High levels of serum IL-18 promote cartilage loss through suppression of aggrecan synthesis
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ABSTRACT INTRODUCTION
Osteoarthritis (OA) is the most common ailment of the aged and closely related to the function of several inflammatory cytokines. Recently, it has been reported that older age was associated with higher serum levels of IL-18 (1). In this present study, we have investigated the long-term role of serum IL-18 in cartilage degradation in vivo using a new strain of IL-18 transgenic mice (Tg) in with a comparison study against wild-type mice (WT). We developed an IL-18 Tg mouse strain that overproduces constitutively soluble mature IL-18. These mice showed high levels of serum IL-18, not of IL-18. Findings showed that the area and cell density of the articular cartilage from the femoral condyle of IL-18 Tg mice were significantly reduced in comparison with the WT mice. Aggrecan was only detected in a few cells in the deep zone of the articular cartilage of Tg mice. The expression of aggrecan mRNA was also decreased significantly in articular aggrecan from Tg mice. With regard to the expression of inflammatory cytokines, endogenous IL-18 mRNA was significantly increased in the chondrocytes of Tg mice in comparison with WT mice. Furthermore, expression of IFN-γ and TNF-α were also slightly increased in the Tg mice. In cultured chondrocytes from WT mice, the expression of aggrecan mRNA was suppressed by recombinant IL-18 in a dosage-dependent manner. In contrast, IL-18 stimulated the expression of endogenous IL-18. These results indicated that high levels of serum IL-18 promoted the over-expression of endogenous IL-18 in articular chondrocytes, resulting in cartilage degradation through suppression in aggrecan. Thus IL-18 may play an important role in the degeneration of articular cartilage in osteoarthritis.

METHODS
Utilizing the human surfactant protein C (SP-C) promoter to drive the expression of mature mouse IL-18 cDNA, we developed an IL-18 TG mice strain that constitutively over-produce IL-18 in the lung. And we established B6 caspase-1 (-/-) SPC-IL-18 Tg mice by backcrossing line A of SPC-IL-18 Tg mice with B6 caspase-1 (-/-) mice. The IL-18 Tg B6 caspase-1-deficient (−/−) SPC-IL-18 Tg mice and original wild-type (WT) mice (C57BL/6N) were sacrificed at the age of 4, 8, 12 and 24weeks of age under anesthesia with phenobarbital and articular cartilage was harvested for histological analysis. Severity of cartilage degeneration was quantified by SafraninO staining and square measure using a computerized color image analysis software system. In addition, the relationship between IL-18 expression and reduction of aggrecan in the extra-cellular matrix was measured by immunohistological analysis. Gene expression of IL-18, IL-1, TNF-α in chondrocytes isolated from TG articular cartilage was examined by real time PCR.

Cultured mouse articular chondrocytes were isolated from cartilages of the femoral condyle and tibial plateau of WT mice. All experiments were performed with chondrocytes in primary culture. Expression of IL-18, IL-1, TNF-α and aggrecan mRNA with or without IL-18 stimulation of IL-18 was measured by real time PCR.

RESULTS
The area of articular cartilage area on the femoral condyle of IL-18 TG mice was significantly less that of WT mice area at 4, 8, 12 or 24-weeks. On the tibial plateau, significant differences were evident only at 12 or 24-weeks. Safranin O staining is revealed no difference between the IL-18 TG and WT mice. No inflammatory changes such as synovitis were evident in the IL-18 TG mice area. The area of the femoral articular cartilage of caspase-1 (−/−) Tg mice stained with Safranin-O-fast green was drastically decreased in comparison with control IL-18 transgenotypic negative WT mice. Although aggrecan was immunostained in all zones of the articular cartilage in WT mice, aggrecan was detected only in cells in the deep zone of the articular cartilage in the IL-18 TG mice. The expression of mRNA for inflammatory cytokines was assessed in articular chondrocytes isolated from the right femur and tibia of the IL-18 TG and WT mice using real time PCR. The expression of aggrecan mRNA was significantly decreased in the IL-18 TG mice. On the contrary, The expression of IL-18 mRNA was significantly increased in the IL-18 TG mice. Furthermore, expression of IFN-γ and TNF-α, which are the main inflammatory cytokines, was also slightly increased in the IL-18 TG mice, whereas no clear the elevation of IL-1 was observed.

In cultured chondrocytes, the expression of aggrecan was also suppressed by IL-18 in a dose-dependent manner. Rambibinant IL-18 (500 ng/ml) stimulated the expression of endogenous IL-18 in comparison with the control.

DISCUSSION
Several studies have indicated that IL-18 contribute to joint inflammation and cartilage destruction (2, 3, 4). We have reported that high serum levels of IL-18 in Ig-IL-18 Tg mice led to a decrease in the turnover rate of trabecular bone, resulting in an aberrant cortical bone structure (5). In the present study, we found that prolonged overexpression of serum IL-18 in SPC-IL-18 Tg mice resulted in a loss of cartilage area in the knee joints. These results were considered attributable to a reduction of aggrecan in the cartilage matrix. In the IL-18 TG mice, the expression of aggrecan mRNA and protein was suppressed in cartilage. Furthermore, the expression of inflammatory cytokines such as IL-18, TNF-α and IFN-γ was also higher in the IL-18 TG mice the osteoarthritis has not been clarified. Therefore, we examined the degree of cartilage degeneration resulting from constitutive stimulation with IL-18 using the IL-18 TG mouse model. The IL-18 TG mice showed in WT mice. And similar results were also obtained in caspase-1 (−/−) SPC TG mice. In conclusion, IL-18 Tg mice were shown to have accelerated development of cartilage degradation characterized by loss in aggrecan and reduction in chondrocytes through overexpression of endogenous IL-18. The SPC-IL-18 Tg mouse is considered to be a good model of high levels of circulating IL-18. The present findings indicate that high levels of serum IL-18 associated with aging are associated with not only cardiovascular disease but also osteoarthritis, one of the common diseases characteristic of aging. Our results indicate that circulating IL-18 might act as a trigger in the development of osteoarthritis.

REFERENCES