Late-Breaking
Basic Science
Abstracts

from the
American Heart Association
Scientific Sessions 2005

Dallas, Texas
November 13 – 16, 2005

Abstract Topics Include:

- Stem Cell and Cardiac Cell Replacement
- Arrhythmia Mechanisms
- Cellular Signaling
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November 13–16, 2005
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Abstracts

Late-Breaking Basic Science: Stem Cells and Cardiac Cell Replacement

5000

Adult Mouse Spermatogonial Stem Cells Differentiate into Cardiovascular Lineages and Generate Functional Cardiomyocytes
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Adult stem cells with the pluripotency of embryonic stem cells (ESCs) would be ideal for organ regeneration strategies. Previous studies in neonatal mice suggest that the germine lineage may retain the ability to generate pluripotent cells. We aimed to evaluate the pluripotency and plasticity of adult spermatogonial stem cells (SSCs), which are responsible for maintaining spermatogenesis throughout life in the male. Using a genetic approach we established the culture condition for isolation of SSCs from adult mouse testis (success rate ~27% of mice). These isolated SSCs respond to the culture conditions and acquire ESC properties. They are able to spontaneously (without co-culture) differentiate into functional cardiomyocytes expressing cardiac-specific genes (α1T, GATA4, Nkx2.5, MEF2C, α-MHC, MLC2v, ANF, SERCA2a and NCX) and proteins (sarcomeric MHC, cardiac troponin T and connexin 43). Four major types of action potentials (APs) characteristic for specialized cells of the ventricle, sinus node, Purkinje fibers and atrium are found in these cardiomyocytes. Functional maturity of ventricle-like cells (n ~11 from 3 independent experiments) is shown by AP characteristics: upstroke velocity (dV/dt max) being 55.8±4.8 V/s, amplitude (APA) being 115.8±3.3 mV, duration at 90% of repolarisation (APD90) being 286.9±55.5 ms and maximum diastolic potential (MDP) being -48.9±3.0 mV. The amplitudes of calcium transients (flux4) measured in SSC-derived ventricle-like cardiomyocytes (n = 10) are similar to those measured in ESC-derived cardiomyocytes (n = 6; 464.77 nm vs. 287.69 nm, respectively). This is also comparable to adult cardiac myocytes. Besides cardiomyocytes, SSCs give rise to vascular smooth muscle (expressing smooth muscle-α-actin, vascular smooth muscle MHC) and endothelial cells (expressing PECAM-1, VEGFR2, and vWF). SSCs also differentiate into other somatic cell types in vitro. Furthermore, we show that SSCs are able to differentiate into cardiac cells and most other somatic cells in vivo after blastocyst microinjection. Thus, adult spermatogonial stem cells exhibit pluripotency like embryonic stem cells and may offer new possibilities in cell based cardiac regeneration strategies.

Introduction: Before replacing damaged myocardium, engineered cardiac tissues have potential as model systems for high-throughput evaluation of therapeutic interventions. However, none have reproduced classical indices of physiologic myocardial pump function. We hypothesize that engineered cardiac tissue chambers (ETCh) can exhibit a functional Frank-Starling mechanism and generate positive stroke work. Methods: Living ETCh (n = 3) were created from neonatal rat cardiac myocytes and fibroblasts suspended in a 3-D collagen and Matrigel scaffold, and cultured in a custom concentric mold with an inflatable central core. After 7–10 days of culture, the spontaneously beating ETCh was removed from the mold and attached to a high-sensitivity Langendorff setup at 37 C. Chamber pressure and area were acquired at 200 Hz and 50 fps, respectively. Results: ETChs (9 mm diameter, 0.3 mm thick) spontaneously contracted at 4–5 Hz. Fluorescence microscopy revealed a homogeneous cell distribution with randomly oriented myocytes having registered and striated sarcomeres. Developed pressure amplitude increased linearly with mean chamber pressure or preload (Fig). Cyclic changes in pressure and area resulted in a counter-clockwise pressure-area loop with a mean stroke work of 0.013±0.004 Pa (Fig). Conclusions: Thus, ETChs exhibited a functional Frank-Starling mechanism and generated positive stroke work in an isolated pressure-volume measurement system.

