Late-Breaking Basic Science Abstracts

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Abstract Topics Include:

- Renewal of Cardiac Cells
- Cardiac and Pulmonary Pathophysiology
- Vessel Homeostasis and Disease
Late-Breaking Basic Science Oral Session: Renewal of Cardiac Cells

Subspecialty: General
Room 291–292
Abstracts 3523–3532

3523
The Turnover of Cardiac Progenitor Cells is Modulated by the Level of p53 Expression
Adriana B Carvalho, João Ferreira-Martins, Marcello Rota. Carlos Rondon, M. Elena Padin-Irugenes, Hangjiao Zheng, Alessandro Gatti, Konrad Urbanek, Jan Kajstura, Toru Hosoda, Piero Anversa, Brigham and Womens Hosp, Boston, MA; Manu Serrano, Spanish National Cancer Resch Ctrn, Madrid, Spain; Annaarosa Lenn; Brigham and Womens Hosp, Boston, MA

The objective of this work was to establish whether the transcription factor p53 modulates the growth and survival of cardiac progenitor cells (CPCs) and whether enhanced p53 function has a positive or negative effect on the aging myopathy. Because of the critical role that p53 plays in cellular senescence, wild type (WT) and super p53 mice (TG) at 24–30 months of age were studied. In this TG model, the gene dosage of p53 is modestly increased and the behavior of the endogenous gene is faithfully reproduced. Additionally, super p53 mice typically show a stronger response to DNA damage, increased cancer resistance and enhanced cardiomyocyte apoptosis at young age. Therefore, CPCs from old TG and WT mice were isolated by enzymatic digestion and following their expansion in vitro, cell proliferation was analyzed at baseline and in the presence of IGF-1 stimulation. In control conditions, BrdU incorporation was 2.7-fold higher in CPCs from TG than in WT. With IGF-1, cell replication increased in both CPC classes; however, this parameter was 2.6-fold larger in super p53. The increased growth behavior of CPCs from TG was associated with a 4.6-fold higher degree of apoptosis at baseline. Oxidative stress induced by xanthine-xanthine oxidase potentiated the difference in CPC apoptosis between TG and WT. In an attempt to define the mechanisms involved in the high level of replication and death of CPCs from TG mice, the expression of genes related to p53 was determined by quantitative RT-PCR: the amount of p21G1, bax, bcl-2, mdm2, c-met and IGF-1 receptor was comparable in the two CPC categories. Conversely, the expression of the senescence-associated gene p16INK4a was significantly lower in CPCs from old TG than WT. Similarly, p16INK4a mRNA, together with the expression of some of the components of the renin-angiotensin system - angiotensinogen and AT1 receptor - was decreased in vertebral myocytes from TG. These molecular modifications were characterized by an increase in CPC and myocyte apoptosis in the old heart of super p53 mice. In conclusion, mild overexpression of p53 enhances CPC and myocyte turnover suggesting that potentiation of cell death leads to preservation of a younger cardiac phenotype possibly increasing lifespan in this model.

3524
The Reparative Benefits of Very Small Embryonic-Like (VSEL) Stem Cells are Sustained During Long-Term Follow-Up
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We have shown that transplantation of adult bone marrow (BM)-derived pluripotent Sca-1+/Lin-/CD45- very small embryonic-like (VSEL) stem cells improves LV function and remodeling at 35 days after myocardial infarction (MI). However, it is unknown whether these beneficial effects are sustained in the long term - an issue of fundamental importance for clinical translation. Moreover, because of the pluripotency nature of VSELS, potential tumor formation in the long term is a concern. Here, we tested the ability of VSELs to regenerate myocardium in an infarcted heart at 6 months post-infarction. VSELs were harvested from a VSEL-producing mouse strain (Sca-1+/Lin-/CD45- VSELs) and injected into the LV cavity at 24 months of age. The treated hearts exhibited improved LV function and decreased LV remodeling. LV function of VSEL-treated mice at 6 months post-infarction was comparable to the LV function of non-infarcted wild-type mice. These results indicate that VSELs are able to regenerate myocardium in the long term and may offer a novel therapeutic strategy for patients with myocardial infarction.

3525
Micro RNA-21 Targeting of Programmed Cell Death 4 is Required for Valve Development
David J Milan, Heather J Kolpa, C. G Burns, Calum A MacRae. Massachusetts General Hosp, Boston, MA

Background: Congenital heart defects are the leading cause of death from birth defects and are estimated to occur in approximately 1% of live newborns and ~5% of pre-term lethalities. Among congenital cardiac defects, valvular abnormalities and endocardial cushion defects are the most common. Despite its medical importance, there remains a limited understanding of the molecular events that govern many of the steps of valvulogenesis. Recently the discovery of a class of biologic molecules, micro-RNAs, has revealed a new regulatory mechanism for gene expression. One micro-RNA, miR-21, is specifically and highly expressed in the developing cardiac valves in zebrafish, while its function there remains unknown. Methods: Two distinct antisense morpholino oligos targeting miR21 and one construct designed to specifically block miR-21 binding to the Programmed Cell Death 4 gene (PDCD4) were injected into zebrafish embryos at the single cell stage and resulting embryos were analysed for effects on cardiac and valve development using light microscopy, in situ hybridization, histology and confocal microscopy. Candidate miR-21 targets were identified using bioinformatics and confirmed with luciferase reporter assays. Results: Knockdown of miR-21 results in atrioventricular regurgitation, loss of cardiac looping, and failure of formation of endocardial cushions and valve leaflets. MiR-21 knockdown in transgenic Tie2:GFP zebrafish revealed defective endocardial activation evidenced by loss of GFP upregulation in AV ring endocardium. Loss of miR-21 also eliminated expression of the cellular migration marker osteopontin, or spo1, in the AV ring endothelium. Luciferase assays in cultured cells confirmed the prediction that PDCD4 is a target of miR21 in vitro. To analyze the in vivo relevance to valvulogenesis we employed a target protector morpholino that specifically interferes with miR21 binding to PDCD4. Injection of the PDCD4 target protector morpholino phenocopied miR-21 knockdown, demonstrating that miR-21 mediated downregulation of PDCD4 is a requirement for early valvulogenesis and cell migration. Conclusion: MicroRNA 21 plays a critical role in cardiac valvulogenesis via its downregulation of the target gene Programmed Cell Death 4.

3526
Turnover of Human Cardiomyocytes

The ability of cardiomyocytes to regenerate in the adult mammalian heart has been a subject of controversy over the last few decades. Although, the heart was viewed as an organ that had lost its ability to regenerate, recent studies suggest that progenitor cells with the potential to generate new myocardial cells are present in the adult heart. However, little is known about
the extent to which such progenitor cells give rise to new cardiomyocytes under normal as well as under pathologic conditions. Traditional methods used for dating cells are limited or not appropriate for human use (e.g. BrdU). We have taken advantage of the integration of 14C (radiocarbon), generated by nuclear bomb tests during the Cold War, into DNA in order to establish the age of cardiomyocytes in the human heart. Using flow cytometry, the nuclei of cardiomyocytes isolated from post mortem hearts (n=12) and the purified blasts were subjected to 14C analysis. Our data show that human cardiomyocytes in the left ventricle are on average 6 years younger than the individual. Mathematical modeling predicts an annual cardiomyocyte turnover of 1% at the age of 20, decreasing to 0.3% at the age of 75. The capability of the adult human heart to generate cardiomyocytes opens up the possibility to develop therapeutic strategies aiming to foster this process in the diseased heart.

