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-ckit+ 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 post-infarction in nearly 50% of HSC injected hearts, 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 17 days, c-kit stained HSC were not labeled by markers of neutrophils, monocytes, -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, -sarcomeric actin, myosin and -actin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures and arterioles. Producing myocytes were found with partially aligned myofilibrils 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, and injected the 5’3Rb 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 at this stage of 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 R1a 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, intramyocardiac myxomas develop in the setting of lentigines and endocrinopathies. We previously localized the Carney complex gene defect to human chromosome 17q24. We now demonstrate that mutations in the chromosome 17 PFKR1a gene encoding the R1a regulatory subunit of cAMP-dependent protein kinase A (PKA) cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions in the PFKR1a gene in three unrelated kindreds (JFStferGst2201 in Family YA, JFStferVst233 in Family YB, JFStferThr163 in Family YF). These mutations were confirmed by bidirectional sequence analysis and by denaturing acrylamide gel electrophoresis. Each mutant produces a frameshift of the conserved Serine 320 which produces a truncated and nonfunctional protein. 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 PFKR1a alleles, and that wildtype R1a but mutant protein is stably expressed. However, western blot analysis demonstrates a reversal in the ratio of PKA regulatory subunits R1a to R1b in the myxoma that can alter PKA activity and contribute to tumor development. Our data, then, suggest that PFKR1a 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, proliferation, and differentiation. 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 PKC has been shown to activate multiple downstream targets in preconditioning, the molecular components that mediate PKC signaling complexes and those that constitute the PKC signaling architecture are unknown. We used a proteomic approach to characterize PKC-dependent signaling complexes. PKC monomeric antibodies were used to immunoprecipitate cardiac tissues from PKC-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 PKC signaling complexes. These include signaling proteins (RACK2, Lck, Src, PKC1, PKC3, p170(65), p38 MAPK, p54(p46) JNKs, ERKs, Hsp27/Hsp71, v-ckstabilin, INOS, eNOS), and structural proteins (cardiac -actin, troponin T, -tropomyosin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKC-immuno complexes. In PKC-Tg mice, altered expression and post-translational modification were evident for 21 known and 5 unknown molecules. These data show, for the first time, (i) that PKCe forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKCe isoform; and (ii) that cardioprotection is coupled with dynamic modulation of PKCe-associated proteins and recruitment of signaling molecules to PKC complexes. Functional proteomic analysis of PKC signaling complexes is a crucial step toward understanding PKCe-dependent signaling architecture and cardioprotection.

Mutations in the Human b-Sarcomeric Actin 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. Autonomic abnormalities are 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 tafazzin), 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 cardiac cell types whose products are found in these structures. Here we report the screening of -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 -sarcoglycan gene was screened for mutations using single strand conformation polymorphism (SSCP), denatured high performance liquid chromatography (D-HPLC) and DNA sequencing. Structural analysis and immunohistochemistry were performed.

Results: Mutation analysis of the DCM pedigree identified a single nucleotide change in exon 8 of the -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. Immunohistochemistry demonstrated significant reduction of -sarcoglycan staining.

Conclusions: Mutation of the -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 common pathway hypothesis which suggests that DCM results from disruption of the cytoskeleton/sarcolemma.

Dual Modulation of Cell Survival and Cell Death by b2-Adrenergic Gi and Gs Signaling in Adult Mouse Cardiac Myocytes

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Cardiac b2-AR activates both Gi and Gi proteins whereas b1-AR couples only to Gi. The goal of this study is to determine whether b1-AR and b2-AR differ in regulating cardiomyocyte survival and apoptosis, if so, to explore underlying mechanisms. To avoid complicated crosstalks between b-AR subtypes, we express b1-AR or b2-AR individually in the null background of b1-2 d2 double knockout mice. Using adult mouse myocyte culture and adenosine gene transfer techniques. Stimulation of b1-AR, but not b2-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 b2-AR to b1-AR in terms of its apoptotic effect, suggesting that Gi is essential for b2-AR-mediated survival responses. To explore the downstream signaling events of b2-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 b-AR subtype increases p38 MAPK activity, this effect is insensitive to PTX, excluding a role of p38 MAPK in b2-AR-mediated cell-survival. In contrast, b2-AR (but not b1-AR) elevates the activity of Akt, a powerful survival signal, this effect is fully abolished by inhibiting Gi with pertussis toxin, scavenging Gi with jw- or jark-c, or blocking PKC with LY294002, indicating that b2-AR activates Akt via Gi3/p38-AKT pathway. The strikingly different effects of b-AR subtypes on cardiac cell survival and apoptosis may have important pathophysiological and therapeutic implications in chronic heart failure.
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