



Mini Review

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LOX-1-deficient mice are resistant to zymosan-induced arthritis: A mini review

Kazuhiko Hashimoto^{1*}, Yutaka Oda¹, Kotaro Yamagishi¹, Ichiro Tsukamoto¹, Masao Akagi¹
Department of Orthopedic Surgery, Kindai University Hospital, Osaka-Sayama City, Osaka 589-8511, Japan

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*Correspondence:

Dr. Kazuhiko Hashimoto, Department of Orthopedic Surgery,
Kindai University Hospital 377-2 Ohno-Higashi, Osaka-
Sayama City, Osaka 589-8511, Japan;
Telephone: +81-072-366-0221;
Fax: +81-072-366-0206;
Email: hazzhiko@med.kindai.ac.jp

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Keywords

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Zymosan
Arthritis
Osteoarthritis
Atherosclerosis

ABSTRACT

Background: Some reports have shown that metabolic syndrome, including hypertension, hyperlipidemia, and diabetes mellitus, contributes to osteoarthritis (OA) development. Further, lectin-like oxidized low-density lipoprotein (ox-LDL) and ox-LDL receptor-1 (LOX-1), which contributes to atherosclerosis, have also been considered factors contributing to OA development. Several studies have suggested that the LOX-1/ox-LDL system is involved in OA development *in vitro*. We have suggested the same and conducted *in vitro* and *in vivo* studies to validate this concept. However, the role of the LOX-1/ox-LDL system in OA development has not been clarified. This study aimed to identify the mechanism of the LOX-1/ox-LDL system to clarify OA development.

Methods: A zymosan-induced arthritis model was used to identify the mechanism of the LOX-1/ox-LDL system using LOX-1-knockout (KO) mice. Zymosan was administered via the intra-articular route to induce arthritis.

Results: From our experiment, we found that the LOX-1/ox-LDL system contributes to OA development through matrix metalloproteinase-3.

Conclusion: Our findings suggest that the treatment of abnormal lipid metabolism may contribute to the prevention and suppression of arthritis.

Introduction

Osteoarthritis (OA), which is characterized by wear and tear of the articular cartilage, was previously believed to be caused by mechanical stress¹. However, recent studies have suggested the role of other systemic factors². One of them is metabolic syndrome^{3, 4}, including hypertension, hyperlipidemia, and diabetes mellitus, all of which lead to atherosclerosis⁵. Hyperlipidemia is caused by various factors, such as low levels of high-density lipoprotein (HDL), high levels of low-density lipoprotein (LDL), and triglycerides⁶. In recent years, Sawamura et al. have reported that lectin-like, oxidized low-density lipoprotein (ox-LDL) and ox-LDL receptor-1 (LOX-1) contribute to atherosclerosis⁷. Moreover, recent studies revealed the epidemiological relationship between atherosclerosis and OA^{8, 9}. However, how atherosclerosis contributes to OA has not been clarified in detail. Interestingly, subsequent studies have shown that the LOX-1/ox-LDL system contributes to the development of rheumatoid arthritis¹⁰⁻¹². Previously, we described that ox-LDL binding to LOX-1 induces stress-induced premature senescence of chondrocytes and results in suppression of telomerase activity by inactivating the PI3K/Akt pathway¹³. Recently, we also demonstrated that OA development is downregulated in LOX-1 knockout (KO)

mice¹⁴. Moreover, development of age-related OA was also found to be reduced in LOX-1 KO mice¹⁵.

Generally, arthritis such as rheumatoid arthritis differs from OA, as OA is induced by mechanical stress^{16,17} while arthritis is mainly induced by inflammation. Although some studies revealed the relationship between arthritis and LOX-1/ox-LDL^{12,18}, the roles of LOX-1/ox-LDL system in arthritis are still not clarified in detail. Therefore, this study aimed to clarify the role of the LOX-1/ox-LDL system in the development of arthritis. In other words, we tried to identify the role of LOX-1/ox-LDL in inflammatory changes such as synovitis. We also hypothesized that LOX-1 KO would inhibit arthritis-associated changes, which include synovitis and cartilage degeneration.