Late-Breaking Basic Science: Cardiac Repair Strategies

5002

Engineered Cardiac Tissue Chambers Demonstrate Functional Frank-Starling Mechanism and Positive Stroke Work
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Background: Cellular cardiomyoplasty using skeletal myoblasts or angiopoietic progenitor cells offers a promising approach for the treatment of ischemic heart failure. Although several studies have shown encouraging results in small settings of acute myocardial infarction, the efficacy of cell therapy on the chronic ischemic heart remains undetermined. Methods: A model of chronic ischemia was created using LAD-ligation in nude rats. Culture medium (C), homologous skeletal myoblasts (BM), human AC-133+ cells (SC) and both homologous skeletal myoblasts and human AC-133+ cells (SM-SC) were injected in the infarct (SM) and peri-infarct area (SC) four weeks after infarction. Assessment of myocardial function was performed using echocardiography 8 weeks after injections. Infarct size, collagen deposits and cardiomyocyte apoptosis were quantified to evaluate the effect cell injections using histology. Results: Echocardiographic studies revealed an amelioration of left ventricular dilation in animals receiving combined cell transplantation (VEDM: SM, SC, SM-SC: C: 7.5±1.5, 7.1±1.6, 5.7±0.8 and 7.7±0.9, p=0.003). Left ventricular ejection fraction improved significantly in all three cell therapy groups but no additional benefit was observed in the SM-SC group (SM, SC, SM-SC: C: 63.5±13.8, 63.3±7.8, 68.2±5.6 vs. control: 48.6±8.9, p=0.0017). Quantification of scar tissue showed a significant reduction of infarct size.
area in the SM-SC group (SM, SC, SM-SC, C: 22.3 ± 9.1%, 19.8 ± 7.6%, 13.2 ± 5.8%, 36.5 ± 8.2%, p < 0.008). Improvement of myocardial function was associated with reduced apoptotic index in animals after cellular cardiomyoplasty (SM, SC, SM-SC, C: 3.2 ± 0.9, 3.1 ± 0.6, 1.8 ± 0.8, 10.3 ± 1.6, p = 0.0002). Conclusions. Combined transplantation of skeletal myoblasts and AC-133 angiopoietic progenitor cells leads to improvement of diastolic and systolic left ventricular function, and reduction of scar size and myocardial apoptosis in a model of chronic ischemia.

**5004 Transplanted Human Cord Blood Derived Unrestricted Somatic Stem Cells (ussc) Improve Left-ventricular Function And Prevent Left-ventricular Dilation And Scar Formation After Acute Myocardial Infarction**

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Objectives. Intra-myocardial transplantation of adult stem cells as a therapy of heart failure is increasingly being discussed and performed in animal and human studies. Human unrestricted somatic stem cells (usssC) are newly discovered cord blood-derived cells with pluripotent differentiation potential. We aimed to assess whether intra-myocardial transfer of USSC would improve global left-ventricular function in a porcine model of myocardial infarction (MI). Methods. USSC were isolated from cord blood (CB) of the umbilical cord vein. 13 to 10 6 cells were transplanted into the acutely ischemic lateral wall of the left ventricle (n = 5). Control animals (n = 5) received medium injection. LV dimension and function were assessed by transesophageal echocardiography (TEE) immediately before MI, after MI followed by cell transplantation and after 6 weeks. The hearts were examined for cell survival, cardiac differentiation and scar formation. Results. Global LV ejection after MI was 32 ± 8% in controls and 36 ± 9% in the cell treated group. After 2 months, mean global LV ejection had decreased to 27 ± 5% in the control group and increased to 52 ± 2% in the cell treated group (p < 0.01). Left ventricular end-diastolic volume (LVEDV) after two months was 77 ± 4 ml in the control group compared to 26 ± 2 ml in the cord blood group (p < 0.01). Myocardial scar was present only in the control group. Conclusion. Transplantation of USCC after myocardial infarction significantly improves LV function, reduces infarct size and prevents LV dilation.

**5005 Reduction Of Fibulin-4 Expression Affects Elastogenesis And Results In Aortic Aneurysm And Dissection**

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The Fibulins are a six-member protein family, prominently expressed in blood vessels and hypothesized to function as intermolecular bridges that stabilize the organization of extracellular matrix structures such as elastic fibers and basement membranes. To examine a potential role of Fibulin-4/EEMF2 in elastic fiber assembly and cardiac vascular disease we generated a mouse model underexpressing Fibulin-4. We decreased the mouse Fibulin-4/ EEMF2 expression through transcriptional interference by targeted integration of the neomycin expression through transcriptional interference by targeted integration of the neomycin selective marker. A reduction in Fibulin-4 RNA expression levels was confirmed by micro-array expression analysis and real-time quantitative PCR. Fibulin-4 heterozygous (Fibulin+/-) and homozygous (Fibulin0/0) mice are viable, born at Mendelian frequencies and appeared indistinguishable from wild-type littermates during the first two weeks. Fibulin+/0 mice (with a 2-fold reduction in Fibulin-4 RNA expression levels) showed abnormalities in the elastic fiber network of the aorta after Elastin staining of cross sections of the ascending aorta but no increased mortality or abnormal appearance during the first year was observed in these mice. In contrast, more than 80% of the Fibulin0/0 mice (with a 4-fold reduction in Fibulin-4 RNA expression levels) died suddenly after 2 weeks. Pathological analysis showed that these mice died from aortic dissection resulting from severe abnormalities in the elastic fiber network of the aorta. We pathologically analyzed heart and aorta of newborn, 2-week old and 10–20 week old surviving Fibulin0/0 mice and determined hemodynamic parameters. We found multiple heart and aortic defects, including a tortuous aorta and a 2-fold dilatation of the left ventricle and ascending aorta. Furthermore, the 10–20 week old surviving Fibulin0/0 mice had a thickened and stiffened aorta. Heart rate and mean aortic pressure were not different between Fibulin-4 wild-type, Fibulin-4+/- and Fibulin-40/0 mice. However, in three surviving Fibulin-40/0 mice aortic pulse pressure was increased more than 2-fold consistent with a stiffening of the thickened and widened aorta compared to wild-type and Fibulin-4+/- mice. Also, Fibulin-40/0 mice had thickened aortic valvular leaflets that were associated with an increased systolic pressure-gradient between left ventricular pressure (154 ± 4 mmHg) and aortic pressure (117 ± 7 mmHg) and transvalvular blood flow velocity suggestive for the presence of a mild aortic valve stenosis. Altogether Fibulin-4 heterozygous and homozygous mice provide a unique model to follow the pathogenetic sequence for aneurysm. In addition, we implemented a functional genomics approach and determined the aorta transcriptome of wild-type, Fibulin-4+/- and Fibulin-40/0 animals using full mouse genome Affymetrix arrays. This approach enables us to identify those biological processes that were significantly over represented including apoptosis, blood pressure and coagulation and extracellular matrix organization as well as to identify several gene targets implicated in the response to aortic failure.

