Late-Breaking
Basic Science Abstracts From
the American Heart Association’s
Scientific Sessions 2009
Deletion of GSK-3

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Cardiac progenitor cells (CPCs) delivered to the infarcted heart generate a large number of small cardiomyocytes which fail to acquire the differentiated phenotype. In contrast, activation of the β-isoform has been largely ignored. All of the work done in the heart to date implicating GSK-3α in various processes has relied on gain of function approaches, dominant negative strategies, or non-selective small molecule inhibitors, none of which allow one to define the true biology of the GSK-3 family, nor do they allow an understanding of isoform-specific effects. We now present the first studies defining the role of differential microRNA regulation in these cells.

Late-Breaking Basic Science Abstracts

Deletion of GSK-3α, but Not GSK-3β, Defines an Essential Role in β-Adrenergic Responsiveness and Identifies a Novel Strategy for β-Blockade


The glycogen synthase kinase-3 (GSK-3) family of protein kinases consists of two highly related isoforms, α and β. Since its discovery, GSK-3β has been reported to regulate an astonishing variety of cellular processes, whereas GSK-3α has been largely ignored. All of the work done in the heart to date implicating GSK-3α in various processes has relied on gain of function approaches, dominant negative strategies, or non-selective small molecule inhibitors, none of which allow one to define the true biology of the GSK-3 family, nor do they allow an understanding of isoform-specific effects. We now present the first studies defining the role of GSK-3α and β in the mouse heart using gene targeting. We find that miR-499 is a central regulator of atrioventricular nodal dysfunction. The mice also develop profound abnormalities of mitochondrial structure on transmission electron microscopy, consistent with opening of the permeability transition pore. Following thoracic aortic constriction in young mice, we find marked enhanced early contractile response but not at later timepoints. Thus, GSK-3α is a central regulator of the GSK-3 family, with a profound effect on atrioventricular nodal function.

DSAGE Identifies the Orphan Nuclear Receptor, Nur77, as a Downstream Effector of Integrin-Linked Kinase (ILK) in Cardiac-Specific ILK Knockout Mice

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Integrin-linked kinase (ILK) has been implicated in the regulation of cardiomyocyte (CM) hypertrophy, and ILK sequence variants have been associated with human heart failure. However, the responsible downstream mechanisms have not been delineated. Here we generated cardiomyocyte-specific ILK knockout mice (CSILK-KO), characterized their phenotype, and used a novel nanotechnology to identify downstream effectors. CSILK-KO mice were generated using CRE expression driven by the α-MHC promoter, which effectively and specifically deleted ILK in CMs. CSILK-KO developed spontaneous dilated cardiomyopathy (DCM), with heart failure, dying at 3–6 weeks of age (n=10). At 10 days post-natal, CSILK-KO mice had normal cardiac function by echocardiography. By 21 days, CSILK-KO mice developed heart failure in association with a dramatic increase in apoptosis (TUNEL positive/nuclei 6.36% ± 0.31% (KO) vs 0.06% ± 0.02% (WT), n=5, p<0.05). To identify pathways altered prior to the development of cardiac dysfunction, we used deep sequence analysis of gene expression (DSAGE), to compare comprehensive transcriptional profiles of hearts from 10 day old ILK-WT and CSILK-KO mice. ~2x10^6 cDNA clones from each genotype were sequenced, corresponding to 3.2/274 independent transcripts. 93 genes was significantly altered, using nominal threshold p>0.14 fold change and p<0.0001. DSAGE revealed a 2.1-fold decrease in miRNA (p<0.0001) for the orphan nuclear receptor, Nur77, in CSILK-KO hearts compared to WT littermates, which was confirmed by qRT-PCR (KO decrease −4.5-fold vs WT, n=5, p<0.05). Cell fractionation revealed that Nur77 nuclear protein expression was also dramatically decreased in 10 day old CSILK-KO hearts, and increased after adenosine expression of ILK-WT in rat neonatal CMs. In addition, adenosine expression of Nur77 reduced hypoxia-induced apoptosis in rat neonatal CMs (cell death detection ELISA, OD405 WT vs KO:1.57 ± 0.02 vs 1.10 ± 0.06, n=4, p<0.05). These results demonstrate that genetic deletion of ILK in CMs leads to apoptosis and the development of a lethal cardiomyopathy. Moreover, our study provides the first evidence that the nuclear receptor, Nur77, is a downstream effector of ILK and the heart contributes to its anti-apoptotic effects.

Impaired In Vivo Vascular and Cardiac Repair Capacity of Endothelial Progenitor Cells From Patients With Ischemic Cardiomyopathy: Role of Differential MicroRNA Regulation