Summary of the current study

Material and methods

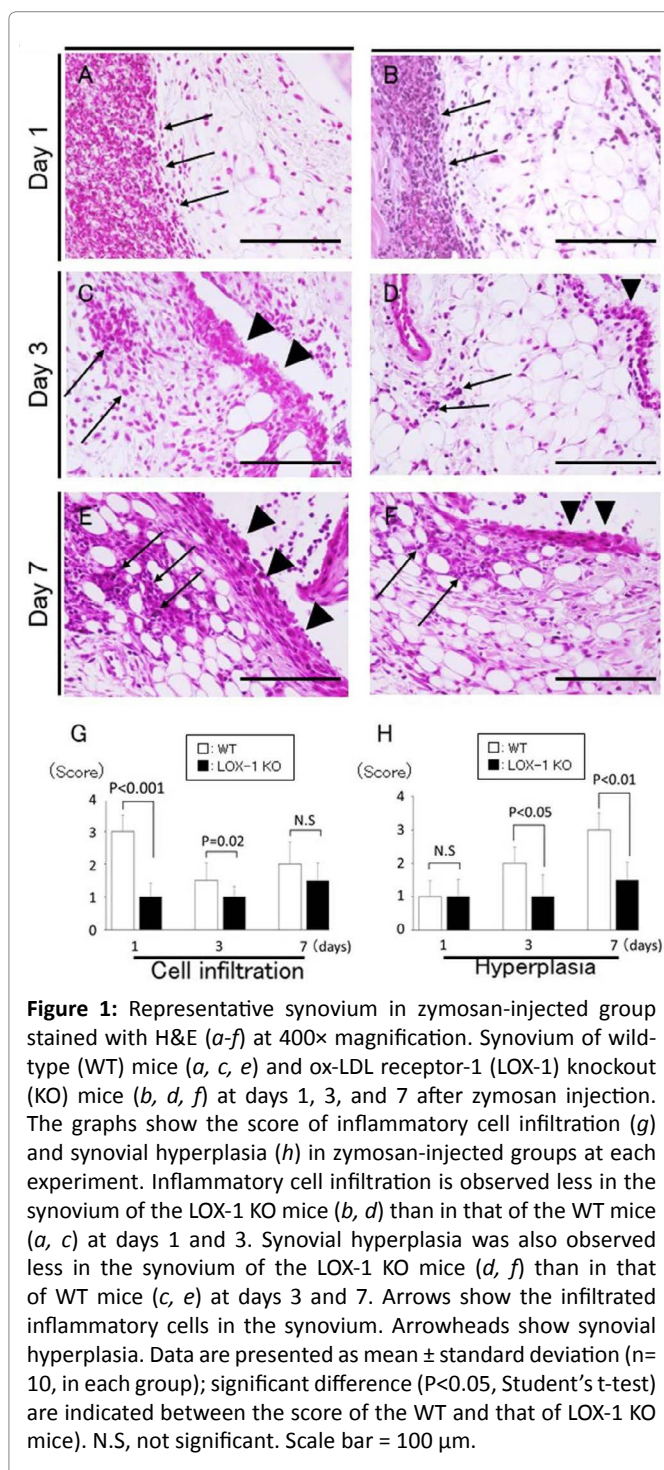
We induced arthritis by administering zymosan intra-articularly. Zymosan is an insoluble fraction of yeast cell walls, which are composed predominantly of polysaccharides. It induces arthritis, which is characterized by cartilage degeneration and synovitis in rodents when injected intraperitoneally or intra-articularly. Zymosan is often used to induce arthritis, especially to induce synovial inflammation¹⁹. Zymosan was used, as it is the most commonly used method. In addition, in the this study, the model was used to evaluate the role of LOX-1/ox-LDL system on arthritis, especially synovitis.

Wild-type (WT) and LOX-1 KO mice (n=10, in each) were used, and we injected with zymosan to induce arthritis (180 µg/6 µL; Sigma-Aldrich, St. Louis, MO, USA). We observed at the time point of 24, 48 and 72 hours after zymosan injection. As a control group, we conducted saline injection to contralateral knee joint with observation at the same time point. Immunohistochemical analysis was performed to evaluate the expression of matrix metalloproteinase-1 and 3 (MMP-1 and 3) and to determine its role in arthritis development.

