S5-5

Structural biology and evolution of telomere binding proteins

Heeyoung Park[1,3], Jung-Sue Byun[2], Hansol Kim[2], Sung-Geon Ko[1] , Woong Han[1], Woo Taek Kim[2] , Hyun-Soo Cho[2,3] and Weontae Lee[1,3*]

[1]Department of Biochemistry, Yonsei University, Seoul 120-749, Korea; [2]Department of Biology and [3]Protein Network Research Center, Yonsei University

Telomeres, which are essential for the maintenance of chromosome integrity and for protection from exonucleolytic degradation or end-to-end fusion with other chromosomes, are the specialized nucleoprotein complexes at the ends of linear eukaryotic chromosomes. They consist of tandem repeats of short G-rich sequence elements, such as TTAGGG in human or TTTAGGG in higher plants. In mammalian cells, telomerase activity is intimately associated with the program of cell proliferation, dedifferentiation and immortalization. Due to their essential role on telomere structure and functions, the mode of binding of telomere-associated proteins have attracted much interest. In addition to telomerase activity, specific telomere-binding proteins are also important for telomere integrity and function. Recently, protein components of the telomere complex have been identified and extensively characterized in several organisms ranging from ciliates to humans. In this presentation, structural evolution of the telomere binding proteins from plants to human will be discussed based on three-dimensional structural information. The structural and sequence information of telomere binding proteins derived from different organisms will serves as a framework for understanding their various functions in a sense of evolution.

S6-1

Treatment of ischemic cardiovascular disease: present and future

Myung Ho Jeong

The Heart Center of Chonnam National University Hospital, Chonnam National University Research Institute of Medical Sciences, Gwangju, Korea

The prevalence of ischemic heart disease has been rapidly increased in Korea. With the advent of percutaneous coronary intervention (PCI), invasive treatment of ischemic heart disease is known to be effective in many patients, especially with acute coronary syndrome. One of major limitations of PCI is restenosis. The introduction of drug-eluting stent (DES) has rapidly and profoundly affected the field of PCI. Local delivery antiproliferative agents using DES has dramatically reduced restenosis rate. DES are now used in a majority of PCI procedures. DES has emerged as a potential solution for restenosis so far. Currently two DES have proven effective in large randomized trials: the sirolimus-eluting stent (Cypher stent, Cordis/Johnson & Johnson) and the paclitaxel-eluting stent (Taxus, Boston Scientifics). These polymer-regulated deliveries of both paclitaxel and sirolimus at the site of arterial injury has been shown to reduce clinical and angiographic restenosis rates after stent implantation in de novo coronary lesion. However, delayed endothelization and hypersensitivity reaction due to drug and/or polymer remain as major problems of DES. New DES should be developed for the prevention of stent thrombosis and restenosis using more physiologic drugs and/or more biocompatible coating technologies than those of Cypher or TAXUS stents. We have developed new DES, anti-oxidant and anti-platelet coated stents, with anti-inflammatory, anti-proliferative, anti-thrombotic activities in our animal and clinical cardiac catheterization laboratories.

S6-2

New paradigm in endothelial progenitor cells for vasculogenesis 'cell-and-gene hybrid therapy' of GSK3β and β-catenin axis

Hyo-Soo Kim, MD, PhD

Department of Internal Medicine, National Research Laboratory of Cardiovascular Stem Cell,Seoul National University College of Medicine, Seoul, Korea

Endothelial Progenitor Cells (EPC) are the bone marrow-derived circulating progenitor cellsthat are involved in the postnatal vasculogenesis in physiologic, such as wound healing, as well as pathologic conditions, such as tumor angiogenesis. These cells are most promising candidate of clinical application for patients with ischemic heart or limb diseases. Before clinical application, the limitations of number, vitality, or function of EPCs should be overcome to get the satisfactory efficacy. I will present the practical strategy to improve the therapeutic efficacy of EPC, namely, 'Cell-and-Gene Hybrid Therapy' by targeting GSK3b and b-catenin axis.

1] Genetic Modification of EPC with GSK-3β (glycogen synthase kinase-3β)

Previously we reported inhibition of glycogen synthase kinase-3β (GSK3β), a key regulator in many intracellular signaling pathways, enhances the survival and migration of vascular endothelial cells. Here we investigated the effect of inhibition of GSK3β activity on the angiogenic function of endothelial progenitor cell (EPC) and demonstrated a new therapeutic angiogenesis strategy using genetically modified EPC. As we previously reported, biologically distinct two types of EPC, spindle-shaped 'early EPC' and cobble-stone shaped 'late EPC'could be cultivated from human peripheral blood. Catalytically inactive GSK3β gene was transduced into both EPCs. Inhibition of GSK3β signaling pathway led to increased nuclear translocation of β-catenin, and increased secretion of angiogenic cytokines (VEGF, IL-8). It enhanced the survival and proliferation of early EPC, whereas it promoted the survival and differentiation of late EPC. Transplantation of either of these genetically modified EPC into ischemic hindlimb model of athymic nude mouse significantly improved blood flow, limb salvage, and tissue capillary density compared to non-transduced EPC. Inhibition of GSK3β signaling of either of these genetically modified EPC augmented the *in vitro* and *in vivo* angiogenic potency of these cell populations. These data provide evidence that GSK3β has a key role in the angiogenic properties of EPC. Furthermore, the genetic modification of EPC to alter this signaling step can improve the efficacy of cell-based therapeutic vasculogenesis.

2] Effect of local β¬catenin gene transfer to ischemic limb of nude mouse

GSK3β knock-out significantly increased the availability of β-catenin in the experimnt mentioned above. In order to assess the downstream effect of GSK3β knock-out on angiogenesis in ischemic limb, we analyzed the effect of b-catenin gene transfer on the angiomyogenesis of ischemic limb. β-Catenin signaling plays a critical role in directing cell fate during embryogenesis, and uncontrollable activation of β-catenin leads to various human cancers, suggesting its importance in cell survival and proliferation. However, little is known regarding its role in endothelial cell (EC) and skeletal muscle proliferation, and progenitor cell mobilization. Adenovirus-mediated β-catenin gene transfer enhanced EC proliferation, protected ECs from serum-deprivation-induced apoptosis, and increased the capillary forming capabilities, which was completely blocked by inhibition of its nuclear translocation with NCad $\triangle$C cotransfection. In addition, the increased proliferation of ECs by β-catenin was associated with increased expression of cyclin E2, but not cyclin D1. In skeletal muscle cells, β-cateninoverexpression increased proliferation with cyclin D1 expression, decreased apoptosis, and induced hypertrophy. Furthermore, β-catenin induced the expression of VEGF in skeletal muscle cells, resulting in EC proliferation. In a mouse hindlimb ischemia model, β-cateningene transfer significantly increased recovery of blood perfusion, capillary density along with enhanced VEGF expression, and number of proliferating ECs and myocytes. Local delivery of β-catenin gene also promotes angiogenic progenitor cell mobilization from bone marrow and increases the number of satellite cells in muscle. β-Catenin may be an important modulator of angiogenesis and myocyte regeneration in ischemic tissue not only by directly enhancing proliferation and survival of ECs and skeletal myocytes, but also by inducing VEGF expression and promoting progenitor cell mobilization.