The Anabolic Effects Of Hif-1α-induced Hsp70 In Rabbit Articular Chondrocyte Under Hypoxic Conditions

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Introduction: Articular cartilage is an avascular tissue that derives its nutrition and oxygen supply via diffusion from synovial fluid and subchondral bone. It has been estimated that chondrocytes at the articular surface are exposed to approximately 6-10% oxygen (O2), whereas chondrocytes in the deepest layers of the articular cartilage are exposed to only 1-5% O2. Several studies have shown that chondrocytes can survive hypoxic conditions by adjusting their metabolism. Oxygen is an important modulator of gene expression, with hypoxia-inducible factor (HIF) being the primary regulatory factor responding to variations in O2 levels. The transcription factor HIF-1alpha (HIF-1α) is important in maintaining proper cellular functions under hypoxic conditions. In addition, HIF-1α is essential for chondrogenesis, including chondrocyte growth arrest, survival, maturation, and apoptosis. HIF-1α also promotes the synthesis of relevant extracellular matrix (ECM) components. However, these HIF-1α-dependent anabolic pathways in chondrocytes remain poorly understood. Among the genes regulated via the HIF-1α pathway under hypoxic conditions are the highly conserved heat shock proteins (HSPs), which act as cellular chaperones for proteins misfolded by cellular stress. We also reported that HSP70 overexpression promotes the metabolic activity of chondrocytes and protects these cells under various conditions. Nevertheless, the role of HSP70 in articular chondrocytes exposed to hypoxic conditions remains unclear. The aim of this study is to analyze the expressions of HIF-1α and HSP70 in articular chondrocytes under hypoxia, and to investigate whether HSP70 implicate HIF-1α-dependent anabolic pathways.

Methods: This study was conducted according to the regulations regarding animal research of Kyoto Prefectural University of Medicine. The cartilage was aseptically collected from Japanese white rabbits. The isolated chondrocytes were cultured as monolayers for 1 week at 37°C in 5% CO2 /95% humidified air. Pre-designed si-RNAs targeting rabbit HIF-1α(si-HIF-1α) and HSP70(si-HSP70) were used. A non-targeting scramble si-RNA were served as a negative control (si-neg). The cells were seeded in six-well plates at a density of 1 × 106 cells/well (n = 4). Twenty-four hours later, si-HIF-1α or siHSP70 were added to each well. The cells were cultured under 1% (hypoxia) or 20% (normoxia) oxygen, respectively, balanced with N2 in in a humidified incubator. In a simulated hypoxic condition, CoCl2 (150 μM), a chemical inducer of HIF-1, was added to the cells incubated under normoxia. The mRNA levels of HIF-1α, HSP70 were measured by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). The protein production were subjected to Western blotting for HIF-1α and HSP70. Cell viability
was assessed by lactate dehydrogenase (LDH) release and tetrazolium salt-based assays. Statistical significance was defined as a p-value of less than 0.05 by Tukey’s test.

Results: Cells cultured under hypoxic conditions showed significantly higher expression of HIF-1α and HSP70 mRNA and a notably higher expression of HIF-1α and HSP70 proteins compared with cells cultured under normoxia. Treatment of cells under normoxia with CoCl2 also caused enhanced the levels of HIF-1α and HSP70 mRNA and proteins. Although si-HIF-1α significantly reduced chondrocyte expression of HSP70 under hypoxia, si-HSP70 did not affect the expression of HIF-1α. These findings indicated that HIF-1α regulates hypoxia-induced HSP70 expression in chondrocytes. The levels of expression of PG and Col II mRNA were significantly higher under hypoxia or simulated hypoxia than under normoxia. Each si-RNA knocked down the expression of its mRNA and protein under hypoxia, but neither si-RNA induced the overexpression of PG and Col II mRNAs under hypoxia. These findings showed that hypoxia-induced PG and Col II mRNAs were regulated by HIF-1α-induced HSP70 expression. LDH release was significantly lower in chondrocytes cultured under hypoxia or simulated hypoxia than under normoxia. Moreover, LDH release was completely abrogated in chondrocytes transfected with si-HIF-1α or si-HSP70. Cell viability in tetrazolium salt-based assay was significantly higher in chondrocytes cultured under hypoxia or simulated hypoxia than under normoxia. But, cell viability was significantly inhibited in chondrocytes transfected with si-HIF-1α or si-HSP70. Taken together, these assays showed that HIF-1α and HSP70 expression positively correlated with chondrocyte viability under hypoxia.

Discussion: We observed that the levels of HSP70 mRNA and protein were higher under hypoxia and simulated hypoxia than under normoxia, but that these effects were abrogated by inactivation of HIF-1α. These finding suggested that HIF-1α regulates the expression of hypoxia-induced HSP70 in chondrocytes. To determine whether HSP70 expression affects ECM metabolism in chondrocytes under hypoxic conditions, we also analyzed the mRNA levels of ECM genes, such as PG and Col II. We found that the levels of PG and Col II mRNAs were significantly increased under hypoxia, but not in cells transfected with si-HIF-1α or si-HSP70. These findings suggest that the ECM metabolism in chondrocytes may be regulated via HIF-1α-induced HSP70. We observed that si-HIF-1α or si-HSP70 almost completely abolished the chondroprotective effects induced by hypoxia, suggesting that HIF-1α-induced HSP70 may increase chondrocyte viability under hypoxic conditions. Here we showed that HIF-1α-induced HSP70 increased the expression of ECM mRNAs and chondrocyte viability. These finding suggest that HIF-1α may regulates interaction with HSP70. Chronic hypoxia in the joints of OA patients has been associated with increased levels of HIF-1α in chondrocytes. In addition, the levels of expression of HSP70 in chondrocytes could also be correlated with the histological severity of OA. In chondrocytes of OA patients, however, the levels of expression of HIF-1α and HSP70 may not be high enough to eliminate stress-induced damage to cartilage, resulting in the progression of this disorder. Control of HIF-1α-induced HSP70 expression could provide a novel therapeutic strategy for OA.

Significance: This study showed, for the first time, that HIF-1α-induced HSP70 expression, at least partially, was positively correlated with HIF-1α-dependent anabolic pathways.
ORS 2015 Annual Meeting
Poster No: 0337