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

BASIC ABSTRACTS

Exogenous Hematopoietic Stem Cells Can Generate 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-Kit transgenic mice were harvested from transgenic mice expressing green fluorescent protein (GFP) and injected in the region bordering the infarcted zone. c-kit/GFP positive HSC were found in the infarcted area shortly after injection with BrdU, once a day for 4 days, to establish the extent of cell proliferation in the infarcted area. HSC were BrdU positive. These three levels of BrdU labeling were statistically different. Additionally, c-kit stained HSC were not labeled by BrdU in the non-infarcted area. In conclusion, HSC, when injected in the heart, rapidly differentiate into myocardium indicating that DCM is a disease of the cytoskeleton and sarcolemma and have focused our studies on characterizing DAPC members that may provide therapeutic opportunities. 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 indicating that β2-AR-mediated cell-survival. In contrast, β2-AR, but not β1-AR, markedly induces myocyte apoptosis, as indicated by increased TUNEL or Hoechst staining 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 coupled Gi, we first examined the possible involvement of p38 MAPK, since recent studies propose that p38 MAPK mediates β2-AR-mediated cell survival. Future studies will focus on understanding the role of PKCε in the heart may ultimately foster new therapeutic strategies for cardiac cell 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 PKCe has been shown to activate multiple downstream targets in preconditioning, the molecular components that mediate PKCe signaling complex formation and thus Reperfusion-stimulated architecture are unknown. We used a proteomic approach to characterize PKCe signaling complexes. PKCe monoclonal antibodies were used to immunoprecipitate cardiac tissues from PKCe 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 proteins in PKCe signaling complexes. These include signaling proteins (RACK2, Lck, Src, Pw2, Pk23, pia3 pi107/p65), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, v-raf/cbaltis, INOS, eNOS), and structural proteins (cardiac α-actin, tropinin T, α-tropomyosin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKCe-immuno complexes. In PKCe Tg mice, altered expression and post-translational modification were evident for 27 known and 12 unknown antibodies. These data show for the first time, that PKCe forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKCe isomere; and that cardioprotection is coupled with dynamic modulation of PKCe-associated proteins and recruitment of signaling molecules to PKCe complexes. Functional proteomic analysis of PKCe signaling complexes is a crucial step toward understanding PKCe-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


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 deficiency, which is 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 characterizing DAPC members that may provide therapeutic opportunities. 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 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 coupled Gi, we first examined the possible involvement of p38 MAPK, since recent studies propose that p38 MAPK mediates β2-AR-mediated cell survival. Future studies will focus on understanding the role of PKCε in the heart may ultimately foster new therapeutic strategies for cardiac cell regeneration.
Mutations in the R1α Regulatory Subunit of Protein Kinase A Cause Familial Cardiac Myxomas

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