

Prevention of Cartilage Destruction With Intraarticular Osteoclastogenesis Inhibitory Factor/Osteoprotegerin in a Murine Model of Osteoarthritis

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Objective. To investigate the effect of osteoclastogenesis inhibitory factor/osteoprotegerin (OPG) on chondrocytes in the development of osteoarthritis (OA) in vivo.

Methods. To determine the role of endogenous OPG in the progression of OA, OA was surgically induced in OPG^{+/-} mice and their wild-type (WT) littermates. To determine the role of exogenous OPG, knee joints of C57BL/6J mice with surgically induced OA were injected intraarticularly with recombinant human OPG (rHuOPG) or vehicle 5 times a week. All mice were euthanized 4 weeks after OA induction; joints were harvested and evaluated immunohistochemically.

Results. Although OA changes were induced in both WT and OPG^{+/-} mice, the degenerative changes in the articular cartilage were significantly enhanced in OPG^{+/-} mice. In C57BL/6J mice with surgically induced OA, intraarticular OPG administration protected the articular cartilage from the progression of OA. The Mankin and cartilage destruction scores in OPG-treated animals were ~50% of those seen in the control group. Furthermore, OPG administration significantly protected articular cartilage thickness. Findings of the

TUNEL assay indicated that rHuOPG prevented chondrocyte apoptosis in joints with surgically induced OA. Results of immunostaining indicated that OPG protein was detected in the synovium and in resident chondrocytes at higher levels in the OPG-treated group than in the control group.

Conclusion. These data indicate that endogenous OPG had a protective effect against the cartilage destruction that occurs during OA progression. Furthermore, direct administration of rHuOPG to articular chondrocytes prevented cartilage destruction in an experimental murine model of OA via prevention of chondrocyte apoptosis.

Osteoarthritis (OA), a chronic degenerative joint disorder characterized by articular cartilage destruction and osteophyte formation, is a major cause of disability worldwide (1). OA risk factors identified by previous epidemiologic studies are age, history of trauma, occupation, and sex. Since these factors are closely related to the mechanical load placed on joints, OA is thought to be induced primarily by accumulated mechanical stress (2). Although several symptomatic therapies have been attempted for OA, no radical treatment methods have been established, with the exception of arthroplasty. In OA, articular chondrocytes appear to be eliminated by apoptosis (2,3). The number of apoptotic cells in the articular cartilage of OA patients was found to be significantly higher than the number in healthy subjects (4). In addition, chondrocyte apoptosis has been reproduced in animals with experimentally induced OA (5).

Osteoclastogenesis inhibitory factor/osteoprotegerin (OPG) is a heparin-binding basic glycoprotein that was originally purified from the conditioned medium of the human embryonic lung fibroblast line IMR-90 (6). OPG is a secreted member of the tumor

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Dr. Asou is submitting a patent application for an adaptation of osteoprotegerin.

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necrosis factor (TNF) receptor family that functions as a decoy receptor for RANKL (6–8), serving to inhibit osteoclastogenesis and accelerate osteoclast apoptosis (9,10). OPG deficiency in mice causes severe bone loss and destruction of internal bone structures through an unbalanced shift in favor of osteoclast differentiation, but without other abnormalities (11–13). Homozygous OPG knockout (OPG^{-/-}) mice also exhibit unusual bone formations associated with severe destruction of growth plate cartilage (14,15). The proximal epiphyses of the femurs and humeri in OPG^{-/-} mice exhibit resorption of subchondral bone and collapse of the joint surface resulting from mechanical damage at the end of the bone (13). Inactivating mutations in *TNFRSF11B*, the gene that encodes OPG, result in juvenile Paget's disease (16). Polymorphisms in OPG also increase the risk of developing Paget's disease (16). Patients with Paget's disease exhibit a wide range of clinical manifestations, including bone pain, fracture, hearing loss, syndromes of neurologic compression, and secondary OA (17).

RANK, RANKL, and OPG messenger RNA (mRNA) and proteins are expressed in normal cartilage. Cartilage from patients with OA contains increased levels of OPG mRNA, and the expression of these 3 proteins extends into the midzone of the cartilage (18,19). OPG is expressed in the synovial tissues of patients with rheumatoid arthritis, spondylarthropathies, and OA (19). OPG expression by chondrocytes is increased in response to in vitro stimulation with interleukin-1 β , the proinflammatory cytokine expressed in OA joints (18), implying the existence of OPG targets within the joint space, in addition to the subchondral area.

