Inhibition of Shedding of Low-Density Lipoprotein Receptor–Related Protein 1 Reverses Cartilage Matrix Degradation in Osteoarthritis

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Objective. The aggreganase ADAMTS-5 and the collagenase matrix metalloproteinase 13 (MMP-13) are constitutively secreted by chondrocytes in normal cartilage, but rapidly endocytosed via the cell surface endocytic receptor low-density lipoprotein receptor–related protein 1 (LRP-1) and subsequently degraded. This endocytic system is impaired in osteoarthritic (OA) cartilage due to increased ectodomain shedding of LRP-1. The aim of this study was to identify the LRP-1 sheddase(s) in human cartilage and to test whether inhibition of LRP-1 shedding prevents cartilage degradation in OA.

Methods. Cell-associated LRP-1 and soluble LRP-1 (sLRP-1) released from human cartilage explants and chondrocytes were measured by Western blot analysis. LRP-1 sheddases were identified by proteinase inhibitor profiling and gene silencing with small interfering RNAs. Specific monoclonal antibodies were used to selectively inhibit the sheddases. Degradation of aggregan and collagen in human OA cartilage was measured by Western blot analysis using an antibody against an aggregan neoepitope and a hydroxyproline assay, respectively.

Results. Shedding of LRP-1 was increased in OA cartilage compared with normal tissue. Shed sLRP-1 bound to ADAMTS-5 and MMP-13 and prevented their endocytosis without interfering with their proteolytic activities. Two membrane-bound metalloproteinases, ADAM-17 and MMP-14, were identified as the LRP-1 sheddases in cartilage. Inhibition of their activities restored the endocytic capacity of chondrocytes and reduced degradation of aggregan and collagen in OA cartilage.

Conclusion. Shedding of LRP-1 is a key link to OA progression. Local inhibition of LRP-1 sheddase activities of ADAM-17 and MMP-14 is a unique way to reverse matrix degradation in OA cartilage and could be effective as a therapeutic approach.

Osteoarthritis (OA) is the most prevalent age-related joint disorder, but there is no disease-modifying treatment available except for joint replacement surgery (1). The main cause of the disease is degradation of articular cartilage due to elevated activities of matrix metalloproteinases (MMPs) and ADAMTS. While both ADAMTS-4 and ADAMTS-5 have been considered to participate in aggregan degradation in human OA (2,3), recent studies by Larkin et al with neutralizing monoclonal antibodies have shown that ADAMTS-5 is more effective than ADAMTS-4 in aggregan degradation in human OA cartilage and nonhuman primates in vivo (4). Collagen fibrils are mainly degraded by collagenolytic MMPs, and MMP-13 is considered to be the major collagenase in OA cartilage (5–7).

We have recently found that both ADAMTS-5 and MMP-13 are constitutively produced in healthy human cartilage, but they are rapidly taken up by the chondrocytes via the endocytic receptor low-density lipoprotein receptor–related protein 1 (LRP-1) and degraded intracellularly (8–10). These findings suggest that they probably function for a very short period of time to maintain normal
homeostatic turnover of extracellular matrix (ECM) components of the tissue. Other proteins that are endocytosed by LRP-1 include ADAMTS-4 (11) and tissue inhibitor of metalloproteinases 3 (TIMP-3) (12,13), indicating that LRP-1 is a key modulator of cartilage matrix degradation systems. This endocytic pathway is impaired in OA cartilage because of the reduction of protein levels of LRP-1 in chondrocytes without any significant changes in the level of messenger RNA (mRNA) for LRP-1, resulting in increased extracellular activity of ADAMTS-5 (8). We thus proposed that the loss of LRP-1 in OA cartilage is due to proteolytic shedding of the receptor, and that this process shifts normal homeostatic conditions of cartilage to a more catabolic environment, leading to the development of OA.

The aim of this study was to identify the “sheddase” activities that cleave LRP-1 and release the soluble form of LRP-1 (sLRP-1) in human cartilage. We also aimed to test whether inhibition of the sheddase(s) prevents the degradation of cartilage in OA.

