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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 atherosclerosis[1]. Lectin-like ox-LDL receptor-1 (LOX-1) is an important receptor for ox-LDL, which was originally cloned from cultured bovine aortic endothelial cells[2]. LOX-1 has also been widely shown to participate in degeneration of the articular cartilage both *in vivo* and *in vitro*[3–12]. 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 expression[10]. 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 et al.[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[2]. Knee OA was evaluated using the OARSI scoring system which is a semi-quantitative scoring system[14]. 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[15, 16]. Interestingly, telomere shortening has also been detected in chondrocytes isolated from the articular cartilage of older adults[17]. We previously reported that ox-LDL binding to LOX-1 promotes stress-induced premature senescence in chondrocytes, resulting in suppressed telomerase activity[9]. Furthermore, oxidative changes are important for chondrocyte senescence in cartilage degeneration[18, 19]. The ox-LDL–LOX-1 interaction induces reactive oxygen species (ROS) production in bovine articular chondrocytes[8].

Recent studies have indicated that endochondral ossification signals, which cause hypertrophy and apoptosis in chondrocytes, are involved in age-related OA development[20, 21]. A hypertrophic phenotype was also observed in an age-related OA mouse model and in human OA chondrocytes[22, 23]. We previously reported that ox-LDL-LOX-1 binding induces ROS production[8], and ROS were recently shown to induce chondrocyte hypertrophy[24]. It is also well established that cartilage degeneration involves various enzymes, including MMPs[25, 26]. Specifically, MMP-13 is a major cartilage degradation enzyme that contributes to OA progression[27, 28], particularly for that occurring in age-related OA[29, 30].

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.

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