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-ki/ct-HSC were harvested from transgenic mice expressing green fluorescent protein (GFP) and infused in the region bordering the infarct, 3-5 hours after coronary artery occlusion in mice. A band of closely packed cells was identified in 20% of hearts 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 cardiomyocytes, 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 30% death or non-fatal MI and the trial has 80% power to determine non-inferiority for the EPISSTENT Trial. The primary endpoint will be presented along with the key subgroups such as diabetics. Follow-up data for the trial to 1 year will also be performed.

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 a mutation in the gene encoding the R1α regulatory subunit of the catalytic subunit of PKA, which leads to constitutive activation of the PKA enzyme. The R1α subunit contains a conserved domain that binds and promotes the inactivation of phosphatase 2A (PP2A), a ubiquitous phosphatase that normally dephosphorylates the catalytic subunit of PKA. In this study, we show that a series of mutations in the R1α subunit, including a common frameshift and premature stop codon with consequent loss of the catalytic subunit of PKA, cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions and single nucleotide changes in the R1α gene in 7 of 10 Carney complex cases, indicating that mutations in the R1α subunit are a common cause of familial cardiac myxomas. These findings suggest that PP2A may be a target for therapeutic intervention in familial cardiac myxomas.

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 sporadically occurring. Amongst 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 candidates whose products are found in these structures. Here we report the screening of α-sarcomeric actin, 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 α-sarcomeric actin gene was screened for mutations using single strand conformation polymorphism (SSCP), denaturing high performance liquid chromatography (DHLPC) and sequencing. Results: Protein structural analysis and immunohistochemistry were performed. Results: Mutation analysis of the DCM pedigree identified a single nucleotide change in exon 6 of α-sarcomeric actin 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 235, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of α-sarcomeric actin staining. Conclusions: Mutation of the α-sarcomeric actin 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.

Duplication of Modulation 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 Gs. 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 (β1-AR; β2 double knock out) in cardiomyocytes. Using adult mouse myocyte culture and adenovirus 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-mediated survival effects. To explore the downstream signaling events of β2-AR, we 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 is fully abolished by inhibiting Gi with pertussis toxin, scavenging Gi by JARK-c1, or blocking PI3K with LY294002, indicating that β2-AR activates Akt via Giβ3-PI3K pathway. Most importantly, inhibition of the Giβ3-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 Giβ3-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.

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 cardiopro-
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