Human Vein-derived Progenitors Give Rise to Pericyte-like Cells Supporting In vitro and In vivo Angiogenesis
Paola Campagnolo, Nicolle Kraenkel, Gianni D Angelini, Paolo Madeddu; Univ of Bristol, Bristol, United Kingdom

The dogma of bone marrow as a unique source of adult stem cells has been recently overruled by demonstration of stem cell niches in peripheral tissues. We evaluated the presence, plasticity and functions of progenitor cells from saphenous veins (SV) of patients undergoing coronary bypass (age 69.5±9). Immunohistochemistry identified non-endothelial (vWF-) CD34+ cells surrounding adventitial vessels of SV. Flow cytometry showed low coexpression of CD45, CD133, KDR and NG2 on CD34+ cells. Cells obtained by collagenase digestion of SV were either cultured in NeuroCult, where they form floating spheres mainly composed of CD34+ (56±16%) and KDR + cells (42±1%), or separated using anti-CD34 magnetic beads (CD34+ = 55±18%). Cells selected either way were cultured in presence of serum and VEGF-A. Under these conditions, they gained adherence and proliferated, losing CD34 expression and acquiring mesenchymal (CD90 and CD144) and pericyte markers (NG2 and Desmin), but remained negative for endothelial and smooth muscle antigens. We thus defined those cells as SV pericytes (SVp). When cocultured with SV-endothelial cells (vEC) on Matrigel, SVp assumed pericytic position, stimulating the growth of longer, thicker and more stable tubes as compared to the two cell types alone. Similarly, SVp conditioned medium (collected without additional VEGF) stimulated tube growth (cumulative length: 126±3.9 vs control 71±0.5 mm, P<0.001) increasing vEC proliferation (BrdU: 0.82±0.04 vs 0.57±0.13 RFU, P<0.05), preventing starved-induced apoptosis in vEC (apoptosis: 116±2.9 vs 65±3.8 RFU, P<0.001) and promoting gap closure in a scratch assay (73±18 vs 45±5%, P<0.05). In a nude mouse model of limb ischemia, SVp transplantation (8x104) improved blood flow recovery compared to both circulating endothelial progenitor cells and vehicle (P<0.05). Capillary density increased by 1.4 fold in muscles injected with SVp (P<0.05 vs vehicle). We newly demonstrated the presence of pericyte-committed progenitors in human SV able to support angiogenesis in vitro through direct interaction and paracrine stimulation of vEC and promote in vivo neovascularization in a model of limb ischemia. Highly proliferative SVp may be valuable for the treatment of ischemic disease.

Cells Cultured Directly From Heart Biopsies Express Stem Cell Markers And Improve Function After Myocardial Infarction
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The adult heart contains small reservoirs of cardiac progenitor cells (CPCs) that express both embryonic and stem cell related antigens. While these cells represent promising candidates for autologous cellular therapy, clinical application is hampered by their limited abundance and the tedious methods for enriching such cells while maintaining regenerative potency. Here we investigate the regenerative capacity and cellular/molecular signature of cells directly expanded from biopsy specimens from human heart disease. Human heart biopsy specimens were minced and cultured from 5 separate regions (atria, LV-free wall, RV-free wall, LV-septum apex, LV-septum base). Loosely adherent CPCs were harvested weekly for a total of five harvests. RT-PCR revealed expression of CD34, CD133, KDR and NG2 on CD34+ cells (42±18%) and cytokine secretion (IGF-1 and VEGF). Rats injected with CO or CDCs after LAD ligation had improved ejection fraction (EF) compared to vehicle injected animals (27±4 vs 18±4%, P<0.05). These differences in ejection fraction were maintained at 6 weeks with superior invasive functional benefit of CDCs (71±10 vs 55±10%, P<0.05). Preconditioned supernatant (PCS) was collected from the infarcted myocardium and was associated with improved cardiac function (EF: 27±4 vs 12±0.7%, P<0.05) increasing vEC proliferation (BrdU: 0.82±0.13 RFU, P<0.05) and promoting gap closure in a scratch assay (73±18 vs 45±5%, P<0.05). In vivo, NPY induced cardiomyocyte cell-cycle reentry and mitosis in the infarcted myocardium and was associated with improved cardiac function (EF: 27±4 vs 12±0.7%, P<0.05) increasing vEC proliferation (BrdU: 0.82±0.13 RFU, P<0.05) and promoting gap closure in a scratch assay (73±18 vs 45±5%, P<0.05). In a nude mouse model of limb ischemia, SVp transplantation (8x104) improved blood flow recovery compared to both circulating endothelial progenitor cells and vehicle (P<0.05). Capillary density increased by 1.4 fold in muscles injected with SVp (P<0.05 vs vehicle). We newly demonstrated the presence of pericyte-committed progenitors in human SV able to support angiogenesis in vitro through direct interaction and paracrine stimulation of vEC and promote in vivo neovascularization in a model of limb ischemia. Highly proliferative SVp may be valuable for the treatment of ischemic disease.

Neuropeptide Y (NPY) Promotes Adult Cardiomyocyte Proliferation And Differentiation Following Transplantation Of Stem Cells In Infarcted Myocardium
Yigang Wang, Dongsheng Zhang, Tiemin Zhao, Tao Wang, Atif Ashraf, Meifeng Xu, Muhammad Ashraf; Ambikaipakan Balasubramanian; Univ of Cincinnati, Cincinnati, OH

Background: Adult cardiomyocytes were thought to be incapable of proliferation. In this study we show that NPY induces both reentry of differentiated rat adult cardiomyocytes into the cell cycle and differentiation of bone marrow-derived-mesenchymal stem cells (MSCs) into cardiomyocytes following transplantation into infarcted myocardium. Method: In vitro: Isolated rat adult cardiomyocytes were treated with vehicle, NPY or FGF (100ng/ml), or FGF plus NPY. DNA synthesis, mitosis, and cytokinogenesis were determined by immunocytochemistry. NPY-induced gene expression in MSCs was analyzed by microarray and RT-PCR. In vivo: Vehicle or male GFP-MSCs (2x10^5) pretreated with vehicle or with NPY (10^-7) Mi for 72 hours were injected into border zone of myocardial infarction (MI) of female rats following LAD ligation. In addition, vehicle or NPY (10^-7) was delivered continuously for 14 days into the myocardium by a micro-pump. On day 30, heart function was measured and then hearts were harvested for immunohistochemical analyses. Results: NPY increased BrdU incorporation, promoted cytokinogenesis, and mitosis in adult cardiomyocytes. NPY also upregulated several genes required for DNA synthesis, such as NPY (Figure 1). In vivo, NPY induced cardiomyocyte cell-cycle reentry and mitosis in the infarcted myocardium and was associated with improved cardiac function (EF: 27±4 vs 12±0.7%, P<0.05) increasing vEC proliferation (BrdU: 0.82±0.13 RFU, P<0.05) and promoting gap closure in a scratch assay (73±18 vs 45±5%, P<0.05). In conclusion: NPY directly induced cardiomyocyte cell-cycle reentry and enhanced the proliferation of differentiating cardiomyocytes from transplanted stem cells in the infarcted myocardium.

