Accelerated Development of Aging-Associated and Instability-Induced Osteoarthritis in Osteopontin-Deficient Mice

INTRODUCTION: The main causes of OA are considered to be aging and excessive mechanical stress against joints due to joint instability or obesity. However, the mechanisms for the development of OA have not been clearly elucidated. The synthesis and degradation of cartilage extracellular matrix (ECM) is particularly regulated by mechanisms that depend on interactions of chondrocytes with the ECM proteins. Thus, it is increasingly recognized that these ECM proteins contribute to maintaining articular cartilage homeostasis and are also involved in the pathogenesis of OA. Osteopontin (OPN) was originally identified as a noncollagenous ECM protein in bone. Previous studies have demonstrated that the expression of OPN proteins and genes correlates well with the severity of OA such as disintegration of the cartilaginous matrix. However, the functional roles of OPN in the development of cartilage degradation have not been elucidated. The hypothesis of this study was that OPN would regulate cartilage degradation in response to both aging and excessive mechanical stress against a joint. To test this hypothesis, we performed a functional analysis of OPN in the cartilage degradation process using OPN-deficient (OPN−/−) mice. The objectives of this study were to determine the functional role of OPN in the development of aging-associated and instability-induced OA, and to clarify the relationships between OPN and matrix metalloprotease (MMP) expressions in the cartilage degradation process.

METHODS: Animals. Six-week-old male OPN−/− mice [1], backcrossed nine generations to C57BL/6 mice and age-matched male C57BL/6 (wild type, WT) mice as a control were used in the present experiments approved by the institutional animal care committee.

Induction of OA model: An instability-induced OA model was created in 8-week-old WT or OPN−/− mice. The right knee joint was destabilized by resection of the medial collateral ligament and removal of the cranial half of the medial meniscus [2]. Mice were kept for an additional 8 weeks after surgery. As an age-associated model, WT or OPN−/− mice were followed for the spontaneous development of OA up to an age of 15 months [3].

Histological and immunohistochemical evaluation: Serial sagittal sections 5 µm thick were cut through the knee joints. Sections were stained with Safranin O-fast green to assess the presence of proteoglycan and to measure the cartilage layer thickness in OA joints. The severity of OA changes was evaluated according to the Mankin scoring system. For the detection of OPN protein, the sections were stained with anti-mouse OPN rabbit IgG.

In vitro cartilage degradation: Femoral head articular cartilage was harvested from 4-week-old WT and OPN−/− mice. Cartilage samples were cultured as explants for 48 hours in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum. For the induction of cartilage degradation, the explants were then cultured for an additional 72 hours in serum-free DMEM. Conditioned medium and cultured cartilage were collected at the end of the culture period. To quantify the proteoglycan release from cartilage explants with cartilage degradation, the proteoglycan content in the conditioned medium and cultured cartilage were measured using a dimethylmethylen blue assay.

cDNA array and real-time RT-PCR analyses: We collected the femoral head articular cartilages cultured in DMEM as mentioned above and uncultured articular cartilages. After the cDNA synthesis and biotin d-UTP labeling, GEArray Q Series Mouse Extracellular Matrix Adhesion & Molecule Gene Array (Super Array Bioscience, Frederick, MD) was used to analyze the gene expression profile of matrix metalloprotease (MMP). Furthermore, real-time RT-PCR was performed to monitor the MMP expression.

Statistical analysis: All data were represented as mean ± SD. Significant differences between the two groups were determined using an unpaired Student’s t-test. The significance level was set at 0.05.

RESULTS: Localization of OPN in cartilage: In cartilage of WT mice, OPN expression was detected in calcified zones, but absent in uncultured zones of superficial layers after sham operations (Fig.1E). With OA progression, strong expression of OPN extended to uncultured areas of cartilage (Fig.1F).

Appearance of severe OAr lesions in OPN−/− mice: The Mankin score at 8 weeks postoperatively was significantly higher in OPN−/− mice than WT mice (n = 6, 11.0 ± 1.1 vs 9.7 ± 0.8, P < 0.05). This structural alteration was reflected by the finding that the tibia and femur cartilage layer thickness in OPN−/− mice, relative to their sham side, was significantly thinner than those in the WT mice (n = 6, 34.6 ± 9.3% vs 49.9 ± 10.8 in the tibia, 66.6 ± 16.9% vs 84.4 ± 8.0 in the femur, p < 0.05; Fig.1 and 2).

Facilitated development of aging-associated spontaneous OA lesions in OPN−/− mice: In the knee joints obtained from 2 month-old mice, no significant difference in the Mankin scores was detected between OPN−/− and WT mice (n = 8). However, at 15 months, the accelerated progression of OA lesions was detected in OPN−/− mice, as compared with WT mice (n = 10, 10.2 ± 2.7 vs 7.9 ± 1.9, p < 0.05; Fig.3).

Accelerated cartilage degradation and augmented expression of cartilage MMP-13 mRNA in OPN−/− mice during in vitro culture: A significant increase in total proteoglycan release from OPN−/− cartilage was observed under in vitro conditions, compared with that from WT mice (n = 8, 20.3% ± 5.0 vs 14.4 ± 4.8, p < 0.05). Explant histology also revealed that Safranin O-fast green staining was reduced in OPN−/− cartilage (Fig.4A). Among 18 distinct MMP genes (MMP-2, 3, 7-17, 19, 20, 23 and 24), the expression of MMP-3 and -13 genes were elevated during the in vitro culture. In OPN−/− mice, a significant up-regulation in cartilage MMP-13 gene was detected, as compared to WT mice. Finally, real-time RT-PCR analysis suggested that cartilage MMP-13 mRNA expression was significantly augmented in OPN−/− mice cartilage, as compared to that in WT mice (n = 3, 3.0 ± 0.8 vs 1.1 ± 0.7, p < 0.05) (Fig.4B).

DISCUSSION: The purpose of this study was to elucidate the functional roles of OPN in OA pathogenesis using both instability-induced and aging-associated OA models. This study showed that OPN was involved in development of OA related to these major etiologic factors. A therapeutic effect of OPN administration on cartilage degradation was not directly proven in this study. However, we may conclude that OPN is a crucial intrinsic regulator of cartilage degradation via its inhibitory effect on MMP-13 expression and is a potential molecule for preventing cartilage degradation. Future study will be performed to develop a novel strategy based on the current results for the treatment of OA.