dothelium or exposed matrix, transmigration through the endothelium, and, finally, migration and invasion into the target tissue. The capacity to migrate and invade may be pivotal to functional integration even when cells are injected intramuscularly. Particularly in patients, who lack the endogenous stimuli incited by acute ischemic injury, the enhancement of local homing signals or cells' ability to respond may be of critical importance. Cytokines (e.g. SDF-1), integrins and proteases (e.g. cathepsin L) are critical regulators of these steps. Influencing endothelial differentiation of progenitor cells may be another option to improve vascular regeneration. Epigenetic control mechanisms as well as transcription factors including the hoemobox protein HoxA9 are regulating these complex events.

1

Apoptotic, Oncotic and Autophagic Cell Death in Cardiovascular Diseases

L. Maximilian Buja
The University of Texas Health Science Center at Houston, USA

Modes of cell death are apoptosis and oncosis in acute cardiovascular disease, and also autophagic cell death in chronic disease, with necrosis as the final stage of cell degradation. Apoptosis is pre-programmed (suicidal), energy-dependent, initiated by extrinsic (TNF/Fas receptor) and/or intrinsic (mitochondrial) pathways, and mediated by caspases in cells with intact membranes. Oncosis is accidental injury, initiated by energy depletion, caspase independent, and mediated by progressive membrane damage. Apoptosis contributes to progression of atherosclerosis, including rupture of vulnerable plaques. Recent studies utilizing mutant mice underexpressing proapoptic molecules (Fas, Bid) or overexpressing antiapoptotic molecules (Bcl-2) have yielded 50-60% reductions in infarct size versus controls, indicating that apoptosis as well as oncosis contributes to myocardial ischemia-reperfusion injury, with the rate and magnitude of ATP depletion as a major determinant of apoptosis or oncosis. The progression of heart disease to heart failure is associated with very low but abnormal and persistent levels of cardiomyocyte cell death. The progression to heart failure is accelerated or blocked in transgenic mice with overexpression of proapoptotic (procaspase-8, Nix/BNip3L) or antiapoptotic (Bcl-2) molecules, respectively, demonstrating the importance of recurrent low levels of cell death in heart failure. Long-term outcome is determined by the relative magnitude of cell death versus regeneration by endogenous and circulating stem cells. These observations raise the potential for new forms of targeted therapy for cardiovascular diseases.

2

Cardiac Stem Cells and Myocardial Regeneration

Hidemasa Oh
Hidemasa Oh, Kento Tateishi, Tetsuya Nomura, Hiroaki Matsubara
Department of Experimental Therapeutics, Kyoto University Hospital, Kyoto

Recent reports raised hope that cardiac progenitor cells exist within the adult heart and suggested the possibility that the cardiac repair is not so far fetched. Although the origin and cellular regulation of mouse cardiac progenitor cells as wells as of postnatal cardioblasts are just begun to be identified, less is known about those in human as translational investigation. Here, we report the clonal isolation of pluripotent stem cells in adult mouse and human from cardiac and skeletal muscles. These cells are clonogenic and proliferative in vitro to form replatable colonies that expressed telomerase reverse transcriptase, which is associated with self-renewal potential, as well as BrdU incorporation and markers characteristic of endodermal, mesodermal and ectodermal precursors. Cardiac and skeletal stem cells colonies purified side population cells and partially expressed markers of embryonic stem cells but none of that as skeletal satellite cells. Upon differentiation, single cardiac stem cells produced distinct population of cardiac-, skeletal-, and vascular smooth muscle cells, and endothelial, glial cells, and adipocytes by colony-dependent specificity. Moreover, the newly differentiated cardiac muscle cells demonstrated spontaneous beating characterized by calcium transient and single cell action potential analyses. Genetically tagged cells could survive after transplantation and differentiated into striated cardiac muscle following acute infarction. These cells represented a previously unidentified adult intrinsic cardiac stem cell population from mammalian heart, and are a promising candidate for cell-based therapeutic strategies to treat human heart failure.

