2 and SUR2A are significantly higher in N-iPSC-CMs compared to T1DM-iPSC-CMs and T2DM-iPSC-CMs. These differences in channel kinetics contribute to compromised cardiac preconditioning in type 2 diabetic patients.

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Key Words: Stem cells; Potassium channel; Type 1 Diabetes; Type 2 Diabetes; Cardioprotection

22904 Notch Inhibition Prevents the Progression of Small Abdominal Aortic Aneurysms in Angli-Induced Mouse Model

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Progression of abdominal aortic aneurysm (AAA) is characterized by activation of the inflammatory pathway and an imbalance between the synthesis and degradation of aortic wall structural components including elastin and collagen. Our previous studies have demonstrated that loss of Notch reduces the incidence of AAA in the angiotensin II (Angli) mouse model by preventing the influx of inflammatory macrophages. The present study was performed to determine if pharmacological inhibition of Notch (DAPT) prevents the progression of AAA in an Angl-Induced mouse model.

Active aneurysm was introduced in Apoe<sup>−/−</sup> mice (n=36) by administering Angli. DAPT (10mg/kg, 3 times a week) was injected in these mice starting either 3 days (Group II; n=12) or 8 days (Group III; n=12) after Angli infusion. Group I (n=12) received vehicle (10% alcohol in corn oil) only. DAPT treatment significantly reduced luminal expansion of the abdominal aorta in both the groups as compared to vehicle-treated Angli<sup>−/−</sup> mice (p<0.05) as detected by echocardiography. DAPT treatment also prevented characteristic aneurysmal traits of elastin fragmentation and aortic remodeling in both the groups as detected by histology. Marginal increase in macrophage content (CD-68) was observed in Group III compared to Group II, but was significantly less than Group I. However, the increased macrophage content in Group III was not associated with increased monocyte chemotactic protein–1 suggesting that DAPT treatment prevents continuous influx of macrophages. In vitro data suggest that Notch inhibition promotes the M2 polarization of macrophages. Interestingly, increased expression of newly synthesized tropoelastin and collagen staining was detected in Group I suggesting that the vascular injury was repaired in the absence of recruitment of macrophages. Expressions of MMP2 and MMP9 were also decreased in the aorta of Group II and Group III compared to Group I (vehicle) as determined by quantitative real-time PCR and immunohistochemistry. The present study demonstrates that if Notch inhibition stabilizes the progression of AAA and is associated with increased elastin and collagen regeneration and decreased MMP activity suggesting the potential use of Notch inhibitors in the treatment of AAA.

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Key Words: Abdominal aortic aneurysm; Angiostatin; Inflammation; Extracellular matrix

22993 TNF/TRNFR1 Signaling Inhibits Cardiomyogenic Differentiation Of Cardiac Stem Cells And Promotes A Neuroadrenergic Phenotype

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Heart failure (HF) progression in humans occurs despite augmented tissue resident cardiac stem cells (CSCs), suggesting that the HF microenvironment opposes endogenous cardiac repair. Tumor necrosis factor-α (TNF) levels are increased in HF; its effects are mediated through the tumor necrosis factor receptor (R) specific, such that TNFFR1 promotes whereas TNFFR2 alleviates pathological remodeling. We hypothesized that TNF modulates the CSC differentiation and function in an analogous manner. c-Kit sort-<sup>+</sup>/Sca1<sup>+</sup> CSCs were isolated from wild type (WT) and TNFR1<sup>−/−</sup> mice. WT CSCs constitutively expressed TNFR1 and exhibited intact downstream TNF signaling. CSCs were then differentiated in the presence and absence of TNF (20 ng/mL) using 5-azacytidine and TGF-β1. Differentiated WT CSCs developed striated morphology and increased abundance of multinucleated cells. In contrast, WT CSCs exposed to TNF acquired morphological features of monocytic lineage. However, TNF-exposed TNFR1<sup>−/−</sup> CSCs also exhibited increased (p<0.05) expression of the neuroadrenergic marker tyrosine hydroxylase (TH). Also, culture supernatants from TNF-exposed WT CSCs revealed 3-4 fold increased expression of epinephrine and norepinephrine. These changes were significantly attenuated in TNFR1<sup>−/−</sup> CSCs. Intramyocardial injection of CSCs in mice following reperfused infarction indicated increased abundance of TH staining in hearts (border zone and scar) injected with WT GFP+ CSCs as compared to vehicle-injected hearts. TH staining, however, was significantly diminished, and cardiac function significantly improved in TNFR1<sup>−/−</sup> CSC-injected hearts. Conclusion: TNF/TRNFR1 signaling inhibits cardiomyogenic differentiation of CSCs and promotes a neuroadrenergic phenotype that can serve as a local source of catecholamines. Modulation of TNFR1-specific signaling in CSCs may be a novel approach for augmenting endogenous cardiac repair and reducing adrenergic activation in HF, and may also enhance the efficacy of exogenous stem cell therapy.


Key Words: Stem/progenitor cells; Stem cell therapy; Inflammation
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