MATERIALS AND METHODS

Reagents and antibodies. The sources of materials used were as follows; mouse monoclonal anti-LRP-1 α-chain antibody (8G1), mouse anti-LRP-1 β-chain monoclonal antibody (SA6) that recognizes the ectodomain, BC-3 mouse monoclonal antibody that recognizes the N-terminal 374ARGSVbody (5A6) that recognizes the ectodomain, BC-3 mouse monoclonal antibody (8G1), mouse anti–LRP-1 also aimed to test whether inhibition of the sheddase(s) soluble form of LRP-1 (sLRP-1) in human cartilage. We thus examined whether messenger RNA (mRNA) for LRP-1, resulting in degradation of cartilage to a more catabolic environment, leading to the development of OA.

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Western blot analysis of LRP-1 in cartilage. To analyze LRP-1 in the cartilage, medium was removed after incubation for various periods of time, and then total protein was extracted by adding 50 μl of 4× sodium dodecyl sulfate (SDS) sampling buffer (200 mM Tris pH 6.8)/8% SDS and 20% glycerol) to each explant in a 96-well plate. After 1 hour of incubation, the sample buffers were pooled from each condition (3 explants per condition), and 10 μl of samples was analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) under nonreducing conditions and Western blotting using anti–LRP-1 α-chain, anti–LRP-1 β-chain, and antitubulin antibodies. Immune signals of LRP-1 and tubulin were quantified using ImageJ software (National Institutes of Health), and the relative amounts of LRP-1 α- and β-chains in the cartilage extracts were estimated using tubulin as an internal control.

Immunofluorescence staining of LRP-1. OA and normal cartilage samples (n = 3 each) were rested in culture with DMEM for 2 days. Explants were then snap-frozen and sectioned (5-μm sections) using a CM1900 cryostat (Leica Microsystems). Each sample was fixed with methanol and incubated with anti–LRP-1 β-chain antibody for 3 hours at room temperature. Incubation with Alexa Fluor 568-conjugated anti-mouse IgG (Molecular Probes) for 1 hour at room temperature was used to visualize the antigen signals. Nuclei were stained with DAPI. Samples were viewed using an Eclipse TE2000-U confocal laser scanning microscope (Nikon). Data were collated using Velocity software (Improvision).

Quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR). Quantitative RT-PCR was carried out as described previously (8). Briefly, RNA was extracted and isolated from 50 mg of ground cartilage tissue using an RNaseasy kit (Qiagen), and complementary DNA was then generated using a reverse transcription kit following the guidelines of the manufacturer (Applied Biosystems). Complementary DNA was then used for real-time PCR assays using TaqMan technology. The ΔΔCt method of relative quantitation was used to calculate relative mRNA levels for each transcript examined. The 60S acidic ribosomal protein P0 (RPLP0) gene was used to normalize the data. Predesigned primer/probe sets for LRP-1, ADAM-12, and RPLP0 were purchased from Applied Biosystems.

Western blot analysis of cellular LRP-1 and sLRP-1. Chondrocytes (5 × 10⁴) were cultured in 12-well plates in 2 ml of DMEM containing 10% FCS for 2 days. Cells were rested in 1 ml of DMEM for 24 hours, and the medium was replaced with fresh DMEM. Samples were then collected and analyzed by Western blotting.
with 1 ml of DMEM and used in the experiments. After incubation for various periods of time, the medium was collected and concentrated 20-fold using spin filters (Microcon YM-30; Merck Millipore), and 20 μl of 4× SDS sampling buffer was added to 50 μl of each concentrated medium. The cells were lysed with 200 μl of 2× SDS sampling buffer, and 10 μl of samples were analyzed by SDS-PAGE under nonreducing conditions and Western blotting using anti-LRP-1 α-chain, anti-LRP-1 β-chain, and antitubulin antibodies. Immune signals of LRP-1 and tubulin were quantified using ImageJ software, and the relative amounts of LRP-1 α-chain in the medium and LRP-1 α- and β-chains in the cell lysate were estimated within the linear range of measurements (see Supplementary Figures 1A and B, available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40080/abstract) and normalized using tubulin, and those in the standard cell lysates of chondrocytes were used as internal controls. An absolute number of LRP-1 molecules released into medium was estimated by comparing various concentrations of purified LRP-1 within a reasonable linear range.