Figure 1

Figure 2

Genetic Manipulation for CXCR4 overexpressing MSCs (CXCR4+MSC) Promotes Rat Adult Cardiomyocyte Proliferation
Yigang Wang, Dongsheng Zhang, Tiemin Zhao, Tao Wang, Atif Ashraf, Meifeng Xu, Muhammad Ashraf; Univ of Cincinnati, Cincinnati, OH

Background: Adult cardiomyocytes were thought to be incapable of proliferation. In this study we show that injection of CXCR4+MSCs into the damaged myocardium increased myocyte proliferation. Method: In vitro: MSCs were divided into following groups: MSCs, CXCR4+MSCs, or miRNA-targeting CXCR4 gene. Preconditioned supernatant (PCS) was collected from the above groups under hypoxic conditions for 8 hours, then concentrated and added to adult myocytes. Cell proliferation was measured by BrdU incorporation and CXCR4 levels were measured by flow cytometry. Results: NPY injection into CXCR4+MSCs resulted in significantly increased cell proliferation (P<0.05). In vivo: Female rats underwent LAD ligation and were divided into four groups. Saline, 5x10^5 MSCs, CXCR4+MSCs, or MSCs treated with miRNA was injected into border zone of MI. Heart function was measured and then hearts were harvested for histology and immunohistochemistry analyses. Results: CXCR4+MSCs promoted both reentry of differentiated rat adult cardiomyocytes into the cell cycle and differentiation of bone marrow-derived-mesenchymal stem cells (MSCs) into cardiomyocytes following transplantation into infarcted myocardium.
Increased Availability But Defective Homing Capacities Of Circulating Cells In Patients With Acute Myocardial Infarction

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Background: Cell-based strategies to repair the injured heart have been achieved by e.g. mobilizing circulating progenitor cells (CPCs) with cytokines or drugs, such as statins. Besides CPC mobilization, adequate cell homing is a prerequisite for optimal regeneration. The chemokine stromal cell-derived factor-1 (SDF-1α) is important for homing of CPCs expressing SDF-1α on the cell surface. We hypothesize that statin treatment early after AMI enhances the number of CPCs in the circulation. The objective of this work was to establish whether statins affect the CPC axis which may be critical in cell proliferation, survival and lineage commitment. The SDF-1 system consists of two ligands, SDF-1 and IP-10, which the RBP-Jk binding site was mutated in a single nucleotide. Reporter gene activity was measured 30 days after LAD ligation and hearts were harvested for analyses. Results: In vitro: BrdU, phosphorilated histone, and Aurora B kinase positive CPCs were significantly higher in the CXCR4+MSCs group. In vivo: BrdU, phosphorilated histone, and Aurora B kinase positive CPCs were significantly higher than other groups, and displayed significant increase in heart function with reduced fibrosis (Figure 2). When co-cultured with neonatal myocytes, CXCR4-MSCs significantly increased the number of differentiated myocytes as compared with control group. Conclusion: CXCR4-MSs induced both differentiation of MSCs into myocytes and stimulation of myocytes cell-cycle reentry could repopulate an infarcted myocardium.

Late-Breaking Basic Science Poster Session

Subspecialty: General

Hall A-B1

Abstracts 1540–1559

Notch1 Regulates the Fate of Cardiac Progenitor Cells

João Ferreira-Martins, Brigham & Women’s Hosp, Boston, MA; Konrad Urbaneck, Alessandro Boni, Toru Hosoda, Hanqiao Zheng, Francesca Delucchi, Katsuya Amano, Aranba Gonzalez, Serena Vitali, Caroline Gajami, Katherine E Yubey, Marcelo Rota, Piero Anversa, Annarosa Lea, Brigham & WOMENS Hosp, Boston, MA; Jan Kajstura; Brigham & Women’s Hosp, Boston, MA

The Notch receptor mediates stem cell fate decision in multiple organs. The objective of this work was to establish whether Notch promotes stemness or differentiation of cardiac progenitor cells (CPCs). In the niches, CPCs expressed Notch1 receptor and the surrounding microenvironment exhibited the Notch ligand Jagged1. Notch1 was found to be expressed in nearly 60% of freshly isolated CPCs while Notch2–4 were present in ~10% of CPCs, pointing to Notch1 as the major Notch isoform in CPCs. Following stimulation with Jagged1, the active fragment of Notch1, N1ICD, translocated to the nucleus in CPCs. N1ICD was consistently associated with the expression of the early marker of myocyte commitment Nkx2.5. In contrast, transfection expression of vectors were downregulated by Jagged1. The expression of Nkx2.5 did not interfere with the capacity of the cells to proliferate. Developing myocytes expressed Nkox1, had a thin layer of sarcomeric proteins, and labeled by BrdU or Ki67. In the search for the mechanism by which Notch1 regulates CPC differentiation into myocytes, we established first whether N1ICD and the Notch-dependent protein RBP-Jk interact in CPCs. N1ICD was consistently associated with the expression of the early marker of myocyte commitment Nkx2.5. In contrast, transfection expression of vectors were downregulated by Jagged1. The expression of Nkx2.5 did not interfere with the capacity of the cells to proliferate. Developing myocytes expressed Nkox1, had a thin layer of sarcomeric proteins, and labeled by BrdU or Ki67. In the search for the mechanism by which Notch1 regulates CPC differentiation into myocytes, we established first whether N1ICD and the Notch-dependent protein RBP-Jk interact in CPCs. N1ICD and the Notch-dependent protein RBP-Jk protein complex was 4-fold greater in Jagged1-treated CPCs than in untreated cells. By gel-shift and chromatin immunoprecipitation, we identified a binding site for RBP-Jk in the Nkox2.5 promoter. Reporter gene assays were performed in CPCs which were transfected with luciferase plasmids carrying the wild-type Nkox2.5 promoter or an Nkox2.5 promoter in which the RBP-Jk binding site was mutated in a single nucleotide. Reporter gene activity was ~3-fold higher in CPCs transfected with the wild-type promoter than with its mutated form. Importantly, Nkox2.5 transactivation in CPCs did not necessitate co-factors including GATA4 and Smad4. Inhibition of Notch1 in infarcted mice impaired the commitment of resident CPCs to the myocyte lineage opposing cardiomyogenesis. These observations indicate that Notch promotes the early specification of CPCs to the myocyte phenotype but maintains the newly formed cells in a highly proliferative state. In conclusion, Nkox2.5 is a novel target gene of Notch1 which may have critical implications in the control of heart homeostasis and its adaptation to pathologic states.
**D1275N SCN5A Mutation Causes Dilated Cardiomyopathy and Arrhythmia: Mechanisms Revealed in Mice but Absent by Heterologous Expression**

Hiroshi Watanabe, Tao Yang, Nagesh Chopra, Thomas Atack, Hyun Hwang, Brenda Leake, Ninamaka Apochuku, Prince Kannankeril, Sabina Kupershmidt, Björn Knöllmann, Dan Roden; Vanderbilt Univ, Nashville, TN