The function of OPG that is expressed during OA pathogenesis is poorly understood. In this study, we investigated the effects of OPG on chondrocytes during OA development in vivo. We demonstrated that endogenous OPG functions in the prevention of articular cartilage degradation in a mechanical stress-induced animal model of OA. Furthermore, we found that direct administration of exogenous OPG to articular chondrocytes effectively retarded the progression of OA via suppression of chondrocyte apoptosis.

MATERIALS AND METHODS

Animals. C57BL/6J mice (8–10 weeks old) were purchased from Sankyo Labo (Tokyo, Japan). Mice heterozygous for the OPG gene mutation, OPG/Jcl, on a C57BL/6J background were purchased from Japan Clea (Tokyo, Japan).

Surgical induction of OA. All experiments were performed according to a protocol approved by the Animal Care

and Use Committee of Tokyo Medical and Dental University. With the mice under general anesthesia, the right knee joint was surgically exposed. The medial collateral ligament was transected, and the medial meniscus was removed using a surgical microscope with microsurgical technique, as previously reported (1). The left knee joint was sham-operated, without ligament transection or meniscectomy.

Reagents. Recombinant human OPG (rHuOPG) was kindly provided by Biological Research Laboratories, Daiichi Sankyo (Tokyo, Japan).

Experimental design. *Surgical induction of OA in OPG^{+/-} mice.* OPG^{+/-} mice (n = 7) and their wild-type (WT) littermates (n = 7) (ages 8–12 weeks) were surgically induced to develop OA by medial collateral ligament transection and medial meniscectomy. Four weeks after surgery, the mice were euthanized.

Intraarticular administration of rHuOPG. After surgical induction of OA, C57BL/6J mice (n = 14) were divided into 2 groups. The OPG-treated group (n = 7) was administered 100 ng of rHuOPG in 10 μ l of phosphate buffered saline (PBS) intraarticularly 5 days a week beginning on postoperative day 1 and continuing for 4 weeks after the operation. The control group (n = 7) received 10 μ l of PBS intraarticularly according to the same schedule as in the OPG-treated group. Four weeks after surgery, the animals were euthanized.

Assessment of the severity of OA. Whole knee joints were removed by dissection, fixed in 4% paraformaldehyde, and decalcified in EDTA. After dehydration and paraffin embedding, we cut serial 5- μ m sagittal sections from the whole medial compartment of the joint. Two sections obtained at 100- μ m intervals from the weight-bearing region of each knee joint were stained with Safranin O-fast green. OA severity in the tibial plateau was evaluated according to Mankin's histologic grading system (20,21), and a cartilage destruction score was also assigned (1). The thickness of the articular cartilage layer was measured as the average distance from the superficial layer to the osteochondral junction of the tibia. Quantitative determination of the articular cartilage thickness and bone volume in subchondral bone was made using Image-Pro Plus 4.1 software (Media Cybernetics, Carlsbad, CA).

Immunohistochemical analysis. Expression of OPG and TRAIL at the protein level was examined by immunohistochemistry using an anti-mouse OPG antibody (N-20; catalog no. sc-8468) or an anti-mouse TRAIL antibody (K-18; catalog no. sc-6079) according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA). Briefly, sections were blocked with 5% normal rabbit serum for 30 minutes, then incubated overnight with anti-mouse OPG antibody (1:100 dilution) or with anti-mouse TRAIL antibody (1:20 dilution) at 4°C in a humidified chamber. Sections were incubated for 30 minutes at room temperature with a biotinylated rabbit anti-goat IgG and visualized by peroxidase-conjugated avidin and diaminobenzidine using a Vectastain kit (Vector, Burlingame, CA).

TUNEL assay. The TUNEL assay was performed using a TUNEL detection kit according to the manufacturer's instructions (Takara Shuzo, Kyoto, Japan). Briefly, sections were incubated with 15 μ g/ml of proteinase K for 15 minutes at room temperature, then washed with PBS. Endogenous peroxidase was inactivated with 3% H₂O₂ for 5 minutes at room temperature. After washing with PBS, sections were immersed

in buffer containing deoxynucleotidyl transferase and biotinylated dUTP and incubated for 90 minutes at 37°C in a humid atmosphere. After washing in PBS, signals were examined by fluorescence microscopy.

Statistical analysis. Data are expressed as the mean \pm SD. Statistical analysis was performed with the Mann-Whitney U test. *P* values less than 0.05 were considered significant.

RESULTS

Enhancement of cartilage destruction by OPG heterozygous deficiency in an experimental OA model.

