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

EXOGENOUS HEMATOPOETIC 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. Low-density 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 loosely packed cells was identified at 17 days in nearly 50% of HSC within the borderzone, between the endocardial and epicardial surface of the infarcted ventricle. This band occupied 50% 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-smooth muscle actin or myosin, endothelial cells, factor VIII, and smooth muscle cells, smooth muscle actin. The band of tissue included in the infarcted zone was partially aligned myofibrils and resembled late fetal-neonatal cells. GFP-positive replicating myocytes 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 stage of repair: 18% myocytes, 10% endothelial cells and 8% smooth muscle cells were in G1, G-S, G2. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocardiocytes 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 subendoocardium. In autosomal dominant Carney complex, intracardiac myxomas develop in the setting of lentigines and endocrine overgrowth. The target cell of the primary genetic defect of human chromosome 17q24. We now demonstrate that mutations in the chromosome 17 P19KAR1α 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 P19KAR1α gene in three unrelated kindreds (JStfer-Thr163 in Family YA, JStfer-Val253 in Family YB, JStfer-Thr163 in Family YF). These mutations were confirmed by bidirectional sequence analysis and by denaturing acrylamide gel electrophoresis. Each mutant produces a frameshift and premature stop codon with consequences for 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 P19KAR1α alleles, and that wildtype R1α but not mutant protein is stably expressed. However, western blot analysis demonstrates a reversed, mutant to wildtype ratio of PKA regulatory subunits in R1α to R1β in the myxomas that can alter PKA activity and contribute to tumor development. Our data, then, suggest that P19KAR1α 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. This reorientation of the role of PKA in the heart may ultimately foster new therapeutic strategies for cardiac cell regeneration.

FUNCTIONAL PROTEOMIC ANALYSIS OF PROTEIN KINASE C AND 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 (PKC) exhibit resistance to ischemic injury, a cardioprotected phenotype analogous to that observed during preconditioning. Although PKCβ has been shown to activate multiple downstream targets in preconditioning, the molecular components that PKC signaling complexes with those that constitute the signaling architecture are unknown. We used a proteomic approach to characterize PKC signaling complexes. PKCβ monomeric antibodies were used to immunoprecipitate cardiac tissues from PKCβ 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 PKCβ signaling complexes. These include signaling proteins (RACK1, Lck, Src, PKCβ, Pi3 kinase (p170/60), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, β-catenin, INOS, eNOS), and structural proteins (cardiac α-actin, tropinin T, α-tropomyosin, prohibitin, desmin, Lap2, cavelin-3). Many of these proteins were not previously suspected to be in PKCβ immuno-complexes. In PKCβ Tg mice, altered expression and post-translational modification were evident in 16 known and 10 unknown molecules. These data show for the first time, (i) that PKCβ forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKCβ isozyme; and (ii) that cardioprotection is coupled with dynamic modulation of PKCβ-associated proteins and recruitment of signaling molecules to PKCβ complexes. Functional proteomic analysis of PKC signaling complexes is a crucial step toward understanding PKCβ-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 with the remainder occurring sporadically. Among the known causes of familial DCM, the most common form of DCM, although X-linked disease is also well described. Two genes have been identified for the X-linked forms (dystrophin and tubulin), whereas three genes have been identified in autosomal dominant DCM (actin, lamin A/C, desmin). We have hypothesized that DCM is a disease of the cytoskeleton and sarcolemma and have focused our studies on candidate genes 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 gradient gel electrophoresis (DGGE) and DNA sequencing. Results: Protein structural analysis and immunohistochimistry were performed. Results: Mutation analysis of the DCM 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 deletes lysine at position 238, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochimistry 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 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 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. Using β1 and β2 double knock-out mouse cardiomyocytes, using adult mouse myocyte culture and adenosine gene transfer techniques. Stimulation 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 (but not β1-AR) in terms of its apoptotic effect, suggesting that Gi is essential for β2-AR (but not β1-AR), activates concurrent apoptotic and survival signals in cardiomyocytes, and the β2-AR activates apoptotic effect, suggesting that Gi is essential for β2-AR (but not β1-AR) in terms of its apoptotic effect, suggesting that Gi is essential for β2-AR (but not β1-AR). We further confirmed the observation of p38 MAPK, since recent studies propose that p38 MAPK underlies Gi-dependent anti-apoptotic effect. We found that although stimulation of β2-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 Gij with JARK-ch, or blocking PKC with LY294002, indicating that β2-AR activates Akt via Giβ3-PSK pathway. Most importantly, inhibition of the Giβ3-PSK-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 Giβ3-PSK-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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