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Background: Endothelial progenitor cells (EPCs) promote vascular and cardiac repair mechanisms. However, functional repair capacity of EPCs may be altered in cardiovascular disease, not only limiting endogenous repair capacity, but at the same time representing an important potential limitation for autologous cell-based treatment approaches. We therefore characterised in vivo cardiovascular repair capacity of EPCs from patients with ischemic cardiomyopathy (ICM) as compared to healthy subjects (HS) and analysed the functional role of differential microRNA regulation in these cells. Methods and Results: Early EPCs and CD34+ cells were isolated from peripheral blood from patients with heart failure due to ICM. In vivo cardiovascular repair capacity of EPCs was examined after transplantation into nude mice with cardiac injury and myocardial infarction. The microRNA profile of EPCs was characterized using a microRNA array, results of array were confirmed by quantitative RT-PCR. The functional role of microRNAs was examined by transfection with antagonims. In vivo cardiovascular repair capacity of EPCs derived from patients with heart failure due to ICM was markedly reduced as compared to HS. Cardiac function improved after transplantation of EPCs from patients with ICM as determined by cardiac MRI and haemodynamic analyses. MicroRNA profiling indicated a markedly reduced expression of the miR126 and miR130a in EPCs and CD34+ cells from patients with ICM. Furthermore, levels of the respective targets, SHED-1 and HOXA-5, were upregulated in these cells. Moreover, down-regulation of miR126 and miR130a impaired the pro-angiogenic effects of EPCs but not their pro-inflammatory effects. By down-regulation of miR130a, but not of miR126, abolishes the vascular repair capacity of EPCs. Altered microRNA regulation may also represent an interesting target for the optimisation of cell-based treatment approaches.

Late-Breaking Basic Science Abstracts: Translational Studies

Room W304ab

Abstracts 5285–5294

Vol 105, No 12 December 4, 2009
Migration of Human Cardiac Progenitor Cells in the Infarcted Mouse Heart

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One of the major factors responsible for successful implementation of cell therapy in the diseased heart involves the migratory ability of the delivered cardiac progenitor cells (CPCs). The possibility exists that the mechanisms that govern the egress of CPCs from their niches are also implicated in the translocation of CPCs to the injured myocardium. We report that stem cell niches in the mouse heart are composed of CPCs expressing the EphA2 receptor and myocytes displaying the ephrin A1 ligand. We then tested whether the EphA2 receptor-ephrin A1 ligand system is implicated in the motility of human CPCs (hCPCs) in vivo. EGF-tagged hCPCs were pre-treated for 15 minutes with ephrin A1 and subsequently injected in the border zone acutely after infarction. Two days later, the translocation to the necrotic myocardium of EGF-positive hCPCs was measured quantitatively by confocal microscopy. Ephrin A1-activated hCPCs showed a polarized morphology and were aligned in proximity and within the infarct. Conversely, untreated hCPCs preserved a round shape and were confined to the site of injection. Treatment with recombinant ephrin A1 promoted internalization of EphA2 receptor, enhanced actin bundling and increased the spontaneous motility of hCPCs. Moreover, EphA2 activation by ephrin A1 potentiated the chemotactic response of hCPCs. The differential expression of Ephrin A2 and ephrin A1 in CPCs and myocytes, respectively, was confirmed at the RNA and protein level by quantitative RT-PCR. Western blotting and immunocytochemistry. Ephrin A1, an established regulator of cell adhesion and chemotaxis, was detected in thoracic CPCs and its ligand ephrin A1 was restricted to human cardiomyocytes. In the absence of ephrin A1 treatment, serially passaged hCPCs synthesized ephrin A1 which accumulated at the cell trailing edge. However, senescent hCPCs showed negligible levels of ephrin A1 and failed to acquire an increased motional morphology. We confirm, in addition, that EphA2/ephrin A1 favors CPC motility mediating their migration to areas of injury. In situ activation of resident hCPCs with ephrin A1 or its ex vivo manipulation prior to delivery to the myocardium may improve cell targeting to sites of damage, providing a novel strategy for the treatment of heart failure.

Dramatic Increase of Micro-RNAs Targeting Cell Cycle, Angiogenesis, and Epidermal Differentiation in CD34+/Lin- Bone Marrow Stem Cells From Patients With Coronary Artery Disease

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Introduction/Hypotheses: Defective homing and reparative function of bone marrow (BM) derived endothelial progenitor stem cells may be underlying causes of coronary artery disease (CAD). Such defects may also decrease the therapeutic potential of these cells in the context of autologous stem cell therapy for the treatment of ischemic cardiomyopathy. Objectives: To compare gene expression and miR profiles in CD34+/Lin- BM cells from CAD patients, age-matched non-CAD patients and healthy controls. Methods: BM was collected from 5 patients with CAD, 4 patients undergoing cardiovascular surgery for non-CAD-related conditions, and 5 healthy volunteers. Mononuclear cells (MNCs) were isolated and CD34+/Lin- cells were purified by Lin depletion and FACS. All samples were treated identically. Immediately before RNA isolation, CD34+/Lin- cells within each group were pooled and cultured for 24h in α-MEM, 20% human serum, 2 μm hydrocortisone. The same RNA samples were used for gene and miR-arrays, and RT-PCR. Results: The yield of MNCs, representation of CD34 +/Lin- cells (~93%), and expression of surface markers were similar between groups. Micro-RNA array analyses identified 12 miRs that were increased 3-fold or more in cells from CAD versus non-CAD or healthy controls. These included miRs 16, 21, 26a, 92a, and 155. Gene expression analyses identified 12 miRs that were increased 3-fold or more in cells from CAD versus non-CAD or healthy controls. These included miRs 16, 21, 26a, 92a, and 155. Gene expression array analyses identified targets for each of these miRs. The mRNA of cell cycle regulator genes CCNB1 and CCND2, known targets of miR-16 were decreased ~3-fold in CAD samples. VEGFA mRNA, another target of miR-16 also decreased ~3-fold. The mRNA for BMPR2, the initiating receptor for BMPs that regulate stem cell self-renewal and differentiation, and a known target for miR-21 was decreased ~3-fold in CAD versus both non-CAD groups. MIR-92a, recently shown to negatively regulate angiogenesis and functional recovery from ischemia in mice was increased 3-fold in the CAD samples; the miR92a target integrin subunit alpha5 was correspondingly decreased in the CAD samples. Conclusions: BM CD34+/Lin- cells from CAD patients have dramatically amplified miRs that target cell cycle, self-renewal, differentiation, apoptosis, and inflammation. As a result, angiogenic functions and hence therapeutic efficacy of these cells is likely to be severely compromised.