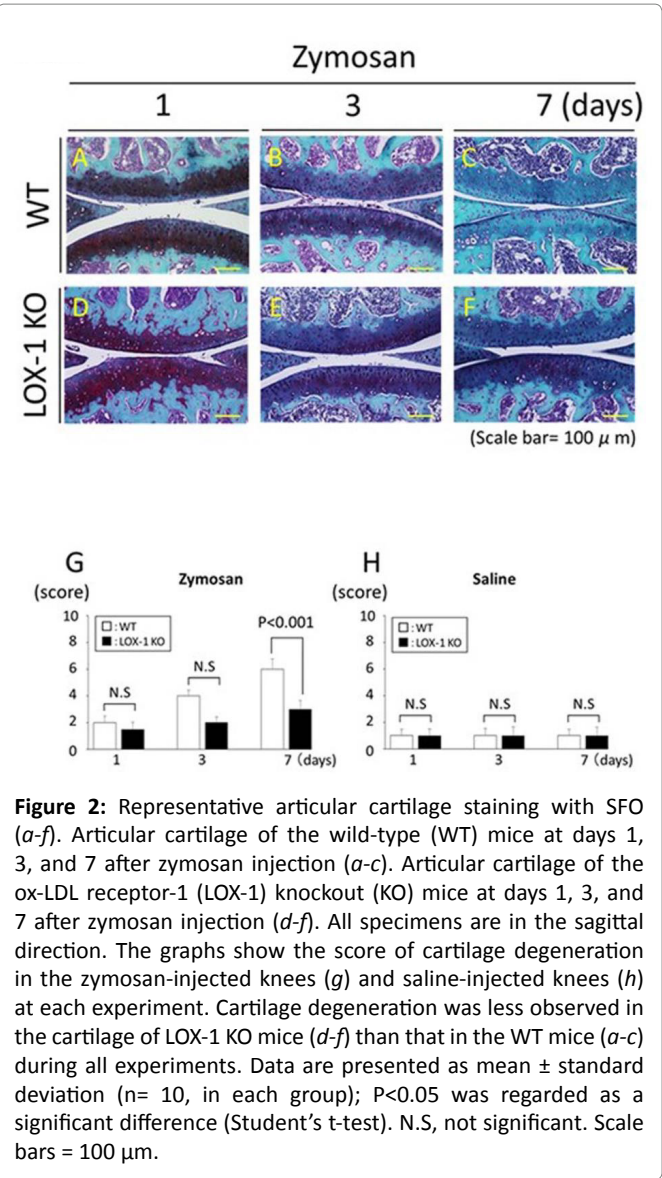
All data have been presented as mean ± standard deviation. The scores of each group (n= 10) were compared using Student's t-test. P-values less than 0.05 were considered significant. All data were analyzed with Stat Mate (Atms, Tokyo, Japan) software for Windows, version 4.01.15,16). The correlation between the LOX-1 or ox-LDL positive cell rate and cartilage degeneration score was examined using Pearson's correlation (Excel 2010, Microsoft, Tokyo, Japan).

Results

We compared zymosan-induced arthritis in wild-type (WT) and LOX-1 KO mice and found it to be more