**Background:** The D1275N SCN5A mutation has been associated with a range of unusual phenotypes, including conduction disease and dilated cardiomyopathy (DCM) as well as atrial and ventricular arrhythmias. However, when D1275N is studied in heterologous expression systems, sodium current amplitudes are unchanged (245 ± 42 pA/pF at -30 mV) vs 252 ± 52 for wild-type (WT) in CHO cells, and only minor differences in recovery from inactivation are seen. Thus the mechanisms for these phenotypes remain underdetermined. **Methods:** The scn5a locus was modified to knock out the mouse gene, substitute full-length WT (H) or mutant (DN) human alleles, and retain physiologic regulation of sodium channel expression. Littermates from DN/H x DN/H matings were studied at 3–12 weeks. **Results:** Pup distribution was in Hardy-Weinberg equilibrium (22 H/H, 38 DN/H, and 20 DN/DN mice). ECGs and echocardiograms in H/H mice were not different from WT mice. However, the DN allele caused slow conduction and a DCM phenotype (Table). Telemetry in unanesthetized mice revealed spontaneous heart block and ventricular tachycardia in DN/H and DN/DN (but not H/H) animals, and EP studies identified prolongation of HV interval by the DN allele. Mechanistic studies revealed no increased fibrosis by histology and no evidence for impaired EC coupling and calcium handling in ventricular cells. SCN5A mRNA abundance was also unaffected using real-time PCR, but Western blotting revealed gene-dose dependent reduction in sodium channel protein. Sodium current in ventricular cells was strikingly reduced by the DN allele, with slowing of fast inactivation (Table). **Conclusions:** The D1275N SCN5A mutation generated conduction slowing, arrhythmias, and a DCM phenotype in mice. The data strongly support the idea that these phenotypes reflect a reduction in channel protein abundance at the post-transcriptional level and a change in inactivation that were absent when the mutant was studied in heterologous expression systems.

<table>
<thead>
<tr>
<th>ECG</th>
<th>Heart rate (bpm)</th>
<th>PR (ms)</th>
<th>QRS (ms)</th>
<th>QTc (ms)</th>
<th>Pup distribution</th>
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<tbody>
<tr>
<td>DN/H</td>
<td>398±8</td>
<td>13.0±0.6</td>
<td>9.8±0.2</td>
<td>38.9±1.6</td>
<td>0.05 vs. control</td>
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<tr>
<td>DN/DN</td>
<td>354±18</td>
<td>17.0±0.3</td>
<td>11.8±0.3</td>
<td>38.5±0.8</td>
<td>0.1 vs. control</td>
</tr>
<tr>
<td>DN/DN</td>
<td>335±15</td>
<td>19.4±0.9</td>
<td>22.3±2.7</td>
<td>50.2±2.7</td>
<td>0.05 vs. control</td>
</tr>
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Erg was recorded in anesthetized animals. *P < 0.05 vs. DH/H. †P < 0.05 vs. DN/H; QTC: 0.25±0.01/0.5 (mouse-specific); LV: left ventricle; VM: ventricular myocyte; BSA: cardiac sodium current.

Phosphatase-1-Inhibitor-1 Improves Contractile Performance but Increases Propensity toward Arrhythmias and Contractile Dysfunction after Pathological β-adrenergic Stimulation in Mice

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**Inhibitor-1 (I-1) acts as an amplifier of β-adrenergic signaling and is downregulated in failing hearts. Overexpression of a constitutive active mutant reverses contractile dysfunction and has been suggested as a therapeutic approach, but potential risks are unknown. To study I-1 cause-effect-relationships, we generated conditional double-transgenic (dTG) mice (doxycy- cline [DOX]-controlled Tet-off system) on a complete I-1-KO background with cardiomyocyte-restricted expression of constitutive active (I35D, truncation) I-1 (dTGI-1). Littersmates having only the transactivator (TAI–I-1-KO) served as controls. In vivo analysis by echo revealed both hypercontractility in dTG-I-1* at baseline was converted into hypocontractility in older mice (16 months, FAS% 8.7 ± 0.9 vs. 1.6 ± 4.3). Isoproterenol-treated dTG-I-1* mice showed significant increases of Hw/Bw ratio and systolic function compared to the WT group (ΔHw/Bw ratio 0.7 ± 0.3* vs. 0.6 ± 0.2*). Importantly, phosphorylation levels of p38 and its downstream target MAPKAPK-2 were dramatically elevated in the heart of Dusp1,4 double null mice compared to control mice. Dusp1,4 double null embryonic fibroblasts also showed elevated p38 phosphorylation, thus emphasizing the ubiquitous role of these 2 Dusps in regulating the p38 pathway. Dusp1,4 null mice were challenged with isoproterenol infusion or coarctation of the aorta (TAC) for 2 weeks to examine the disease relevance of p38 in more detail. Isoproterenol-treated Dusp1,4 null mice showed significant increases of Hw/Bw ratio (7.5 ± 0.5* vs. 3.0 ± 0.6*). Importantly, Dusp1,4 null mice died suddenly (65% VS none for WT) within an hour following TAC. Dusp1,4 double null mice who survived the TAC procedure showed greater increases of Hw/Bw ratio than controls (10.7 ± 1.2 VS 8.0 ± 0.4 for WT). **Conclusion:** These observations suggest that Dusp1 and Dusp4 together are essential for the maintenance of cardiac function and compensation by restraining p38 MAPK pathological signaling.

Genetic Activation of Endogenous p38-MAPK upon Deletion of Dusp1 and Dusp4 Triggers Cardiac Maladaptive Remodeling

Manxh Auger-Messier, Orlando F Bueno, Allen J York, Jeffery D Molkentin; CCHMC, Cincinnati, OH

**Background:** Although p38-mitogen-activated protein kinase (MAPK) has been implicated in regulating cardiomyocyte hypertrophy and survival, use of cardiac-specific transgenic animals to dissect its function in vivo have revealed conflicting results. MAPKs are inactivated by dephosphorylation mediated by dual-specificity phosphatases (Dusp), of which more than 10 family members have been described with varying specificity for p38, ERK1/2 and JNK1/2. Dusp1 and Dusp4 are each expressed in the heart where they regulate the strength and duration of p38 MAPK signaling. We hypothesized that targeted disruption of either or both Dusp1 and Dusp4 would enhance p38 signaling within physiological limits and trigger maladaptive remodeling of the heart. **Methods and Results:** Targeted disruption of Dusp1 and Dusp4 completely abrogated their respective transcript levels in the mouse heart. However, neither Dusp1 nor Dusp4 single null mice showed an alteration in cardiac p38 activation or a cardiac phenotype compared to wild-type (WT) controls. In contrast, Dusp1,4 double null mice showed a marked increase in baseline heart weight to body weight ratio (Hw/Bw) at 2 months of age (6.5 ± 0.3 VS 4.8 ± 0.1 for WT). Importantly, phosphorylation levels of p38 and its downstream target MAPKAPK-2 were dramatically elevated in the heart of Dusp1,4 double null mice compared to control mice. Dusp1,4 double null embryonic fibroblasts also showed elevated p38 phosphorylation, thus emphasizing the ubiquitous role of these 2 Dusps in regulating the p38 pathway. Dusp1,4 null mice were challenged with isoproterenol infusion or coarctation of the aorta (TAC) for 2 weeks to examine the disease relevance of p38 in more detail. Isoproterenol-treated Dusp1,4 null mice showed significant increases of Hw/Bw ratio (7.5 ± 0.5* vs. 3.0 ± 0.6*). Importantly, Dusp1,4 null mice died suddenly (65% VS none for WT) within an hour following TAC. Dusp1,4 double null mice who survived the TAC procedure showed greater increases of Hw/Bw ratio than controls (10.7 ± 1.2 VS 8.0 ± 0.4 for WT). **Conclusion:** These observations suggest that Dusp1 and Dusp4 together are essential for the maintenance of cardiac function and compensation by restraining p38 MAPK pathological signaling.
Apollipoprotein A1 Mimetic Peptide L-4F Prevents Weight Gain Resulting in Adipose Tissue Remodeling and Preservation of Muscle Mass in a Rat Model of Obesity-Induced Insulin Resistance