**5006 Elevated Plasma Transforming Growth Factor-Beta 1 (TGF-1) Reduces Aortic Atherosclerosis, Aortic Root Dilation and Pseudoeurymena Formation in Apolipoprotein E-null (ApoE-) Mice**

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INTRODUCTION The “protective cytokine” hypothesis predicts that elevated plasma TGF-1 would limit atherosclerosis. But, in humans high plasma TGF-1 has been associated with both protection against and predisposition to severe atherosclerosis. An animal model of regulable TGF-1 expression would permit a direct test of the hypothesis. METHODS We generated ApoE-/- mice that express active TGF-1 in a “tet-off” system (Mx-CRTA tk-TetO-TGF-1). Dietary doxycycline (dox) suppresses transepression expression. Removal of dox from the diet at 6 weeks of age activates cardiac-specific expression of the TGF-1 transgene and stabilizes an elevated plasma TGF-1 within two weeks. We compared atherosclerosis in doubly transgenic mice off dox (DT-Off) to doubly transgenic mice on dox (DT-On) and singly transgenic mice off dox (ST-Off). All mice were littermates, ApoE-/-, and fat-fed from age 6 –18 weeks. TGF-1 transgene expression was evaluated by Northern analysis and by ELISA of plasma (n = 7–14). Plasma size and histology were analyzed in aortic root sections (n = 14–17). Plasma lipid measurements and PLEC were performed (n = 8–13). RESULTS Only the hearts of DT-Off mice expressed TGF-1 transgene mRNA. Hearts of DT-Off mice secreted more total TGF-1 to yield a 10-fold elevation of plasma total TGF-1. DT-Off mice had significant reductions in aortic root plaque area (±SEM) and lipid content (±SEM), aortic root circumference (±SEM), and fewer pseudoeurymena (±SEM). CONCLUSIONS Elevated plasma TGF-1 retards atherosclerosis, limits aortic root dilation, and inhibits pseudoeurymena formation. Elucidation of the mechanisms of these effects may suggest novel therapies for atherosclerosis and aneurysms.

**5007 Scavenger Receptor Bi Prevents Nitric Oxide-mediated Cytotoxicity And Endothoxin-induced Animal Death**

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Nitric oxidative stress contributes to a variety of diseases, such as atherosclerosis, Alzheimer’s disease and diabetes. Although numerous mechanisms have been described controlling the production of nitric oxide (NO), for example, endothelial nitric oxide synthase (eNOS) activity is regulated by membrane targeting and by caveolin-1 binding, the mechanisms to prevent NO cytotoxicity post NO synthesis are largely unknown. Several laboratories including ours recently demonstrated that scavenger receptor BI (SR-BI) stimulates eNOS to produce 5-fold more NO in caveolae in the presence of HDL. The dramatic increase in NO levels, especially within caveolae, may be a problem because NO can cause cell damage at high level. However, the fact is that NO does not cause endothelial cell damage at physiological conditions, implying that a detoxicification mechanism exists in endothelial cells. In this study we assessed the hypothesis that SR-BI prevents NO-induced cytotoxicity. Using genetically manipulated mice, we demonstrate that SR-BI null mice have a 3–4-fold increase in tyrosine nitrosylated proteins in aorta and in the liver compared to wild type littermates, indicating that expression of SR-BI prevents peroxynitrite formation in vivo. Using Lipopolysaccharide (LPS)-challenged mice as an in vivo model of nitric-oxide-induced cytotoxicity, we found that a single low-dose of LPS (120,000 units/kg, ip) induced 90% fatality of SR-BI null mice within 3 days while all of the wild type littermates survived (20 mice in each group), demonstrating that SR-BI is highly protective against NO-cytotoxicity in vivo. Using CH0 cell lines expressing wild type and single site mutant SR-BI protein, we further demonstrate that SR-BI functions in caveolae and that the active site responsible for SR-BI’s anti-N0 cytotoxicity is a highly conserved CXXS redox motif. In conclusion, we demonstrate a novel regulatory mechanism in caveolae to prevent NO-induced oxidative stress, which most likely will affect human cardiovascular diseases.

