Membrane type 1 matrix metalloproteinase promotes LDL receptor shedding and accelerates the development of atherosclerosis

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Plasma low-density lipoprotein (LDL) is primarily cleared by LDL receptor (LDLR). LDLR can be proteolytically cleaved to release its soluble ectodomain (sLDLR) into extracellular milieu. However, the proteinase responsible for LDLR cleavage is unknown. Here we report that membrane type 1-matrix metalloproteinase (MT1-MMP) co-immunoprecipitates and co-localizes with LDLR and promotes LDLR cleavage. Plasma sLDLR and cholesterol levels are reduced while hepatic LDLR is increased in mice lacking hepatic MT1-MMP. Opposite effects are observed when MT1-MMP is overexpressed. MT1-MMP overexpression significantly increases atherosclerotic lesions, while MT1-MMP knockdown significantly reduces cholesterol accumulation in the aortas of apolipoprotein E (apoE) knockout mice. Furthermore, sLDLR is associated with apoB and apoE-containing lipoproteins in mouse and human plasma. Plasma levels of sLDLR are significantly increased in subjects with high plasma LDL cholesterol levels. Thus, we demonstrate that MT1-MMP promotes ectodomain shedding of hepatic LDLR, thereby regulating plasma cholesterol levels and the development of atherosclerosis.
Atherosclerotic cardiovascular disease is one of the main causes of morbidity and mortality in Western societies. Plasma levels of low-density lipoprotein cholesterol (LDL-C) are positively correlated with risk of atherosclerosis1. LDL receptor (LDLR) mediates LDL uptake and plays an essential role in removing plasma LDL-C. Upon LDL binding, LDLR is internalized via clathrin-coated pits and delivered to endosomes, where LDL is released from the receptor and delivered to the lysosome for degradation, LDLR is then recycled to the cell surface2. Mutations in LDLR cause familial hypercholesterolemia and increase risk for atherosclerosis and coronary heart disease4.

LDLR is transcriptionally regulated by the sterol regulatory element-binding protein 2 (SREBP-2). Statins inhibit 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCRC) and consequently activate the transcriptional activity of SREBP-2, leading to increased LDLR expression1. Posttranslationally, proprotein convertase subtilisin/kexin 9 (PCSK9) binds to LDLR and redirects the receptor for lysosomal degradation3–5, while the inducible degrader of LDLR (IDOL) reduces LDLR levels via the polyubiquitination and lysosomal degradation pathway3–6. In addition, the ectodomain of LDLR can be cleaved by proteases with the released extracellular domain detected in cell culture media and in human plasma as a soluble form (sLDLR)4,5. Serum levels of sLDLR are positively correlated with plasma LDL-C levels11,14. It has been shown that ectodomain cleavage of LDLR is inhibited by metalloproteinase inhibitors10,15. Knockdown of a disintegrin and metalloproteinase 17 (ADAM17), a metalloproteinase responsible for the shedding of many transmembrane proteins, however, has only a marginal effect on ectodomain cleavage of LDLR in HepG2 cells15. Thus, the metalloproteinase(s) responsible for the bulk of LDLR shedding is unknown.

Membrane type-I matrix metalloproteinase (MT1-MMP/ MMP14), a Zn2+-dependent endopeptidase, belongs to a six-member family of membrane-type MMPs that includes four transmembrane type MMPs (MT1-, MT2-, MT3-, MT5-MMP), and two glycosyl phosphatidylinositol-anchored membrane-associated MMPs (MT4-, MT6-MMP)16. MT1-MMP is widely expressed in various tissues and cell types and plays key roles in both physiological processes and disease progression through remodelling of extracellular matrix and pericellular proteolysis17–19. MT1-MMP has been reported to cleave transmembrane proteins such as death receptor-6, neuropilin-1, and LDLR-related protein 1 (LRP-1) in breast cancer cells20,21. Cleavage of LDLR is enhanced by 4β-phorbol 12-myristate 13-acetate that can induce trafficking of MT1-MMP to the cell surface and enhance its cleavage function10. The exact role of MT1-MMP in LDLR shedding, however, is unclear. Here, we found that reducing MT1-MMP expression increased LDLR levels in cultured cells. Furthermore, specific knockout of MT1-MMP in mouse hepatocytes increased hepatic LDLR levels and reduced plasma levels of lipoprotein cholesterol. An opposite phenotype was observed when the wild-type MT1-MMP, but not the enzymatically dead mutant E240A, was overexpressed in cultured cells and mice. Consequently, overexpression of MT1-MMP significantly increased atherosclerotic lesion area in aortic sinus, while knockdown of MT1-MMP reduced cholesterol accumulation in the aortas in apolipoprotein E knockout (apoE–/–) mice. Mechanistically, we found that MT1-MMP directly associated with LDLR and promoted its ectodomain cleavage. Taken together, these findings demonstrate that hepatic LDLR ectodomain is shed by MT1-MMP and that MT1-MMP regulates plasma LDL-C metabolism and the development of atherosclerosis.