To determine the role of endogenous OPG in the progression of OA, we compared histologic features in the knee joints of OPG-deficient mice with those in their WT littermates. Histologic sections of the knee joints of young adult homozygous OPG-knockout (OPG^{-/-}) mice (8 weeks old) exhibited significantly thinned articular cartilage layers, active infiltration of vessels into subchondral bone, and irregularity of the osteochondral junction as compared with knee joints from OPG^{+/-} mice and WT littermates (Figures 1A–C). With aging, cartilage degradation was found to be enhanced in OPG^{-/-} mice and even in OPG^{+/-} mice (Figures 1D–F), which suggests that sufficient levels of OPG expression are essential for the prevention of age-dependent cartilage degradation. However, it was unclear whether OPG affected chondrocyte metabolism directly or whether it was affected indirectly through osteoclastic erosion of subchondral bone via RANK signaling, since subchondral bone was apparently reduced in OPG^{-/-} mice.

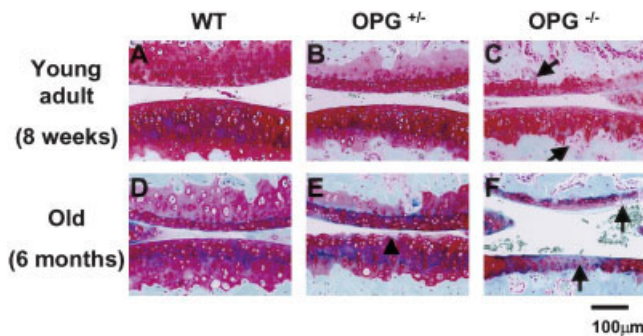


Figure 1. Histologic findings in the knee joints of young adult (8-week-old) and old (6-month-old) osteoprotegerin (OPG)-deficient mice and their wild-type (WT) littermates. Compared with the OPG^{+/-} (B) and WT (A) littermates at 8 weeks of age, the OPG^{-/-} mice (C) exhibited thinning of the articular cartilage layers, active infiltration of vessels into subchondral bone (arrows), and irregularity of the osteochondral junction. At 6 months of age, signs of enhanced cartilage degradation were observed in the OPG-deficient mice, such as superficial fibrillation (E) (arrowhead) and proteoglycan defects (F) (arrows), as compared with their WT littermates (D).

Therefore, we used young adult OPG^{+/-} mice in our experimental model of OA to avoid the effect of the subchondral bone defect and to examine the effects of OPG insufficiency on cartilage.

We compared the rates of progression and the severity of OA in OPG^{+/-} mice subjected to medial collateral ligament transection and medial meniscectomy to induce OA (1) with those in their WT littermates. Both the structure of the articular cartilage and the total bone volume were similar in OPG^{+/-} mice and WT littermates at the ages examined (8–12 weeks old). Mice were euthanized 4 weeks after the operation, and the knee joints were harvested and evaluated histologically.

Destruction of the medial tibial cartilage was observed in WT littermates, as reported previously (1) (Figure 2A, parts a and c). Histologic evaluation revealed that degenerative changes of the articular cartilage were enhanced in OPG^{+/-} mice as compared with WT littermates (Figure 2A, parts b and d). Both the Mankin scores (Figure 2B) and the cartilage destruction scores (Figure 2C) in OPG^{+/-} mice were 25% higher than those in the WT littermates (*P* < 0.05). The morphology of subchondral bone structures was not affected by OPG haploinsufficiency (Figure 2A, parts c and d). Cartilage thickness, however, was significantly reduced (*P* < 0.05) in OPG^{+/-} mice (Figure 2D), indicating that endogenous OPG plays an important role in the maintenance of articular cartilage during the development of mechanical stress-induced OA.

Prevention of cartilage destruction in an experimental OA model by exogenous OPG administration.

To examine whether exogenous OPG prevents cartilage destruction independently of the protection of subchondral bone structures, we administered rHuOPG by intraarticular injection to induce OA surgically in C57BL/6J mice. We chose this method because systemic administration of OPG may affect subchondral bone metabolism via the suppression of osteoclastogenesis. Medial collateral ligament transection and medial meniscectomy to induce OA were performed on the right knees of all mice; sham operations were performed on the left knees. OPG or vehicle alone was injected intraarticularly 5 days a week beginning the day after the operation, and all mice were euthanized 4 weeks after the operation.

