Overexpression of cathepsin K in human osteoarthritic cartilage

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INTRODUCTION
Cathepsin K (CatK) is one of the cysteine proteases and mainly degrades collagen [1]. CatK is known to be expressed in osteoclasts, chondrocytes and synovial fibroblasts. It also shows collagenolytic activity against type II collagen, suggesting the involvement in degradation of articular cartilage. We had presented that progression of OA was delayed in CatK−/− mice at the 2011 ORS meeting. We here investigated the CatK expression and activation in human articular cartilage using clinical specimens.

MATERIALS AND METHODS
Human specimens: Human osteoarthritic (Collins score 3–4) cartilage was obtained during surgery of total hip arthroplasty (n=10), and control cartilage was from that of femoral head prosthesis for femoral neck fracture (n=10). Synovium was also collected in each group.

Cell culture: Chondrocytes were isolated with a 1-hr treatment with 0.2% pronase followed by an overnight treatment with 0.025% collagenase P. The chondrocytes were cultured in alginate beads 3D culture for 7 days [2].

Immunohistochemistry: Formaldehyde fixed tissues were embedded in paraffin. The sections were subjected to immunohistochemical analysis. Rabbit anti-cathepsin K polyclonal antibody (Proteintech Group Inc.) and Goat anti-Cathepsin B, L and S polyclonal antibody (SantaCruz) were used as primary antibodies. After washing, sections were incubated with Histofine Simple Stain MAX PO (R or G) (Nichirei Bio.). The number of stained chondrocytes was counted in each OA and control cartilage (positive staining cells per 50 cells).

RNA isolation and quantitative real-time PCR: Total RNA was extracted using Trizol. Cathepsins and Cystatin C (CysC) mRNA expression was assessed by real time PCR.

ELISA analysis for protein expression of Cathepsin K: Protein concentration of CatK in chondrocytes was quantified using a human Cathepsin K enzyme-linked immunosorbent assay (ELISA) kit.

Intracellular Cathepsin K activity: Fluorescence staining was performed using a MagicRed™ Cathepsin K Detection Kit (Cresyl violet) to investigate intracellular CatK activity.

IL-1 treatment: Control cartilage was dissected into 5mm slices, and subjected to explant culture. CatK expression was evaluated with or without IL-1β (0.01-10ng/ml) treatment for 6 hours.

Statistical analysis: Mann-Whitney U test was used to assess differences between groups. P-values of < 0.05 were considered as statistically significant. All analyses were performed using SPSS 18.0 for Windows software.

RESULTS
Cathepsins immunostaining and expression: OA chondrocytes and synovial cells were strongly-stained with CatK, particularly in superficial layer and more damaged area (Fig.1A). Significant large number of chondrocytes was stainable in OA compared with control chondrocytes (p<0.001) (Fig.1B). CatB, L, S and CysC were weakly stained in both groups (Fig.2A). CatK mRNA expression increased significantly in OA group compared to that in control group (p=0.043), whereas CatB, L, S and CysC expression showed no significant difference between groups (Fig.2B). There was positively strong correlation between CatK and CysC expression (R²=0.81) (data not shown).

Intracellular Cathepsin K protein concentration and activity: Mean CatK concentration (4.83 pmol/g protein) in OA chondrocytes was higher than that (3.91) in control chondrocyte (p=0.001) (Fig.3A). CatK was significantly activated in OA chondrocytes compared with control chondrocytes (Fig.3B).

Control articular cartilage explants cultured with IL-1: Increased protein and mRNA expression of CatK was observed with immunostaining and real time PCR in a dose-dependent manner (Fig.4A,B), respectively.

DISCUSSION
It has been reported that CatK overexpression transgenic mice has resulted in destruction of articular cartilage, and CatK has been expressed in synovial cells of human RA and OA. However, no study has ever reported characteristics of CatK expression in human OA cartilage.

Although CatK is basically lysosomal protein, there is a report that secreted CatK from synovial fibroblasts degrades type II collagen in cartilage. Hou et al demonstrated that CatK is capable to degrade cartilage proteoglycans at specific cleavage sites [3]. CatK is expressed in chondrocytes of human OA. Together with our previous results that OA progression was delayed in CatK−/− mice, CatK plays crucial roles in the pathogenesis of OA cartilage degeneration.

REFERENCES