Loss of Plakoglobin Leads to Remodeling of the Intercalated Disc and Activation of β-Catenin Signaling in the Heart

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Arthrogrypomogenic right ventricular cardiomyopathy (ARVC) is an inherited disorder characterized by cardiac dysfunction, heart failure and sudden cardiac death. A recessive coronary-artery truncation (Naxos disease) and a dominant insertion mutation in plakoglobin (JUP) gene have been identified in ARVC patients and mice, respectively, and such mutations lead to cardiac dysfunction and subsequent development of structure and conduction abnormalities that are poorly understood. Similar to β-catenin, plakoglobin has dual functions as both an adhesion protein at the intercalated disc (ICD) and signaling protein via its ability to modulate the Wnt pathway. In addition to its adhesive function, we hypothesize that cisplakoglobin and β-catenin interactions are critical for the stability and function of the ARVC. To analyze the role of plakoglobin in the working myocardium, we generated an inducible cardiac-restricted knockout (KO) of the plakoglobin gene in mice. We demonstrated that inactivation of plakoglobin in the adult mouse heart resulted in progressive loss of cardiac myocytes, extensive inflammatory infiltration, fibrosis, and lung edema. Echocardiography exhibited decreased left ventricular end-diastolic- and -systolic dimension and decreased LV fraction shortening in the CKO hearts (CKO 22.17% ± 7.28 vs WT 28.65% ± 4.47; n = 11; p < 0.01). Electron microscopy showed significant reduction in the number and length of desmosomes (n = 10; p < 0.01), whereas the number and length of adherens junctions (n = 10; p < 0.02) were significantly increased at the ICD. Accordingly, desmosomal proteins plakoglobin-2 and desmoplakin were decreased consistent with the ultrastructural ICD defects. Furthermore, the expression and distribution of the gap
Late-Breaking Basic Science Poster Session: Vascular and Cardiac Biology
Poster Hall A2-A3, Late-Breaking Basic Science
Abstracts 5172–5191

5172
An Essential Role of the E2F3 Transcription Factor in Ischemic Angiogenesis
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Background: E2F family of transcription factors play a central role in cell-cycle control and provide an ideal target for therapeutic modulation of vascular growth. However, the PREVENT trial, targeting E2F activity in the autologous vein grafts of CABG surgery to prevent graft overgrowth and failure, generated negative results presumably due to the non-specific inhibition of both activating and repressive E2F species; therefore, it is imperative to elucidate the specific function of individual E2F members in the vascular biology. Recently, we have shown that E2F1 suppresses ischemic angiogenesis while E2F2 regulates vascular contractility and blood-pressure homeostasis. Methods and Results: To generate endothelial specific E2F3-knockout mice, we crossed Tie2-Cre/E2F2fl/fl (male) and E2F3fl/fl (female) mice. The resultant Tie2-Cre/E2F3fl/fl (E2F3 KO) mice were embryonic lethal with a penetrance equivalent to the global E2F3-knockout mice (live birth at ~ 8% of the expected frequency), suggesting that E2F3 expression in ECs is essential for embryonic development. The survival E2F3 KO mice grew to adulthood relatively normally, however, with a greater body weight and higher body fat percentage as compared to their Tie2-Cre/E2F3fl/fl (WT) littermates (WT). We then induced hindlimb ischemia (HLI) in E2F3 KO and WT mice (n=8 per group) by surgical excision of the left femoral artery. E2F3 KO but not WT mice exhibited necrosis in the limbs and toes (25% and 50%, respectively) at d7 post-HLI. Laser Doppler imaging revealed an impaired blood-flow recovery in E2F3 KO group (p<0.01 at d7 and d14, n=8 for WT, n=6 for E2F3 KO). Immuno-histological analyses confirmed a significantly lower capillary density (Lectin perfusion and staining, p<0.01 at d7 and d14) and EC proliferation rate (BrDU/U31 double-staining, P<0.001 at d7 and d14) in the ischemic limb of E2F3 KO mice than in WT controls. In vitro, the primary ECs isolated from E2F3 KO mice expressed lower levels of cell-cycle-associated genes (e.g., cyclin D1, cdc2, cdc6, and DHFR) and proliferated at a slower rate than ECs isolated from E2F1 KO, E2F2 KO, and WT mice. Conclusion: Among E2F family members, E2F3 plays a major role in promoting endothelial cell-cycle progression and ischemic angiogenesis.