Histologic investigation indicated that OPG administration protected the articular cartilage from proteoglycan depletion, alterations of surface structure, and

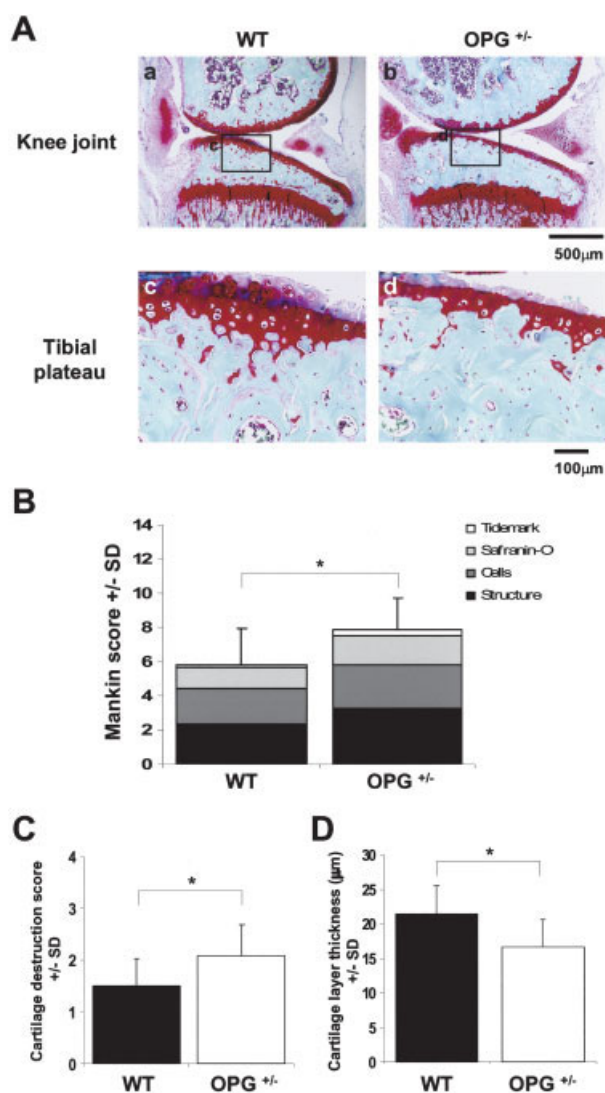


Figure 2. Histologic analysis of surgically induced osteoarthritis (OA) in the knee joints of young adult osteoprotegerin (OPG)-deficient mice and their wild-type (WT) littermates. OA was surgically induced in mice ages 8–12 weeks, and knee joints were harvested 4 weeks later. **A**, Sections of articular cartilage from WT (**a** and **c**) and OPG^{+/-} (**b** and **d**) mice were stained with Safranin O to detect proteoglycans. Degenerative changes in the articular cartilage were enhanced in OPG^{+/-} mice (**b**) as compared with their WT littermates (**a**). Morphologic features of the subchondral bone were similar in the WT (**c**) and OPG^{+/-} (**d**) mice. Boxed and labeled areas in **a** and **b** are shown at higher magnification in **c** and **d**, respectively. **B** and **C**, Histologic changes in the OA joints were assigned Mankin scores (**B**) and cartilage destruction scores (**C**). Scores in the OPG^{+/-} mice were 25% higher than those in their WT littermates. **D**, Mean cartilage thickness in OA joints was measured as the average distance from the superficial layer to the osteochondral junction of the tibia. The mean cartilage thickness was significantly reduced in OPG^{+/-} mice as compared with their WT littermates. Values in **B–D** are the mean and SD of 7 mice per group. * = $P < 0.05$ by Mann-Whitney U test.