Results

MT1-MMP regulates LDLR expression. MT1-MMP activates pro-MMP217,22. We have previously reported that active MMP2 can cleave PCSK923. Thus, we initially assumed that inhibition of MT1-MMP could reduce the amount of active MMP2 and suppress MMP2-induced cleavage of PCSK9, thereby enhancing PCSK9-promoted LDLR degradation and consequently decreasing LDLR levels. To test this hypothesis, we knocked down expression of MT1-MMP in human hepatoma-derived Huh7.5 cells. The two MT1-MMP siRNAs that targeted different regions in the MT1-MMP mRNA efficiently reduced the levels of MT1-MMP but not MT2-MMP (the closest family member to MT1-MMP) (Fig. 1a). Addition of PCSK9 reduced cellular LDLR levels in scrambled siRNA-transfected cells, as well as in cells transfected with MT1-MMP siRNA (Fig.1b). We then co-transfected Huh7.5 cells with MT1-siRNA and plasmid containing PCSK9 cDNA and found that overexpression of PCSK9 efficiently stimulated LDLR degradation in cells transfected with either scrambled or MT1-MMP siRNA (Supplementary Fig. 1a). Thus, knockdown of MT1-MMP did not affect PCSK9-promoted LDLR degradation. Surprisingly, LDLR levels were markedly increased in MT1-MMP knockdown cells in the absence of PCSK9 (Fig. 1b, lanes 3 and 5 vs. 1). We noticed that knockdown of MT1-MMP appeared not to markedly affect the levels of LDLR in the presence of exogenous PCSK9 (Fig. 1b, lanes 4 and 6 vs. 2). It is of note that the experiment was performed in the presence of excess PCSK9 and under a non-physiological condition. First, Huh7.5 cells express endogenous PCSK9. Second, the cells were incubated in medium containing 5% NCLPPS that is known to increase endogenous PCSK9 expression and enhance PCSK9-promoted LDLR degradation. Third, the cells were supplied with additional 2 μg/ml of recombinant human PCSK9. Thus, it was likely that PCSK9-promoted LDLR degradation became overwhelming under this condition. To further confirm the impact of MT1-MMP on LDLR expression, we knocked down MT1-MMP expression in another human hepatoma-derived cell line (HepG2) and found that LDLR levels were significantly increased in MT1-MMP siRNA-transfected cells, whereas the levels of MT2-MMP, LRP-1 and transferrin receptor were comparable in cells transfected with scrambled or MT1-MMP siRNA (Fig.1c, lanes 2 and 3 vs. 1). Similar results were observed in mouse hepatocytes, Hepa1c1c7 (Supplementary Fig. 1b). Next, we overexpressed HA-tagged MT1-MMP in Huh7.5 cells and observed that MT1-MMP reduced cellular LDLR levels in a dose-dependent manner (Fig. 1d). Overexpression of MT1-MMP, however, had no significant effect on the levels of endogenous PCSK9 (Supplementary Fig. 1c, lanes 4–6 vs. 1–3). LDLR resides on plasma membrane, where it binds to and mediates LDL internalization. Thus, cell surface proteins in Huh7.5 cells were assessed using biotinylation. As shown in Fig. 1e, expression of MT1-MMP in both whole-cell lysate and the cell surface fraction was reduced by its siRNA. Conversely, expression of LDLR was increased in whole-cell lysate (lane 2 vs. 1) and the surface fraction (lane 4 vs. 3) in MT1-MMP-knockdown cells. Calnexin (an ER protein) was undetectable in the surface fraction. Analysis of basal LDL uptake by these cells demonstrated a role for MT1-MMP in this process, with knockdown of MT1-MMP showing significantly increased cellular LDL uptake (Fig. 1f). Together, these findings demonstrate that MT1-MMP regulates LDLR expression and LDL uptake in cultured hepatocytes.

