Heat Shock-Induced ROS Uregulates MMP-1 and MMP-9 Expressions via MAP Kinase Pathway in HaCaT Cells

Department of Dermatology, Seoul National University College of Medicine, Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Institute of Dermatological Science, Seoul National University
Mi Hee Shin, Young Ji Moon, Kwang Hyun Cho, Kyu Han Kim, Hee Chul Eun, Jin Ho Chung

Human skin is exposed to infrared radiation (IR) as well as ultraviolet radiation (UVR) from natural sunlight. IR typically causes an increase of skin temperature. In the present study, we have investigated whether heat shock-induced reactive oxygen species (ROS) stimulates matrix metalloproteinases (MMPs) in immortalized human keratinocyte HaCaT cells. In cultured HaCaT cells, heat treatment induced MMP-1, and MMP-9, but not MMP-2 at the mRNA and protein levels. Moreover, heat shock caused the rapid activation of three distinct mitogen-activated protein kinases (MAPKs), ERK, JNK, and p38 kinase. The heat shock-induced MMP-1 and MMP-9 expressions were prevented by the pretreatment of inhibitors of ERK, JNK, or p38 kinase, respectively. Furthermore, heat shock increased intracellular ROS levels, including hydrogen peroxide and superoxide, and pretreatment with antioxidant NAC, or catalase significantly suppressed heat shock-induced MMP-1 and MMP-9 expression. On the other hand, SOD inhibited only MMP-9 induction, but not MMP-1, by heat shock. Also, pretreatment of NAC attenuated the phosphorylation of ERK, JNK and p38 kinase by heat shock. These results indicate that ROS produced by heat shock may play an important role in the heat-induced activation of MAPKs signaling pathways, which can lead to the induction of MMP-1 and MMP-9 in cultured HaCaT cells.
The increase of reactive oxygen species (ROS) levels in human skin may lead to skin damage through altering the expression of a variety of genes as well as the structure and/or function of a variety of proteins. These features are also observed during skin aging or photoaging processes. Thus, treatment of antioxidants to the skin is suggested to be a potential therapeutic method for reducing ROS and skin aging/photaging. UV is known to regulate the expressions of type I procollagen and MMPs, and may lead to collagen deficiency in photoaged human skin. Here, we investigated the protective effect of synthetic chemical compounds, SOD mimics, on the UV-induced expression of type I procollagen and MMPs in cultured human dermal fibroblasts and hairless mouse skin. In this study, two SOD mimics (M40403, and a new synthetic compound, S50907) were used. Cultured dermal fibroblasts were pretreated with SOD mimics (0.01 uM) for 24 h before UV irradiation. Pretreatment of M40403 and S50907 inhibited the UV-induced ROS formation in the fibroblast at 0.1 uM. They also prevented the UV-induced expression of type I procollagen and MMPs in cultured dermal fibroblasts. Both SOD mimics were applied topically to the dorsal skin of the hairless mice immediately before UV irradiation (2 MED). By western blotting and zymography, we demonstrated that topical treatment of M40403 or S50907 inhibited UV-induced MMP-2, MMP-9 and MMP-13 in mouse skin. Also, these compounds provided protective effect against sunburn cell formation on UV-induced acute sunburn reaction. Our results show that the synthetic SOD mimics may prevent harmful effects of UV on the expression of type I procollagen and MMPs, and may be used as potential anti-skin aging agents.

**P011**

Cholesterol Inhibits Matrix Metalloproteinase-1 (MMP-1) Expression in Human Dermal Fibroblasts

Department of Dermatology and Biochemistry and Molecular Biology, Seoul National University College of Medicine, Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Institute of Dermatological Science

Sangmin Kim, Nok-Hyun Park, Kyung A Cho, Sang Chul Park, Jin Ho Chung

Cholesterol is a major component of specialized membrane microdomains known as lipid rafts or caveolae, which modulate the fluidity of biological membranes and membrane cholesterol has a direct role in cell signaling and vesicular transport. Here, we investigated the effects of cholesterol on MMP-1 expression in human dermal fibroblasts. We observed that cholesterol significantly decreased MMP-1 expression dose-dependently in the fibroblasts. In contrast, cholesterol depletion of human dermal fibroblasts with a cholesterol binding agent, methyl-beta-cyclodextrin (MβCD) or a cholesterol neosynthesis blocker, fluvastatin, increased MMP-1 protein expression in a dose-dependent manner, and depletion of cholesterol suppressed MβCD-induced MMP-1 expression. Depletion of cholesterol by MβCD activated ERK1/2 and JNK, but not p38 MAPK, and the inhibition of ERK or JNK using specific chemical inhibitors prevented MβCD-induced MMP-1 expression, which indicates that ERK and JNK play an important role in cholesterol depletion-mediated MMP-1 induction. In addition, cholesterol depletion by MβCD activated c-Src and the inhibition of c-Src activity with specific chemical inhibitor significantly reduced MβCD-induced MMP-1 expression. However, these effects is prevented by cholesterol depletion. Taken together, our results suggest that cholesterol regulates MMP-1 expression in human dermal fibroblasts.

**P012**

Autologous, Allogeneic and Heterogeneic Cultured Dermal Fibroblasts Transplantation to Guinea Pig

Department of Dermatology, The Catholic University of Korea

Gyung Moon Kim, Sei Yeon Kim, Su Jean Chong, Hee Su Kim, Si-Yong Kim

The advantage of allogeneic cells over autologous cells are reduced patient donor site, decreased operating time, and avoidance of a delay in wound treatment by the time required for autologous cell isolation and multiplication. And because skin substitutes containing allogeneic fibroblasts have become more commercially available, there are attempts to replace autologous fibroblasts with allogeneic fibroblasts. In previous reports, allogeneic fibroblasts induced more inflammation and scar formation than autologous fibroblasts but another report indicate that cryopreserved allogeneic fibroblasts seeded to diabetic ulcer improved the reepithelialization. So we compared the use of autologous versus allogeneic fibroblasts transplantation to full thickness defect of guinea pig wounds and also compared the use of heterogeneous human keloid fibroblasts transplantation to full thickness defect of guinea pig wounds for the purpose of possibility of heterogeneous transplantation. In both allogeneic and autologous transplantation models, there are good wound healing with