3

Distinct Features in Apoptosis and Regeneration between Myocytes and Nonmyocytes of the Heart

Genzou Takemura
Hisayoshi Fujiwara
Cardiology & Respirology, Regeneration & Advanced Medical Sciences, Graduate School of Medicine, Gifu University, Gifu

Although cardiac myocyte apoptosis has been demonstrated experimentally, for example with Fas stimulation, its significance in actual heart disease remains controversial. Some myocytes in diseased hearts clearly show DNA nicks, but these may be phantoms, actually reflecting biological phenomena other than apoptosis: oncosis with secondary DNA fragmentation in acute myocardial infarction or DNA repair or RNA synthesis in heart failure. Most importantly, ultrastructural evidence of myocyte apoptosis is still lacking. By contrast, myocardial interstitial cell (nonmyocyte) apoptosis has been ultrastructurally demonstrated and has recently attracted significant attention, as its inhibition following myocardial infarction appears to mitigate postinfarction ventricular remodeling and dysfunction. Indeed, the target of antiapoptosis therapy may be shifting from myocytes to nonmyocytes. Also controversial is whether cardiac myocytes are really regenerated in diseased hearts. Some researchers have reported substantial transdifferentiation of bone marrow cells into cardiac myocytes after myocardial infarction, but others attribute the beneficial effects of so-called regenerative therapies such as bone marrow cell transplantation and administration of hematopoietic cytokines to the effects of angiogenetic cytokines or other cardioprotective factors. This controversy arises mainly from differences in interpretation of confocal microscopic findings, which underscores the need for an alternative method of assessing whether or not cardiac myocyte regeneration has occurred. In the present session, we show the ultrastructure of bone marrow-derived cardiac myocytes observed within infarcted hearts, although the incidence of such cells was very rare. A better understanding of the regulation of death and regeneration of both cardiac myocytes and nonmyocytes could form the basis of new approaches to the treatment of heart disease.

4

Concurrent Vasculogenesis and Cardiomyogenesis by Somatic Stem/ Progenitor Cell Transplantation for Functional Regenerative Recovery Post Myocardial Infarction

[1]Atsuhiko Kawamoto
[2]Hiroto Iwasaki, [7]Toshinori Murayama, [3]Makoto Kinoshita, [3]Minako Katayama, [5]Nobuhiro Handa, [5]Yukikatsu Okada, [4]Yasuki Kihara, [4]Shigefumi Morioka, [6]Masanori Fukushima, [1]Takayuki Asahara
[1]Stem Cell Translational Research, Kobe Institute of Biomedical Research and Innovation, Kobe, [2]Stem Cell Translational Research, RIKEN Center for Developmental Biology, Kobe, [3]Department of Cardiology, Kobe Institute of Biomedical Research and Innovation, Kobe, [4]Department of Cardiology, Kobe City General Hospital, Kobe, [5]Department of Cardiovascular Surgery, Kobe City General Hospital, Kobe, [6]Department of Clinical Trial Management, Kobe Translational Research Institute, Kobe, [7]Translational Research Center, Kyoto University Graduate School of Medicine, Kyoto

Circulating CD34+ cells were initially identified as endothelial progenitor-enriched fraction and their therapeutic potential for neovascularization was confirmed in animals with hindlimb and myocardial ischemia. A clinical trial of autologous CD34+ cell transplantation for critical limb ischemia has been performed in our institution. No patients have experienced severe adverse events. Wong-Baker pain rating scale, toe brachial pressure index and pain-free walking distance were significantly improved following cell transfer. Regenerative therapy for myocardial infarction (MI) has been attempted to induce vasculogenesis and/or cardiomyogenesis. However, the ideal strategy to efficiently regenerate both cell lineages has never been established. We have recently proved significant potential of human CD34+ cells for concurrent vasculogenesis and cardiomyogenesis in rats with MI. Actually, quantitative double immunohistochemistry for human nuclear antigen and cardiomyocyte markers (BNP, troponin I) indicated dose-dependent cardiomyogenesis, whereas that for smooth muscle actin, human leukocyte antigen or ulex europaeus lectin type 1 revealed dose-dependent vasculogenesis following CD34+ cell transplantation. RT-PCR also indicated dose-dependent expression of human-specific cardiomyocyte, smooth muscle and endothelial cell marker genes in infarcted myocardium. On the basis of above achievements, a clinical trial of CD34+ cell transplantation for chronic coronary artery disease will start in this autumn. Recently, somatic unrestricted stem cells have been identified in skeletal muscle (Young, et al) and cord blood (Kogler, et al). In collaboration with these groups, we have confirmed in vivo differentiation of the stem cells into