**5008 ApoE-Null Mice Lacking Thrombospondin-1 or Its Receptor CD47 Are Protected Aga inst Neointimal Thickening of Injured Arteries But Not Against Diet-induced Atherosclerosis**

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Thrombospondin (TSP1) is widely expressed by circulating and mural vascular cells and a vast amount of in vitro data suggests that TSP1 can influence vascular patho-physiology. CD47
Urokinase Accelerates Atherosclerosis Through a Plasminogen-Dependent Pathway

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BACKGROUND: Urokinase-type plasminogen activator (uPA) is expressed in human atherosclerotic lesions, primarily by macrophages. To test whether macrophage-expressed uPA accelerates atherosclerosis (athero), we generated Apo E null (ApoE-/-) mice with a macrophage-targeted uPA transgene (SR-uPA mice). SR-uPA mice had accelerated atherosclerosis but no changes in plaque size or composition. uPA has both catalytic activity (e.g., activation of plasminogen) and noncatalytic activity (e.g., cell surface receptor binding). Genetic ablation of uPA catalytic activity is impaired atherosclerosis (PAI-1-/-) mice (69.7% vs 5.4% p=0.001). Hence, uPA is a player in human atherosclerotic disease.

RESULTS: SR-uPA plg-/- mice had elevated uPA expression compared to the wild type, whereas SR-uPA Lipc-/- mice had reduced uPA expression compared to the wild type. In both of these mice, the lack of uPA did not affect the ratio of liver to total body weight or the ratio of liver to total body weight. In addition, the lack of uPA did not affect the ratio of liver to total body weight. In both of these mice, the lack of uPA did not affect the ratio of liver to total body weight. In both of these mice, the lack of uPA did not affect the ratio of liver to total body weight. In both of these mice, the lack of uPA did not affect the ratio of liver to total body weight.

CONCLUSIONS: Increased expression of uPA in the liver accelerates atherosclerosis, which may be a valuable therapeutic intervention strategy for the treatment of hyperlipidemias.
 upon solidification in tissue becomes a hydrogel. The aim of the present study was to test the hypothesis that an injection of this novel alginate solution mimicking the extracellular matrix (ECM) into an old scar tissue, late after myocardial infarction (MI), will provide physical and biological scaffolding, promote tissue rejuvenation and will prevent progressive LV dysfunction.

**Methods and results:** Rats (n = 40) were subjected to extensive anterior myocardial infarction (MI). Two months later, the survivors were randomized to injection of cross-linked alginate solution (n = 15) or PBS (control, n = 12) into myocardial scar. Four months after MI and two months after injection, postmortem morphometric analysis and histological examination of the hearts revealed that alginate solution injection promoted increased neovascularization and increased vessel density (106 ± 2 vs. 43 ± 9 mm²; p < 0.0001), increased scar thickness (0.73 ± 0.27 cm vs. 1.63 ± 0.31 cm; p < 0.01) and significantly reduced expansion index (0.56 ± 0.08 vs. 1.05 ± 0.26; p < 0.0001), as compared with controls. Serial echocardiography studies showed that alginate solution injection attenuated the progressive deterioration in LV fractional shortening, as compared with control (12.6 ± %FS vs. 37.9%; p < 0.05). Furthermore, while control animals developed restrictive LV filling pattern, as assessed by Doppler echocardiogram, diastolic function improved in alginate-treated hearts (E/A wave ratio of 1.73 ± 0.09 for control vs. 3.34 ± 0.5 for control; p < 0.0001). Conclusions: Our work shows, for the first time, that injection of in situ gelable cross-linked alginate into an old scar tissue provides both physical and biological scaffolding, and preserves LV systolic and diastolic function. Our work enables a minimally invasive, catheter-based, acellular option to repair old scar tissue and to prevent heart failure.

**PKC and p38MAPK Mediate the Paracrine Cardioprotective Effect of Bone Marrow Cells in Man**

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Background: Bone marrow cells (BMC) protect the human heart against ischemic injury but the mechanism of this effect is unknown. Here we have investigated whether some of the beneficial effect of BMC against ischemic injury is mediated by PKC and p38MAPK, essential elements of the signal transduction of preconditioning. Methods and Results: Myocardium obtained from the right atrial appendage (N = 6/group) during cardiac surgery was subjected to 90min of normothermic ischemia followed by 120min of reperfusion. The bone marrow was aspirated from the iliac crest of the same patients, the mononuclear fraction was separated by density gradient and then 10⁶ cells/mg tissue were co-incubated with the muscles during the entire experimental period. Muscles incubated for the same time period under aerobic conditions served as control. Some groups were treated with the PKC inhibitor chelerythrine (CH, 10μM) or the p38MAPK inhibitor SB203580 (SB, 10μM). The creatine kinase released into the media during the reperfusion period (after subtraction of aerobic control values) was significantly reduced by BMC from 1.3 ± 0.11 to 0.33 ± 0.06 IU/mg wet tissue (p < 0.05), an effect that was abolished by CH (0.96 ± 0.19; p < 0.05) and SB (0.93 ± 0.12; p < 0.05). Cell death (Figures 1a & 2a) by necrosis (assessed by propidium iodide) and by apoptosis (assessed by TUNEL) caused by ischemia/reperfusion was prevented by BMC, an effect that was partially or totally reversed by CH and SB (p < 0.05). Conclusion: BMC have a cardioprotective effect against ischemia/reperfusion injury which is mediated, at least in part, by the kinases PKC and p38MAPK, a mechanism shared by preconditioning.