5173
Molecular, Cellular, and Functional Phenotypes of Human Cardiac Stem Cells Dependent Upon Monolayer Versus Three-Dimensional Culture Conditions
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Background: Autologous cardiac stem cells are promising candidates for myocardial regenerative therapy. Previous reports have shown that cardiac stem cells can be grown directly from adult myocardial tissue, and expanded as three dimensional (3D) spheres or as monolayers. We investigated the molecular and cellular profiles of cardiac stem cells cultured under 3D sphere and monolayer conditions, and compared their therapeutic potency in myocardial repair. Methods and Results: Cardiac stem cells and supporting cells were grown directly from human endomyocardial biopsies, and then cultured under 3D sphere or monolayer conditions for 3 days. Compared to monolayer culture, 3D sphere culture inhibited overall cell-cell proliferation. The expression of c-kit increased in the 3D sphere culture condition (10.4±1.7 vs 5.7±1.0% at baseline, P<0.01), but decreased under the adherent monolayer condition (3.6±0.8%, P<0.01 vs baseline and 3D culture). Microarray, qPCR, and immunostaining analyses revealed that 3D sphere culture enhanced the expression of stem cell factors, and adhesion/extracellular-matrix molecules, including IGF-1, HDAc2, Tert, integrin-β1, laminin-β1, and MMPs. Implantation of 3D sphere-cultured cells into SCID mice after coronary ligation resulted in higher cell engraftment (Figure A) and better functional benefit (Figure B), as compared to monolayer-cultured cells. Conclusions: Cultures of adult cardiac stem cells as 3D spheres improves the “stemness” and expression of adhesion/extracellular-matrix molecules. This benefit enhanced cell survival and myocardial function following implantation into the infarcted heart.

5174
Genetically Engineered Sca-1+ Cardiac Mesenchymal Cells Overexpressing Netrin-1 Shows Enhanced Survival and Promote Angiogenesis in Infarcted Heart
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Background: We have previously shown that cell based sonic hedgehog (Shh) gene delivery to the infarcted heart promotes angiogenesis and regional blood flow via up-regulation of Netrin1 and INGS. Here we report that genetically engineered Sca-1+ cells over expressing Netrin1 promotes angiogenesis. Methods and Results: Sca-1+ cells isolated from rat heart using magnetic beads (Easy Sep™) were positive for Tert, Nestin, Met2, and Gata4. pLP-Adeno-X Viral/Trans vector containing netrin1 cDNA from rat MSCs was constructed and transfected into 293 cells to produce Netrin1 adenoviral vector which was used for transduction of Sca-1+ cells (Sca-1+/Netrin1). Successful transduction of Netrin1 in Sca-1+ was confirmed by Immunostaining, real time PCR and Western blotting which showed ~200 fold higher Netrin1 expression as compared with adenoviral null transduced cells (Sca-1+/Netrin1) and non-transduced Sca-1+ cells (Sca-1+/Sca-1+). Cardiomyocytes, HUVEC, and Sca-1+/Netrin1+ (all positive for netrin-1 receptor UNC5B) cultured in conditioned medium (CM) from Sca-1+/Netrin1+ showed significantly higher survival (p<0.01) under anoxia as compared with the ones cultured with CM from Sca-1+/Sca-1+ and Sca-1+/Sca-1+. HUVEC treated with CM from Sca-1+/Netrin1+ showed higher migration in Transwell system and tubule formation on matrigel (magn.: 2.4 x 4.9 in vitro (p<0.01 vs. Sca-1+/Sca-1+ and Sca-1+/Sca-1+). Western blots showed higher activation of Akt and ERK44/214 in Sca-1+Netrin1+. In vivo studies, rat model of myocardial ischemia was developed in female Wistar rats by LAD occlusion for 45 min followed by reperfusion. The animals were grouped to receive 70UG of DMEM without cells (group-1) or containing 2x10^6 Sca-1+/Netrin1+ cells (group-2) or Sca-1+/Sca-1+ cells (group-3) labeled with PKH26 dye. Echocardiography at 6 weeks showed significantly improved global heart function (EF 57.2±3.6% FS 25.7±2.1% in group-3 (p<0.05 vs. group-1 and 2). Blood vessel density was significantly higher in group-3 (p<0.05 vs. other groups). Markedly higher survival of PKH26 labeled Sca-1+ cells was observed in the infarcted heart which showed myogenic differentiation. Conclusion: Netrin1 transgene delivery to the heart using cardiac derived Sca-1+ cells promote angiogenesis in the heart.