MT1-MMP promotes ectodomain cleavage of LDLR. We then examined what mechanisms mediated the effect of MT1-MMP on LDLR expression. Iломаst (GM6001), a collagen-based peptidomimetic hydroxamate that can inhibit MT1-MMP activity24,
did not alter expression of MT1-MMP but increased endogenous LDLR levels in Huh 7.5 cells (Supplementary Fig. 1d). Leupeptin (an inhibitor of serine and cysteine proteases) and pepstatin (an inhibitor of aspartyl proteases), however, had no effect on LDLR levels (Supplementary Fig. 1e). These findings were consistent with previous reports that LDLR cleavage depends on MMPs10. Moreover, MT1-MMP could efficiently promote LDLR degradation in the absence and presence of MG132, a proteasome inhibitor, or chloroquine, a lysosome inhibitor (Fig. 2a), indicating a negligible role of the two pathways in MT1-MMP’s action on the
receptor. qRT-PCR data showed that both MT1-MMP siRNAs efficiently reduced mRNA levels of MT1-MMP but not that of MT2-MMP, LDLR, SREBP-2, PCSK9, or IDOL (Fig. 2b), indicating that MT1-MMP had no dramatic effect on transcription of LDLR. We then generated a catalytically inert mutant MT1-MMP by replacing Glu at position 240 (E240) with Ala (MT1-E240A) since E240 is critical for MT1-MMP proteolytic activity. As shown in Fig. 2c, overexpression of the wild-type MT1-MMP but not MT1-E240A markedly reduced LDLR levels in HepG2, Huh7.5, Hepa1c1c7 and rat hepatoma-derived McArdle cells, indicating the requirement of the proteolytic activity of MT1-MMP for its effect on LDLR expression. We then collected culture medium from Huh7.5 cells transfected with scrambled or MT1-MMP siRNA to measure cleaved ectodomain of LDLR. As shown in Fig. 2d, knockdown of MT1-MMP significantly reduced the intensity of an LDLR band with a molecular weight of ~100–120 kDa in culture medium. Concomitantly, LDLR abundance in whole-cell lysate was increased in MT1-MMP knockdown cells (Fig. 2d, lane 2 vs. 1). On the other hand, co-expression of N-terminal Myc-tagged LDLR and HA-tagged
MT1-MMP in HEK293 cells significantly reduced LDLR levels in whole-cell lysate but increased cleaved ectodomain of LDLR in culture medium that was detected by an anti-HA antibody (Fig. 2e, lane 2 vs. 1). The size of the cleaved extracellular domain of LDLR was consistent with the size of the soluble ectodomain of LDLR reported in previous studies. We also measured sLDLR levels in culture medium using ELISA and found that knockdown of MT1-MMP significantly reduced (Fig. 2f), while co-expression of MT1-MMP and LDLR significantly increased the levels of sLDLR in culture medium (Fig. 2g). Thus, MT1-MMP can proteolytically cleave LDLR.

Our next experiments were to investigate the interaction between MT1-MMP and LDLR. As shown in Fig. 3a, LDLR was immunoprecipitated from whole-cell lysate isolated from HepG2 cells by its specific monoclonal antibody, but not a monoclonal anti-Myc antibody. MT1-MMP was present only in the LDLR-immunoprecipitated sample (lane 1). Transferrin receptor was not detectable in the immunoprecipitated pellets. A reciprocal immunoprecipitation using an anti-MT1-MMP antibody to pull down MT1-MMP revealed that LDLR co-immunoprecipitated with MT1-MMP but not the anti-Myc antibody (Fig. 3b, lane 1 vs. 2). Similarly, immunoprecipitation of MT1-MMP from whole-cell lysate of Huh7.5 cells pulled down LDLR (Fig. 3c, lane 2 vs. 1) and vice versa (Fig. 3d, lane 1 vs. 2). We also immunoprecipitated LDLR from Huh7.5 cells overexpressing the HA-tagged wild-type or E240A mutant MT1-MMP. Consistently, LDLR was only pulled down by its specific antibody but not the anti-Myc antibody or protein-G beads only (Supplementary Fig. 1f, lanes 3, 6 and 9). We found that HA-tagged E240A was co-immunoprecipitated with LDLR (lane 9). A long exposure of the membrane showed that a small amount of the wild-type MT1-MMP was present in the LDLR-immunoprecipitated sample, but not in others (lanes 6 vs. 4 and 5). It was likely that overexpressed wild-type MT1-MMP cleaved LDLR when they were co-localized since LDLR levels in the wild-type MT1-MMP-expressing cells were much lower than that in the control and MT1-E240A-expressing cells (Supplementary Fig. 1f, lane 11 vs. 10 and 12). To further confirm these findings, we performed confocal microscopy and found that a majority of endogenous MT1-MMP (green) and LDLR (red) could be detected on the cell periphery and co-localized in Huh7.5 cells (Fig. 3e, yellow in Merged panel). Similarly, HA-tagged MT1-E240A mainly resided on the cell surface and colocalized with endogenous LDLR when expressed in HepG2 cells (Supplementary Fig. 1g). Together, these findings indicate that MT1-MMP associates with LDLR.

We next determined the effects of MT1-MMP on LDLR expression in human primary hepatocytes that are more representative of the functions of human liver than immortalized human hepatoma-derived cell lines, such as HepG2 and Huh7.5. As shown in Fig. 3f, MT1-MMP siRNA reduced mRNA levels of MT1-MMP but not that of LDLR. Conversely, LDLR protein levels were markedly increased in MT1-MMP knockdown cells (Fig. 3g) but reduced in MT1-MMP-overexpressing cells (Fig. 3h). Consistently, sLDLR in the culture medium was reduced when expression of MT1-MMP was silenced (Fig. 3i) but increased when MT1-MMP was overexpressed (Fig. 3j). Collectively, these findings demonstrate that MT1-MMP promotes ectodomain cleavage of LDLR.

