EPISTENT trial. The primary endpoint data will be presented along with the key subgroups such as tirofiban with an expected event rate in the control (abciximab) group of 5.3% based on the

dultly foster new therapeutic strategies for cardiac cell regeneration.

growth and differentiation. Further research into the specific role of PKA in the heart may

ultimately lead to the partial alignment of myofibrils and resembled late fetal-neonatal cells. GFP-positive replicating muscle cells, smooth muscle actin. The band of tissue included in the infarcted zone was

in the PRKAR1 (PKA) cause inherited cardiac myxomas and Carney complex. We detect heterozygous deletions.

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 cardiopro-
tected phenotype analogous to that observed during preconditioning. Although PKCε has been shown to activate multiple downstream targets in preconditioning, the molecular components that mediate the signaling complex and the specific subcellular architecture are unknown. We used a proteomic approach to characterize PKCε signaling complexes. PKCε-

monoclonal antibodies were used to immunoprecipitate cardiac tissues from PKCε 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 PKCε signaling complexes. These include signaling proteins (RACK2, Lck, Src, Pw2, P3k kinase p170/95), p38 MAPK, p54/p46 JNKS, ERKs, Hsp27/Hsp71, a-actinfilin, INOS, eNOS, and structural proteins (cardiac α-actin, troponin T, α-tropomyosin, prohibin, desmin, Lap2, caveolin-3). Many of these proteins were not previously suspected to be in PKCε-immuno-

plexes. In PKCε Tg mice, altered expression and post-translational modification were evident for at least 21 known and an additional 28 potentiated molecules. These data show, for the first time, (i) that PKCε forms signaling complexes with multiple proteins in multiple subcellular compartments, suggesting heretofore-unrecognized functions of PKCε isozyme; 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.

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

BASIC ABSTRACTS

Exogenous Hematopoietic Stem Cells Can Regenerate Infarcted Myocardium

Donald Orlic, Jan Kajstura, Stefano Cimmino, Baozhong Li, Stacie Anderson, David Bodine, James Pickel, AnnaRosa Corse, Bernadette Grand, Piero Anversa, New York Medical College, Valhalla, NY; NHLBI/NHS, Bethesda, MD

To determine whether hematopoietic stem cells (HSC) can transform into cardiomyocytes with the potential to repair dead myocardium after infarction, Lin-ckit 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 post-infarction 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-ckit/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 marking with GFP. 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 constituted 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 and scattered arteries. Proliferating myocytes were seen 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 percent 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 myocardium resulting in significant recovery of muscle mass after infarction.

Mutations in the R1α Regulatory Subunit of Protein Kinase A Cause Familial Cardiac Myxomas

Carl J. Vaughan, Minaed Casey, Je He, Cathy Hatcher, Jordan M. Winter, Stanislawa Weremowicz, Kate Montgomery, Jiangui Chen, Cynthia C. Morton, and Craig T. Basson, Cardiology Division, Dept. of Medicine, Weill Medical College of Cornell University, New York, NY

Cardiac myxomas arise from primitive pluripotent mesenchymal cells within the subendocardial-dia-

myocardium. In autosomal dominant Carney complex, intramyocardial myxomas develop in the setting of lentigines and endocrinopathy. We previously localized the Carney complex gene defect to the R1α (FSterGly208 in Family YA, FSterThr163 in Family YF, FSterThr163 in Family YF). The R1α gene in three unrelated kindreds (JSterGly208 in Family YA, JSterVal253 in Family YB, JSterThr163 in Family YF). The R1α gene mutations were confirmed by bidirectional sequence analysis and by denaturing acrylamide gel electrophoresis. Each mutant produces a truncated product that is present in nearly 50% of R1α transcripts. DNA and protein analysis of an atrial myxoma resected from an affected individual in Family YA reveals that the tumor cells retain both the wildtype and mutant PRKAR1α alleles, and that wildtype R1α but not mutant protein is stably expressed. However, western blot analysis demonstrates a reverse shift in the ratio of PRKAR1α levels to R1α in the myxoma that can alter PKA activity and contribute to tumor development. Our data, then, suggest that PRKAR1α acts as a tumor suppressor gene in the heart via regulation of PKA activity. These novel findings implicate the PKA-dependent PIA signaling pathway as a critical modulator of cardiac cell growth and differentiation. Further research into 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.

Peipei Ping, Jun Zhang, Roberto Bollt, Cardiology Division, University of Louisville, KY.

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 cardioprotect
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Circulation. 2000;102:2672
doi: 10.1161/01.CIR.102.21.2672-g
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/21/2672.9

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