5175
Improved Myocardial Mechanics After GRK2 Inhibition Using Molecular Cardiac Surgery With Recirculating Delivery (MCARD™) to Deliver AAV6-GRK2cT in Sheep
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Background: Genetic modulation of ventricular function may offer a novel therapeutic strategy for patients with acute and chronic heart failure (HF). Manipulation of molecular targets has shown therapeutic potential in HF models. Among these is the G protein-coupled receptor kinase-2 (GRK2 or βARK1), which is up-regulated in failing human myocardium. The carboxyl-terminal portion of GRK2 (βARKct) is up-regulated in failing human myocardium. The carboxyl-terminal portion of GRK2 (βARKct) has been shown to be an effective inhibitor of GRK2 activity in myocytes. This present study focuses on the effects of AAV6-mediated GRK2cT gene delivery on LV function using a novel gene delivery platform, MCARD™. Methods & Results: MCARD™ provided an isolated cardiac circuit using cardiopulmonary bypass that allowed for cardiac-specific delivery of 10^12 gc of saRNA6.CMV.βARKcT in 6 normal ovine subjects. All animals survived. The hemodynamics were evaluated using MRI-mediated LV contractility (LV dP/dtmax and dP/dtrelaxation) and relaxation (LV dP/dtrelaxation) which was assessed at baseline and after administrating isoproterenol (ISO) before gene delivery (control group) and at 4 and 8 weeks post gene delivery. Iso-stimulated LV systolic performance remained elevated at 4 and 8 weeks compared with pre-gene delivery, demonstrating significant augmentation of myocardial β-adrenergic reserve [ISO (control) vs. ISO (4wk) mean ΔdP/dtrelax = -30.1%, p<0.05; base vs. ISO (4wk): ΔdP/dtrelax = -50.7%, p<0.05]. Conclusion: βARKcT expression improves global LV systolic performance relative to controls. These results in normal ovine
of Cx43 revealed a decrease of voltage gating with increasing voltage across the GJ. Single channel analysis showed the presence of various channel populations: In Cx43+/− and Cx43−/− >70% of channels were heteromeric/heterotypic channels formed by Cx43/Cx40/Cx45 and Cx40/Cx45, respectively. Homomeric/homotypic Cx40/Cx40 channels increased from 4.9% to 14% with Cx40 ablation, 9% were homomeric/homotypic Cx45/Cx45 channels in both genotypes. Importantly, genetic ablation of Cx43+/− in atrial myocytes was associated with a 47% decrease in peak inward Na current, I<sub>Na</sub> = 280.6 ± 52.2 pA/PF vs. −147.3 ± 25.8 pA/PF.

Conclusions: Our data demonstrate that genetic ablation of atrial Cx43 has multiple effects on the electrical phenotype: (1) it decreases the expression of Cx40, indicating regulatory interaction between Cx40 and Cx45 in gap junctions; (2) it unveils a mixture of various GJ channel types composed of Cx45 and Cx40, and (3) it reduces significantly peak inward Na current. Thus, the arrhythmogenic effect of atrial Cx43 remodeling involves both reduction in intercellular electrical conductance and ion current driving propagation.
Nitroxy Enhances Contractility in Failing Isolated Mouse Cardiomyocytes

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Nitroxy (HNO) enhances contractility and accelerates relaxation in normal mouse myocytes. These actions do not require β-adrenergic/PKA signaling or the recruitment of extracellular Ca2+. Rather, HNO modifies critical cysteines in key E-C coupling proteins, enhancing Ca2+ cycling in the sarcoplasmic reticulum and inducing myocardial Ca2+ sensitization. Whether HNO retains its positive inotropic/lusitropic action in failing myocardium is unknown. Therefore, we isolated myocytes from sham-operated mice (C57BL6, 3–4 mo. old) and from mice subjected to 2 weeks of transverse aortic constriction (TAC). Left ventricular (LV) dilation and dysfunction in TAC hearts were confirmed by echo. Cells were field-stimulated (0.5 Hz, 22°C, Ca2+ 1mM); 2% (ISO, both p<0.05 vs base). Consistently, Ca2+ transients (Fura-2 fluorescence), time to baseline from peak shortening and from peak Ca2+ (t to bl) decreased by 11%, 16% (HNO) vs 8% ± 2% (ISO, both p<0.05 vs base). Relaxed was also equally accelerated by HNO and ISO (t to bl decreased by 11% ± 4% (HNO) vs 8% ± 2% (ISO, both p<0.05 vs base). Consistently, Ca2+ transients increased: 21% ± 9% (HNO) vs 21% ± 5% (ISO, both p<0.05 vs base), with a faster Ca2+ decay (−13% ± 5% vs −11% ± 2%, both p<0.05 vs base). Echo measurements showed enlarged LV end-systolic diameter (2.7 ± 3mm vs 1.2 ± 2mm, p<0.01), and FS decrease (34% ± 4% vs 64% ± 1%, p<0.01) in TAC vs sham hearts. Moreover, TAC myocytes displayed a profound β-adrenergic des-sensitization, with only 29 ± 13% increase in FS (p<0.02 vs sham) and no change in relaxation after 2.5 μM ISO. In stark contrast, the positive inotropic/lusitropic action of the HNO donor CXL-1020 was fully preserved in TAC cells: FS increased by 116 ± 27%, t to bl diminished by 16 ± 3% (both p<0.001 vs base); Ca2+ transients also increased (23 ± 11%) and Ca2+ t to bl was shortened by CXL-1020 (−13 ± 5%, both p<0.05 vs base). Thus, HNO donors such as the novel agent CXL-1020 display full positive inotropy and lusitropy in failing myocytes with altered β-adrenergic signaling. This evidence further supports the use of HNO donors as a class of promising pharmacological agents in the treatment of heart failure.