MT1-MMP promotes LDLR cleavage in vivo. We further sought to understand the regulatory role of MT1-MMP in LDLR expression in vivo. Considering that MT1-MMP null mice die at 3–4 weeks and that the catabolism of plasma LDL-C is mainly mediated via hepatic LDLR, we generated MT1-MMP liver-specific knockout mice (MT1LKO) through crossing MT1Flox mice with Alb-Cre mice that express Cre recombinase under the control of a hepatocyte-specific albumin promoter (Supplementary Fig. 2a). The MT1Flox mice, in which exons 2 and 4 of the MT1-MMP gene (containing prodomain and catalytic domain) are flanked by LoxP sites, have been successfully used to generate knockout of MT1-MMP in monocytes/macrophages and epidermis. The expression of MT1-MMP in primary hepatocytes isolated from mouse livers was markedly reduced in MT1LKO mice (Fig. 4a). Genotyping results also revealed that the MT1-MMP gene was essentially undetectable in the liver of MT1LKO but not MT1Flox mice (Supplementary Fig. 2b) or other tissues isolated from MT1LKO mice (Supplementary Fig. 2c). mRNA levels of MT1-MMP were also drastically reduced in the liver but not in other tissues of MT1LKO mice (Fig. 4b). MT1LKO mice were active, fertile and indistinguishable from floxed littermates (Supplementary Fig. 2d).

Body weight and plasma ALT activities were also comparable in MT1LKO and MT1Flox mice (Supplementary Fig. 2e, f). We also did not observe a significant difference in mRNA levels of liver MT2-MMP and Aadam17 (Fig. 4c), indicating that the loss of hepatic MT1-MMP was not compensated by these metalloproteinases. MT1LKO mice, however, did not display a significant collagen difference in Mason’s trichrome staining of liver sections (Supplementary Fig. 3a) and plasma active MMP2 (Supplementary Fig. 3b). Thus, knockout of MT1-MMP in hepatocytes did not cause obvious liver damage. Consistent with our findings in cultured cells, the protein levels of LDLR but not LRPI in liver homogenate were significantly increased (Fig. 4d), while the levels of plasma sLDLR were significantly reduced in MT1LKO mice (Fig. 4e). On the other
hand, the levels of PCSK9 in the liver homogenate and plasma were comparable in MT1LKO mice and MT1Flox mice (Supplementary Fig. 3c, d). We have previously reported that active MMP2 inhibited PCSK9-promoted LDLR degradation in hepatic1c7 cells23. However, the levels of plasma pro- and active forms of MMP2 were not significantly altered in MT1LKO mice (Supplementary Fig. 3b), implying that MMP2 did not play an important role in the action of MT1-MMP on LDLR in mice. Furthermore, mRNA levels of Ldlr, Srebp2, Hmgcr and Pcsk9 in mouse liver were also not affected by hepatic deficiency of MT1-MMP (Fig. 4f). On the other hand, MT1LKO mice displayed a mild but significant reduction in plasma levels of total cholesterol (Fig. 4g). FPLC data showed that cholesterol levels in both LDL and HDL fractions were mildly reduced (Fig. 4h). Conversely, plasma TG levels were comparable in MT1LKO and MT1Flox mice (Supplementary Fig. 3e). Similarly, the lack of MT1-MMP in hepatocytes did not significantly affect the levels of liver TG or total cholesterol (Supplementary Fig. 3f, g). To confirm the
Fig. 3 Effects of MT1-MMP on LDLR. Immunoprecipitation of LDLR (a and d) and MT1-MMP (b and c). Whole-cell lysate from HepG2 (a and b) or Huh7.5 cells (c and d) was subjected to immunoprecipitation using protein G beads and a mouse anti-LDLR (LDLR) or anti-Myc (Myc) antibody (a and d), or protein G beads and a mouse anti-MT1-MMP antibody, a mouse anti-Myc (Myc) antibody or rabbit IgG (RigG) (b and c). The immunoprecipitated proteins (IP-Beads) and whole-cell lysate (Input) were subjected to immunoblotting. Image representation was shown. Similar results were obtained from three independent experiments. e Confocal microscopy. hU7.5 cells were fixed, permeabilized, and then incubated with a mouse anti-LDLR monoclonal and a rabbit anti-MT1-MMP monoclonal antibody. MT1-MMP: green; LDLR: red, DAPI: blue. An x-y optical section of the cells illustrates the cellular distribution of molecules (magnification: 100×). f, g MT1-MMP knockdown (n = 3 independent experiments). Primary human hepatocytes were transfected with scrambled (Scram) or MT1-MMP siRNA (MTI). The relative mRNA levels were the ratio of the mRNA levels of the target genes to that of GAPDH at the same condition (f). Same amount of total proteins in whole-cell lysate was subjected to immunoblotting. Cal: calnexin. Relative densitometry was the ratio of the densitometry of LDLR to that of calnexin at the same condition.

