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-Kit4^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 after injection in nearly 50% of HSC hearts, between the endocardial and epicardial surface of the infarcted ventricle. This band occupied 50 ± 7.5% 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 for monocytes, 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 75% by GFP, α-sarcomeric actin, myosin and α-actin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures. Injured myocytes were partially myofibrotic and resembled late fetal/neonatal cells. GFP-positive replicating myocytes, endothelial cells and smooth muscle cells were c-Ki negative. Infarct mice were injected with BrdU, once a day for 4 days, to trace the β37% of cell proliferation in the regenerating myocardium: 29% 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-, G2-M. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocardial cells 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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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. Variants in the cardiac sarcomplast are specifically associated with DCM. Identification of genetic defects in 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 DFM (actin, lamin A/C, desmin). We have hypothesized that DCM is a disease of the cytoskeleton and sarcolemmas and have focused our studies on cardiac sarcomplast whose products are found within sarcolemmas. We now demonstrate that mutations in the β-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 heart transplantation or autopsy. The β-actin gene was screened for mutations using single strand conformation polymorphism and DNA sequencing. Protein structural analysis and immunohistochemistry were performed. Results: Mutation analysis of the DCM pedigree identified a single nucleotide change in exon 6 of the β-actin gene causing an amino acid change from a polar (serine) to non-polar amino acid (alaine) altering the protein secondary structure. In 2 of the 50 sporadic cases, a 3bp deletion in exon 9, which deletes lysine at position 203, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of β-actin staining. Conclusions: The β-actin gene causes autosomal dominant DCM. As mutations in 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 current pathogenesis 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 of β2-12 double knock out (DKO) 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 cell survival. 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 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 effect is fully abolished by inhibiting Gi with pertussis toxin, scavenging GJ with JARK61, or blocking β3 with LY294002, indicating that β2-AR activates Akt via Gβγ-Pi3K pathway. Most importantly, inhibition of the Gβγ-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 Gβγ-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.
Mutations in the Human δ-Sarcoglycan Gene in Familial and Sporadic Dilated Cardiomyopathy, a Disease of the Cytoskeleton and Sarcolemma
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