AdAtg7 Induction of Autophagy Reduces CryABR120G Aggregates and Cytotoxicity in Cardiomyocytes

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Introduction: Protein conformation and aggregation are increasingly being recognized as playing major roles in the development of cardiac disease and heart failure. Several cardiomyopathies, including the CryAB(R120G) model of desmin-related cardiomyopathy (DMR), accumulate cytotoxic misfolded proteins and protein aggregates. Those misfolded and aggregated proteins can be cleared by a process of bulk protein degradation known as autophagy. Thus inducing autophagy is a potential means for clearing intracellular protein aggregates and reducing cardiac pathology. Autophagy is induced in many disease and stress states; however, the mode and cellular context of autophagic activation are important determinants of whether autophagy is beneficial or detrimental. Atg7, a known mediator of autophagy, is induced in many disease and stress states; however, the mode and cellular context of autophagic activation are important determinants of whether autophagy is beneficial or detrimental. Atg7, a known mediator of autophagosome biogenesis, is a putative regulator of autophagic function. Hypothesis: Autophagic induction by AdAtg7 overexpression will reduce aggregate content and cellular toxicity in CryAB(R120G) expressing cardiomyocytes. Methods: Rat neonatal cardiomyocytes were infected with adenoviral constructs expressing wild-type CryAB, CryAB(R120G), which forms intracellular aggregates and pre-amyloid oligomers, AdAtg7 to induce autophagy or LacZ control. Results: Our data show that AdAtg7 is an effective inducer of autophagy. AdAtg7 expression significantly increases the number of GFP-Atg3 and GFP-Atg8 expressing cardiomyocytes. Autophagic flux experiments using Bafilomycin A1 show AdAtg7 significantly upregulates LC3-III levels (autophagosome synthesis) relative to controls. Induction of autophagy by AdAtg7 overexpression has no detrimental effects on cell survival, suggesting that Atg7-activates beneficial autophagy. Co-expression of AdAtg7 and CryAB(R120G) significantly reduces aggregate content and cardiomyocyte cytotoxicity in a dose-dependent manner. Conversely, use of an AdAtg7 siRNA with CryAB(R120G) significantly reduces autophagosome synthesis and increases CryAB(R120G) aggregate content and cytotoxicity. Conclusions: Inducing autophagy by AdAtg7 is beneficial to CryAB(R120G) expressing cardiomyocytes and may represent a therapeutic tool for the treatment of cardiomyopathies caused by protein misfolding and aggregate-forming diseases.
MicroRNAs are a distinct class of single-stranded noncoding RNAs that post-transcriptionally repress expression of target genes. It has been shown that microRNAs are involved in various biological processes, however, little is known about the role of microRNA on hypoxic/surgical paracrine factors of stem cells, which is critical to the heart repair by stem cells. To elucidate the roles of MicroRNA in the establishment of hypoxia-mediated paracrine effects, we first examined the cytokine profiles by protein array analysis in cardiosphere-derived progenitor cells (CPC) via hypoxia preconditioning, and observed that 245 of the 308 cytokines are up-regulated, and most of them are HIF-1α mediated paracrine factors, which are involved in various biological processes of heart repair after ischemic injury, including angiogenesis, anti-apoptosis, cell proliferation/survival and extracellular matrix metabolism. Of interest, we observed expression of paracrine factors in hypoxia preconditioning in hypoxia-exposed CPCs was 4-fold higher than that of normoxia. Using echocardiography, we further demonstrate that cardiac ejection fraction were higher in mice treated with hypoxia preconditioned CPCs vs. normoxia CPC. Using echocardiography, we further demonstrate that cardiac ejection fraction were higher in mice treated with hypoxia preconditioned CPCs than in mice treated with normoxia cells 4 weeks after MI. These data demonstrated the beneficial effect of cardiac progenitor cell derived paracrine factors, which are regulated by HIF-1α induction via suppression of miR-17. This is first report to highlight the involvement of microRNA-transcriptional factor crosstalk in hypoxia-induced paracrine factor expression in stem cells.

Angiogenesis and Noncardiomyocyte Signaling in Pressure Overload Hypertrophy: Novel Role of Bmx Tyrosine Kinase

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Bmx nonreceptor tyrosine kinase was recently established as a novel regulator of cardiac phenotype but the mechanisms underlying this role are unknown. The objective for the current study is to investigate the role of non-cardiomyocyte signaling by Bmx as well as its role in cardiac angiogenesis. Methods: Bmx knockout mice and wild type (WT) controls were subjected to transverse aortic constriction (TAC). Immunoblotting and immunohistochemistry were used to assess protein expression and microvasculature density, respectively. siRNA was used to knockdown Bmx in bovine aortic endothelial cells (BAECs). Results: In comparison with WT mice, which progressively developed massive hypertrophy following TAC, Bmx KO mice were resistant to TAC-induced cardiac growth at the organ and cell level. Bmx deficient hearts exhibited less developed networks of microvasculature following TAC as compared to their WT counterparts, suggesting that Bmx regulates angiogenesis in response to increased afterload. Western blotting was used to measure the relative abundance of the known angiogenic factors Ang-1, VEGF, HIF-1α, and STAT3 at various time points following TAC. Contrary to expectation, we observed significantly higher levels of VEGF in KO versus WT mice after TAC, suggesting that absence of Bmx enhances VEGF expression. To identify a vasculature-specific phenotype affected by Bmx following angiogenic stimuli, we used siRNA to knockdown Bmx in BAECs followed by challenge with a wound healing assay, in which the ability of the BAECs to proliferate following injury is a surrogate for angiogenesis. Knockdown of Bmx substantially impaired endothelial cell proliferation after wounding, supporting a role for this kinase in angiogenesis. Lastly, we have examined the role of known angiogenic signaling molecules released in the setting of cardiac hypertrophy, particularly nitric oxide, on Bmx-dependent endothelial cell proliferation. Conclusion: These findings significantly expand the recently established role for the TEC family of tyrosine kinases in the heart. Our data indicate that Bmx participates in cardiac hypertrophy through non-cardiomyocyte signaling (including in endothelial cells) and implicate the molecule in stress-induced cardiac angiogenesis.