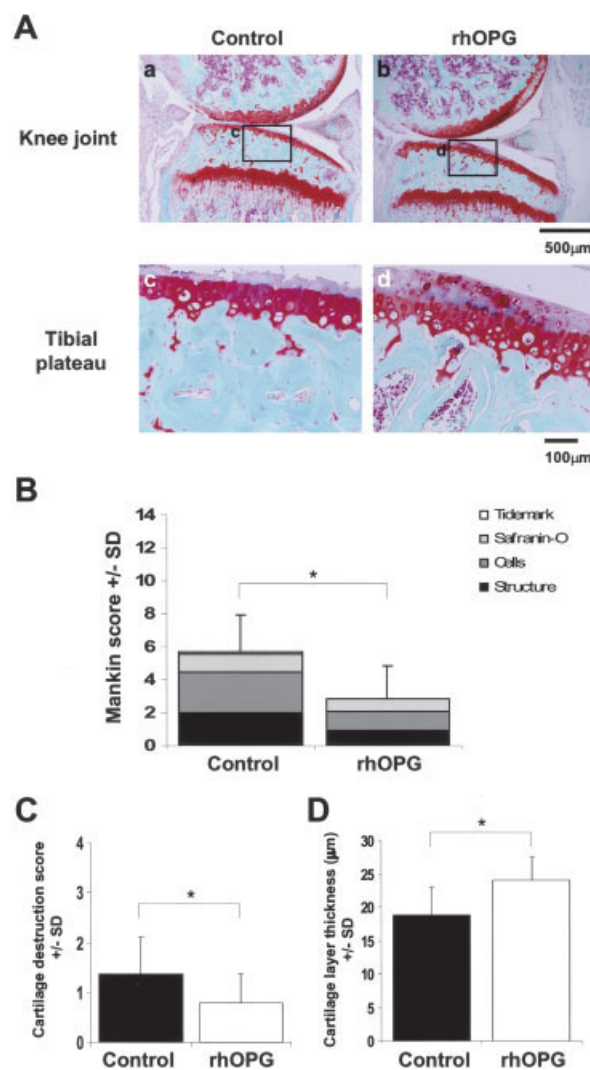


Figure 3. Histologic analysis of surgically induced osteoarthritis (OA) in the knee joints of mice after administration of recombinant human osteoprotegerin (rhHuOPG) or vehicle. Intraarticular injection of rhHuOPG (rhOPG) or vehicle alone (control) into mouse knee joints was performed 5 times a week beginning the day after surgery and continuing for 4 weeks thereafter. **A**, Sections of articular cartilage from the knee joints of control (**a** and **c**) or OPG-treated (**b** and **d**) mice were stained with Safranin O to detect proteoglycans. Degenerative changes in the articular cartilage were reduced in OPG-treated mice as compared with the controls (**a–d**). Morphologic features of the subchondral bone were similar in the control (**c**) and OPG-treated (**d**) mice. Boxed and labeled areas in **a** and **b** are shown at higher magnification in **c** and **d**, respectively. **B** and **C**, Histologic changes in the OA joints were assigned Mankin scores (**B**) and cartilage destruction scores (**C**). Scores in the OPG-treated mice were less than 50% of those in the controls. **D**, Mean cartilage thickness in OA joints was measured as the average distance from the superficial layer to the osteochondral junction of the tibia. The mean cartilage thickness was significantly reduced in the OPG-treated group as compared with the controls. Values in **B–D** are the mean and SD of 7 mice per group. * = $P < 0.05$ by Mann-Whitney U test.

clustering of chondrocytes (Figure 3A, parts a–d). At this time point, Mankin scores (Figure 3B) and cartilage destruction scores (Figure 3C) in OPG-treated animals were ~50% of those seen in the control group ($P < 0.05$). Thus, OPG administration significantly protected the articular cartilage thickness (Figure 3D). The structure and bone volume (mean \pm SD bone volume/total volume $56.79 \pm 12\%$ in the control group versus $59.18 \pm 22\%$ in the OPG-treated group) of subchondral bone were not affected by intraarticular administration of OPG, as was expected (Figure 3A, parts c and d). The number of osteoclasts in the subchondral region (mean \pm SD $4.4 \pm 1.1/\text{mm}$ in the control group versus $4.4 \pm 1.2/\text{mm}$ in the OPG-treated group) was also similar between these groups, indicating that exogenous OPG protected the articular cartilage from degradation in a manner that was independent of the protection of subchondral bone.

Prevention of chondrocyte apoptosis in an experimental OA model by exogenous OPG administration. Chondrocyte apoptosis is increased in OA cartilage and is anatomically linked to proteoglycan depletion (2,3). These observations prompted us to investigate the effect of OPG administration on chondrocyte apoptosis. We injected rHuOPG or vehicle alone into the knee joints of C57BL/6J mice with surgically induced OA for 5 days a week beginning on postoperative day 1 and continuing for 4 weeks. Knee joints were then examined after TUNEL staining. TUNEL-positive cells were abundant among the chondrocytes present in control mice with surgically induced OA that had received only PBS injection (Figure 4A, part a). In contrast, TUNEL-positive cells were rare in joints injected with rHuOPG (Figure 4A, part b). The number of TUNEL-positive chondrocytes in the joints of the OPG-treated group was almost one-third of that in the control group ($P < 0.05$) (Figure 4B). These data indicated that the anti-apoptotic effect of OPG functions to protect the articular cartilage.

