Lectin-like, oxidized low-density lipoprotein receptor-1-deficient mice show resistance to age-related knee osteoarthritis: A Mini review

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ABSTRACT

The lectin-like, oxidized low-density lipoprotein (ox-LDL) receptor-1 (LOX-1)/ox-LDL system contributes to atherosclerosis and thus may play a role in cartilage degeneration. The purpose of this study was to determine whether the LOX-1/ox-LDL system contributes to the pathogenesis of age-related osteoarthritis (OA) in vivo, using LOX-1 knockout (LOX-1 KO) mice. Knee cartilage samples from 6-, 12-, and 18-month-old (n = 10 per group) C57Bl/6 wild-type (WT) and LOX-1 KO mice were compared for OA-related changes with Safranin-O staining. At 12 and 18 months, the OA changes were significantly reduced in LOX-1 KO mice compared to those in WT mice. Moreover, an immunohistological analysis showed that the expression levels of Runt-related transcription factor-2, type-X collagen, and matrix metalloproteinase-13 in the articular chondrocytes were significantly decreased in LOX-1 KO mice compared with those in WT mice. Overall, this study indicates that the LOX-1/ox-LDL system in chondrocytes plays a role in the pathogenesis of age-related knee OA, highlighting a novel potential target for preventing OA progression.

Introduction

Oxidized low-density lipoprotein (ox-LDL) is produced by LDL oxidation at sites of oxidative stress and inflammation, and thus, plays an important role in the pathogenesis of atherosclerosis1. Lectin-like ox-LDL receptor-1 (LOX-1) is an important receptor for ox-LDL, which was originally cloned from cultured bovine aortic endothelial cells2. LOX-1 has also been widely shown to participate in degeneration of the articular cartilage both in vivo and in vitro3-12. However, the influence of LOX-1 on the age-related progression of OA in vivo remains unclear. In particular, we previously reported that the LOX-1/ox-LDL system induces chondrocyte hypertrophy in vitro via Runt-related transcription factor-2 (Runx2) expression, and that LOX-1 knockdown reduced Runx2 expression10. These previous findings led us to hypothesize that the LOX-1/ox-LDL system is involved in age-related cartilage degeneration via chondrocyte hypertrophy in vivo. Therefore, in the present study, we conducted histological observations of OA development in the articular cartilages of wild-type (WT) and LOX-1 knockout (KO) mice, which were maintained for up to 18 months of age.

Material and Methods

Mice

LOX-1+/+ C57BL/6 Jcl mice (WT) were provided by Nihon CLEA (Tokyo, Japan). The LOX-1−/− C57BL/6 Jcl mice (KO) were originally generated by Sawamura etal.13 and provided by the National Cerebral
and Cardiovascular Center (Osaka, Japan). Mice were housed in cages with access to food and water ad libitum in a temperature-controlled room with a 12-h dark/12-h light cycle. All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of our hospital.

**Safranin O staining and immunohistochemistry**

To monitor age-related OA, articular cartilage samples of WT and LOX-1 KO mice were examined at 6, 12, and 18 months of age (n = 10, in each). We also recorded the body weights of the mice at the same three time points.

The knee samples were stained with Safranin O (WAKO, Japan) for histological evaluation of cartilage degeneration during OA progression at 6, 12, and 18 months of age. Knee OA was evaluated using the OARSI scoring system which is a semi-quantitative scoring system. We also divided the prevalence of OA by the OARSI score in both WT and LOX-1 KO mice at each time point.

To visualize LOX-1 and ox-LDL expression and investigate the involvement of cartilage cell hypertrophy in OA progression, we performed immunohistochemistry against LOX-1, ox-LDL, Runx2, and type-X collagen (COL X). Immunohistochemistry was also used to detect matrix metalloproteinase-13 (MMP-13), which is a cartilage matrix-degrading enzyme.

**Statistical analysis**

All data are expressed as the mean ± standard deviation. The scores of each group were compared using Student’s t-test. P-values of less than 0.05 were considered statistically significant.

**Results**

**OA development**

At 6 months age, there was no significant difference between the OA changes of WT and LOX-1 KO mice. However, at 12 and 18 months, there were significant differences between OA changes of the two groups, and LOX-1 KO mice showed significantly reduced scores reflecting OA than WT mice. The mean body weights were not significantly different between WT and LOX-1 KO mice at any time point tested.

**Prevalence of OA**

The prevalence of OA in LOX-1 KO mice was lower than that in WT mice at 12 and 18 months of age (40% vs. 70%, and 70% vs. 90%, respectively; n = 10).

**Time course of LOX-1, ox-LDL, Runx2, COL X, and MMP-13 expression in the cartilage**

In the WT mice, the staining intensity for LOX-1 and ox-LDL increased at 12 and 18 months of age, compared to that detected at 6 months. However, no LOX-1 or ox-LDL staining was observed in articular cartilage sections of LOX-1 KO mice. Similar results were obtained for Runx2, COLX, and MMP-13 immunohistochemical staining at 6 months in the two groups. However, at 12 and 18 months, LOX-1, ox-LDL, Runx2, and MMP-13 expression was increased in both groups of mice, although significantly lower expression was observed in LOX-1 KO mice.

**Discussion**

We demonstrated that the loss of LOX-1 prevented the progression of age-related cartilage degeneration in the murine knee. Furthermore, LOX-1/ox-LDL expression increased with OA progression in WT mice. These findings suggest that LOX-1 plays an important role in cartilage degeneration during age-related OA progression in vivo.

Chondrocyte senescence is known to drive the development of age-related OA. Interestingly, telomere shortening has also been detected in chondrocytes isolated from the articular cartilage of older adults. We previously reported that ox-LDL binding to LOX-1 promotes stress-induced premature senescence in chondrocytes, resulting in suppressed telomerase activity. Furthermore, oxidative changes are important for chondocyte senescence in cartilage degeneration. The ox-LDL–LOX-1 interaction induces reactive oxygen species (ROS) production in bovine articular chondrocytes.

Recent studies have indicated that endochondral ossification signals, which cause hypertrophy and apoptosis in chondrocytes, are involved in age-related OA development. A hypertrophic phenotype was also observed in an age-related OA mouse model and in human OA chondrocytes. We previously reported that ox-LDL–LOX-1 binding induces ROS production, and ROS were recently shown to induce chondrocyte hypertrophy. It is also well established that cartilage degeneration involves various enzymes, including MMPs. Specifically, MMP-13 is a major cartilage degradation enzyme that contributes to OA progression, particularly for that occurring in age-related OA.

Our present results expand on these known mechanisms by pointing to a novel role of the LOX-1/ox-LDL system in cartilage degeneration via mediating MMP-13 expression in vivo. Since LOX-1 deficiency suppressed OA development in a murine model of age-related OA, addressing or treating atherosclerosis may help to prevent OA.

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**Conflicts of interest**

The authors have no conflict of interest to declare.
References


