Introduction
Osteoarthritis (OA), a chronic degenerative joint disorder characterized by articular cartilage destruction and osteophyte formation, is prevalent in society as a major cause of disability [1]. Risk factors of OA so far identified by previous epidemiologic studies are limited to age, trauma history, occupation and gender. Although several symptomatic therapies have been applied to OA, radical treatment method is not established except arthroplasty.

Osteoprotegerin (OPG) is a secreted TNF receptor member that functions as a decoy receptor for RANKL [2], thereby inhibiting osteoclastogenesis and accelerating osteoclast apoptosis [3]. Exogenous OPG has been shown to prevent bone loss in rodent models of adjuvant-induced arthritis (AIA) [4], collagen-induced arthritis (CIA) via conservation of subcondral bone [5]. On the other hand, OPG is known to bind to TRAIL, a death domain-containing type II transmembrane protein of the TNF superfamily. Recent studies showed that TRAIL induced chondrocyte apoptosis and expression of TRAIL was increased in chondrocytes from rats with experimentally induced OA [6]. These results support the notion that in vivo blocking of TRAIL prevents the induction of chondrocyte apoptosis in experimentally induced OA and that TRAIL and its receptor play a crucial role in cartilage generation and destruction [6]. OPG therapy is quite effective and promising in ameliorating the pathological bone destruction including osteoporosis and rheumatoid arthritis (RA). However, advantages of OPG in cartilage destruction in OA are poorly known. The purpose of this study was to evaluate the direct effects of OPG on chondrocyte in the process of development of OA in vivo, by intra-articular administration to experimental murine OA model.

Materials and Methods

Surgical procedures: Under general anesthesia, the right knee joint was exposed and the medial collateral ligament (MCL) was transected, and the medial meniscus (MM) was removed using a surgical microscope and microsurgical technique, which established as medial model by Kamelakura et al. [1]. The contralateral knee joint was sham-operated through the same approach without any ligament transaction or meniscectomy.

Intraarticular injection of OPG: After surgical induction of OA, C57BL/6J mice (n=10) were divided into two groups. In OPG-treated group (n=5), 100ng recombinant human OPG (rhOPG) / 10μl PBS was administered intra-articularly to mice 5 days a week from the next day to 4 weeks after the operation. In control group (n=5), 10μl PBS was administered intra-articularly.

Histological analysis: All mice were sacrificed at 4 weeks. The whole knee joints were dissected, fixed in 4% paraformaldehyde, and decalcified. After dehydration and paraffin embedding, 5-μm sagittal serial sections were cut from the whole medial compartment of the joints. Sections were stained with Safranin-O fast green, and the OA development in the tibial plateau was quantified by Mankin’s histological grading and cartilage destruction score [1]. OPG and TRAIL protein expression was examined by immunohistochemistry, and Terminal Deoxynucleotidyltransferase-Mediated UTP End Labeling (TUNEL) assay was conducted to detect the chondrocyte apoptosis in the joints.

Statistical analysis: Mann-Whitney’s U-test was used for assessing differences. A value of p<0.05 was considered significant.

Results
The combination of MCL transection and medial meniscectomy caused the cartilage destruction in the medial tibial cartilage at 4 weeks. While the cartilage destruction at the tibia of the medial model showed typical osteoarthritic changes, including a loss of the superficial zone, a decrease in Safranin-O staining and an increase in cellularity in the middle zone at 4 weeks in control group (Fig. 1-A), OPG administration prevented these osteoarthritic changes (Fig. 1-B). Articular cartilage thickness was protected in OPG-treated animals. Mankin scores (Fig. 2-A) and cartilage destruction scores (Fig. 2-B) of OPG-treated animals were less than 50% of control group, as a result of reduced severity and surface area involvement. Subchondral bone density was not affected by OPG administration.

Immunostaining indicated that OPG protein was clearly detected in the synovium and superficial layers of articular cartilage in OPG administered joints whereas OPG expression were slightly observed in the deep layers of control chondrocytes. TRAIL expression was also observed in the chondrocytes of OA cartilage predominantly in the superficial layers regardless of OPG administration. TRAIL and OPG were co-expressed in superficial chondrocytes in OPG-treated group. TUNEL assay indicated that apoptotic chondrocytes were predominantly observed in the superficial layers of OA cartilage in control group. As expected, TUNEL-positive chondrocytes were significantly reduced in OPG-treated group. When surgical induction of OA was conducted to OPG−/− mice, degenerative changes of the articular cartilage were enhanced in OPG−/− mice compared with wild-type mice. Mankin scores and cartilage destruction scores of OPG−/− mice were significantly elevated (more than 25%) compared with wild-type littermates. Subchondral bone density was similar between wild type and OPG−/− mice. This result indicated that endogenous OPG expression in chondrocyte was essential for cartilage maintenance.

Discussion
Our present data provide novel function of exogenous OPG in the progression of OA. In this experiment, we performed intra-articular administration of rhOPG to investigate its direct effect on chondrocytes, rather than indirect effect including defending the integrity of subchondral bone. The severity of disease in OPG-treated animals was significantly reduced 4 weeks after induction of joint instability. In addition, rhOPG prevented chondrocyte apoptosis in experimental OA model. These data indicate that rhOPG protects articular cartilage by preventing chondrocyte apoptosis. In the present study, OA-induced OPG−/− mice showed severe degeneration of the articular cartilage compared with WT mice, indicating that loss of single OPG allele results in significant enhancement of cartilage destruction in the process of OA.

Our results provide some clues that OPG prevents TRAIL-induced chondrocyte apoptosis through a direct effect on chondrocyte in vivo. These results support potential therapeutic application of rhOPG for human OA.

References

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