**Myostatin regulates Cardiac Hypertrophy through a Novel p38-Akt Mechanism**

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Myostatin(MSTN) is a negative regulator of skeletal muscle growth, however the role of MSTN in the heart is not known. We have previously shown that transgenic mice expressing activated Akt(myr-Akt) in the heart exhibit hypertrophy and upregulation of MSTN. To determine the role of MSTN in cardiomyocyte(CM) hypertrophy we infected mice with adenoviral vectors carrying control(Ad.GFP), MSTN(Ad.MSTN), dominant negative MSTN(Ad.dMSTN), dominant negative Akt(Ad.dAkt), constructs, or myr-Akt and cultured them in the +/- 100μM PE for 24 hours. Ad.MSTN blocked PE-mediated increases in CM size(p < 0.001) and protein synthesis(> 0.001), while ad.dMSTN was sufficient to increase CM size(p < 0.001) and protein synthesis(p < 0.01). Furthermore ad.dMSTN was sufficient to activate Akt(p < 0.05), and PE-stimulated Akt phosphorylation was inhibited by ad.dMSTN(p < 0.01). Ad.dAkt attenuated PE-stimulated increases in protein synthesis(+4.9 ± 0.001) and CM size(+ 2 ± p < 0.001). Ad.dMSTN also attenuated PE-mediated increases in CM size(< 0.001), and ad.MSTN inhibition of PE-mediated CM size increase was rescued by ad.myrAkt(28% p < 0.001) demonstrating the functional importance of Akt. PE-stimulated phosphorylation of p38 was inhibited by ad.MSTN injection(n = 5). Inhibition of p38 with 10μM SB239063 blocked Akt phosphorylation by PE(n = 4), suggesting that MSTN may inhibit hypertrophy via direct inhibition of p38 which is necessary for PE-induced Akt activation. Interestingly increased protein synthesis stimulated by LIF(FN) and IGF-I(X100nm), two hypertrophic agonists known to activate Akt, was not inhibited by ad.MSTN(n = 8, 11). Additionally ad.MSTN did not inhibit LIF or GGF-I-induced Akt phosphorylation(n = 4). Moreover mice subjected to 14 days of chronic PE injection showed increased heart weight and cardiomyocyte cell size compared to vehicle-treatment. Acute in vivo stimulation with PE led to greater Akt activation in MSTN-/ - hearts versus MSTN+/ + hearts, and increased p38 phosphorylation. Thus, MSTN negatively regulates PE-mediated CM hypertrophy both in vitro and in vivo. MSTN inhibition of p38 and Akt appear to contribute to this effect, and explain the observations seen with different hypertrophic stimuli.

**Myobrilligenesis**

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**Background:** The Z-line is a highly specialized structure of striated muscle that serves as the anchoring site for contractile filaments, and acts as the interface between the sarcomere, the cytoskeleton and the nucleus providing the elements necessary to transmit and coordinate the contraction-relaxation cycle between individual myofibrils. Increasing number of reports recently had identified genes mutations associated with autosomal dominant CMD in the sarcomeric Z-line proteins complex. Objective: To investigate whether the Q9R-α-actinin-2 (Q9R-Actn2) mutation lead to CMD and study the CMD-associated mechanism. Methods: A transgenic mouse model with cardiac-restricted expression of Q9R-Actn2 driven by α-Mycosis heavy chain promoter was generated and characterized by echocardiography, histopathology, immunohistochemistry and western blot analysis. Myoblast cell lines including C2C12 (skeletal myoblasts) and HL-1 (trialar myoblast) were used to explore the role Q9R-Actn2 in myofibrillogenesis, transcription factors expression and nuclear trafficking of Z-lines proteins. Results: Echocardiographic analysis demonstrated systolic dysfunction in Q9R-Actn2 mutants with significant reduction in left ventricular fractional shortening (%FS = 18.71 ± 2.21) compared to the transgenic mouse expressing the WT-Actn2 (%FS = 30.2 ± 2.8) and the non-transgenic control mice (%FS = 32.67 ± 1.63). Congenital septal and left ventricular wall hypertrophy in Q9R-Actn2 mutant mice that progresses to dilated cardiomyopathy over time. Western blot analysis reveals decreased in Myf-2 and myogenin expression and increased levels of α-actinin-2 binding protein Zyxin in mutant mice. Studies in C2C12 and HL-1 cell lines show that Q9R-Actn2 expression block myofibrillogenesis and expression of transcription factors including MyoD, Myf-2 and myogenin. Myobrilligenesis and transcription factors expression are rescue by Zyxin expression. Conclusion: The Q9R mutation in the Z-line protein α-actinin-2 plays a causative role in the development of CMD by a mechanism associated with nuclear transcription factor expression that regulate myobrilligenesis and heart remodeling.

