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 cardiac myocytes 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 closely packed cells was identified by c-kit+ c-kit+ cells 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. 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 and smooth muscle 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-smooth muscle actin, and α-actinin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures and arterioles. Prelabeling myocytes were found with partially aligned myofibrils 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, to establish the percentage 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 myocytes resulting in significant recovery of muscle mass after infarction.

Mutations in the R1α Regulator 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 mutations in the chromosome 17q24. We now demonstrate that mutations in the chromosome 17q24 gene encoding the R1α isoform of cyclic AMP-dependent protein kinase A (R1α) cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions in G1–G2M. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocytes resulting in significant recovery of muscle mass after infarction.

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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 (PKC-ε) exhibit resistance to ischemic injury, a cardioprotected phenotype analogous to that observed during preconditioning. Although PKC-ε has been shown to activate multiple downstream targets in preconditioning, the molecular components that mediate PKC-ε signaling following ischemia and those that confer cardioprotection are unknown. We used a proteomic approach to characterize PKC-ε signaling complexes. PKC-ε monomeric antibodies were used to immunoprecipitate cardiac tissues from PKC-ε 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-ε signaling complexes. These include signaling proteins (RACK1, Lck, Src, PKCδ, PKCε, gene p170(956), p38 MAPK, p54/p56 JNKS, ERKs, Hsp27/Hsp71, β-cystatin, inos, eNOS), and structural proteins (cardiac α-actin, trophin T, α-tropomyosin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKC-ε immuno-complexes. In PKC-ε Tg mice, altered expression and post-translational modification were evident in 21 known and 12 unknown non-cardiac proteins. These data show that (i) 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 families have not been specifically studied. Autosomal recessive and autosomal dominant inheritance are the most common form of DFCM, although X-linked disease is also well described. Two genes have been identified for the X-linked forms (dystrophy 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 cardiomyopathies 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. Myocardiay samples were obtained after transplantation or autopsy. The δ-sarcoglycan gene was screened for mutations using single strand conformation polymorphisms (SSCP), denaturing gradient gel electrophoresis (DGGE), and DNA sequencing. Protein structural analysis and immunohistochemistry were performed.

Results: Mutation analysis of the DFCM pedigree identified a single nucleotide change in exon B 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 213, 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 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 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 of β11 and β2 double knockout mice, with confirmed absence of β1-AR and β2-AR individually. Using adult mouse myocyte culture and adenovirus gene transfer techniques. Stimulations of β1-AR, but not β2-AR, markedly induces myocyte apoptosis, as indicated by increased TUNEL and 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 survivals and apoptotic cell death. 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 β1-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 G i with JHk, or adding JHk, or blocking PKC with LY294002, indicating that β2-AR activates Akt via Giβγ-Pi3K pathway. Most importantly, inhibition of the Giβγ-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 Giβγ-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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