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

EXHIBITION

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 infarcted zone. GFP-positive cells were identified in 17 days after an ischaemic injury 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 7 days. c-kit stained HSC were not labeled by markers of muscle and myelomonocytic lineage. GFP-positive cells were organized in nascent capillary structures and arterioles. GFP-expressing myocytes were positive for regulatory subunit of protein kinase A, a protein that is specifically expressed in cardiomyocytes, suggesting that these cells had the capacity to differentiate into cardiomyocytes and contribute to tumor development. Our data, then, suggest that PRKAR1a and PRKAR1b regulatory subunits can transform HSC into cardiomyocytes, and that those transformed cells have the potential to repair myocardium with the potential to repair dead myocardium after infarction.

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

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Background: Familial (FDCM) or sporadic (SFCM) cardiac myxomas are developmental tumors composed of mesenchymal cells, and the underlying molecular defects remain to be established. Mutations in the FGFR3 gene cause autosomal dominant FDCM (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 β-sarcoglycan, a member of the dystrophin-associated protein complex (DAPC). Methods: Blood was drawn and DNA extracted from one 4-generation family and 5 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 (DHPLC) and DNA sequencing. Protein structural analysis and immunohistochemistry were performed. Results: Mutation analysis of the FDCM pedigree identified a single nucleotide change in exon 6 of the β-sarcoglycan gene that causes amino acid change from a polar (serine) to nonpolar amino acid (alanine) altering the protein secondary structure. Conclusions: Mutation of the β-sarcoglycan gene causes autosomal dominant FDCM. As mutations of this gene are also known to cause the Syrian hamster hamartoma/hyperplasia 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 current final pathway hypothesis which suggests that DCM results from disruption of the cytoskeleton/sarcoclemma.

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 Gi 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. The β1- and β2 double knockout (DKO) mice have cardiac hypertrophy. 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 in the ratio of the PRKAR1α and PRKAR1β regulatory subunits R1α to R1β in the myxoma that can alter PKA activity and contribute to tumor development. Our data, then, suggest that PRKAR1α acts as a tumor suppressor gene in the heart via regulation of PKA activity. This novel finding implicates the R1α-dependent PKA signaling pathway as a critical modulator of cardiomyocyte cell death and cardiac hypertrophy. The role of PKA in the heart may ultimately foster new therapeutic strategies for cardiomyocyte 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 underlie PKCε signaling complex-dependent preconditioning are unknown. We used a proteomic approach to characterize PKCε signaling complexes. PKCε monoclonal 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, Pm2, P3 kinase (p170/p65), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, vα-crystallin, INOS, eNOS), and structural proteins (cardiac α-actin, tropomin T, α-tropomyosin, prohibitin, desmin, Lap2, cavelin-3). Many of these proteins were not previously suspected to be in PKCε immune-complexes. In PKCε Tg mice, altered expression and post-translational modification were evident in 21 known and 9 uncharacterized molecules. 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.
TARGET: Do Tirofiban And ReoPro Give Similar Efficacy Trial?
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Circulation. 2000;102:2672
doi: 10.1161/01.CIR.102.21.2672-l
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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