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Background. Obesity, an inflammatory condition, can cause insulin resistance. Obese adipose tissue is characterized by enlarged adipocytes and increased expression of the cannabinoid CB-1 receptor, a mediator of lipogenesis. L-4F, an apollipoprotein A1 mimetic peptide, increases heme oxygenase-1 (HO-1) and adiponectin levels, reducing inflammation and improving insulin resistance. We hypothesize that L-4F administration to Zucker diabetic fat (ZDF) rats will alter adipose tissue on a cellular level, reduce adiposity and prevent weight gain without loss of muscle mass.

Methods. 12 week old lean (ZL) and ZDF rats were divided into 4 groups: ZL, ZL-L4F, ZDF and ZDF-L4F. Control groups were administered 20μL vehicle and treatment groups 200μg/100g L4F daily for 6 weeks. At 18 weeks rats were sacrificed and gastrocnemius, subcutaneous and visceral adipose tissues were harvested. Adipocyte size was measured and immunostaining was used to detect HO-1 and CB-1 proteins. Subcutaneous and adipose tissue volumes were measured using magnetic resonance imaging (MRI). Gastrocnemius muscle weight and fiber diameter were measured and Oil Red O staining was used to determine muscle adiposity. Results. Compared with ZDF controls, L4F decreased subcutaneous and visceral adipose tissue volumes by 27.9% (p < 0.05) and 18.5% (p < 0.001) respectively, as measured by MRI, as well as reducing the adipose tissue/body volume ratio from 56% to 53.5% (p < 0.05). Adipocyte cell size was reduced in ZDF-L4F rats in subcutaneous and visceral adipose tissues by 6.12% and 17.27% compared to ZDF controls. Although food intake was the same in L4F and control groups, L4F decreased CB-1 levels in subcutaneous and visceral adipose tissues (p < 0.05 respectively). Gastrocnemius muscle weight was unchanged between L4F and control groups (352 ± 48 mg and 350 ± 52 mg, respectively), as was muscle fiber diameter and gastrocnemius tissue fat content. Conclusion. This study demonstrates that L4F slows weight gain, reduces adipose tissue volume and causes adipose tissue remodelling by decreasing adipocyte size and CB-1 expression, without loss of muscle mass. These findings suggest apollipoprotein mimetic peptides as a potential treatment for obesity and its associated health risks.

A Nitric Oxide-Soluble Guanylyl Cyclase-Cyclophilin Guanosine Monophosphate Signaling Lipid Raft Microdomain in Cardiac Myocytes is Altered by Pressure-Overload and Restored with Chronic Sildenafil Treatment

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Soluble guanylyl cyclase (sGC) is co-localized with cGMP-dependent kinase (PKG) in caveolae at plasma membrane in endothelium, a microdomain that is suggested to be vital to NO signaling by modulating NOS-NO-cGMP. However the role and regulation of sGC localization in cardiac myocytes (CMs) is unknown. Here we characterized the sub-cellular localization and function of sGC1 (dominant cardiac isoform) in CMs from normal murine hearts and hearts with cardiac hypertrophy and failure induced by pressure-overload (TAC), in sham CMs, basal sGC1, localized to the outer membrane, particularly in lipid raft (LR) fractions containing caveolin 3. This localization became diffuse after 5-wk TAC (hyper trophy stage, Fig A, B), declining in LRs. After 8-wk TAC (failure stage), sGC1, was again observed at the plasma membrane, though still not in LRs (Fig B, C). Interestingly, cGMP-stimulating treatment by sildenafil during the latter 5 weeks (TAC8wk/H11001) restored sGC1 in LRs at the plasma membrane. The alteration in localization had functional consequences. NO-stimulated cGMP synthesis was reduced in CM LRs from TAC hearts but restored in TAC8wk hearts (Fig D). These results provide the first evidence for a LR specific NO-sGC-cGMP microdomain.

Similar Metabolic Mechanisms in Two Different Models of Longevity: Caloric Restriction and the Adenylate Cyclase Type 5 Knockout Mice

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Caloric restriction (CR) is the most established model of longevity. Mice lacking type 5 adenylate cyclase (AC5) knock out (AC5 KO) also exhibit a ~32% increase in lifespan as compared to wild-type (WT) littermates. AC5 KO, similar to CR mice, weigh less than wild type (WT), but opposite to CR, AC5 KO eat more than WT. We therefore examined potential metabolic mechanisms that AC5 KO might have in common with CR. In CR mice GLUT1 mRNA increased by 84%, and by 100%, in AC5 KO. AMPK, which mediates an energy-sensing FOXO pathway, and is also known to result in lifespan extension, was activated as indicated by the increase in P-AMPKalpha in the heart, p<0.05 in CR (2.1 fold), and in AC5 KO mice (1.5 fold). The aim of this study was to determine the effects of combining AC5 KO and CR in mice. AC5 KO mice, subjected to CR resulted in rapid death after 10 days on the diet. There were no deaths recorded in the AC5 WT mice under CR, whereas 50% of the AC5 KO mice were dead within 15 days, with the remainder dying within 35 days. Blood glucose levels normal under a decrease during CR; however, glucose levels under CR were not different in AC5 KO mice to control mice (50% and 64% at days 14 and 21, respectively). Blood insulin levels were also significantly decreased in AC5 KO CR mice as compared to WT CR mice (45% and 28% at days 14 and 21, respectively). Physical examination of the mice during sacrifice showed that AC5 KO mice had no remaining adipose tissue deposits visible. Histopathological examination of the liver of the CR mice showed that there was a decrease in glycogen levels as compared to mice on a normal diet. Surprisingly, liver glycogen was already at CR levels in AC5 KO, and could fall no further when these mice were subjected to CR. Thus, the AC5 KO mice share many of the features and common metabolic mechanisms with CR. Since both models rely on glucose metabolism resulting in exhaustion of glycogen stores, the superimposition of CR on AC5 KO cannot be tolerated and rapidly leads to death. Late Breaking Basic Science, Control # 08-SS-LBS-2425-AHA

