D in Family YB, resulting in significant recovery of muscle mass after infarction. That the tumor cells retain both the wildtype and mutant PRKAR1 protein analysis of an atrial myxoma resected from an affected individual in Family YA reveals ultimately foster new therapeutic strategies for cardiac cell regeneration. Growth and differentiation. Further research into the specific role of PKA in the heart may cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions in the PKA gene encoding the R1(α) subunit, and that wildtype but not mutant protein is stably expressed. However, western blot analysis demonstrates a reversal of the ratio of PKA regulatory subunits R1b to R1a in the myxomas that can alter PKA activity and contribute to tumor development. Our data, then, suggest that PKA-R1b acts as a tumor suppressor gene in the heart via regulation of PKA activity. These novel findings implicate the CAMP-dependent PKA signaling pathway as a critical modulator of cardiac cell growth and differentiation. These findings further the role of PKA 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 (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 complex formation and those that constitute the PKC-ε signaling architecture 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 (RACK2, Lck, Src, Ptk2, PI3 kinase p170/p85), p38 MAPK, p54/p46 JNKs, ERKs, Hsp27/Hsp71, α-actin, inos, eNOS, and structural proteins (cardiac α-actin, tropinin T, α-tropomysin, prohibitin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKC-ε immune-complexes. In PKC-ε Tg mice, altered expression and post-translational modification were evident for at least 21 known and 10 uncharacterized molecules. These data show, for the first time, (i) that PKC-ε forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKC-ε isoform; and (ii) that cardioprotection is coupled with dynamic modulation of PKC-ε-associated proteins and recruitment of signaling molecules to PKC-ε complexes. Functional proteomic analysis of PKC-ε signaling complexes is a crucial step toward understanding PKC-ε-dependent signaling architecture and cardioprotection.

Mutations in the Human β1-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 are predominantly inherited as autosomal recessively. Identification of the most common form of DCM, although X-linked disease is also well described. Two genes have been identified for the X-linked form (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 cardiac sarcomere proteins whose functions are not clearly understood. Among the most common form of DFCM, 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 β1-sarcoglycan gene was screened for mutations using single strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), and sequencing. Protein structural analysis and immunohistochemistry were performed. Results: Mutation analysis of the DFCM pedigree identified a single nucleotide change in exon 8 of the β1-sarcoglycan causing an amino acid change from a polar (serine) to nonpolar amino acid (alanine) altering the protein secondary structure. In 1-1.5 of the DCM sporadic cases, a 3bp deletion in exon 9, which deletes lysine at position 258, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of β1-sarcoglycan staining. Conclusions: Mutations of the β1-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 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, β3-1 double knock out mouse cardiomyocytes. Using adult mouse myocyte culture and adenoviral 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 function. 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 β2-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 Gβγ with βARK1, or blocking PI3K 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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