The role of cathepsin K in development of knee osteoarthritis: analysis of cathepsin K knockout mice

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INTRODUCTION
Cathepsin K (CatK) is one of the cysteine proteases predominantly expressed in osteoclasts and cleaves bone matrix protein. It is also synthesized by chondrocytes and synovial fibroblasts, however few studies showed the involvement of CatK in osteoarthritis (OA). We investigated the roles of CatK in pathological process of OA using OA model of CatK knockout (CatK-) mice.

MATERIALS AND METHODS
Mice: We developed knee osteoarthritis model in mice. Right knees of 8 wild-type control mice (C57BL/6) and 8 CatK- mice (C57BL/6/129S background), were destabilized by partial resection of the medial meniscus (MM), transaction of the medial collateral ligament (MCL) and anterior cruciate ligament (ACL). Skin and joint capsule were incised in left knees (Sham side) of 6 controls and 6 CatK- mice. At 8 weeks postoperatively, knee joints were excised and subjected to histological analysis.

OA evaluation: The cross-sections of knee joints were graded according to a modified Mankin histological scoring system for articular cartilage.

Immunohistochemistry: Mice were transcardinally perfused with cold 4% paraformaldehyde (PFA), and the knee joint were post-fixed in 4% PFA at 4 °C for 12 h. The samples decalcified were embedded in paraffin. The sections were subjected to immunohistochemical analysis. Rabbit anti-cathepsin K polyclonal antibody (Proteintech Group Inc.) and Mouse anti-MMP-13 monoclonal antibody (Thermo Scientific) were used as primary antibodies. After washing, sections were incubated with either Histofine Simple Stain MAX PO (R) (Nichirei Biosciences, Tokyo, Japan) or Histofine Mouse Stain Kit (Nichirei Biosciences, Tokyo, Japan).

The number of stained chondrocytes and synovial cells was counted in each group of mice (positive staining cells / 30 cells).

Tartrate-resistant Acidic Phosphatase (TRAP) staining: Tissue sections were deparaffinized, and TRAP staining was performed using a commercial acid phosphatase leukocyte kit (Sigma, St Louis, MO). The number of TRAP positive osteoclasts in epiphyseal to metaphyseal region was counted in each mouse.

Bone histomorphometry: Perimeters, areas and thickness of bone trabeculae or cortex in the epiphysis, metaphysis and diaphysis of the OA mouse model were morphologically analyzed with Scion Image software. Osteoclast number / bone perimeter (N.Oc/B.Pm:per100mm) was measured as an expression levels of osteoclasts. 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 17.0 for Windows software.

RESULTS
Modified Mankin grading: Scores were significantly lower in the CatK- compared with those in WT (P=0.045), indicating the delay of OA progression in CatK- mice.

CatK immunostaining: The number of chondrocytes and synovial cells stainable with CatK was increased in Op side, but did not reach significant (Fig.1A-D). Significant large number of osteoclasts in metaphysis was stainable in Op side compared with Sham side (P=0.004).

MMP13 immunostaining: The number of positive chondrocytes and synovial cells was significantly higher in WT mice compared with CatK- mice (P=0.024, 0.057, respectively, Fig.2A, B). In WT mice, the number of positive chondrocytes and synovial cells was significantly higher in right knees (Op side) compared with that in left knees (Sham side) (P=0.002, 0.005, respectively, Fig.2C, D).

Bone histomorphometry: There was no morphological difference in epiphysis, whereas the thickness of bone cortex in diaphysis and that of bone trabeculae in metaphysis were higher in CatK- mice than that in WT mice. N.Oc/B.Pm of tibia and femur metaphyses was much higher for CatK- mice than WT mice (Fig.3A-C).

DISCUSSION
CatK has been shown to be expressed within osteoclasts and maintain a bone metabolism. Recently, it has been studied that the correlation between CatK and OA, while it has been suggested that subchondral bone remodeling plays significant roles in the progression of OA.

CatK deficiency in human develop pycnodysostosis due to bone resorption disorder, which characterized by short limbed dwarfism, skeletal deformities and bone brittleness. Because of compensatory mechanism for maintenance of bone metabolism, CatK deficiency mice have significantly milder osteoporosis and grow normally. Mice used in the current study were previously reported as normal bone property, suggesting that bone property itself had lesser impact on OA progression in this model.

The number of CatK stainable chondrocytes and synovial cells was higher in Op side than in Sham side in WT mice, indicating the involvement of CatK in pathogenesis of OA.

Moreover, MMP13, which is a crucial enzyme associated with OA, was down-regulated in CatK- mice. Given that a previous report indicated the association of CatK with MMP13 expression, suppression of CatK may have inhibitory effects on OA progression via perturbation of disorder of bone and cartilage metabolism in OA.

REFERENCES