Human Induced Pluripotent Stem Cells Give Rise to Functional Cardiomyocytes

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The recent generation of induced pluripotent stem (iPS) cells from human somatic cells has opened new avenues for the generation of patient- and disease-specific iPS cells. However, the ability of human iPS cells to differentiate into functional cardiomyocytes (CMs) has not been characterized. The aim of this study was to explore the cardiac differentiation potential of human iPS cells, and to characterize the CMs at molecular, structural and electrophysiological functions. CMs derived from normal-derived human iPS cells (H9 and H1) and hES cell derived CMs were compared. Using embryoid body (EB) method, the human iPS cells (iPS-IMR90 clone 4; iPS-f sorensen clone 1) and hES cells (H1 and H9) were differentiated for 60 days. The IPS and ES EBs developed contractile outgrowths over a similar time course, and the contraction rates were comparable. However, IPS and ES cell lines exhibited differences in the absolute percentages of contracting EBs (N ~ 750 – 4121 EBs observed per group); H9, 20.0 ± 1.5%; H1, 11.3 ± 2.0%; IMR90, 10.6 ± 2.2%; and foreskin, 3.0 ± 0.4%. RT-PCR demonstrated similar expression of cardiac genes including Nkx2.5, cTnT, a-MHC, α-actin, ANF, Myl2, Myl7 and Pim. Immunoocytochem- istry of contracting EBs and isolated single CMs confirmed the expression of cardiac proteins including cTnT, α-actin, and MLCL2a. Co-labeling of BrDU with MF20 and MLCL2a revealed similar proliferative CMs from IPS and ES cells at day 10 of differentiation. Sharp microscopy recordings indicated that IPS cells have a capacity similar to hESCs for differentiation into nodal-, atrial-, and ventricular-like phenotypes, and myocytes with a given EB exhibited similar characteristics. Action potentials of MR60 and foreskin derived CMs (N = 32, 36) were indistinguishable from those of H9 and H1 derived cells (N = 57, 41) in all major features, including maximum diastolic potential, amplitude, dV/dtmax and duration. We conclude that the efficiency of forming CMs varies among IPS and hESCs lines; however, the cardiac gene and protein expression patterns, cell division, and functional properties appear quite similar as does the range of different CMs types formed. Therefore, IPS cells hold great promise as an autologous cell source for cardiac repair and for research applications.

Adiponectin Inhibits Allograft Rejection in Murine Cardiac Transplantation

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Objective: Low levels of adiponectin, an anti-inflammatory and anti-atherogenic adipokine associated with obesity, correlate with higher prevalence of several cardiovascular diseases.

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Obesity constitutes a risk factor for allograft rejection following cardiac transplantation. This study tested the hypothesis that adiponectin attenuates allograft rejection in mice. Wild-type (APN+/+) mice received heterotopic Bm12 allografts. Four weeks later, the transplants in APN+/+ mice showed severe acute rejection rate to those in wild-type hosts (APN+/+). (Parenchymal rejection score: APN+/+ vs. APN−/−: 2.15±0.24 vs. 3.92±0.08, p<0.0001, n=10 and 6, respectively). Allograft rejection in APN−/− mice coincided with increased accumulation of CD4- and CD8-positive T lymphocytes and Mac3-positive macrophages (APN+/+ vs. APN−/−: CD4: 283±13.8 vs. 628±14.6 cells/mm², p<0.05; CD8: 296±114.2 vs. 850±83.2 cells/mm², p<0.01; Mac3: 3.62±1.19 vs. 4.81±0.35 %, p<0.05, n=4 and 6 respectively). The rejected allograft in APN−/− mice expressed significantly higher levels of mRNAs that encode cytokines/chemokines associated with the immune and inflammatory responses including TNFα, IFNγ, and RANTES (301%, 441%, 289% vs. 100% for allograft in APN+/+ vs. APN−/−, respectively). Adiponectin proven by adenovirus in APN−/− mice reversed these exacerbated responses to allografting. Moreover, adiponectin 10 μg/ml significantly suppressed both proliferation by 78.2% assessed by [3H]thymidine incorporation, p<0.001, n=6 and cytokine/chemokine production of T lymphocytes stimulated in vitro with anti-CD3 antibodies (TNFα, IFNγ, RANTES with ELISA), by 96.0, 99.5, 85.2% vs. anti-CD3 stimulation alone, p<0.01, <0.0001, <0.001, n=3, respectively. Conclusion: Adiponectin inhibits cardiac allograft rejection in conjunction with reduced lymphocyte proliferation and recruitment and inhibition of cytokine/chemokine production. The present study provides new mechanistic insight into immunoregulation in allograft recipients relating to an increasingly prevalent risk factor.

**1550 Novel Role of Elastolytic Cathepsin Activity in Accelerated Osteogenesis in Atherosclerosis and Inflamed Aortic Vessels Determined by Molecular Imaging**

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Westernized societies face growing burden of cardiovascular calcification. However, despite its vast clinical significance, the mechanisms of calcification, particularly in chronic kidney failure (CKF), remain obscure. We recently established in vivo that inflammation triggers arterial and valvular calcification. In vitro evidence also indicates that elastin degradation products may promote osteogenesis. We thus hypothesized that cathepsin(s) (catS), a macrophage-derived potent elastolytic proteinase, accelerates calcification in elastoticetic mice with CKF. 10 weeks post-nephrectomy, mice had high serum phosphate, creatinine and cystatin C levels, characteristics of CKF. To visualize catS activity, inflammation and osteogenesis, we administered catS-, macrophage- and calcification-targeted molecular imaging agents via i.v. injection. Imaging colocalized increased catS and osteogenic signals in inflamed aortas and aortic valves of CKF apoE−/−/catS−/− mice, while CKF apoE−/−/catS−/− mice showed insignificant signals (Figure). Quantitative histological assessment showed greater elastin degradation associated with catS, macrophage accumulation and calcification in CKF apoE−/−/catS−/− versus CKF apoE−/−/− mice, confirmed imaging findings. Elastolytic imaging findings suggested increased calcification in smooth muscle cells in vitro, which was further amplified in the phosphate-enriched media. Our study demonstrated the first direct in vivo evidence that catS-induced elastolysis accelerates arterial and valvular calcification in CKF, providing new insight into the pathophysiology of cardiovascular calcification.

SMC-specific inactivation of Pten, a negative regulator of PI3-kinase/Akt signaling, is an early and critical trigger driving vascular lesion formation. To explore the direct role of Pten in mediating SMC function, we generated inducible, SMMHCreER22-mediated SMC-specific Pten mutant mice (Pten iko) which carry the R26R allele allowing fate mapping of SMHC-expressing SMC in response to injury. Induced Pten iko mice exhibited enhanced neointima formation after injury. Induced Pten iko mice exhibited characteristic tubular cell morphology typical of SMC. AcLDL knock-in showed uniform X-Gal staining throughout the media of uninjured vessels, indicating efficient Cre-mediated recombination and ablation of Pten in virtually 100% of medial SMC. After injury, a combination of replicating X-Gal-positive, medial-derived SMC and X-Chromosome material are transferred to the forefront of remodeling and healing media establishing that both SMC and non-medial-derived cells contribute to injury-induced vascular remodeling. Pten iko mice reconstituted with GFP-labeled bone marrow exhibited increased accumulation of bone marrow-derived cells in the developing neointima compared to WT mice. Pten deletion in cultured SMC resulted in autocrine SMC proliferation and upregulation of the cytokines, SDF-1α, MCP-1, IL-6, and CCLX1/1C. Inhibition of NFκB or HIF-1α blocked cytokine upregulation and the induction of an autocrine growth loop mediated by Pten deletion. Our data suggest that a change in SMC Pten expression may be considered as a key player in the complex remodeling of the injured vessel leading to dysfunction and fibrosis therapy and remodeling media establishing that both SMC and non-medial-derived cells contribute to injury-induced vascular remodeling.

