CONCLUSIONS: Physiological tonicity provides a simple, yet effective, means to improve marker expression during cytokine-free isolation and in vitro expansion of human articular chondrocytes. Compared to FK506 alone, elevated tonicity significantly improved chondrogenic marker expression. Combining FK506 and elevated tonicity significantly improved chondrogenic marker expression even further, while suppressing the few hypoxia-induced catabolic and hypertrophic marker genes.

Our findings will lead to the development of improved cell-based repair strategies for chondral lesions and may provide novel insights into mechanisms underlying osteoarthritic progression.

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EXPRESSION OF P35R2 IN CHONDROCYTES IS REGULATED BY SHEAR STRESS

Kobe Univ. Graduate School of Medicine, Kobe, Japan

Purpose: Chondrocyte apoptosis plays an important role in cartilage degeneration in osteoarthritis (OA), and mechanical injury to cartilage induce chondrocyte apoptosis. In response to DNA damage, p53 expression is up-regulated and regulates the p53-regulated apoptosis-inducing protein 1 (p53AIP1). We previously showed that mechanical stress induced chondrocyte apoptosis via p53 and p53AIP1 pathway. While, p35R2 expression is regulated in response to DNA damage. However, p35R2 repairs damaged DNA, and it protects from catabolism of chondrocytes. In this study, we evaluated the p35R2 expression of OA and normal chondrocytes in response to shear stress.

Methods: OA cartilage samples were obtained from total knee replacement surgery, and normal cartilage samples were from femoral neck fracture. Chondrocytes were isolated from cartilage. Two, five and ten% shear stress was introduced to chondrocytes for 12 hours by using Flexer cell system. Expression of p35R2 in chondrocytes was detected by western blotting.

Results: The expression of p35R2 in OA chondrocytes was increased by 2 and 5% shear stress but decreased by 10% shear stress in comparison with control (non stress).

Conclusions: We previously demonstrated that phosphorylation of p53 was elevated in OA chondrocytes in comparison with normal chondrocytes. In vivo, p35R2 expression was increased in OA chondrocytes when mild shear stress was introduced. However, p35R2 expression was decreased when excessive shear stress was introduced. Therefore, excessive shear stress may spend all the p35R2 in OA chondrocytes and allow the induction of apoptosis. We are doing further investigation to analyze p35R2 expression and function in chondrocytes.

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IL-6, A HIF-2α TARGET GENE, REGULATES OSTEOARTHRITIC CARTILAGE DESTRUCTION BY MODULATING MATRIX METALLOPROTEINASES (MMP)3 AND 13

J.-H. Ryu, Y. Shin, J.-S. Chun
Gwangju Inst. of Sci. and Technology (GIST), Gwangju, Korea, Republic of

Purpose: Recently we have identified that hypoxia-inducible factor (HIF)-2α is a novel transcription factor of catabolic genes involved in cartilage destruction. In an attempt to understand further regulatory mechanisms of HIF-2α in cartilage, direct target gene of HIF-2α was selected and its function in cartilage was elucidated.

Methods: For finding novel target genes of Hif-2α, cDNA microarray assay was performed. As a result of microarray, interleukin (IL)-4 is selected as a novel target gene which is dramatically up-regulated cytokine by HIF-2α in articular chondrocytes. To further elucidate the role of IL-6 in cartilage, the expression of IL-6 in several arthritic cartilages from both human and mice was determined by RT-PCR and immunohistochemistry. Recombinant IL-6 protein was injected into knee joint for gain-of-function study, and neutralizing anti-IL-6 antibody was used for loss-of-function study in vivo.

Results: HIF-2α directly regulates IL-6 expression by binding to its promoter region. We determined that HIF-2α mediated IL-1β-induced IL-6 expression and recombinant IL-6 treatment in articular chondrocytes caused MMP3 and MMP13 production, which induced cartilage destruction. Moreover, we observed that IL-6 is up-regulated in arthritic cartilage from both