Angiogenesis and Regeneration Medicine in Cardiovascular Medicine (M)

FRS2

March 27 (Sat)

Room 6 (Hall B5(2))

8 : 20—10 : 26

Keynote Lecture:

Gene and Cell Therapy for Tissue Regeneration: A New Era in Cardiovascular Disease Management

Douglas Losordo
St. Elizabeth's Medical Center, Boston, U.S.A.

Preclinical studies performed in animal models of limb and myocardial ischemia have documented that certain cytokines, including vascular endothelial growth factor (VEGF) and fibroblast growth factor-1 and -2, administered as recombinant protein or cDNA, may promote neovascularization of ischemic tissues. More recently, preliminary clinical trials have established that the results of these animal studies may extend to human subjects with limb and myocardial ischemia, and large scale clinical trials are no w underway to test the utility of direct myocardial gene therapy with VEGF-2 plasmid for patients with severe myocardial ischemia and no other options for revascularization. In the context of preclinical studies of angiogenic gene therapy, the contributi on of circulating bone marrow derived endothelial progenitor cells to new blood vessel formation was documented. This discovery suggested that these cells could be employed as therapeutic tools for revascularization of ischemic tissue, either on their own, or in combination with other approaches. Preclinical studies demonstrated the potency of these cells, selected as CD34+ cells from the circulation or bone marrow, for neovascularization. Pilot clinical trials are planned to establish the safety and evidence for bioactivity of these cells as autologous therapy. Together these approaches define a new modality of tissue revascularization, i.e. biologic revascularization.

FRS-009

Close relationship between Wnt and PI3-kinase pathway during earliest cardiomyocyte differentiation

[1]Atsuhiko Naito
[1]Hiroshi Akazawa, [1]Issei Komuro
[1]Department of Cardiovascular Science and Medicine, Chiba University, Chiba, [2]Department of Cardiovascular Science and Medicine, Chiba

We previously reported that inhibiting the PI3-kinase activity only at the earliest stage of cardiomyocyte differentiation inhibited differentiation of multipotent teratocarcinoma cell line, P19CL6, into functional cardiomyocytes. However, the molecular mechanisms of how PI3-kinase is involved in cardiomyocyte differentiation remain unknown. In *Drosophila, wingless*, a homologue of mammalian Wnt, regulates the expression of cardiac transcription factor Csx/Nkx-2.5 homologue *tinman*. It has been reported using P19CL6 that Wnt plays an important role in mammalian cardiomyocyte differentiation through the canonical Wnt/ β -catenin pathway. Here we examined a novel crosstalk between the PI3-kinase/Akt and Wnt/ β -catenin pathways. Forced expression of dominant-negative Akt at the earliest stage of culture suppressed cardiomyocyte differentiation of P19CL6 cells. We found that the canonical Wnt/ β -catenin pathway was activated at the earliest stage of culture and that inhibition of the PI3-kinase/Akt pathway blocked both canonical Wnt/ β -catenin pathway and cardiomyocyte differentiation. Dominant-negative Akt decreased the phosphorylation of GSK-3 β which was associated with a decrease in expression levels of cytoplasmic and nuclear β -catenin. Blockade of cardiomyocyte differentiation by dominant-negative Akt was rescued by activating canonical Wnt/ β -catenin pathway through forced expression of non-phosphorylated, constitutively active form of β -catenin. These results suggest that PI3-kinase/Akt pathway positively regulates cardiomyocyte differentiation through suppressing the GSK-3 β activity and in turn activating the canonical Wnt/ β -catenin pathway.