Expression of OPG and TRAIL in chondrocytes of mice with experimentally induced OA. Immunohistochemical analysis indicated that while OPG could be detected in synovial cells and chondrocytes, OPG protein was observed at higher levels in the peripheral layers of OA joint cartilage and synovial tissue following OPG administration (Figure 5A). One of the OPG ligands, TRAIL, has also been observed in chondrocytes and synovial tissues from OA joints (18,19,22). Moreover, TRAIL is known to induce chondrocyte apoptosis in vitro (22,23). Our immunohistochemical analysis also

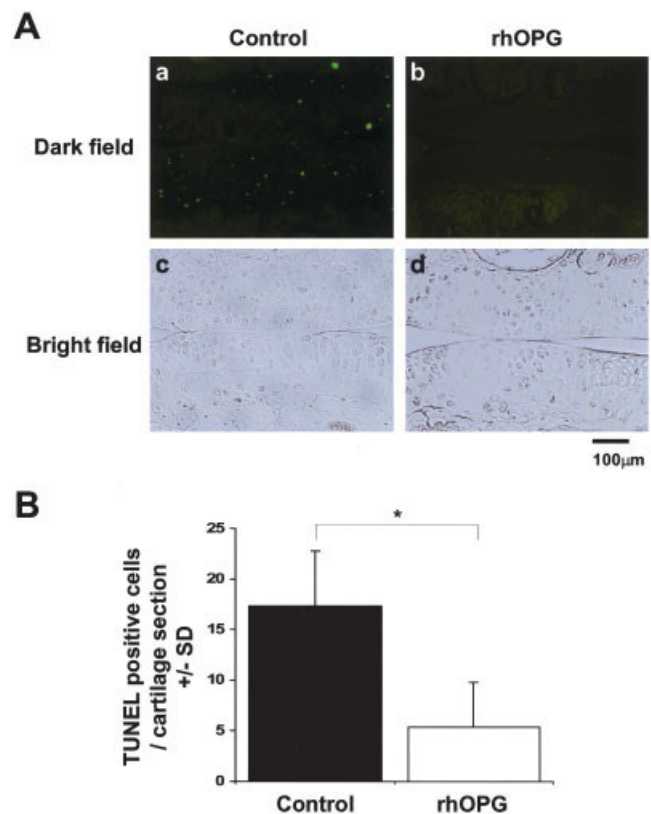


Figure 4. Analysis of apoptosis in TUNEL-stained sections of cartilage from mice with surgically induced osteoarthritis (OA) after intraarticular administration of recombinant human osteoprotegerin (rHuOPG) or vehicle. Intraarticular injection of rHuOPG (rhOPG) or vehicle alone (control) into mouse knee joints was performed 5 times a week beginning the day after surgery and continuing for 4 weeks thereafter. **A**, TUNEL staining of OA cartilage sections was examined by darkfield (a and b) and brightfield (c and d) microscopy. The number of TUNEL-positive cells was increased in knee joint cartilage from control mice (a) but was significantly reduced in knee joint cartilage from mice injected with rHuOPG (b). **B**, The number of TUNEL-positive cells per section of OA cartilage was determined under fluorescence microscopy. Values are the mean and SD of 5 mice per group. * = $P < 0.05$ by Mann-Whitney U test.

indicated that TRAIL was expressed in chondrocytes. TRAIL-positive chondrocytes were primarily detected in the periphery of the joint cartilage in OPG-treated animals (Figure 5B, part b), whereas they were present in the middle and deep zones of the joint cartilage, with hypertrophic differentiation, in control animals (Figure 5B, part a). The expression patterns of OPG and TRAIL overlapped significantly in the OPG-treated group (Figure 5A, part f, and Figure 5B, part b). These results suggest that exogenous OPG protected the articular chondrocytes by inhibiting TRAIL-induced apoptosis in vivo.