Metabolic Remodeling Precedes Left Ventricular Remodeling in Cardiac Hypertrophy: Early Detection by Noninvasive Imaging

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Introduction: In left ventricular hypertrophy (LVH) there is a shift away from fatty acid to glucose metabolism. However, the temporal relationship between metabolic changes and hypertrophy is not known. Hypothesis: Metabolic remodeling precedes structural and functional remodeling of left ventricular myocardium subjected to pressure overload. Methods: 8 C57/B6J adult male mice were subjected to transverse aortic constriction (TAC) surgery to induce pressure overload LVH. 4 additional sham animals were subjected to the same surgical protocol, without tying off the suture. Dynamic cardiac gated 18F-FDG PET imaging was performed using a Siemens microPET scanner, at baseline (BL), 1 day, 2 and 4 weeks post surgery, first on the Siemens scanner and done with a 7T Siemens scanner. Results: PET images in figure 1A shows a gradual enlargement in LV lumen over 4 weeks. Figure 1B shows the corresponding short-axis MR images. A plot of % Ki (ml/g/min) in figure 1C shows a 5 fold increase in the rate of myocardial glucose uptake by day 1 indicative of the adaptive response to stress and by about 1.5–2 fold increase from day 1 to 4 weeks, indicating a compensatory phase of metabolic adaptation (p < 0.01). Sham animals were imaged at 4 weeks. Heart weight to body weight ratios (HW/BW) and end-diastolic wall thickness (EDWT) measured using MRI increased over 4 weeks (p < 0.01) to maintain ejection fraction (EF), which shows a moderate lowering between baseline to day 1 up to 2 weeks post surgery (figures 1 D-F). The measured percentage changes in Ki, EF, HW/BW and EDWT from baseline to day 1 were 407.6%, 20.9%, 9.8% and 14.1% respectively. Conclusion: The results support the stated hypothesis.

PKA Inhibition Prevents Myocyte Death and Hypertrophy Induced by Adrenergic Agonists and Ameliorates Adverse Remodeling Upon Cardiac Stress Impression

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Adrenergic (AR) overstimulation causes adverse remodeling during heart disease development. Protein kinase A (PKA) is the major downstream molecule of AR pathway. However, to date, few studies explored the possibility of using PKA inhibition as a therapy for heart disease. Methods: Adrenergic overstimulation in vivo was achieved in PKA-deficient mice (PKAβ-/-Cre) and wild type (WT) mice. PKAβ-/- mice were subjected to transverse aortic constriction (TAC) surgery and compared to their WT littermates. Results: TAC-induced cardiac remodeling such as increase in the rate of myocardial glucose uptake by day 1 indicative of the adaptive response to stress and by about 1.5–2 fold increase from day 1 to 4 weeks, indicating a compensatory phase of metabolic adaptation (p < 0.01). Sham animals were imaged at 4 weeks. Heart weight to body weight ratios (HW/BW) and end-diastolic wall thickness (EDWT) measured using MRI increased over 4 weeks (p < 0.01) to maintain ejection fraction (EF), which shows a moderate lowering between baseline to day 1 up to 2 weeks post surgery (figures 1 D-F). The measured percentage changes in Ki, EF, HW/BW and EDWT from baseline to day 1 were 407.6%, 20.9%, 9.8% and 14.1% respectively. Conclusion: The results support the stated hypothesis.

PKA inhibition prevents myocyte death and hypertrophy induced by adrenergic agonists and ameliorates adverse remodeling upon cardiac stress impression.
remodeling were evaluated in TG after ISO infusion, transaortic constriction (TAC), and coronary artery ligation (MI). Results: 1. PKA activity stimulated with cAMP in the crude protein extract from PK-FAVMs or TG heart was suppressed. 2. The iNOS, contraction, Ca transient, and SR content in PK-FAVMs or TG VMs did not respond to ISO stimulation; 3 PKI blocked NFRM hypertrophy induced by isoproterenol and PE. 4. ISO, dobutamine + ICI 111851 induced 65–69% of GFP-FAVMs to die but no cell death in PK-FAVMs at 24 hours; 5. AFVM death was prevented by taspisagin, nifedipine and KCl-87; 6. Atalvi2.2(p2a) (MD-5) did not cause significant AFVM death but ISO precipitated the death of j2a-FAVMs (62.7%) at 24 hours, which can be blocked by PKI completely. 8. The significantly higher % of AFVMs of TUNEL+ and Apoptosis+ were observed in TG, ISO, dobutamine + ICI and beta2a+5 ISO were significantly reduced by PKI-8. PKI-1 was able to prevent the phosphorylation of phospholamban at both Ser16 (PKA site) and Thr17 (CaMKII site). 10. PKA-1 TG mice had normal basal cardiac function (EF = 65% in both cTg and Tg) but had blunted response to ISO; 11. MI caused 37% death in control mice (32.9% ± 2.0% v.s. 42.6% ± 2.6%) at 26 days post surgery; PKA-1 TG mice had better cardiac function (EF: 53.3 ± 4.8%, n = 5 vs. cTg: 40.3 ± 2.5%, n = 42) at 28 days post MI without significant wall thinning and dilation. 12. ISO infusion and TAC caused cardiac function depression, myocardium fibrosis and hypertrophy in cTg but not in Tg. 13. Less myocyte apoptosis was determined in Tg in response to myocardium fibrosis in TAC and ISO. PKI inhibition mitigates the adverse remodeling during heart disease development, probably by preventing myocyte death and hypertrophy.