**A Novel Sex-Specific Role for Androgens in Angiogenesis**

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Background: The relationship between androgens and cardiovascular disease (CVD) remains poorly understood. Recent evidence suggests low testosterone levels in men are associated with increased mortality from CVD. While androgens participate in maintenance and renewal of body tissues (e.g. bone and muscle), their role in cardiovascular repair/regeneration, is unknown. Methods: Male and female donor endothelial cell (EC) migration, proliferation and tubulogenesis were assessed with [3H]thymidine incorporation (DHT, 4 – 400nM) and [3H]thymidine incorporation (AcLDL, 0.08 – 4 – 400nM) in non-aromatized androgen specific for the androgen receptor (AR) antagonist hydroxyflutamide (HF). In vivo angiogenesis was assessed using Matrigel plug and hindlimb ischemia (HI) assays in castrated and sham castrated male C57Bl/6J mice. DHT, hydroxyflutamide, or vehicle was injected IP at 0, 24, and 48h, with a final dose of 10μg/mouse on day 4. Male and female-donor endothelial cell migration, proliferation and tubulogenesis were assessed with [3H]thymidine incorporation (DHT, 4 – 400nM) and [3H]thymidine incorporation (AcLDL, 0.08 – 4 – 400nM) in non-aromatized androgen specific for the androgen receptor (AR) antagonist hydroxyflutamide (HF). In vivo angiogenesis was assessed using Matrigel plug and hindlimb ischemia (HI) assays in castrated and sham castrated male C57Bl/6J mice. DHT, hydroxyflutamide, or vehicle was injected IP at 0, 24, and 48h, with a final dose of 10μg/mouse on day 4. Male and female-donor endothelial cell migration, proliferation and tubulogenesis were assessed with [3H]thymidine incorporation (DHT, 4 – 400nM) and [3H]thymidine incorporation (AcLDL, 0.08 – 4 – 400nM) in non-aromatized androgen specific for the androgen receptor (AR) antagonist hydroxyflutamide (HF). In vivo angiogenesis was assessed using Matrigel plug and hindlimb ischemia (HI) assays in castrated and sham castrated male C57Bl/6J mice. DHT, hydroxyflutamide, or vehicle was injected IP at 0, 24, and 48h, with a final dose of 10μg/mouse on day 4.

**1553 Smooth Muscle Pten Depletion Promotes Pulmonary Hypertension Through Increased Cytokine Production Which Enhances Smooth Muscle Cell Proliferation and Promotes Inflammatory Cell Recruitment**

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Hypoxia-induced pulmonary hypertension (PH) involves vascular cell proliferation leading to thickened pulmonary arteries and muscularization of small peripheral pulmonary arteries. Data suggest that cell signaling changes in resident vascular cells and more recently circulatory progenitor/immunological cell (lichen, lichen, lichen) pulmonary vascular remodeling. Pten, a negative regulator of P3-kinase/Akt signaling, is an endogenous inhibitor of smooth muscle cell (SMC) proliferation. We previously showed that hypoxia leads to activation of Akt in pulmonary artery SMC and that SMC-specific Pten mutant mice spontaneously develop pulmonary vascular remodeling and lung hypertension (LH) in a hypoxic environment. Hypoxia significantly increased pulmonary artery SMC proliferation and tubulogenesis and bone marrow-derived progenitor/immunological cell accumulation upon exposure to hypoxia compared to WT mice. Fate mapping for tamoxifen-induced LacZ knock-in revealed unique X-gal staining throughout the media of normoxic mice, indicating efficient Cre-mediated recombination and ablation of Pten in medial SMC. X-gal staining verified that increased muscularization of small pulmonary arteries derived in part from SMHC-expressing SMC. Double BrdU-X-gal staining showed a combination of replicating X-gal-positive medial-derived SMC and X-gal-negative cells not from the arterial media populated the remodeling pulmonary vascular space. Induced Pten iko mice establishing that non-medial-derived cells contribute to hypoxia-induced pulmonary vascular remodeling. These data support the concept that physiological regulation of the Pten/Akt pathway in SMC by hypoxia is a key mediator of PAH.
controlling SMC growth and production of systemic factors involved in recruitment and homing of progenitor and inflammatory cells.

Prevention Of Monocrotaline-induced Pulmonary Hypertension By Lenti-viral Mediated Gene Delivery Of Angiostatin (1–7)

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INTRODUCTION: Activated vasoconstrictive, proliferative and fibrillar axis of the renin-angiotensin system (RAS, ACE-AngII/AT1) has been implicated in pulmonary vascular remodeling leading to pulmonary hypertension (PH). The recent discovery of a counter-regulatory axis of the RAS comprising of ACE2-Ang-(1–7)-Mas has led us to examine the role of this vasoactive axis on pulmonary vasculature including PH. We have hypothesized that overexpression of Ang-(1–7) in the lungs would reduce PH, would exert protective effects against monocrotaline induced pulmonary hypertension. METHODS: Lenti-virus producing Ang-(1–7)-fusion protein (3x10^8 TU) was intra-tracheally administered into the lungs of male SD rats (200 –250g). PH was induced by a single subcutaneous injection of monocrotaline (MCT, 50mg/kg), two weeks following gene transfer. After 28 days of MCT administration, rats were instrumented to measure right ventricular systolic pressure (RVSP), followed by heart excision to right ventricular hypertrophy (RVH). A subset of animals received the Mas antagonist (A-779, 2.5 µg via osmotic pumps) to determine if the beneficial effects of Ang-(1–7) were mediated through stimulation of Mas. RESULTS: MCT administration caused a significant increase in RVSP (30.8 ± 2.6 mmHg) in control and the beneficial effects of Ang-(1–7) were more marked through stimulation of Mas. RESULTS: MCT administration caused a significant increase in RVSP (30.8 ± 2.6 mmHg) in control rats, while the beneficial effects of Ang-(1–7) were more marked through stimulation of Mas. RESULTS: MCT administration caused a significant increase in RVSP (30.8 ± 2.6 mmHg) in control rats, while the beneficial effects of Ang-(1–7) were more marked through stimulation of Mas. RESULTS: MCT administration caused a significant increase in RVSP (30.8 ± 2.6 mmHg) in control rats, while the beneficial effects of Ang-(1–7) were more marked through stimulation of Mas. RESULTS: MCT administration caused a significant increase in RVSP (30.8 ± 2.6 mmHg) in control rats, while the beneficial effects of Ang-(1–7) were more marked through stimulation of Mas.