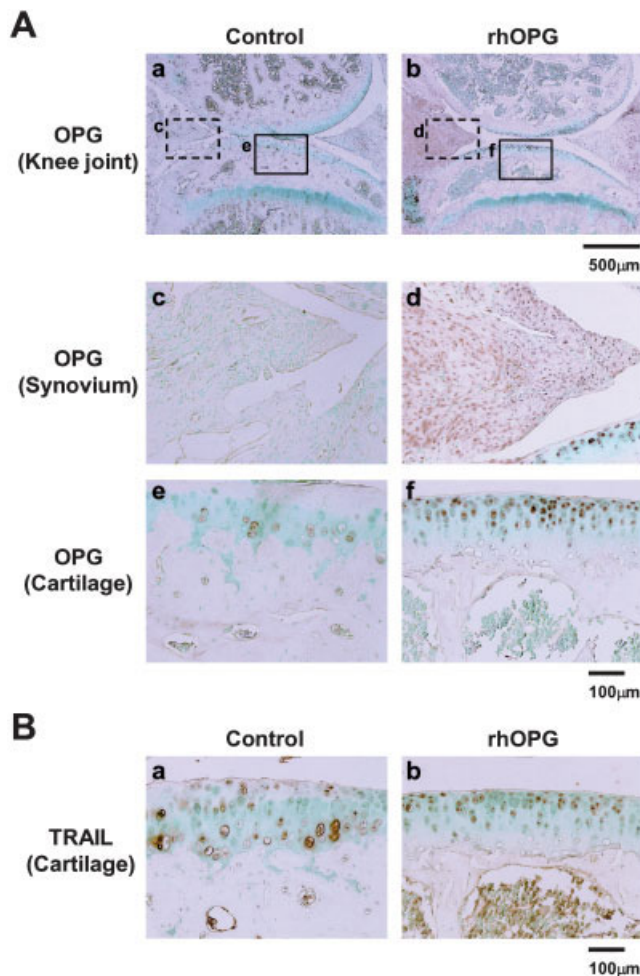


Figure 5. Expression of osteoprotegerin (OPG) and TRAIL in chondrocytes from mice with surgically induced osteoarthritis (OA) after intraarticular administration of recombinant human osteoprotegerin (rHuOPG) or vehicle. Intraarticular injection of rHuOPG (rhOPG) or vehicle alone (control) into mouse knee joints was performed 5 times a week beginning the day after surgery and continuing for 4 weeks thereafter. **A**, OPG protein was present at high levels in synovial cells (**d**) and cartilage chondrocytes (**f**) in sections from rHuOPG-treated mice, whereas only trace amounts of OPG protein were observed in control animals (**a**, **c**, and **e**). Control chondrocytes (**e**) showed hypertrophic changes as a result of OA progression, as compared with chondrocytes from rHuOPG-treated mice (**f**). Boxed and labeled areas in **a** and **b** are shown at higher magnification in **c** and **e** and in **d** and **f**, respectively. **B**, TRAIL expression was also observed in the cartilage chondrocytes of control (**a**) and OPG-treated (**b**) mice with surgically induced OA.

DISCUSSION

This study revealed the effects of reductions of endogenous OPG activity on the progression of instability-induced cartilage destruction in mice heterozygous for an OPG gene mutation. Previous studies have indicated that OPG^{-/-} mice demonstrate severe

destruction of growth plate cartilage and growth plate cartilage loss-induced epiphyseal and metaphyseal trabecular bone formation (14,15). Histologic examination revealed that at 8 weeks of age, OPG^{-/-} mice exhibited irregular articular cartilage, including markedly thinned cartilage layers and invasion of the vasculature into the calcified layer (Figure 1C); in contrast, the articular cartilage of young adult OPG^{+/-} mice was intact at this age (Figure 1B). Although OPG^{+/-} mice exhibit a significant loss of total bone density compared with their WT littermates by the age of 6 months (mean \pm SD 487.6 \pm 27 mg/cm³ versus 521.2 \pm 29 mg/cm³; $P < 0.05$), bone volume is comparable in young adult OPG^{+/-} and WT mice at the ages evaluated in these experiments (13).

Since subchondral bone metabolism is important for the maintenance of articular cartilage (24), we chose young adult OPG^{+/-} mice as our experimental OA model in which to examine the effects of OPG haploinsufficiency in chondrocytes on cartilage metabolism. After induction of OA in OPG^{+/-} mice and their WT littermates, the OPG^{+/-} mice exhibited severe articular cartilage degeneration as compared with the WT mice. This observation indicated that adequate OPG was required for the maintenance of cartilage and the prevention of mechanical stress-induced cartilage degeneration. The observation that the subchondral bone structures in OPG^{+/-} mice and their WT littermates were histologically indistinguishable suggested that endogenous OPG likely plays only a minimal role in subchondral bone turnover in the acute phase of OA progression.

We also demonstrated that intraarticular administration of exogenous OPG effectively protected the articular cartilage from degradation. Although previous reports suggested a protective effect of OPG on cartilage in arthritis models, systemically administered OPG protected both the articular cartilage and articular bone (25–28). Therefore, it was unclear whether in arthritis, OPG affected chondrocyte metabolism directly or indirectly through osteoclastic bone erosion of subchondral bone via RANK signaling (27). To study this, we administered rHuOPG intraarticularly, which revealed the direct effect of this substance on articular chondrocyte metabolism.