**Flow cytometric analysis of LRP-1.** Cells were plated in 6-well plates in DMEM containing 10% FCS and incubated until 80% confluent. Cells were rested in 2 ml of DMEM for 1 day, detached using a cell scraper, and fixed for 5 minutes at 4°C with ice-cold methanol followed by 2 washes with fluorescence-activated cell sorting (FACS) buffer (phosphate buffered saline containing 5% goat serum and 3% bovine serum albumin [BSA]). Cells were stained for 30 minutes at 25°C with anti-LRP-1 β-chain antibody, washed with FACS buffer, and then further incubated with allophycocyanin-conjugated goat antimouse IgG (BD PharMingen) and isotype control in FACS buffer for 20 minutes at 25°C. Cells were then washed with FACS buffer and analyzed using an LSRII flow cytometer (BD Biosciences), and postacquisition data analysis was performed using FlowJo software version 7.6.1 (Tree Star).

**Analysis of endocytosis of ADAMTS-5 and MMP-13.** Cells (5 × 10^5) cultured in 24-well plates were rested in 500 μl of DMEM for 1 day. The medium was replaced with 500 μl of fresh DMEM with or without 10 ng/ml IL-1 in the absence or presence of various concentrations of combinations of the control antibodies (human IgG for the anti–ADAM-17 antibody [D1A12] and antidesmin intracellular domain antibody for the anti–MMP-14 antibody [E2C6]) or the anti–ADAM-17 and the anti–MMP-14 antibodies. After 24 hours of incubation, the cells were lysed with 100 μl of 2× SDS sampling buffer, and then LRP-1 α- and β-chains were detected as described above. To test the effect of inhibitory antibodies against ADAM-17 and MMP-14 on LRP-1 levels in OA chondrocytes and cartilage in culture, cells and cartilage explants were cultured for 24 hours with DMEM in the absence or presence of combinations of 250 nM each of the control antibodies, the anti–ADAM-17 antibody and the antidesmin antibody, the anti–MMP-14 antibody and IgG, or the anti–ADAM-17 and the anti–MMP-14 antibodies. LRP-1 α- and β-chains were then detected as described above.

**Analysis of aggrecan degradation in OA cartilage.** OA cartilage was cultured in a round-bottomed 96-well plate (1 explant per well) and rested in DMEM for 1 day. The cartilage was further cultured in DMEM in the absence or presence of a combination of 2 antibodies (250 nM each) (i.e., combination of the anti–ADAM-17 antibody and the antidesmin antibody [control], combination of the anti–MMP-14 antibody and IgG [control], combination of the anti–ADAM-17 and the anti–MMP-14 antibodies, or combination of the antidesmin antibody and IgG), or 250 nM of anti–ADAMTS-5 or N-terminal domain of human TIMP-3. After 12 hours of incubation, the medium was replaced with fresh DMEM containing the antibodies or TIMP and incubated further for 0–48 hours. For Western blotting, the conditioned media were pooled from each condition (3 explants per condition) and deglycosylated as described above, and immunoreactivity was measured within the linear range of the assay based on standard samples (see Supplementary Figure 1E, http://onlinelibrary.wiley.com/doi/10.1002/art.40080/abstract).
OA cartilage was cultured with various antibodies or with 250 nM of TIMP-1 as described above for aggreccan degradation studies, and media were harvested after 96 hours. The extent of type II collagen degradation in OA cartilage was assessed by measuring the amount of hydroxyproline released into the media using a modification of the assay described by Bergman and Loxley (24). The hydroxyproline contents in cartilage explant remnants after culture were also determined by digesting them in 500 μl of papain digest solution (0.05M phosphate buffer [pH 6.5]/2 mM N-acetylcysteine/2 mM EDTA/10 μg/ml papain) at 65°C for 24 hours. The relative amount of collagen degradation was estimated by dividing the amount of hydroxyproline released into the medium by the summed amount of hydroxyproline in the medium and in the papain digests.

