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−c-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 completely packed cells was identified at 17 days in nearly 50% of the hearts, between the endocardial and epicardial surface of the infarcted 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-sarcomeric actin and myosin, endothelial cells, factor VIII, and smooth muscle cells, smooth muscle actin. The band of tissue included in the infarcted zone was constituted 75% by GFP, α-sarcomeric actin, myosin and α-actin 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 also identified 7 to 17 days later in nearly 50% of HSC injected hearts, between the endocardial and epicardial surface of the infarcted ventricle. This band occupied 50−75% of the damaged portion of the wall. 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 number of cell proliferation in the regenerating myocardium. 28% myocytes, 17% endothelial cells and 12% smooth muscle 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 that divide in this site and repair: 18% myocytes, 10% endothelial cells and 8% smooth muscle cells were in G1-G2M. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocardium resulting in significant recovery of muscle mass after infarction.

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

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Cardiac myxomas arise from primitive pluripotent mesenchymal cells within the subendocardium. In autosomal dominant Carney complex, intracardiac myxomas develop in the setting of lentigines and endocrine overactivity. We previously localized the Carney complex gene defect to human chromosome 17q24. We now demonstrate that mutations in the chromosome 17 PRKAR1A gene encoding the R1α regulatory subunit of cAMP-dependent protein kinase A (PKA) cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions in the PRKAR1A gene in three unrelated kindreds (JFstavr263 in Family YA, JFstavr265 in Family YB, JFstavr163 in Family YF). This finding was confirmed by bidirectional sequence analysis and by denaturing acrylamide gel electrophoresis. Each mutant produces a frameshift mutation in the cAMP response element-binding protein (CREB)-binding protein (CBP) and a fusion protein with CREB-binding activity. DNA and protein analysis of an atrial myxoma resected from an affected individual in Family YA reveals that the tumor cells retain both the wildtype and mutant PRKAR1A alleles, and that wildtype R1α but not mutant protein is stably expressed. However, western blot analysis demonstrates a reversal of the ratio of PKA regulatory subunits R1α to R1γ in the myxomas that can alter PKA activity and contribute to tumor development. Our data, then, suggest that PRKAR1A acts as a tumor suppressor gene in the heart via regulation of PKA activity. These novel findings implicate the cAMP-dependent PKA signaling pathway as a critical modulator of cardiac cell growth and differentiation. Further delineation into the role of PKA in the heart may ultimately foster new therapeutic strategies for cardiac cell regeneration.

Functional Proteomic Analysis of Protein Kinase C ε Signaling Complexes in Preconditioning.

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Studies from our laboratory and others have shown that transgenic (TG) mice expressing low levels of active protein kinase C ε (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 mediate PKCε signaling complex formation and those that constitute PKCε-regulated architecture are unknown. We used a proteomic approach to characterize PKCε signaling complexes. PKCε monomeric antibodies were used to immunoprecipitate cardiac tissues from PKCε-Tg mice and wild type mice (n=10 each). Combining 2-D electrophoresis, MALDI mass spectrometry, and immunoblotting, so far we have identified 27 known and 12 unknown molecules in PKCε signaling complexes. These include signaling proteins (RACK2, Lck, Src, PKCδ, PKCε, p38 kinase (p170/p65), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, v-8-cystatin, INOS, eNOS), and structural proteins (cardiac α-actin, tropinin T, α-tropomyosin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKCε-immuno complexes. In PKCε-Tg mice, altered expression and post-translational modification were evident in 21 known and 10 known and 10 unknown proteins. These data show, for the first time, (i) that PKCε forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKCε isoform; and (ii) that cardioprotection is coupled with dynamic modulation of PKCε-associated proteins and recruitment of signaling molecules to PKCε complexes. Functional proteomic analysis of PKCε signaling complexes is a crucial step toward understanding PKCε-dependent signaling architecture and cardioprotection.

Mutations in the Human δ-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 and many are caused specifically by mutations in sarcomeric and cytoskeletal genes. Nonetheless, the most common form of DCM, although x-linked disease is also well described. Two genes have been identified for the X-linked form (dystrophin and tafazzin), whereas three genes have been identified in autosomal dominant DFCM (actin, lamin A/C, desmin). We have hypothesized that DCM is a disease of the cytoskeleton and sarcolemma and have focused our studies on candidates whose products are found in these structures. Here we report the screening of δ-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 δ-sarcoglycan gene was screened for mutations using single strand conformation polymorphism (SSCP), denaturing high performance liquid chromatography (DHPLC) and DNA sequencing. Protein structural analysis and immunohistochemistry were performed. Results: Mutation analysis of the DFCM pedigree identified a single nucleotide change in exon 8 of δ-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 delete lysine at position 218, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of δ-sarcoglycan staining. Conclusions: Mutation of the δ-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 present 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 Cardiac Myocytes

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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 double knock out mice were generated using adult mouse myocyte culture and adenoviral gene transfer techniques. Stimulation of β1-AR, but not β2-AR, markedly induces myocardite 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 effects. 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 β-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 Akt is fully abolished by inhibiting Gi with pertussis toxin, scavenging Gβγ with βARK1-c, 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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