TRAIL, one of the ligands for OPG, was also expressed in chondrocytes, as reported previously (22), regardless of OPG administration. OPG binds to TRAIL, a death domain-containing type II transmembrane protein member of the TNF superfamily (29,30). TRAIL constitutes a family of ligands that transduces death signals through a death domain-containing receptor (31). OPG inhibits TRAIL-induced apoptosis in

Jurkat cells (29) and endothelial cells (32). TRAIL also induces chondrocyte apoptosis *in vitro*; its expression is increased in the chondrocytes of rats with experimentally induced OA (22). In this study, OPG was observed in TRAIL-expressing chondrocytes and synovium in OPG-treated animals. These findings are consistent with the hypothesis that exogenously administered OPG prevents chondrocyte apoptosis in our model of surgically induced OA. Although inhibition of the TRAIL pathway by OPG may be one of the potent mechanisms of OA prevention of OPG, the target of OPG is still to be elucidated.

The concentration of OPG we used was determined according to previous observations in endothelial cells (32), where endothelial cell apoptosis induced by serum deprivation was blocked by OPG concentrations $>0.5 \mu\text{g/ml}$. The appropriate concentration of OPG will need to be determined for any future clinical applications.

TUNEL staining revealed that OPG administration significantly suppressed chondrocyte apoptosis. Exogenous OPG was confined to the cartilage and synovium; the subchondral bone volume and vascular invasion were not affected by intraarticular OPG administration. These observations indicated that the chondroprotective effect of OPG was independent of the subchondral bone protection.

To investigate the function of OPG in articular chondrocyte metabolism, we used an experimental stress-induced murine model of OA that is reproducible and closely resembles OA in humans (1). The combination of medial collateral ligament transection and medial meniscectomy induced medial tibial cartilage destruction within 4 weeks. The early changes in the articular cartilage after surgery resulted from a defect in the superficial zone and corresponded to a decrease in Safranin O staining. These initial findings were followed by progressive cartilage destruction in a manner identical to that reported for OA pathology in humans as determined by arthroscopic and histologic analyses (33,34). Along with the catabolic changes, the anabolic reactions of chondrocyte proliferation and subchondral sclerosis were also observed in our model.

The findings of this study cannot rule out the importance of subchondral bone metabolism in articular cartilage protection. Although articular chondrocytes do not have an intact RANK signaling apparatus, RANKL-deficient mice have been shown to be protected from bone erosion in a serum-transfer model of arthritis (35), indicating that OPG protects articular cartilage via maintenance of subchondral bone. In the rat inflamma-

tory arthritis model, systemic administration of OPG preserved articular cartilage (25,27). While significant chondroprotection was observed in mildly inflamed joints following administration of Fc-OPG, no significant protection was seen in the more severely inflamed joints of rats treated with OPG. Similarly, in a serum-transfer model of arthritis, RANKL-deficient mice exhibited cartilage loss despite protection from bone erosion and despite partial inhibition of cartilage destruction (35). In these experiments, OPG was administered systemically; intraarticular OPG levels were not affected (27). Administration of OPG both systemically and intraarticularly may have an additive effect on chondrocyte protection in arthritis.

Bone resorption inhibitors, including bisphosphonates and calcitonin, have been shown to reduce cartilage degradation in experimental arthritis models (36,37). In a rat model of stress-induced OA, alendronate was shown to have a partial chondroprotective effect during the early stages of disease (36). Although the direct target of bisphosphonates is not known, bisphosphonate inhibition of subchondral bone turnover may be a candidate mechanism that would explain this phenomenon. Bisphosphonates may indirectly reduce cartilage breakdown by altering the distribution of mechanical stress. Bisphosphonate inhibition of osteoclastic bone resorption may also reduce the release of inflammatory cytokines and growth factors (36).

In conclusion, we have demonstrated that rHuOPG prevents cartilage destruction in an experimental murine model of OA and that endogenous OPG protects against cartilage destruction during the progression of OA. Our results provide clues that OPG prevents chondrocyte apoptosis via a direct effect on chondrocytes *in vivo*. These results support a potential therapeutic application of rHuOPG in human OA.

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AUTHOR CONTRIBUTIONS

Dr. Asou had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Asou, Chung, Kawaguchi.

Acquisition of data. Shimizu, Itoh.

Analysis and interpretation of data. Asou, Chung, Kawaguchi, Muneta.

Manuscript preparation. Shimizu, Asou, Shinomiya.

Statistical analysis. Shimizu.

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