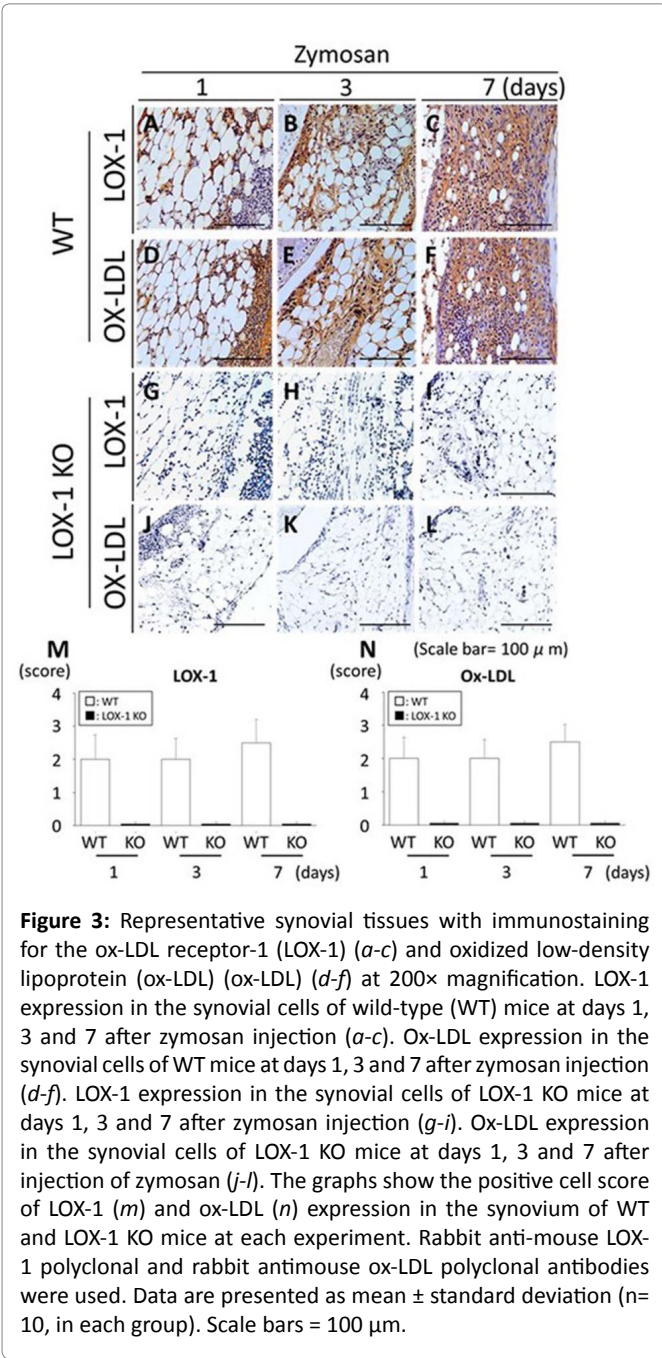
severe in the former (Figures 1, 2). Interestingly, both zymosan-induced cartilage degeneration and synovitis were significantly reduced in LOX-1 KO mice compared to WT mice (Figures 1, 2). In the saline-injected control groups, no difference was observed between WT and LOX-1 KO mice (data not shown). Immunohistochemical staining revealed LOX-1 and ox-LDL expression in the chondrocytes and inflammatory synovial cells in WT mice (Figures 3, 4) but not in LOX-1 KO mice (data not



shown). These findings suggest that LOX-1/ox-LDL in the chondrocytes and inflammatory synovial cells are involved in the development of arthritis. In our evaluation of the expression of matrix metalloproteinase-1 and 3 (MMP-1 and 3) by immunohistochemical analysis, MMP-1 was not detected in both WT and LOX-1 KO mice (data not shown); however, MMP-3 expression was significantly higher in the chondrocytes and inflammatory synovial cells of WT mice than in those of LOX-1 KO mice (Figures 5, 6).

