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

BASIC ABSTRACTS

Exogenous Hematopoietic Stem Cells Can Regenerate Infarcted Myocardium

Donal O’Riain, Jan Kajistr, Stefano Cimberio, Baosheng Li, Stacie Anderson, David Bodine, James Pickel, Annesa Bell, John Marolda-Girand, Peter Anversa, New York Medical College, Valhalla, NY; NIH/NIHR, Bethesda, MD

To determine whether hematopoietic stem cells (HSC) can transform into cardiomyocytes with the potential to repair dead myocardium after infarction, Live/i-15 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-infarct in nearly 50% of HSC mice, between the epicardial and endocardial surface of the infarcted ventricle. This band occupied 50% 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 labelled by markers of myocytes, a-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, a-sarcomeric actin, myosin and a-actin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures and arterioles. Progenitor myocytes were partially aligned by 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 extent 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 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 tissue resulting in significant recovery of muscle mass after infarction.

Mutations in the R1a Regulatory Subunit of Protein Kinase A Cause Familial Cardiac Myxomas

Carl J. Vaughan, Mainrad Case, Je He, Cathy Hatchet, Jordan M. Winter, Stanislawa Wernimowicz, Kate Montgomery, Raja Kucherlapati, 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 subendocardium. In autosomal dominant Cancer, intracardiac myxomas develop in the setting of lentigiosis and endocrinopathy. We previously localized the Carney complex gene defect to the infarct, 3–5 hours after coronary artery occlusion in mice. A band of closely packed cells was identified at 17 days post-infarct in nearly 50% of HSC mice, between the epicardial and endocardial surface of the infarcted ventricle. This band occupied 50% 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 labelled by markers of myocytes, a-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, a-sarcomeric actin, myosin and a-actin positive cardiac muscle cells. Other GFP-positive cell populations were endothelial cells and smooth muscle cells, organized in nascent capillary structures and arterioles. Progenitor myocytes were partially aligned by 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 extent 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 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 tissue resulting in significant recovery of muscle mass after infarction.

Mutations in the Human a-Sarcomeric Actin Gene in Familial and Sporadic Dilated Cardiomyopathy, a Disease of the Cytoskeleton and Sarcolemma

Tsutsui B, Bowles KR, Vatta M, Zintz C, Bowles NE, Towbin JA, Pediatric Cardiology, Baylor College of Medicine, Houston, TX

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 the remaining 70% are sporadic. Autosomal dominant transmitted DCM 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 DFM (actin, lamin A/C, desmin). We have hypothesized that DCM is a disease of the cytoskeleton and sarcolemma and have focused our studies on cardiomyocytes whose products are found in these structures. Here we report the screening of a-sarcomeric, 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 a-sarcomeric gene was screened for mutations using single strand conformation polymorphism (SSCP), denaturing high performance liquid chromatography (DHPLC) and MALDI mass spectrometry. Protein structural analysis and immunohistochemistry were performed.

Results: Mutation analysis of the DCM pedigree identified a single nucleotide change in exon 6 of the a-sarcomeric gene 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 218, occurred. Neither the missense mutation nor deletion mutation was seen in 200 control patients. Immunohistochemistry demonstrated significant reduction of a-sarcomeric staining.

Conclusions: Mutation of the a-sarcomeric 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 common pathway hypothesis which suggests that DCM results from disruption of the cytoskeleton/sarcolemma,

Dual Modulation of Cell Survival and Cell Death by b2-Adrenergic Gi and Gs Signaling in Adult Mouse Cardiac Myocytes

Wei-Zhong Zhu, Ming Zheng, Brian Kobakia, Rui-Ping Xiao, Laboratory of Cardiovascular Science, GRC, MA, NIH, Baltimore, N4D 21224; Howard Hughes Medical Institute, Stanford Univ Med Cent, Stanford, CA 94305.

Cardiac b2-AR activates both Gi and Gi proteins whereas b1-AR couples only to Gi. The goal of this study is to determine whether b1-AR and b2-AR differ in regulating cardiomyocyte survival and apoptosis, if so, to explore underlying mechanisms. To avoid complicated crosstalks between b1-AR and b2-AR subtypes, we express b1-AR or b2-AR individually in the null background. Using b1-2 double knockout mice, we find that b1-AR (but not b2-AR) activates survival signaling from Gi, while b2-AR (but not b1-AR) activates apoptosis from Gs. Using adult mouse myocyte culture and adenoviral gene transfer techniques. Stimulation of b1-AR, but not b2-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 b2-AR to b1-AR in terms of its apoptotic effect, suggesting that Gi is essential for b2-AR-mediated survival signals. To explore the downstream signaling events of b2-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 b2-AR subtype increases p38 MAPK activity, this effect is insensitive to PTX, excluding a role of p38 MAPK in b2-AR-mediated cell-survival. In contrast, b2-AR (but not b1-AR) elevates the activity of Akt, a powerful survival signal in this effect is fully abolished by inhibiting Gi with pertussis toxin, scavenging Gi with Ws-1 or b1-2, or blocking PKC with LY294002, indicating that b2-AR activates Akt via Gi/p38MAPK pathway. Most importantly, inhibition of the Gi/PKA-Pi3K/Akt pathway converts b2-AR signaling from survival to apoptotic. Thus, b2-AR, unlike b1-AR, activates concurrent apoptotic and survival signals in cardiomyocytes, and the survival effect is mediated by the Gi/Pka/Pi3K/Akt pathway. The strikingly different effects of b1-AR subtypes on cardiac cell survival and apoptosis may have important pathophysiological and therapeutic implications in chronic heart failure.
Exogenous Hematopoietic Stem Cells Can Regenerate Infarcted Myocardium
Donald Orlic, Jan Kajstura, Stefano Chimenti, Baosheng Li, Stacie Anderson, David Bodine, James Pickel, Annarosa Leri, Bernardo Nadal-Ginard and Piero Anversa

_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
Copyright © 2000 American Heart Association, Inc. All rights reserved.
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

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/