**The Ras effector Rlf protects from maladaptive hypertrophic signaling**

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Studies in both isolated cardiomyocytes and animals demonstrate an important role for the monomeric G protein Ras in the hypertrophic responses. Ras interacts with multiple intracellular effectors to coordinate the activity of the mitogen activated protein kinases (MAPKs) and phosphoinositide-3-kinase (PI3K). Ras activation of ERK or PI3K is associated with an adaptive hypertrophic response while JNK activation is associated with maladaptive hypertrophy. We identified the Raf guanine nucleotide dissociation inhibitor stimulator like factor (Rlf) as a protein that interacts with Ras in cardiomyocytes. To understand the role of Rlf in regulating cardiac myocyte signaling, we assessed MAPK activation in neonatal rat ventricular myocytes (NRVMs) infected with Rlf or control adenovirus and treated with the hypertrophic agonists endothelin-1 or phenylephrine. Rlf overexpression in NRVMs decreased agonist induced activation of JNK without significantly altering ERK activation. Consistent with the effect of Rlf on agonist-mediated signaling, co-expression of V12-Ras and Rlf attenuated V12-Ras-mediated JNK activation without significantly affecting ERK or PI3K activation. To determine the effect of Rlf on cardiac hypertrophy in vivo, we generated transgenic mice that overexpress Rlf in a cardiac-specific manner. Rlf transgenic mice and nontransgenic littermate controls displayed similar heart weight and myocardial morphology. To examine the role of Rlf in the hypertrophic response, 14-day mini-osmotic pumps with isoproterenol (ISO; 30mg/kg/day) were implanted in 2-month old male transgenic and nontransgenic mice. ISO treatment increased heart weight to body weight and iodial length ratios in transgenic and nontransgenic littermates (p < 0.05). The hearts of Rlf transgenesis showed severe SR deficiency, decreased fibrosis and myocardial cell loss compared to nontransgenic mice. Furthermore, Rlf transgenic mice were protected from ISO-mediated decreased SERCA2a expression, a marker of maladaptive hypertrophy. Overall, our results demonstrate that Rlf attenuates Ras-mediated activation of the JNK pathway and protects from maladaptive cardiac hypertrophy.
fluorescence and confocal microscopy to examine the T-tubular localization of DAP components in the myocardium of F18 (control) and T02 hamsters at 8–15 wks of age. These components included: β-dystroglycan (dβG), dystrophin, δ-, or αG and caveolin-3. Sarcollemmal dβG was observed at high levels in F18 and T02 groups. An increase in T-tubular hypertrophy and JβDg T-tubular localization was observed in the myocardium of 12–15 wk hamsters. This translocation of JβDg co-localized with the T-tubular markers, DHPR α1 and the mediator of T-tubulogenesis, Bin1. The T-tubular localization of these markers was not altered in the F18 or T02 groups. These events corresponded to the development of chamber dilatation, wall thinning, calcifications and fibro-necrosis in the 12–15 wk T02 groups. T-tubular hypertrophy and increased DAP localization was observed in the 8–10 wk T02 or 8–15 wk F18 groups. The observed loss of JβG was accompanied by a loss of αGδ and dystrophin from myocardial t-tubules in the T02 groups. Myocardial T-tubular localization of caveolin-3 was not altered in the F18 or T02 groups. The interaction of JβDg and Bin1 in the T02 group was visualized by co-immunoprecipitation studies, which indicated an association of JβG with Bin1, presumably via the SH3 domain of Bin1 and the proline rich, C-terminal region of JβDg. Discussion: This study suggests that the T-tubular interaction of JβDg with Bin1 in the T02 group initiates T-tubular hypertrophy, potentially by reversing an attenuation of T-tubular growth in mature adult hearts. T-tubular hypertrophy might contribute to increased diffusion of ion fluxes, disrupting physiologic calcium handling and initiating the development of dilated cardiomyopathy.
activity of the NHE-1, a causal link between the decrease in the NHE-1 expression and the protective effect of PDE5A inhibition may be suggested.

### TABLE. LVDD and LVSD: LEFT VENTRICLE DIASTOLIC AND SYSTOLIC DIAMETER RESPECTIVELY.

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<th>LVDD (mm)</th>
<th>LVSD (mm)</th>
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<tbody>
<tr>
<td>MI</td>
<td>45 ± 5</td>
<td>19 ± 4</td>
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<tr>
<td>MI+</td>
<td>46 ± 6</td>
<td>18 ± 3</td>
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Table includes n=5 per group, 2-way ANOVA with Bonferroni post-test. *P < 0.05 vs MI.

### Late-Breaking Basic Science: Arrhythmia Mechanisms and Cellular Signaling

#### 5025

**Mutant Caveolin-3 Alters Cardiac Sodium Channel Function and is Associated with Congenital Long QT Syndrome**

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**Background:** Mutations in the alpha subunits of potassium channels and sodium channels account for approximately 70% of congenital long QT syndrome (LQTS). LQT3–3 while only approximately 1% of LQTS are due to mutations incausing potassium (p) similar to voltage channel subunits (LQT4–6). Recent evidence shows that SCN5A colocalizes with caveolae and that caveolin is directly involved in channel trafficking. Caveolae are specific omega-shaped microdomains of the sarcolemma that are organized by the structural protein caveolin, and are critically involved in cell signaling. We hypothesize that caveolin-3 (CAV3), the major component of caveolae in striated muscle, may be implicated in modulating the cardiac SCN4A-encoded sodium channel activity. We therefore speculated that CAV3 may be a candidate gene for LQTS.

