EXPRESSION OF TRANSDUCED HSP70 GENE PROTECTS CHONDROCYTES FROM APOPTOSIS INDUCED BY NITRIC OXIDE

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Introduction:
Changes of articular cartilage are an essential factor in the development of osteoarthritis (OA). Recent reports demonstrated that the degree of chondrocyte apoptosis is closely related to cartilage destruction and that nitric oxide (NO) induced apoptosis of chondrocytes in OA (1). In this study, we transduced heat shock protein 70 (HSP70) gene which is known to protect cells from various stresses to chondrocytes (2), and investigated the antiapoptotic efficacy of HSP70 gene transduction in vitro.

Methods:
We used two adenovirus vectors, one was AxSHEwt that included wild-type HSP70 gene, another was Ax1w that included no expression gene unit. Chondrocytes were obtained from shoulder, hip, and knee joints of Japanese white rabbits (2kg), and cultured for 1 week in the DMEM with 10% bovine serum and 1% antibiotics at 37°C, 95% humidity and 5% CO². After transfection by adenovirus vectors, apoptosis was induced by 0.5mM sodium nitroprusside dihydrate (SNP). The effect of adenovirus vector mediated - HSP70 gene transfer on the apoptosis was investigated using the lactate dehydrogenase (LDH) activity assay, the Hoechst33342 staining, and Terminal transferase dUTP Nick-End Labeling (TUNEL) staining. The expression of HSP70 gene was confirmed by western blotting.

Results:
In the LDH activity assay, OD at 570 nm (Abs570) - OD at 690 nm (Abs690) were 423.3±30.5 in the control cells, 465.0±15.9 in Ax1w transduced cells, and -26.3±2.3 in AxSHEwt transduced cells (n = 5). In the Hoechst33342 staining assay, the percentages of positive cells were 12.3±3.8% (control), 11.9±2.4% (Ax1w), and 0.8±0.4% (AxSHEwt) (Fig. 1-A, n=5). In the TUNEL-staining assay, the percentages of positive cells were 29.3±13.9% (control), 23.3±9.2% (Ax1w), and 4.4±0.6% (AxSHEwt) (Fig. 1-B, n=10). In these assays, the LDH activity and the percentage of apoptotic cells in the AxSHEwt transduced cells were significantly lower than in other two groups (p<0.001). Western blotting analysis confirmed the expression of transduced HSP70 gene expression in the AxSHEwt transduced cells, but not in the Ax1w transduced cells and control cells.

Discussions:
HSP is a protein family that is induced within cells when various stresses are applied. HSP70 is one of the most important HSPs, and its molecular weight is 70kDa. HSP70 has been known to protect cells against various kinds of environmental stress; hypoxia, ischemic changes, and heat stress. On the other hand, recent studies demonstrated that the apoptosis of articular chondrocytes plays an important role in the pathogenesis of OA and that NO could stimulate this process. This study showed that the HSP70 gene transfer protected chondrocytes from the apoptosis induced by NO. These suggest that the HSP70 gene transfer to chondrocytes could be useful for the treatment of OA.

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References:

Fig 1. Percentage of Hoechst33342 positive cells (A), and TUNEL positive cells (B) quantified by fluorescence microscopy. Data are expressed as mean ± S.E.