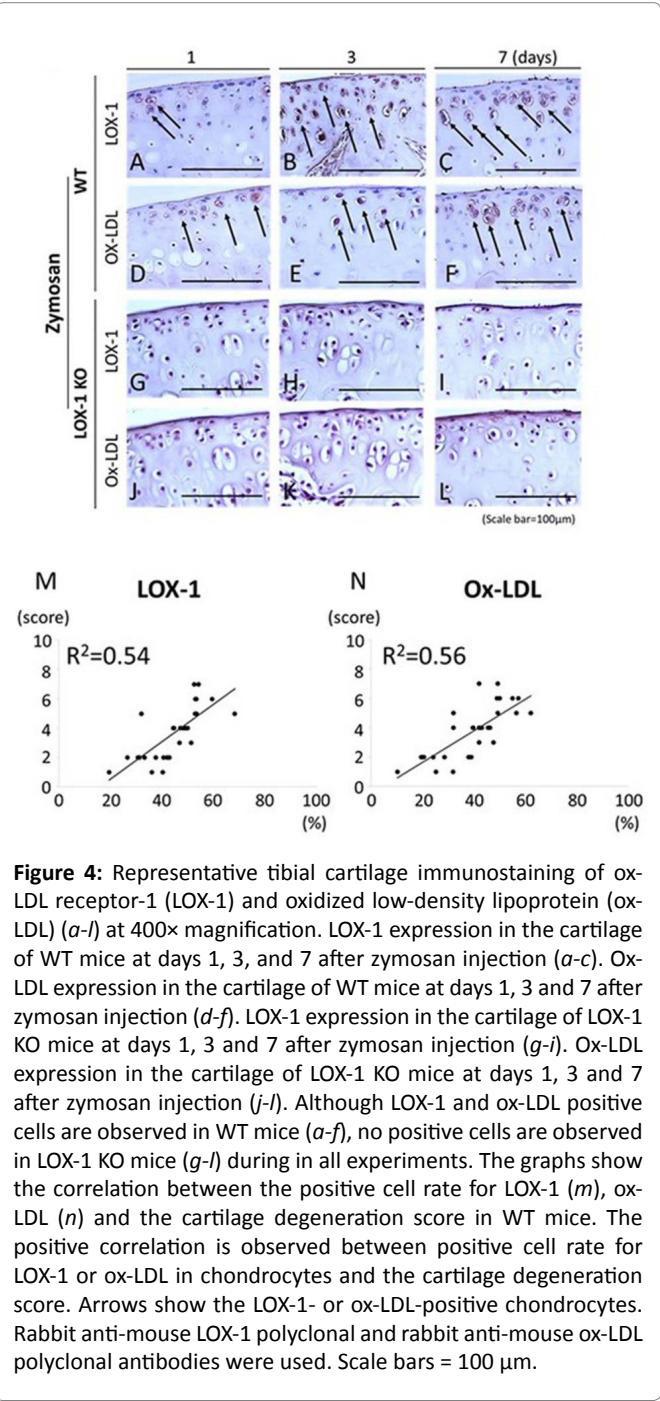
Discussion

The present study was conducted to clarify the role of the LOX-1/ox-LDL system in the development of arthritis, particularly on inflammatory changes such as synovitis. Our findings indicate that MMP-3 plays a role in the development of arthritis downstream of LOX-1/ox-LDL.



These results could provide new findings that LOX-1/ox-LDL system is involved in inflammation such as synovitis and cartilage degeneration induced by synovitis.

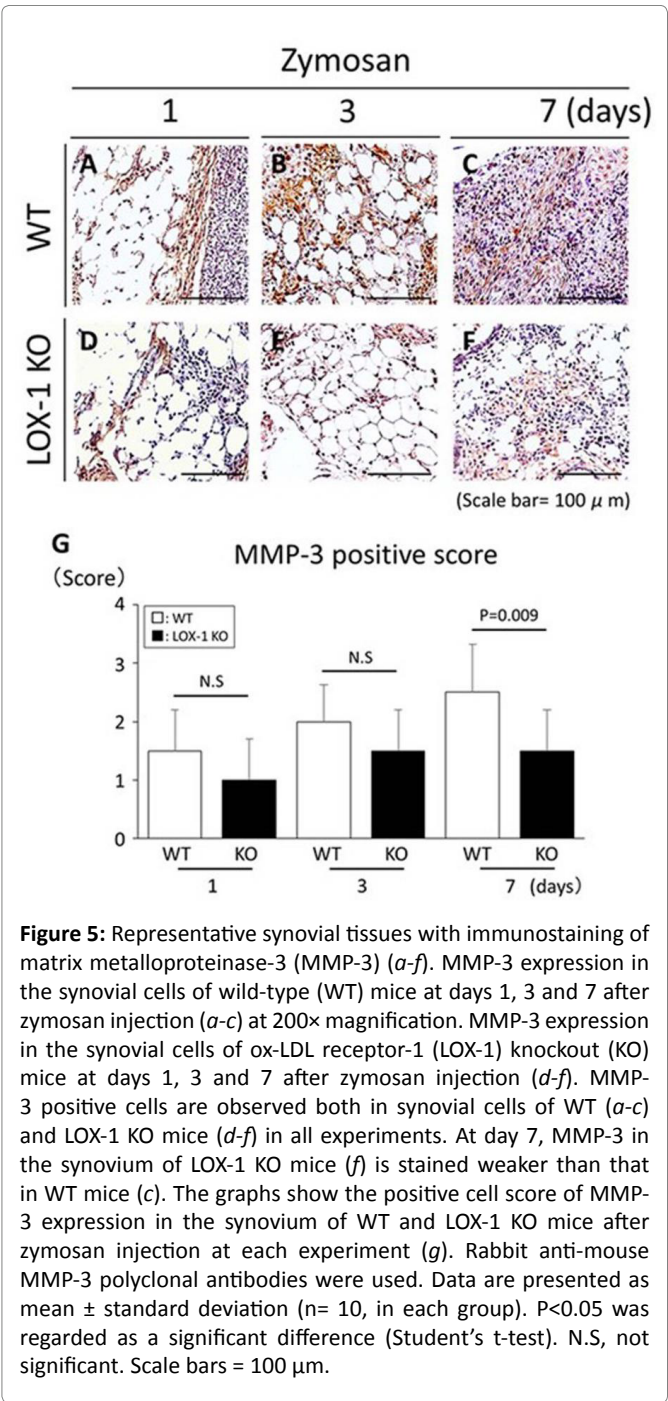
This study has limitations. First, in vitro methods were not employed, which need to be included in future studies. Second, we could not evaluate systemic inflammation. Zymosan could induce systemic inflammation as previously described^{20, 21}. In the current study, systemic inflammation as well as the systemic effects of atherosclerosis was not investigated. Second, atherosclerosis was not evaluated. It