**Methods:** Using denaturing high performance liquid chromatography and direct DNA sequencing. Open reading frame/splice site mutational analysis was performed on CAV3 in 69 unrelated patients referred for LQTS genetic testing. Caveolin-3 mutations were engineered by site-directed mutagenesis and functionally phenotyped by transient expression into stable expressing CAV3-3 HEK293 cells. This is consistent with sustained sodium current observed in prolonged QT intervals in patients with SCN5A alterations.

**Results:** Six non-synonymous single nucleotide polymorphisms were identified in 17 patients (2%), including the common black-specific polymorphisms, G56S and C72W previously linked to limb-girdle muscular dystrophy (LGMD), having heterozygote frequencies of 25% and 2% respectively in this study. The other 4 variants were absent in more than 200 controls, were conserved across species, and were present in 5 subjects (0.6%) lacking mutations in all known LQTS-associated genes. Co-expression of caveolin-3 mutations with SCN5A resulted in a 4-6 fold increase in latex sodium current compared to expression of wild-type caveolin-3 in HEK293 cells. This is consistent with sustained sodium current observed in prolonged QT intervals in patients with SCN5A alterations.

**Conclusions:** We produce the first molecular and functional evidence implicating caveolin-3 in the pathogenesis of LQTS. LQTS-associated caveolin-3 mutations perturb sodium channel function and elicit an LQTS phenocopy by producing a gain-of-function increase in latex sodium current.

#### 5026

**Cardiac-Specific Loss of N-cadherin Leads to Alteration in Connexins with Conduction Slowing and Arrhythmogenesis**

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The remodeling of ventricular gap junctions, as defined by changes in size, distribution, or function, is a prominent feature of diseased myocardium. However, the regulation of assembly and maintenance of gap junctions remain poorly understood. The cell adhesion molecule, N-cadherin, mediates strong cell–cell adhesion via linkage to the actin cytoskeleton. N-cadherin has been implicated in the assembly of gap junctions in cultured cardiomyocytes. To determine whether N-cadherin function is required for maintaining gap junctions in the working myocardium, we utilized a floxed N-cadherin gene in conjunction with a cardiac-specific tamoxifen-inducible Cre transgene. The mutant animals appeared healthy and active until their sudden death about two months after deleting N-cadherin from the heart. Ambulatory ECG monitoring captured the abrupt onset of spontaneous ventricular tachycardia confirming that the deaths were arrhythmic in nature. Electrophysiologic analysis revealed abnormal conduction in the ventricles of mutant animals including diminished QRS complex amplitude consistent with loss of electrical coupling in the myocardium. Hysterograph analysis of CX43 preceded a significant reduction in gap junction proteins, and CX43 and CX40, in N-cadherin depleted cardiomyocytes. Real-time PCR demonstrated an increase in CX43 mRNA levels indicating that N-cadherin regulates CX43 expression posttranscriptionally. Altered connexin function resulted in diminished ventricular conduction velocity as determined by optical mapping. Our data suggest that perturbation of the N-cadherin/catenin complex in heart disease may be an underlying cause leading to the establishment of the arrhythmogenic substrate by destabilizing gap junctions at the cell surface.

#### 5027

**Atrial NAD(P)H Oxidase Activity Predicts the Development of Atrial Fibrillation After On-pump Coronary Artery Bypass Graft Surgery**

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**Background:** Atrial fibrillation (AF) is a frequent complication following coronary artery bypass graft (CABG) surgery and recent evidence associates enhanced human atrial NAD(P)H oxidase activity...
activity with chronic AF. To determine whether enhanced intrinsic atrial oxidative stress was also involved in the pathophysiology of post-operative AF, we investigated whether atrial NAD(P)H oxidase activity could predict the occurrence of AF following CABG surgery. **Methods and Results:** We prospectively measured atrial NAD(P)H oxidase activity in the right atrial appendage (RAA) obtained before cardiopulmonary bypass (CPB) of 172 patients undergoing first-time on-pump CABG surgery using SAMS lucigenin-enhanced chemiluminescence. In a sub-group of patients we compared both basal and NADPH-stimulated superoxide (O2⁻) release in samples of RAA obtained before CPB and 1–3 minutes after repuffusion which revealed no differences, gained data from 23 patients in RLU/sec/mg protein: pre-v. post-CPB basal 97 ± 13 v. 105 ± 22, P = 0.7 and pre-v. post-CPB NADPH stimulated 7224 ± 690 v. 7693 ± 684; P = 0.6). Thiobarbituric acid reactive substances and carbonyls were measured in pre- and post-operative blood samples to assess both lipid and protein oxidation in the plasma. Patients were genotyped for the C242T polymorphism of the p22phox subunit of NAD(P)H oxidase using standard RFLP techniques. 74 patients (43%) developed AF post-operatively and NADPH-stimulated atrial O2⁻ release was significantly higher in this group compared with patients remaining in sinus rhythm (SR), (in RLU/sec/mg protein: 4374 ± 162 v. 3909 ± 144 (n=98 in SR), P = 0.03). Multivariate logistic regression indicated that NADPH-dependent atrial O2⁻ generation was the only significant predictor of post-operative AF (OR, 2.64; 95% CI, 1.02–7.58; P = 0.03). Multivariate logistic regression indicated that NADPH-dependent atrial O2⁻ generation was the only significant predictor of post-operative AF (OR, 2.64; 95% CI, 1.02–7.58; P = 0.03). These findings suggest that atrial NAD(P)H oxidase activity, but not plasma markers of oxidative stress, is a main independent predictor of post-operative AF in patients undergoing CABG surgery.