sLDLR and plasma lipoproteins. The molecular mass of the main form of sLDLR detected in our study was around 100 kDa that should consist of the entire ligand-binding domain of the receptor, indicating that sLDLR might retain the ability to bind LDLR ligands. Thus, we hypothesized that plasma sLDLR could bind to circulating apoB and apoE-containing lipoproteins. To test this possibility, we utilized gel filtration to determine the distribution of sLDLR in fasting mouse plasma. As shown in Fig. 6a, sLDLR was eluted at fractions 8–17 with a wide peak at fractions 11–13 that were the fractions of LDL even though LDL only accounted for a very small portion of plasma cholesterol (Fig. 6b). There was a shoulder at fraction 15 that was partially overlapped with HDL. An additional small peak of sLDLR was present in the VLDL fractions 8–10. These could be caused by binding of sLDLR to apoE in VLDL and HDL particles. We also immunoprecipitated sLDLR from mouse plasma. The antibody efficiently pulled down sLDLR from plasma of the wild-type mice but not the Ldlr−/− mice (Fig. 6c), indicating the specificity of the antibody. Both apoB100 and apoB48, as well as apoE, were co-immunoprecipitated with sLDLR only from the wild-type mouse plasma (Fig. 6d, lane 2) even though the levels of apoB and apoE were much higher in Ldlr−/− mouse plasma (Fig. 6d, lane 1). Next, we analyzed human plasma using gel filtration chromatography. As shown in Fig. 6e, the majority of sLDLR were eluted at the fractions of the VLDL/chylomicron remnants and LDL even though VLDL/chylomicron remnants only accounted for a minor portion of plasma cholesterol (Fig. 6f). In addition, we noticed that, unlike mouse sLDLR, only a small peak of human sLDLR was eluted at the HDL fractions (Fig. 6e, f). We then measured sLDLR levels in purified human lipoproteins. As shown in Fig. 6g, VLDL exhibited the highest levels of sLDLR per μg proteins, followed by LDL and HDL. The levels of sLDLR in VLDL were ~35-fold more than that in LDL. The discrepancy between purified lipoproteins and plasma samples could be simply due to the relatively lower levels of VLDL present in human plasma. Taken together, these findings suggest that sLDLR associates with apoB and apoE-containing lipoproteins in plasma.

Next, we recruited 148 adult Chinese and measured their plasma levels of total cholesterol, LDL cholesterol, and sLDLR. There were 87 men (average age = 52.6), 46 women (average age = 53.7), and 15 individuals whose gender and age were undisclosed. The levels of sLDLR were comparable among the men, women and undisclosed group (Supplementary Fig. 5a). There was no significant correlation between plasma levels of sLDLR and ages (Supplementary Fig. 5b, r = 0.047, p = 0.5925). On the other hand, the correlation between sLDLR and plasma total cholesterol was statistically significant (Fig. 6b, r = 0.41, p = 0.00000228). Plasma LDL-C levels were also significantly correlated to sLDLR levels (Supplementary Fig. 5c, r = 0.198, p = 0.0199), but to a lesser extent than plasma total cholesterol levels. We also divided participants into three groups based on their plasma levels of total cholesterol or LDL cholesterol, group 1) the normal/desirable cholesterol levels (total cholesterol ≤5.2 mM and LDL cholesterol <3.4 mM, N = 87), group 2) the medium/ borderline high plasma cholesterol levels (5.2 mM ≤ total cholesterol <6.1 mM or 3.4 mM ≤ LDL cholesterol < 4.1 mM, N = 40),
and group 3) the high plasma cholesterol levels (total cholesterol ≥ 6.1 mM or LDL cholesterol ≥ 4.1 mM, N = 21). The levels of sLDLR in the medium and high groups were significantly higher than that in the normal group, while there was no significant difference between the medium and high group (Fig. 6i). Given the critical role of PCSK9 in regulation of plasma LDL cholesterol and hepatic LDLR levels, we also measured circulating PCSK9 in these subjects. As shown in Supplementary Fig. 5d, e, plasma levels of PCSK9 did not significantly associate with plasma LDL cholesterol in the whole group or women but did exhibit a positive correlation with plasma LDL cholesterol levels in men (r = 0.2255, p = 0.0303; Supplementary Fig. 5f). On the other hand, there was no significant association between plasma levels of PCSK9 and sLDLR in the whole group, women or men (Supplementary
Fig. 4 Metabolic effects of MT1−/− mice. a MT1-MMP deletion. Whole-cell lysate of primary mouse hepatocytes isolated from MT1−/− mice was subjected to immunoblotting. Similar results were obtained from three independent experiments. b, c Relative expression of target genes. qRT-PCR measurement of mRNA levels of MT1-MMP in different tissues (b) (four or five mice per group) or MT1-MMP, MT2-MMP, and Adam17 in the liver (five or six mice per group) (c). The relative mRNA levels were the ratio of the mRNA levels of the target genes from different tissues to that of Gopdh in the same tissue. WAT, white adipose tissue. d Liver LDLR levels (6 mice per group). Liver homogenate was subjected to immunoblotting. Representative densitometry was the ratio of the densitometry of LDLR of different mice to that of actin of the same mouse. Representative images were shown. Similar results were obtained from the other three mice. e Plasma sLDLR determined by ELISA (6 mice per group). f Relative mRNA levels (4 mice per group). g Plasma levels of total cholesterol (6 mice per group). h Lipid profile. Same amount of plasma from each mouse in the same group was pooled and applied to FPLC analysis of plasma cholesterol (6 mice per group). i–k Expression of human MT1-MMP. MT1−/− mice were injected with AAVs encoding GFP or human MT1-MMP, respectively. Liver homogenate was applied to immunoblotting (6 mice per group). Representative images were shown. Similar results were obtained from the other three mice (i). Plasma samples were used to measure sLDLR levels (6 mice in the control group and 5 mice in MT1-MMP overexpression group) (j), and total cholesterol levels (6 mice per group) (k). Student’s t test (two-sided) was carried out to determine the significant differences between groups (b–g, j, and k). The significance was defined as \( p < 0.05 \). Values of all data were mean ± SD. Source data are provided as a Source Data file.