The Potential Angiogenic Factor Periostin Accelerates Degeneration of the Cardiac Valve Complex

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We previously showed that chondromodulin-I (chm-I) is expressed in cardiac valves and maintains valve function by preventing angiogenesis. Periostin has spliced isoforms and promotes cardiovascular valvular fibrosis. Hence, its role in valvular heart disease (VHD) remains unknown. [Methods and Results] (1) RT-PCR, Quantitative PCR, Western blot, and immuno-staining revealed that peristin was specifically expressed at all cardiac valves and annulus from 10.5 dpc embryo to adult in the rodent heart. The shift from long to short periostin mRNA isoform was observed after birth in mouse heart. The long or short isoform was abundant in adult mitral valve or in the aortic valve, respectively. (2) Human normal (n=8, 52.1 years in average) or degenerated (n=22, 63.0 years) cardiac valves with bicusp, atherosclerotic, and rheumatic VHD were immunostained with periostin, chm-I, VWF, VEGF, and collagen I and von Kossa staining was performed. Semi-quantitative analysis showed that periostin at the aortic valve was augmented in severe VHD cases, while chm-I was seen at the core layer of normal valves. The expression of VEGF being markedly exclusive. The short periostin isoform was mainly augmented in VHD by mRNA and protein levels. Interestingly, the periostin-positive area was markedly increased by 4-fold in the thickened degenerative valves, where the capillary density, collagen I expression, and calcium deposition was also increased. (3) Tube formation assay and migration assay using human coronary artery endothelial cells showed that peristin had angiogenic activity in vitro. (4) Phenotype of wild type (WT) or periostin gene targeting (KO) mice fed with 4 months normal or high fat (HF) diet (n=6 each) was analyzed by 30 MHz echocardiography. Aortic valve thickness was increased, and the hyperechoic area in valve annuli was significantly increased in WT > HF. It was reduced in KO > HF. The hyperechoic area in AoV annulus: 0.38 / 0.38 / 0.61% in WT / WT + HF / KO + HF. Immunostaining revealed that angiogenesis and fibrosis in valve annuli was diminished in KO + HF. [Conclusions] Periostin is a potent angiogenic factor and markedly accelerates degeneration of the cardiac valve complex.

Thiol Oxidative Stress Sensitizes Monocytes to Chemotractants: A New Mechanism Contributing to the Recruitment of Macrophages in Atherosclerosis and Diabetic Nephropathy

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Diabetic complications, including accelerated atherosclerosis and diabetic nephropathy, are characterized by the enhanced recruitment and prolonged persistence of monocyte-derived macrophages at sites of tissue injury, presumably triggered by an increased release of chemotactic factors from inflammatory cells. The goal of this study was to test an alternative hypothesis, i.e. that oxidative stress associated with diabetes may potentiate the sensitization of monocytes to chemottractants and increase chemotaxis. We found that chemotaxis of THP-1 monocytes in response to MCP-1 or PDGF-B was enhanced up to 3-fold and 2.5-fold, respectively, in cells pretreated with H2O2. H2O2 treatment markedly increased protein-5-rich glycoprotein-1 expression, a marker for cellular thiol oxidative stress. Importantly, only the H2O2-induced increase in monocyte migration was prevented in cells overexpressing glutaredoxin 1 (Grx1), suggesting that the H2O2-induced hyperresponsiveness to MCP-1 and PDGF-B was mediated through stimulation of Mas.

Marked Cardioprotective Effects of VEGF-B Overexpression in Chronic Dogs with Non-Ischemic Dilated Cardiomyopathy

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INTRODUCTION: VEGF-B is emerging as an important, selective agonist of VEGFR-1, a receptor primarily expressed on cardiac myofibroblasts and vascular smooth muscle cells. Therefore, VEGF-B is an ideal molecular tool to explore potential direct cardioprotective actions of VEGF-B overexpression, to date, remain poorly understood. We tested the effects of VEGF-B overexpression in a dog model of non-ischemic dilated cardiomyopathy. METHODS: VEGF-B genes were delivered by multiple intramuscular injections of AAV-9 vectors (10^12 viral particles) into the LV free wall during surgical instrumentation. The pacing protocol (210 ±240 beats/min) was started 10 days later. Global cardiac function was assessed by implanted grobes and echocardiography, while contractility in the transduced LV regions was measured with piezoelectric crystals. RESULTS: Before pacing, no significant differences were found between VEGF-B–treated (VEGF-B, n=3) and control dogs transduced with GFP (GFP, n=4). After 4 weeks of pacing, GFP were in overt congestive heart failure, while VEGF-B were still in a well-compensated stage. SMC proliferation and most pro-inflammatory cytokines remained unchanged when compared to baseline. In particular, LV end-diastolic pressure in VEGF-B and GFP dogs was, respectively, 12.2 ±2.2 vs 29.5 ±3.5 mmHg, ejection fraction was 53.3 ±3.0% vs 32.2 ±4.4% and LV regional fractional shortening was 12.1 ±1.7% vs 25.5 ±0.3% (all P <0.03). VEGF-B also prevented LV wall thinning. Molecular analyses revealed that VEGF-B induced negligible angiogenesis, which could be compensated by a cardioprotective hypertrophic transcriptional program, including the up-regulation of genes involved in the intracellular calcium transients such as RYR and SERCA2A, and of PGC-1alpha, a powerful regulator of mitochondriogenesis and cardiac metabolic capacity. CONCLUSIONS: These are the first data to document a remarkable cardioprotective action of VEGF-B overexpression in non-ischemic dilated cardiomyopathy. VEGF-B attenuated both systolic and diastolic dysfunction. Our results suggest that the effects mediated by VEGF

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receptors in the heart extend beyond angiogenesis, and point to VEGFR-1 signaling as a potent inducer of a cardiomyocyte pro-survival program, with clear therapeutic implications.

**Soluble Fms-like Tyrosine Kinase 1: Decreased Levels Promote Atherosclerosis in Renal Dysfunction while Exogenous Administration Reduces Atherosclerosis Progression**

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Chronic kidney disease (CKD) is one of the strongest risk factors for atherosclerotic disease, but the underlying molecular mechanisms remain unknown. We focused on soluble Fms-like tyrosine kinase 1 (sFlt-1), an endogenous inhibitor of vascular endothelial growth factor and placental growth factor, and assessed its relationship to the degree of atherosclerosis in patients with renal dysfunction. Three-hundred and twenty-nine patients who had received cardiac catheterization were divided into five CKD-stage subgroups according to estimated glomerular filtration rate. Arterial plasma levels of sFlt-1 decreased significantly with progression of renal dysfunction (ANOVA, p<0.001, n=329). Supporting this finding, sFlt-1 mRNA expression in human renal biopsy specimens was significantly lower in patients with CKD stages 2, 3 or 4 than in those with normal renal function (ANOVA, p<0.01, n=65). Patients with multivessel coronary artery disease had a significantly higher PlGF/sFlt-1 ratio than did patients with single vessel disease or normal coronary arteries (ANOVA, p<0.05, n=326). We confirmed the role of sFlt-1 in an animal model of renal dysfunction using apolipoprotein E-deficient (ApoE-KO) mice with 5/6 nephrectomy. Mice with renal dysfunction had significantly lower arterial plasma levels of sFlt-1 than did controls (11.2±1.5 ng/ml vs. 13.0±1.3 ng/ml, p<0.001, n=21, 22), as well as significantly more severe atherosclerosis (aortic plaque area: 27.3±3.8% vs. 15.2±2.7%, p<0.001, n=12, 10). Repetitive intraperitoneal administration of recombinant sFlt-1 to mice with renal dysfunction (15 ng/g body weight three times per week, from 12 weeks old to 22 weeks old) significantly reduced aortic plaque area (27.3±3.8% to 21.6±4.8%, p<0.01, n=12). In conclusion, renal impairment is accompanied by decreased production of sFlt-1 in both humans and mice, and administration of exogenous sFlt-1 reduces the progression of atherosclerosis in ApoE-KO mice with renal dysfunction. Soluble Flt-1 could be one of the key molecules that mediate the progression of atherosclerotic disease in renal dysfunction.