would be interesting to investigate the correlation between atherosclerosis and arthritis development. Further research will be necessary.

Conclusion

Our findings indicate that treatment of abnormal lipid metabolism may contribute to the prevention and suppression of arthritis. Hence, we believe that the LOX-1/ox-LDL system is involved in the development of arthritis via MMP-3.



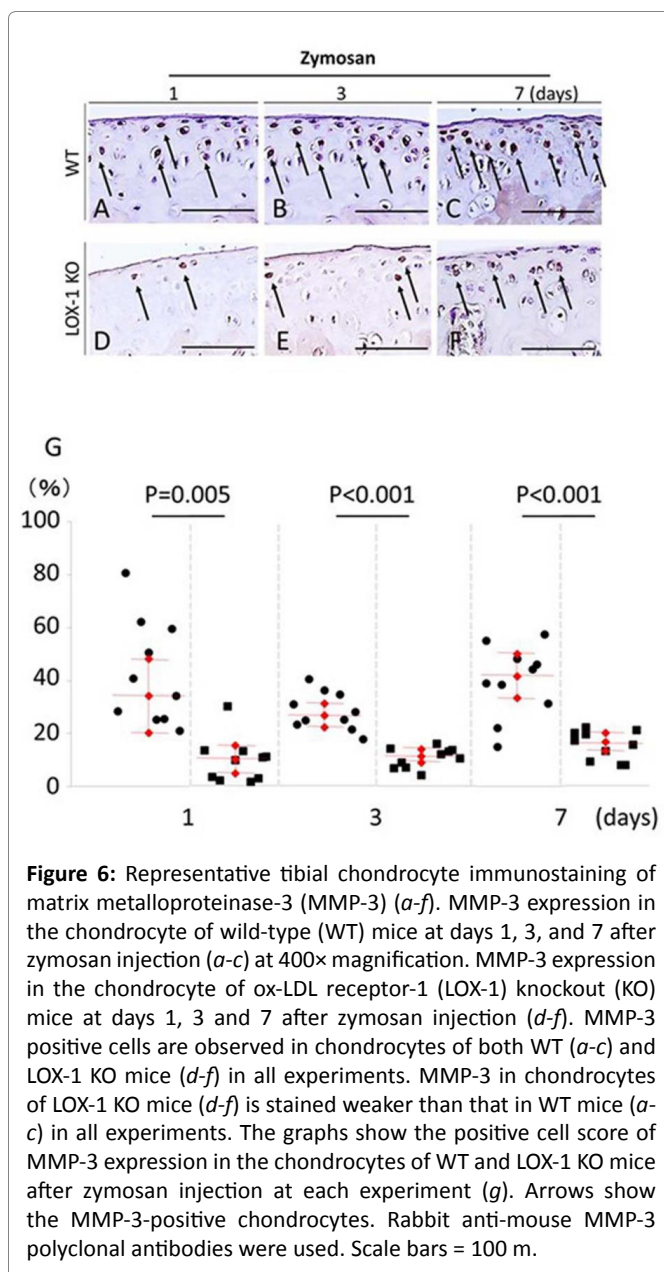
Declarations

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None.



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References

- Gofton JP. Studies in osteoarthritis of the hip. 3. Congenital subluxation and osteoarthritis of the hip. *Can Med Assoc J.* 1971 May 22; 104(10): 911-5.
- Yoshimura N, Muraki S, Oka H, et al. Mutual associations among musculoskeletal diseases and metabolic syndrome components: A 3-year follow-up of the ROAD study. *Mod Rheumatol.* 2015 May; 25(3): 438-48.