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Study approval. Normal human articular cartilage tissue specimens were obtained from the Stanmore BioBank, Institute of Orthopaedics, Royal National Orthopaedic Hospital, Stanmore, following informed consent from patients and approval by the Royal Veterinary College Ethics and Welfare Committee (Institutional approval Unique Reference Number 2012 0048H). Human OA cartilage tissue specimens were obtained from the Oxford Musculoskeletal Biobank and were collected with informed donor consent in full compliance with national and institutional ethical requirements, the United Kingdom Human Tissue Act, and the Declaration of Helsinki.
RESULTS

Increased ectodomain shedding of LRP-1 and reduced endocytic capacity in human OA cartilage. We first verified the loss of LRP-1 protein in human OA cartilage. LRP-1 consists of an extracellular 515-kd α-chain and an 85-kd β-chain that are processed from the precursor by furin. The α-chain contains the ligand-binding domains, and the β-chain has an extracellular domain, a transmembrane domain, and a cytoplasmic domain. Western blotting analyses of α- and β-chains of cartilage extracts with antibodies that recognize the N-terminus of the α-chain and the extracellular domain of the β-chain showed that both chains were reduced in OA cartilage by ~48% and ~65%, respectively, compared with normal cartilage (Figures 1A and B). The reduction of LRP-1 was further confirmed by immunofluorescence staining of Figures 1A and B. The reduction of LRP-1 was further confirmed by immunofluorescence staining of β-chain in the cartilage (Figure 1C). No significant change in the level of mRNA for LRP-1 between normal and OA cartilage (Figure 1D) suggested that the loss of LRP-1 in OA cartilage was due to proteolytic shedding of the receptor.

To further investigate the increased shedding of LRP-1 in OA cartilage, chondrocytes were cultured and LRP-1 proteins were analyzed. Both α- and β-chains were reduced in OA cell lysates compared with normal chondrocytes, which was accompanied by an increased release of full-length α-chain into the medium (Figures 1E and F). Flow cytometric analysis of the β-chain with antiec-todomain antibody further confirmed the reduction of cell surface LRP-1 including the β-chain in OA chondrocytes (Figure 1G), suggesting that the primary shedding site is located in the ectodomain of the β-chain. We estimated that a single normal chondrocyte released ~3.9 ± 2.7 × 10^6 LRP-1 molecules per hour (mean ± SD), while a single OA chondrocyte released 9.6 ± 5.4 × 10^5 LRP-1 molecules per hour. As anticipated, the endocytic capacity of human OA chondrocytes was significantly reduced; the half-life of ADAMTS-5 was ~2.8-fold longer in OA chondrocytes (~210 minutes) than in normal chondrocytes (~75 minutes) (Figures 1H and I).

Soluble LRP-1 ectodomain prevents endocytosis of ADAMTS-5 and MMP-13 without interfering with their activities. We then evaluated whether sLRP-1 alters half-lives of cartilage-degrading metalloproteinases. As shown in Figures 2A and B, endocytosis of ADAMTS-5 and MMP-13 was reduced partially with 2 nM sLRP-1 and almost completely inhibited with 10 nM sLRP-1 to the level that was attained with the LRP ligand antagonist RAP. We also found that sLRP-1–bound ADAMTS-5 and MMP-13 retained activity against their natural substrates, aggrecan and collagen, respectively (Figures 2C and D). It is notable that ADAMTS-5 bound to sLRP-1 was ~3-fold more active on aggrecan cleavage compared with free ADAMTS-5 (Figure 2C). Thus, shedding of the LRP-1 ectodomain impairs the endocytic capacity of the cell not only by reducing the level of cell surface LRP-1 but also by converting membrane-anchored LRP-1 into soluble decoy receptors, leaving excess matrix-degrading proteinases extracellularly.

ADAM-17 and MMP-14 are responsible for shedding LRP-1 in human chondrocytes. To identify the LRP-1 sheddase in human chondrocytes, we first examined whether proinflammatory cytokines such as IL-1 and TNF that stimulate cartilage matrix degradation increase LRP-1 shedding, as this might facilitate characterization of the sheddase in the cartilage. As shown in Figures 3A–C, these cytokines increased LRP-1 shedding ~4.0-fold in normal human chondrocytes. The cytokine-stimulated LRP-1 shedding was inhibited by the hydroxamate metalloproteinases inhibitor CT1746, but not by a serine protease inhibitor (AEBSF) or a cysteine protease inhibitor (E-64) (Figure 3D). Among the 3 TIMPs tested, TIMP-1 was not effective but TIMP-2 and TIMP-3 were, and TIMP-3 showed the strongest inhibition (Figure 3E). Thus, we postulated that the responsible enzyme was likely to be a membrane-anchored ADAM or MMP.

