**Late Breaking Science: Linking Genes to Function in the Heart and Vasculature**

**BASIC ABSTRACTS**

**Exogenous Hematopoietic Stem Cells Can Regenerate Infarcted Myocardium**

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To determine whether hematopoietic stem cells (HSC) can transform into cardiomyocytes with the potential to repair dead myocardium after infarction, Lin-Kit + HSC were harvested from transgenic mice expressing green fluorescent protein (GFP) and injected in the region bordering the infarct, 3–5 hours after coronary artery occlusion in mice. A band of closely packed cells was identified at 17 days in heart in nearly 50% of HSC injected hearts, between the endocardial and epicardial surface of the injured ventricle. This band occupied 50–75% of the damaged portion of the wall. c-kit/GFP positive HSC were found in the infarcted area shortly after coronary ligation and were still detectable at 7 days. c-kit stained HSC were not labeled by markers of myocytes (α-actinin positive cardiac muscle cells, endothelial cells, factor VIII, and smooth muscle cells, smooth muscle actin). The band of tissue included in the infarcted zone was comprised of 75% GFP, α-actinin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures and arterioles. Proliferating myocytes were c-kit negative, and partially myofibrotic and resembled late fetal-neonatal cells. GFP-positive replicating myocytes, endothelial cells and smooth muscle cells were c-kit negative. Infarcted mice were injected with BrdU, once a day for 4 days, to establish the extent of cell proliferation in the infarct. Only 25% of the BrdU-positive cells were BrdU positive. These three levels of BrdU labeling were statistically different. Additionally, the percentages of cells positive to Ki67 were measured to evaluate the fraction of cycling cells at this single time point: 16% myocytes, 10% endothelial cells and 8% smooth muscle cells were in G1, G2-M. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocytes resulting in significant repair of muscle mass after infarction.

**Mutations in the R1α Regulatory Subunit of Protein Kinase A Cause Familial Cardiac Myxomas**

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Patients with familial cardiac myxomas (FDCM) present with dyspnea, syncope and chest pain. A recent study from our laboratory and others has shown that transgenic (Tg) mice expressing low levels of active protein kinase Cε (PKCε) exhibit resistance to ischemic injury, a cardioprotected phenotype analogous to that observed during preconditioning. Although PKCε has been shown to activate multiple downstream targets in preconditioning, the molecular components that PKCε signaling complexes (PKCε complexes) mediate those cardioprotective effects are unknown. We used a proteomic approach to characterize PKCε complexes. PKCε-monoclonal antibodies were used to immunoprecipitate cardiac tissues from PKCε Tg mouse and wild type mice (n=10 each). Combining 2D-electrophoresis, MALDI mass spectrometry, and immunoblotting, we first identified 27 known and 12 unknown molecules in PKCε complexes. These include signaling molecules (RACK2, Lck, Src, PKc, PKε, p38 kinase (p170/p65), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, c-phostin, INOS, eNOS), and structural proteins (cardiac α-actin, troponin T, α-tropomyosin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKCε complexes. In PKCε Tg mice, altered expression and post-translational modification were evident for 21 known and 6 uncharacterized molecules. These data show that (i) PKCε forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore unrecognized functions of PKCε isoforms; and (ii) that cardioprotection is coupled with dynamic modulation of PKCε-associated proteins and regulation of signaling molecules to PKCε complexes. Functional proteomic analysis of PKCε complexes is a crucial step toward understanding PKCε-dependent signaling architecture and cardioprotection.

**Mutations in the Human β-Sarcoglycan Gene in Familial and Sporadic Dilated Cardiomyopathy, a Disease of the Cytoskeleton and Sarcolemma**

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**Background:** Dilated cardiomyopathy (DCM) is a significant cause of morbidity and mortality due to congestive heart failure and rhythm abnormalities. Approximately 30% of cases are familial, with the remainder occurring sporadically. The succinyltransferase (SUcase) domain complex compound to form the β-sarcoglycan, a member of the dystrophin-associated protein complex (DAPC). Methods: Blood was drawn and DNA extracted from one 4-generation family and 50 sporadic cases of DCM after informed consent. Myocardial samples were obtained after transplantation or autopsy. The β-sarcoglycan gene was screened for mutations using single strand conformation polymorphism (SSCP), denaturing high performance liquid chromatography (DHLPC) and DNA sequencing. Results: Mutation analysis of the DCM pedigree identified a single nucleotide change in exon 8 of β-sarcoglycan causing an amino acid change from a polar (serine) to nonpolar amino acid (alanine) altering the protein secondary structure. In 2 of the 50 sporadic cases, a 3bp deletion in exon 9, which deletes lysine at position 236, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of β-sarcoglycan staining. Conclusions: Mutation of the β-sarcoglycan gene causes autosomal dominant DCM. As mutations of this gene are also known to cause the Syrian hamster cardiomyopathy as well as human limb girdle muscular dystrophy, it appears that mutations in this gene place patients at risk for a spectrum of clinical features ranging from cardiomyopathy to skeletal myopathy. This is similar to other DAPC members and dystrophin itself, supporting our final common pathway hypothesis which suggests that DCM results from disruption of the cytoskeleton/sarcolemma,

**Dual Modulation of Cell Survival and Cell Death by β2-Adrenergic Gi and Gs Signaling in Adult Mouse Cardiomyocytes**

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Cardiac β2-AR activates both Gs and Gi proteins whereas β1-AR couples only to Gi. The goal of this study is to determine whether β1-AR and β2-AR differ in regulating cardiomyocyte survival and apoptosis, if so, to explore underlying mechanisms. To avoid complicated crosstalks between β-AR subtypes, we express β1-AR or β2-AR individually in the null background (β2-AR δ1 OR β1-AR δ2 double knockout mice) or in mouse cardiomyocytes. Using adult mouse myocyte culture and adenosine gene transfer techniques. Simulation of β1-AR, but not β2-AR, markedly induces myocyte apoptosis, as indicated by increased TUNEL or Hoechst staining positive cells and DNA fragmentation. Inhibition Gi signaling with pertussis toxin converts β2-AR to β1-AR in terms of its apoptotic effect, suggesting that Gi is essential for β2-AR-mediated survival factors. To explore the downstream signaling events of β2-AR-coupled Gi, we first examined the possible involvement of p38 MAPK, since recent studies propose that p38 MAPK underlies Gi-dependent anti-apoptotic effects. We found that although stimulation of either β1-AR subtype increases p38 MAPK activity, this effect is insensitive to PTX, excluding a role of p38 MAPK in β2-AR-mediated cell-survival. In contrast, β2-AR (but not β1-AR) elevates the activity of Akt, a powerful survival signal; this effect is fully abolished by inhibiting Gi with pertussis toxin, scavenging Gβγ with JAR1-kct, or blocking PI3K with LY294002, indicating that β2-AR activates Akt via Gβγ-PI3K pathway. Most importantly, inhibition of the Gβγ-PI3K-Akt pathway converts β2-AR signaling from survival to apoptotic. Thus, β2-AR, unlike β1-AR, activates concurrent apoptotic and survival signals in cardiomyocytes, and the survival effect is mediated by the Gβγ-PI3K-Akt pathway. The strikingly different effects of β-AR subtypes on cardiac cell survival and apoptosis may have important pathophysiological and therapeutic implications in chronic heart failure.
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