- Sun AR, Panchal SK, Friis T, et al. Obesity-associated metabolic syndrome spontaneously induces infiltration of pro-inflammatory macrophage in synovium and promotes osteoarthritis. *PloS One.* 2017 Aug 31; 12(8): e0183693.
- Niu J, Clancy M, Aliabadi P, et al. Metabolic syndrome, its components, and knee osteoarthritis: The Framingham Osteoarthritis Study. *Arthritis Rheumatol.* 2017 Jun; 69(6): 1194-203.
- Roever L, Resende ES, Diniz ALD, et al. Brazilian Network of Research in Meta-analysis (BRAMETIS). Epicardial adipose tissue and metabolic syndrome: An update protocol for systematic review and meta-analysis. *Medicine.* 2018 Apr; 97(16): e0387.
- Nishikido T, Ray KK. Inclisiran for the treatment of dyslipidemia. *Expert Opin Investig Drugs.* 2018 Mar; 27(3): 287-94.
- Sawamura T, Kume N, Aoyama T, et al. An endothelial receptor for oxidized low-density lipoprotein. *Nature.* 1997 Mar 6; 386(6620): 73-7.
- Hoeven TA, Kavousi M, Ikram MA. Markers of atherosclerosis in relation to presence and progression of knee osteoarthritis: a population-based cohort study.
- Rheumatology (Oxford, England).* 2015 Sep; 54(9):1692-8.
- Yeh HJ, Chou YJ, Yang NP. Association between physical therapy and risk of coronary artery disease and dyslipidemia among osteoarthritis patients: A nationwide database study. *Arch Phys Med Rehab.* 2016 Jan; 97(1): 8-16.
- Akagi M, Nishimura S, Yoshida K, et al. Cyclic tensile stretch load and oxidized low density lipoprotein synergistically induce lectin-like oxidized LDL receptor-1 in cultured bovine chondrocytes, resulting in decreased cell viability and proteoglycan synthesis. *J Orthop Res.* 2006 Aug; 24(8): 1782-90.
- Zushi S, Akagi M, Kishimoto H, et al. Induction of bovine articular chondrocyte senescence with oxidized low-density lipoprotein through lectin-like oxidized low-density lipoprotein receptor 1. *Arthritis Rheum.* 2009 Oct; 60(10): 3007-16. doi: 10.1002/art.24816.
- Ishikawa M, Ito H, Akiyoshi M, et al. Lectin-like oxidized low-density lipoprotein receptor 1 signal is a potent biomarker and therapeutic target for human rheumatoid arthritis. *Arthritis Rheum.* 2012 Apr; 64(4): 1024-34.
- Kishimoto H, Akagi M, Zushi S, et al. Induction of hypertrophic chondrocyte-like phenotypes by oxidized LDL in cultured bovine articular chondrocytes through increase in oxidative stress. *Osteoarthritis Cartilage.* 2010 Oct; 18(10): 1284-90.
- Hashimoto K, Mori S, Oda Y, et al. Lectin-like oxidized low density lipoprotein receptor 1-deficient mice show resistance to instability-induced osteoarthritis. *Scand J Rheumatol.* 2016 Oct; 45(5): 412-22.
- Hashimoto K, Oda Y, Nakamura F, et al. Lectin-like, oxidized low-density lipoprotein receptor-1-deficient mice show resistance to age-related knee osteoarthritis. *Eur J Histochem.* 2017 Feb 14; 61(1): 2762.
- Geenen R, Overman CL, Christensen R, et al. EULAR recommendations for the health professional's approach to pain management in inflammatory arthritis and osteoarthritis. *Ann Rheum Dis.* 2018 Jun; 77(6): 797-807.
- Lorenz J, Grässel S. Experimental osteoarthritis models in mice. *Methods Mol Biol.* 2014; 1194: 401-19.
- Kakinuma T, Yasuda T, Nakagawa T, et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates matrix metalloproteinase 3 synthesis enhanced by oxidized low-density lipoprotein in rheumatoid arthritis cartilage. *Arthritis Rheum.* 2004 Nov; 50(11):3495-3503.
- Nakagawa T, Akagi M, Hoshikawa H, et al. Lectin-like oxidized low-

density lipoprotein receptor 1 mediates leukocyte infiltration and articular cartilage destruction in rat zymosan-induced arthritis. Arthritis Rheum. 2002 Sep; 46(9): 2486-2494.

21. Zhang XT, Ding Y, Kang P, et al. Forsythoside A modulates zymosan-induced peritonitis in mice. Molecules. 2018 Mar 6; 23(3): pii: E593.