ADAM-10, ADAM-12, ADAM-17, and MMP-14 have previously been reported to be LRP-1 sheddases in other cell types (25). We therefore ablated each enzyme individually using a specific siRNA. The ADAM-10 protein level was reduced by ~90%, and the ADAM-12 mRNA level was reduced by ~88% (see Supplementary Figures 2A–C, http://onlinelibrary.wiley.com/doi/10.1002/art.40080/abstract), but their knockdown did not affect LRP-1 shedding (see Supplementary Figure 2D). Small interfering RNAs targeting ADAM-17 and MMP-14 reduced their protein levels by 76% and 84%, respectively (see Supplementary Figures 2E and F), and knockdown of each partially inhibited LRP-1 shedding (Figure 3F). However, knockdown of both MMP-14 and ADAM-17 exhibited a stronger, additive effect, to the level achieved by TIMP-3 (Figure 3F), which suggests that these 2 proteinases function as LRP-1 sheddases. A low level of LRP-1 shedding occurred in unstimulated chondrocytes, and was mainly due to MMP-14.

Combination of inhibitory antibodies against ADAM-17 and MMP-14 blocks LRP-1 shedding in OA cartilage. To verify the role of ADAM-17 and MMP-14 in LRP-1 shedding, we used the recently developed
specific antibodies against ADAM-17 (D1A12) (15) and against MMP-14 (E2C6) (16), which inhibit the target enzymes with inhibition constants of 0.46 nM and 0.11 nM, respectively. The IL-1–induced loss of LRP-1 was partially inhibited by a single antibody, but a combination of the 2 antibodies blocked LRP-1 shedding to the level of IL-1–untreated cells (Figure 4A). They were similarly effective at blocking LRP-1 shedding in OA chondrocytes (Figure 4B). The
restoration of LRP-1 in OA chondrocytes by combining the 2 antibodies was time dependent, and it reached a plateau at 24 hours (Figure 4C). Addition of the 2 antibodies to OA cartilage blocked LRP-1 shedding and increased α- and β-chains 2.6-fold and 2.3-fold, respectively (Figure 4D). This indicates that the antibodies can penetrate the tissue and inhibit the LRP-1 shedding in OA cartilage.

Blocking of LRP-1 sheddases restores endocytic capacity and reduces the degradation of aggrecan and collagen in OA cartilage. We then tested the effect of combining the 2 antibodies on the endocytic capacity of OA chondrocytes. The antibody-treated OA chondrocytes cleared exogenously added ADAMTS-5 from the medium ~2.4-fold faster (half-life ~95 minutes) than the untreated OA chondrocytes (half-life ~210 minutes) (Figure 5A), indicating that blocking LRP-1 sheddases restored the endocytic capacity of OA chondrocytes close to that of normal chondrocytes.

Remarkably, combined antibody treatment reduced the degradation of aggrecan and collagen in OA cartilage. Analysis of the same conditioned media for aggrecanase activity was markedly inhibited by blocking LRP-1 sheddases (Figure 5B) (see Supplementary Figure 3, http://onlinelibrary.wiley.com/doi/10.1002/art.40080/abstract). Potent inhibition with the anti–ADAMTS-5 antibody and TIMP-3 indicated that the primary
aggregan in OA cartilage was ADAMTS-5. Effective inhibition of aggregan degradation by the combination of anti–ADAM-17 and anti–MMP-14 antibodies was further confirmed by Safranin O staining of the cartilage (see Supplementary Figure 4, http://onlinelibrary.wiley.com/doi/10.1002/art.40080/abstract).