Fig. 5g–i). Together, these findings indicate that plasma levels of cholesterol but not PCSK9 are positively correlated to sLDLR levels.

Effects of MT1-MMP on the development of atherosclerosis. We then examined the effect of MT1-MMP on the development of atherosclerosis in mice. ApoE−/− mice (The Jackson Laboratory) were injected with empty AAV or AAV containing human MT1-MMP cDNA and then fed the Western Diet (TestDiet, 5TJN) for 8 weeks. Overexpression of MT1-MMP reduced LDLR levels in the liver (Supplementary Fig. 6a) and significantly increased plasma total cholesterol levels (791 mg/dL in the control, 896 mg/dL in MT1-MMP overexpressing mice, \( p = 0.0283 \); Supplementary Fig. 6b) and lesion area of aortic sinuses in apoE−/− mice (157.5 ± 21.03 \( \mu \)m² \( \times 10^3 \) in the control group and 238.0 ± 21.23 \( \mu \)m² \( \times 10^3 \) in MT1-MMP overexpressing mice, \( p = 0.0228 \); Fig. 6j). Next, we generated MT1−/−/apoE−/− mice through crossing MT1−/− mice with apoE−/− mice. CRE recombinase was then introduced into MT1−/−/apoE−/− mice via AAV under the control of a hepatocyte-specific TBG promoter (AAV-TBG-Cre) to silence hepatic MT1-MMP expression. MT1−/−/apoE−/− mice injected with AAV-TBG-Cre or a control AAV-GFP were then fed the Western Diet from Research Diets Inc. (D12079B) for 8 weeks. The mRNA levels of MT1-MMP were significantly reduced (Supplementary Fig. 6c), while hepatic LDLR levels were significantly increased in mice injected with AAV-TBG-Cre compared to the AAV-GFP injected mice (Supplementary \( p = 0.0385 \), Fig. 6d). However, knockdown of hepatic MT1-MMP did not significantly affect plasma total cholesterol levels (\( p = 0.2578 \), Fig. 6k) or lesion sizes of the aortic sinuses in MT1−/−/apoE−/− mice (Supplementary Fig. 6e, \( p = 0.6081 \)). This was similar to a phenotype reported in the study of PCSK9 that knockdown of PCSK9 in apoE−/− mice did not significantly affect plasma cholesterol levels or atherosclerotic plaque sizes despite of increased hepatic LDLR levels\(^{28}\). Denis et al. reported that knockdown of PCSK9 reduced cholesteryl ester accumulation in the aortas of apoE−/− mice by ~39%, even though the sizes of the entire lesions were not significantly altered\(^{28}\). We employed the same approach and found a similar phenotype, MT1-MMP knockdown caused a significant reduction of ~33% in cholesteryl ester accumulation in the aortas of MT1−/−/apoE−/− mice (mean value: 7.046 and 4.706 \( \mu \)g of cholesteryl ester per aorta in AAV-GFP-injected and MT1-MMP knockdown mice, respectively, \( p = 0.0463 \) Fig. 6l). Together, these findings indicate that MT1-MMP promotes ectodomain shedding of LDLR and accelerates the development of atherosclerosis.

Discussion

LDLR and its family members such as VLDLR, apoER and LRPI undergo ectodomain shedding to release their soluble forms into the extracellular milieu such as cerebrospinal fluid and blood\(^{12,29,30}\). Recently, Girona et al reported that plasma levels of sLDLR were positively associated with non-HDL-C and small LDL numbers in FH children\(^{31}\). Consistently, two independent studies revealed a mild but significant correlation between serum concentrations of sLDLR and LDL-C in healthy adult Japanese and Canadian white population\(^{11,14}\). Here, we also found that sLDLR levels were positively correlated to plasma levels of total cholesterol and LDL cholesterol and significantly increased in subjects with high plasma levels of total or LDL cholesterol in adult Chinese. In addition, we observed a weak positive association between plasma levels of PCSK9 and LDL cholesterol in men but not in the whole group or women, consistent with a previous report\(^{32}\). Conversely, PCSK9 did not significantly correlate to sLDLR in men, women or the whole group. Circulating PCSK9 is mainly secreted from the liver but the underlying mechanism is unclear\(^{33}\). Loss-of-function PCSK9 mutations increase hepatic LDLR levels, which should render more receptors for MT1-MMP-mediated shedding and increase plasma sLDLR levels. Conversely, gain-of-function PCSK9 mutations reduce the amount of hepatic LDLR susceptible for shedding, which should reduce sLDLR in plasma. However, both loss-of-function and gain-of-function mutations can impair PCSK9 secretion and reduce its plasma levels\(^{34–38}\). Thus, a detailed analysis of genotype-phenotype association might be needed to explore the correlation between circulating PCSK9 and sLDLR. Nevertheless, these findings indicate that LDLR shedding plays an important role in lipid metabolism.

