1P-05
Role of Carbonic Anhydrase IX in Chondrocyte Differentiation

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Objective: Carbonic anhydrase (CA) IX is one of the membrane-bound isoforms of CAs which catalyze the reversible hydration of carbon dioxide to bicarbonate. Recently CA IX attracts attention for its high expression in tumor. It is also known that the expression of this enzyme is induced under hypoxic conditions, suggesting its possible distribution in cartilage. Here we investigated the expression and biological roles of CA IX in mouse chondrocytes in vitro.

Methods: Real-time RT-PCR and Western blot analysis were employed to assess the mRNA and protein levels of CA IX in mouse primary chondrocytes as well as in mouse chondrogenic ATDC5 cells. ATDC5 cells transfected with siRNA for CA IX gene or its control were treated with insulin and/or bone morphogenetic protein-2 (BMP-2) to promote their chondrogenic differentiation. Expression of the mRNAs for the marker genes of chondrocyte differentiation, such as type II collagen, type X collagen, aggrecan, Sox9, Sox6, and Sox5 were quantitatively analyzed to examine the role of CA IX in chondrocyte differentiation.

Results: Expression of mRNA and protein for CA IX increased in the primary chondrocytes after treatment with BMP-2 as well as in ATDC5 cells cultured in the presence of insulin. In the same conditions, the gene expression of type II collagen, type X collagen, aggrecan, and Sox6 were also up-regulated in these cells. In ATDC5 cells of which CA IX was knocked down by siRNA, insulin- or BMP-2-induced expressions of type II collagen, type X collagen, aggrecan, and Sox6 were significantly suppressed.

Conclusions: The present results indicate that CA IX functions as a positive regulator of the differentiation of chondrocytes.

1P-06
Reactive Oxygen Species reduce the Expression of BRAK/CXCL14 in Human Head and Neck Squamous Cell Carcinoma Cells

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Objective: It has been previously reported that oxidative stress stimulates gene expression of IL-8/CXCL8 (IL-8), which is ELR-motif angiogenic CXC chemokine in human squamous cell carcinoma (SCC). We recently demonstrated BRAK, which is also known as non-ELR motif angiostatic CXC chemokine ligand 14 (CXCL14) to have anti-tumor activity in human head and neck squamous cell carcinoma (HNSCC) cells. Here we investigated the effects of oxidative stress induced by reactive oxygen species (ROS) such as hydrogen peroxide (H2O2) and hydroxyl radical (HO·), on the expression of both CXCL8 and CXCL14 in HNSCC.

Methods: HNSCC cells were cultured in DMEM-10; and after serum starvation, nearly confluent cells were cultured in the presence or absence of ROS with or without addition of N-acetylcysteine (NAC) or MAPK inhibitors. Messenger RNA levels were measured by quantitative PCR method and protein levels by western blotting. By use of electron spin resonance, we confirmed HO· generation by Fenton’s reaction (H2O2/FeSO4).

Results: When the HNSCC cells were cultured in the presence of ROS, the expression of CXCL14 was significantly decreased; whereas that of CXCL8 was increased. Interestingly, the effects on the expression of both genes in HNSCC cells were much greater with HO· than with H2O2. The effects of ROS on both CXCL8 and CXCL14 expression were attenuated by the pretreatment with NAC or MAPK inhibitors.

Conclusions: Oxidative stress induced by ROS stimulates not only an increase in the expression of CXCL8 but also a decrease in that of CXCL14 in HNSCC cells. These results indicate that oxidative stress induces angiogenesis of tumor progression by regulating the gene expression of both angiogenic and angiostatic factors in HNSCC cells.