Figure 4. Combination of antibodies inhibiting ADAM-17 and matrix metalloproteinase 14 (MMP-14) blocks shedding of low-density lipoprotein receptor–related protein 1 (LRP-1) in osteoarthritic (OA) cartilage. A, Results of culturing human normal chondrocytes (n = 3 donors) with Dulbecco’s modified Eagle’s medium without (None) or with 10 ng/ml interleukin-1 (IL-1) in the absence or presence of combined anti–ADAM-17 and anti–MMP-14 (10–200 nM each) for 48 hours. LRP-1 proteins in the cell lysate were analyzed by Western blotting with antibodies against α- and β-chains of LRP-1. B, Effect of combining anti–MMP-14 and anti–ADAM-17 antibodies (200 nM each) on LRP-1 shedding in human OA chondrocytes (n = 5 donors). C, Time course analysis of the effect of combined anti–MMP-14 and anti–ADAM-17 antibodies on the recovery of cellular LRP-1. D, Effect of combined anti–MMP-14 and anti–ADAM-17 antibodies on LRP-1 shedding in human knee OA cartilage explants (n = 6 donors each). In A, values are the mean ± SD, and the mean value in the cells incubated without IL-1 or the antibodies was set at 1 (dashed line). In B–D, symbols represent individual cartilage donors; bars show the mean ± SD. In B–D, the mean values in the cells or cartilage incubated with the control antibodies for 8 hours (C) or 24 hours (B and D) were set at 1 (dashed lines). * = P < 0.05, by Student’s 2-tailed t-test.

Figure 5. Blocking of low-density lipoprotein receptor–related protein 1 (LRP-1) sheddases restores endocytic capacity and reduces aggregan and collagen degradation in osteoarthritic (OA) cartilage. A, Recovery of endocytic capacity in OA chondrocytes upon treatment with anti–ADAM-17 and anti–matrix metalloproteinase 14 (anti–MMP-14) antibodies (Ab). Endocytosis of ADAMTS-5 (10 nM) was measured as described in Figures 1H and I (n = 3 donors). B and C, Inhibition of aggregan and collagen degradation in human OA cartilage upon treatment with combined anti–ADAM-17 and anti–MMP-14 antibodies. OA knee cartilage explants were incubated with antibodies or tissue inhibitor of metalloproteinases (TIMP) at 250 nM. B, Aggregan degradation detected after 48 hours with an antibody against the aggregan neoepitope 334-ARGSV. The mean value in medium of the cartilage incubated with the control antibodies was set at 100 (dashed line) (n = 6 donors). C, Collagen degradation of cultured OA cartilage after 96 hours of incubation, measured by hydroxyproline assay. The amount of hydroxyproline released with control antibodies was set at 100 (dashed line). In A, values are the mean ± SD. In B and C, symbols represent individual cartilage donors; bars show the mean ± SD. The mean values in media of the cartilage incubated with the control antibodies for 48 hours (B) or 96 hours (C) were set at 100 (dashed lines). * = P < 0.05, by Student’s 2-tailed t-test (A) or one-way analysis of variance followed by Dunnett’s multiple comparison test (C). RAP = receptor-associated protein; N-TIMP-3 = N-terminal domain of human TIMP-3.
Collagen degradation was also inhibited in the presence of anti–ADAM-17 and anti–MMP-14 antibodies by 5% and 10%, respectively, and more effective inhibition (17%) was observed upon combining the 2 antibodies (Figure 5C). In general, anti–MMP-14 showed a stronger effect than anti–ADAM-17, but there was considerable patient-to-patient variation in the effect of each antibody, which may reflect the multifactorial nature of OA. Inhibition was also detected with TIMP-1 treatment, but not with the anti–ADAMTS-5 antibody, indicating that collagen degradation is specific to collagenase. Cell viability analysis indicated that none of these treatments was toxic to chondrocytes (data not shown).

DISCUSSION

In this study, we have shown that the ectodomain shedding of LRP-1 may be an important regulator of the development of human OA. The specific inhibitory antibodies that we recently developed for human MMP-14 and ADAM-17 have allowed us to evaluate the role of LRP-1 shedding in degradation of cartilage matrix in human subjects and thus provided clinically relevant information.