The proteinase responsible for LDLR ectodomain shedding, however, remains unknown. In the present study, we found that (1) MT1-MMP interacted with LDLR and knockdown of MT1-MMP expression in different cell types including human primary hepatocytes increased cellular LDLR levels, but decreased sLDLR levels in culture medium, (2) knockout of hepatic MT1-MMP in mice increased LDLR in the liver and reduced plasma levels of sLDLR, (3) overexpression of MT1-MMP reduced LDLR in cultured cells and mouse liver and increased sLDLR in culture medium and mouse plasma, but had no effect on PCSK9 expression, (4) knockdown of MT1-MMP had no effect on the mRNA levels of LDLR and other SREBP2 target genes including PCSK9, SREBP2 and HMGCR, and (5) lysosomal inhibition with chloroquine that suppresses PCSK9- and IDOL-promoted LDLR degradation had no effect on MT1-MMP-induced reduction in LDLR. MT1-MMP knockdown also had no effects on the mRNA levels of PCSK9 and IDOL. Taken
Fig. 5 The impact of MT1-MMP. a Overexpression of human MT1-MMP. Male C57BL/6J mice were injected with empty or the wild-type MT1-MMP adenoviruses (5 mice per group). Liver homogenate was subject to immunoblotting, followed by quantification of LDLR levels relative to actin in the same mouse. Representative images were shown. Similar results were obtained from other mice. Plasma levels of HDL (b) and non-HDL cholesterol (c) (4 mice per group). d Effect of the Western-type diet (6 mice per group). Mice were fed the Western-type diet for 8 weeks. Liver homogenate was subjected to immunoblotting. Relative densitometry of LDLR was determined as described. Actin was used as a loading control. Representative images were shown. Similar results were obtained from the other mice. Liver section staining. Representative figures and quantification (ImageJ 1.52S) of Masson's Trichrome (e) and oil Red-O staining (f) in cross-sections of the liver (6 mice per group). Similar results were obtained from the other mice (magnification: 400×). Plasma levels of cholesterol content in HDL (g) and non-HDL (h) (4 mice per group). i Lipid profile. Same amount of plasma from each mouse in the same group (2 female and 2 male mice per group) was pooled and applied to FPLC analysis of plasma cholesterol. Student’s t test (two-sided) was carried out to determine the significant differences between groups (a–h). The significance was defined as p < 0.05. Values of all data were mean ± SD. Source data are provided as a Source Data file.
together, our findings show that MT1-MMP regulates LDLR levels via promoting ectodomain cleavage of the receptor. We noticed that knockout of hepatic MT1-MMP in mice markedly reduced the levels of plasma sLDLR by ~67% and significantly increased liver LDLR levels (Fig. 4d, e). This indicates that, at least in the liver, MT1-MMP is the proteinase mainly responsible for the ectodomain shedding of LDLR. Given the ubiquitous expression of LDLR, LDLR shedding in other tissues may contribute to plasma sLDLR detected in MT1LKO mice. However, we cannot rule out the possibility that other hepatic proteinases might also mediate LDLR shedding in the liver. For example, it has been reported that ADAM17 can promote LDLR shedding in HepG2 cells to a small extent. More experiments are needed to define these possibilities.
Hepatic LDLR is critical for the clearance of circulating apoB-100 and apoE-containing lipoproteins. Knockdown of MT1-MMP in cultured cells indeed enhanced cellular LDL uptake. Furthermore, MT1LRKO mice, especially on the Western-type diet exhibited an increase in liver LDLR levels and a significant reduction in plasma levels of total cholesterol, HDL and non-HDL cholesterol. This reduction is likely caused by enhanced LDLR-mediated clearance of LDL, chylomicron remnants, as well as apoE-containing HDL particles as reported in PCSK9 knockout mice.\textsuperscript{5,33,39} Consistently, overexpression of MT1-MMP in apoE\textsuperscript{−/−} mice significantly increased plasma cholesterol levels. However, knockdown of MT1-MMP did not significantly affect plasma cholesterol level in apoE\textsuperscript{−/−} mice. It is of note that majority of plasma cholesterol in apoE\textsuperscript{−/−} mice is remnant cholesterol. apoE\textsuperscript{−/−} mice do not express apoE, the ligand of LDLR. Thus, chylomicron and VLDL remnants cannot be cleared by LDLR. This may explain why knockdown of MT1-MMP significantly reduced plasma cholesterol levels in mice with the wild-type background but not in apoE\textsuperscript{−/−} mice. Similarly, knockdown of PCSK9 does not significantly affect plasma cholesterol levels in apoE\textsuperscript{−/−} mice. It has been reported that overexpression of PCSK9 enhances the development of atherosclerosis and knockdown of PCSK9 reduces the levels of aortic cholesterol in apoE\textsuperscript{−/−} mice.\textsuperscript{28} Consistently, we found that the atherosclerotic lesion area in the aortic sinuses was significantly increased in apoE\textsuperscript{−/−} mice overexpressing MT1-MMP, while MT1-MMP knockdown reduced cholesteryl ester accumulated in the aorta of apoE\textsuperscript{−/−} mice. Thus, MT1-MMP stimulates the development of atherosclerosis most likely through promoting hepatic LDLR shedding.