FRS-010

The Ubiquitin/Proteasome Pathway Modulates the Mode of Cell Death and Ubiquitin Overexpression Protects Against Oxidative Stress in Adult Rat Cardiomyocytes

Yukihiro Hayakawa
Genzou Takemura, Rumi Maruyama, Hisayoshi Fujiwara
Second Department of Internal Medicine, Gifu University School of Medicine, Gifu

The ubiquitin/proteasome (U/P) pathway is a major non-lysosomal route for selective protein degradation, however, its role and the contribution of ubiquitin-mediated autophagic cell death (ACD) in the pathological setting are unclear. To clarify the role of the U/P pathway and ACD in stressed cardiomyocytes, the effects of a selective proteasome inhibitor MG-132 and adenovirus (AdUb)-mediated ubiquitin (Ub) overexpression were determined in oxidatively stressed adult rat cardiomyocytes (OSC) 10 days in culture. Myocytes were treated with MG132 or AdUb before 0.1 mM H2O2 stimulation. Ub was accumulated specifically in monodansylcadaverine (a specific marker of ACD)-positive autophagic OSC. Proteasomal activity (PA) was impaired transiently, but returned to basal levels until 14 hrs after H2O2 stimulation, however, MG-132 repressed the recovery of PA up to 27% compared with control. MG-132-mediated PA inhibiotion enhanced myocardial susceptibility to oxidative stress by increasing apoptosis and necrosis. In contrast, ACD was decreased by MG-132 in a dose dependent manner. AdUb-mediated Ub overexpression revealed cardioprotective effects against oxidative stress by decreasing all 3 modes of cell death. In conclusion, OSC die by 3 different modes (apoptosis, necrosis and autophagy), which are modulated by PA. Ub plays 2 opposite roles : induction of ACD and cardioprotection against oxidative stress. Understanding of the U/P pathway and ACD may provide a novel therapeutic target for heart disease.

FRS-011

High-Mobility-Group Protein A2 Associates with the Smad Transcription Factors and Synergistically Promotes Cardiomyocyte Differentiation

[1]Koshiro Monzen
[1]Yukio Hiroi, [1]Toru Hosoda, [1]Doubun Hayashi, [1]Tsutomu Yamazaki, [1]Ryouzou Nagai, [2]Issei Komuro
[1]Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Tokyo, [2]Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Meicine, Chiba

Members of the high-mobility-group proteins A (HMGA) family have been reported to be expressed ubiquitously in fetus. They regulate expression of numerous genes by participating in specific protein-DNA and protein-protein interactions that induce both structural changes in chromatin substrates and the formation of stereospecific complexes on the promoter/enhancer region of their target genes. By the differential display method, we isolated HMGA2, a member of the HMGA family, whose expression was markedly enhanced during differentiation of the P19CL6 cardiomyogenic cell line. Stable overexpression of wild-type HMGA2 strongly enhanced the differentiation of P19CL6 into cardiomyocytes with increased expresssion of some cardiac-specific genes, while overexpression of the carboxyl terminus-deleted mutant of HMGA2 inhibited it. We also found that Smads, transcription factors which specifically transduce signals from bone morphogenetic proteins (BMPs), and HMGA2 displayed synergistic transacriptional activation of the -3.0 kb Csx/Nkx2-5 promoter, but not of the -1.0 kb promoter. Co-transfection of Smads and HMGA2 into COS-7 cells showed that they associate with each other in mammalian cells. Furthermore, gel mobility shift assays revealed that Smads and HMGA2 bind to an evolutionarily conserved, specific sequence around -2.7 kb upstream of the transcriptional start site on the Csx/Nkx2-5 promoter. These results suggest that Smads and HMGA2 synergistically activate the Csx/Nkx2-5 expression and that HMGA2 promotes cardiomyocyte differentiation at least partly through accelerating the BMP-Smad-Csx/Nkx2-5 cascade.

FRS-012

Myocardin Expression is Regulated by Nkx2.5 and Its Function is Required for Cardiomyogenesis

Tomomi Ueyama
Hideko Kasahara, Takahiro Ishiwata, Nie Qing, Seigo Izumo
Cardiovascular Division, Beth Israel Deaconess Medical Center, Boston, USA

Nkx2.5 (also known as Csx) is an evolutionarily conserved cardiac transcription factor of the homeobox gene family. Nkx2.5 is required for early heart development, as Nkx2.5 null mice die before completion of cardiac looping.