Numerous membrane-anchored proteins are released from the cell surface by the process of regulated proteolysis called ectodomain shedding, and the enzymes responsible for shedding are primarily membrane-anchored proteinases. This process regulates a wide variety of cellular and physiologic functions, and dysregulated shedding is linked to numerous diseases, such as Alzheimer’s disease, inflammation, rheumatoid arthritis (RA), cancer, chronic kidney disease, cardiac hypertrophy, and heart failure (26,27). LRP-1 shedding is increased under inflammatory conditions such as in RA and systemic lupus erythematosus (28), and in cancer (29,30), but the exact pathologic role of LRP-1 shedding in these diseases has not been clearly understood. We propose that LRP-1 shedding in local tissues under inflammatory or chronic pathologic conditions dysregulates normal turnover of ECM and cellular homeostasis, leading to slowly progressing chronic diseases such as in OA.

LRP-1 is widely expressed in different cell types and controls extracellular levels of numerous biologically active molecules to maintain tissue homeostasis (31). Currently, more than 50 ligands have been characterized, including lipoproteins, ECM proteins, growth factors, cell surface receptors, proteinases, protease inhibitors, and secreted intracellular proteins (31). In cartilage, LRP-1 controls not only ECM-degrading proteinases but also the Wnt/β-catenin signaling pathway by interacting with Frizzled-1 (32) and connective tissue growth factor (CCN2), and both regulate endochondral ossification and articular cartilage regeneration (33), emphasizing the

Figure 6. Low-density lipoprotein receptor–related protein 1 (LRP-1)–mediated endocytic pathways in normal and osteoarthritic (OA) cartilage. A, Secreted ADAMTS-5 and matrix metalloproteinase 13 (MMP-13) are largely endocytosed by LRP-1 in normal cartilage. Thus, their activities in the extracellular milieu are limited. B, In OA cartilage, the ectodomain of LRP-1 is shed by ADAM-17 and MMP-14, resulting in impairment of endocytic capacity of chondrocytes. Blocking of ADAM-17 and MMP-14 activities recovers the lost endocytic function of OA chondrocytes, reduces cartilage matrix degradation, and restores cartilage homeostasis.
importance of LRP-1 in skeletal development and in the maintenance of cartilage homeostasis.

Thus, the impairment of LRP-1 function due to increased shedding of the receptor is detrimental to healthy cartilage, as demonstrated in the present study. This is triggered by increased activity of ADAM-17 and MMP-14, but their protein levels were not significantly changed between healthy and OA cartilage (data not shown), which suggests that the activation of these enzymes is regulated posttranslationally. The additive but not synergistic effect of anti–ADAM-17 and anti–MMP-14 antibodies further suggests that these proteinases may be activated by different mechanisms and act independently as LRP-1 sheddases. In addition, MMP-14 and ADAM-17 cleave a number of cell membrane proteins, including growth factors, cytokines, cell adhesion molecules, and mechanosensors (26,34). Therefore, their activation may also affect the integrity of other cell surface molecules in cartilage as well as cellular behavior. We are currently investigating how ADAM-17 and MMP-14 are activated as well as their substrate selectivity in cartilage, as these may indicate additional molecular mechanisms for the development of OA, particularly in the early stages.

Another notable finding of this study is that anti–ADAM-17 and anti–MMP-14 antibodies reduced both aggrecanolytic and collagenolytic activities of human OA cartilage in culture, and this effect was due to restoration of the lost LRP-1 function by blocking the LRP-1 sheddase activities of ADAM-17 and MMP-14 (Figure 6). These results suggest that inhibition of elevated LRP-1 sheddase activities in OA cartilage may be an effective way to prevent cartilage matrix degradation. Although the systematic inhibition of ADAM-17 and MMP-14 as OA therapy may be problematic, as these enzymes are biologically important in the release of growth factors and cell surface receptors in many cell types (27,34), local administration of anti–ADAM-17 and anti–MMP-14 antibodies or small molecule inhibitors of ADAM-17 and MMP-14 may be worth investigating as disease-modifying OA drugs. This approach is an attractive option for OA therapy, as the recovery of the lost endocytic function of chondrocytes would help to maintain cartilage homeostasis. We are currently testing whether this approach is beneficial in early and advanced OA, using preclinical animal models.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Yamamoto 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.

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