**5028**

**Impaired Angiogenesis In β2 Adrenergic Receptor (ar) Knock Out Mice After Chronic Ischemia Is Rescued By Intrapericardial β2ar Gene Transfer**

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We have recently shown in rats that beta 2 adrenergic receptor gene delivery by adenovirus (ADβ2AR) to the endothelium ameliorates the angiogenic response after hindlimb (HL) ischemia. To point out the role of endogenous β2β2AR in neangiogenesis, we removed the right common femoral artery in 6 twelve-week old b2AR knock-out (b2KO) mice and 6 wildtype controls (WT) and evaluated for 15 days blood flow (BF) through both ischemic and non ischemic tibial posterior artery by means of echodoppler probe (100 Mhz, Vevo 770, GE). After 15 days, BF was also assessed by injecting 108 dyed beads into the abdominal aorta and measuring the release of dye in digested muscles of the ischemic and non ischemic HLs. We also counted the capillary (~10 micron diameter) per muscle fiber density on tibial muscle sections stained with CD31 antibody. β2KO and WT presented similar doppler profiles in the non ischemic HL (Vmax, mm/sec; b2KO: 64 ±5; WT: 65 ± 5, n.s.). BF was not assessable in the ischemic HL one day after surgery in both strains. On day 15, WT showed partial restoration of doppler Vmax which was 75 ±15% of the contralateral non ischemic HL. This was significantly less in b2KO (5 ±5%, p<0.02 vs WT). The impaired angiogenic response of b2KO vs WT was also illustrated by a greater occurrence of blistering of the ischemic HL (83% vs 17%, p<0.05, chi squared), reduced dyed microsphere dilution (24 ±15% vs 59 ±15%, p<0.05), and capillary density. In another group of 3 b2KO, at the time of the femoral artery resection we performed intra-arterial ADβ2AR delivery (10^9 PFU) in the ischemic HL, increasing 4fold bAr density as compared to b2KO mice (fmol/mg; b2KO: 31 ± 6; b2KO + ADβ2AR: 133 ± 41, p<0.05). By day 15, BF (70 ± 20%, ns vs WT) and capillary density were increased while blistering was reduced (0%, ns vs WT). Our results suggest that vascular β2ARs play a major role in the regulation of the angiogenic response to ischemia.

**5029**

**First Molecular Evidence that Inositol Triphosphate Signaling Contributes to Infarct Size Reduction with Ischemic Preconditioning**

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Stimulation of G-protein-coupled receptors, followed by production of diacylglycerol (DAG) and activation of protein kinase C (PKC) has been identified as key mechanisms contributing to infarct size reduction with preconditioning (PC). Production of DAG is, however, accompanied by the ‘in parallel’ generation of the second messenger inositol 1,4,5-trisphosphate (IP3). While IP3 signaling (generation of IP3, followed by binding to IP3 receptors) is well-recognized to participate in the regulation of calcium homeostasis, the role of IP3 signaling in PKC-induced cardioprotection is, at present, unknown. **Methods:** To investigate this issue, hearts were harvested from: (1) adult ltp3⁻/⁻ mice displaying spontaneous mutation of the IP3 receptor gene and, thus, reduced expression of IP3 receptor mRNA and protein (heterozygotes were used, as homozgyotes die soon after birth); and (2) age-matched adult C57BL/6J mice (parent strain). All hearts were buffer-perfused in Langendorf mode, and randomized to receive: 2 5-min episodes of PC ischemia; pretreatment with D-my o-IP3 (sodium salt of native IP3; 6 μM); or no intervention (controls). After the treatment phase, all hearts underwent 30 min global ischemia + 2 h reperfusion, and infarct size was delineated by tetrazolium staining. **Results:** Significant cardioprotection was seen with both PC and exogenous D-my o-IP3 in the C57 parent strain (p<0.01 vs C57-Controls). There were no differences in baseline hemodynamics or heart weight/body weight ratios in ltp3⁻/⁻ vs C57BL/6J mice (p-values > 0.15). However, both PC and D-my o-IP3 failed to limit infarct size in IP3 receptor null mice. Post-hoc experiments further revealed that, even when the PC stimulus was amplified to 4 5-min cycles of ischemia, ltp3⁻/⁻ mice remained refractory to PC-induced protection (infarct size: 63%). **Conclusion:** These data provide novel molecular evidence that IP3 signaling contributes to infarct size reduction with preconditioning.

**Infarct SIZE (% of LV)**

<table>
<thead>
<tr>
<th>Infarct Size (%) of LV</th>
<th>Control</th>
<th>PC</th>
<th>D-my o-IP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J; ltp3⁻/⁻</td>
<td>53 ± 4%</td>
<td>56 ± 2%</td>
<td>56 ± 1%</td>
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<tr>
<td>Control</td>
<td>30 ± 6%*</td>
<td></td>
<td>34 ± 7%*</td>
</tr>
<tr>
<td>D-my o-IP3</td>
<td>59 ± 7%*</td>
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