5190 Stem Cells Transduction With Ferritin as a Reporter Gene to Track Their Fate by 1.5 Tesla MRI in the Beating Heart

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The methods so far utilized to localize implanted stem cells by cardiac magnetic resonance imaging (MRI) require cell loading with materials that are potentially cytotoxic and are not retained after replication cycles. Our aim was to develop a MRI-based reporter gene technology for long-term tracking of differentiating stem cells, in beating hearts, complemented by morpho-functional measurements. Cardiosphere-forming stem cells were obtained from pig left ventricle using a retrovirus complemented by morpho-functional measurements. Cardiospheres containing stem cells were implanted in the anterior left ventricular wall by a sterile saline solution (control infarct). By employing clinical standard 1.5 Tesla MRI scanner and a multilistec multislice 12 T2w sequence, we could precisely localize iron-accumulating cells only in areas with ferrous oxide particles. The absence of ferrous oxide in controls, referred to 1 week after MI and its size did not change significantly after 4 weeks (33.5 ± 3.05 vs. 41.4 ± 3.38 mm2). Based on MRI measurements, LV ejection fraction and end-systolic wall thickening of the infarct border zone were, respectively, 60 ± 7.2% and 45.7 ± 5.5% higher in MI vs. control and MI with MRI scanner, while both CV and MI without MRI had a 5 ± 13.2% reduction of the scar size. Importantly, the beneficial effects of CV on scar size were not significantly different compared to those of non-transfused CV. Histological analysis of the LV regions implanted with FHT-CS revealed the presence of iron-accumulating (Pearl’s stain) cardiomyocytes and myocardialfibroblasts, whose porcine origin was confirmed using pig-specific anti-mitochondria antibodies. In conclusion, this is the first evidence that a MRI-based reporter gene technology can be utilized to track the fate of differentiating stem cells in the beating heart, while simultaneously monitoring cardiac morpho-functional changes.

5191 CD36 Ligands Trigger Apoptosis in ER-Strressed Macrophages by TLR2-Dependent NADPH Oxidase Activation

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The ER stress pathway known as the Unfolded Protein Response (UPR) has been associated with apoptotic Mφs in advanced atherosclerotic plaques of both humans and mice, and mice lacking Cd36 are protected against lesional macrophage apoptosis and plaque necrosis. We recently reported reduced macrophage apoptosis and plaque necrosis in mice lacking both types A and B scavenger receptors SRA and CD36. The goal of this study was to explore how CD36 promotes Mφ apoptosis. CD36 is a pattern recognition receptor that binds atherosclerotic lipids on monocytes, which is translated into 37 a fatty acid receptor. Here we show that various CD36 ligands, such as oxidized phospholipids (oxPLs), oxidized LDL and saturated fatty acids (SFAs) can combine with low levels of various ER stressors such as peroxynitrite, 7-ketocholesterol, and taspisagin to trigger Mφ apoptosis. ER-stressed Mφs from SRA-/- and TLR2-/- mice were protected from apoptosis triggered by CD36 ligands. Lipopolysaccharide (LPS) is a known pro-inflammatory factor for expression of SRA and CD36. We therefore show that LPS can be a carrier for oxPLs. We found that LPS also triggers apoptosis during ER stress in a C20-dependent manner. CD36 ligand-induced Mφ apoptosis was blocked in Mφs lacking Nox2 indicating an important role for NADPH oxidase. During ER stress, CD36 ligands promoted sustained generation of reactive oxygen species (ROS) and p47, a marker for NADPH oxidase activation. NADPH oxidase activation was dependent on a TLR2-ERK signaling pathway, as both TLR2 deficiency and an ERK inhibitor blocked p47 clustering, ROS production, and apoptosis. The CD36-TLR2 model of Mφ apoptosis could be recapitulated in vivo mouse fed a diet high in SFAs for 2 weeks, and subsequently exposed to an ER stressor had a significantly higher level of Mφ apoptosis in the peritoneal cavity and spleen when compared with mice treated with the ER stressor or SFA diet alone. This increase in apoptosis in vivo was not observed in TLR2/- or CD36/-/- mice. In summary, CD36 trigger apoptosis in Mφs undergoing ER stress, and in vivo through a pathway involving TLR2, ERK, and NADPH oxidase. These findings identify a potentially new pathway for Mφ apoptosis and plaque necrosis in advanced atherosclerosis.