The shed ectodomains of VLDL and apoER contain the ligand-binding domain and can bind to their ligand, Reelin, functioning as a competitive negative regulator to block binding of Reelin to the cell surface receptors.\textsuperscript{29,30,40} Here, we also found that mouse plasma sLDLR co-eluted with VLDL, LDL and part of HDL on the size-exclusion chromatography and co-immunoprecipitated with apoB and apoE. Similarly, the majority of human sLDLR were present in the fractions of VLDL and LDL. A small amount of human sLDLR was also present in the HDL fractions of human plasma, but to a much lesser extent as compared to mouse sLDLR. This might be caused by the fact that mouse HDL is more enriched in apoE compared to human HDL.\textsuperscript{41} For example, knockout of PCSK9 in mice dramatically reduces plasma levels of LDL and HDL due to enhanced LDLR-mediated clearance of LDL and apoE-HDL.\textsuperscript{5,39} On the other hand, inhibition of PCSK9 in human markedly reduces plasma levels of LDL but not HDL cholesterol.\textsuperscript{42} It will be of interest to examine if sLDLR-bound LDL can be taken up by cell-surface LDLR as efficiently as sLDLR-free LDL.

MT1-MMP plays an essential role in tissue remodelling by cleaving extracellular matrix components and many non-extracellular matrix substrates.\textsuperscript{16,20,21,43} However, compared to its roles in collagenolysis,\textsuperscript{44} our understanding of the non-extracellular matrix targets of MT1-MMP and their related physiological roles is poor. Recently, MT1-MMP has been shown to shed LRP1 in breast carcinoma MCF7 cells as well as in vascular smooth muscle cells and in cartilage.\textsuperscript{21,45} However, we found that MT1-MMP had no detectable effect on LRP1 expression in cultured hepatocytes and mouse liver. It has been reported that MT1-MMP efficiently mediated LRP1 shedding in fibroblastoid type but not the epithelial variant of HT1080 cells. Thus, MT1-MMP appears to cleave LRP-1 in a cell-type-dependent manner.

Taken together, our findings uncover the fact that hepatic LDLR is cleaved by MT1-MMP. It is well documented that MT1-MMP is highly expressed in various types of cancer cells and promotes cancer metastasis and angiogenesis.\textsuperscript{16,44,47–50} In addition, MT1-MMP is expressed in human atherosclerotic plaques and promotes plaque rupture by enhancing degradation of collagens.\textsuperscript{51} We further demonstrated that overexpression of MT1-MMP increased the development of atherosclerosis while knockdown of MT1-MMP reduced aortic cholesteryl ester accumulation in apoE\textsuperscript{−/−} mice. Collectively, these findings indicate that MT1-MMP may act as a shared risk factor for both cardiovascular disease and cancers, the two leading causes of global morbidity and mortality. Thus, inhibition of MT1-MMP is a very promising and valuable therapeutic target as it has the potential to increase hepatic LDLR levels, lower circulating LDL-C levels, increase atherosclerotic plaque stability, and reduce the risk of cancer metastasis and invasion.

**Methods**

**Materials.** Minimum Essential Medium α (MEM α) without nucleosides, RPMI 1640 medium, Opti-MEM\textsuperscript{TM}, penicillin-streptomycin, trypsin EDTA solution, Dulbecco\textsuperscript{®} Modified Eagle\textsuperscript{®} Medium (DMEM), fetal bovine serum (FBS), bovine serine albumin (BSA), Bovine insulin, penicillin-streptomycin, trypsin-EDTA solution, Dil-labeled human LDL, unlabeled human LDL, Lipofectamine\textsuperscript{®} 3000, Lipofectamine\textsuperscript{®} RNAiMAX, High Capacity Complementary DNA (cDNA) Reverse Transcription Kit, SYBR\textsuperscript{®}Select Master Mix, GeneJet and PureLink\textsuperscript{TM} HiPure plasmid miniprep kit, Bicinchoninic acid (BCA) protein assay kit, and TRIzol\textsuperscript{®} were obtained from ThermoFisher Scientific. HDL and LDL/very low-density lipoprotein (VLDL) Cholesterol Assay Kit were purchased from Cell Biolabs Inc. Total Cholesterol Assay Kit was from Wako Life Sciences. Human and mouse LDLR or PCSK9 DuoSet\textsuperscript{TM} and human LDLR Quantikine\textsuperscript{®} ELISA kits were from R&D Systems. Alkaline transaminase colorimetric activity assay kit was obtained from Cayman Chemical. RNeasy\textsuperscript{®} Mini kit was from Qiagen. Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), bovine serum albumin (BSA), Complete\textsuperscript{TM} EDTA-free protease inhibitors, and X-tremeGENETM HP DNA
transfection Reagent were purchased from Millipore Sigma. All other reagents were obtained from Fisher Scientific unless otherwise indicated.

Recombinant human PCSK9 was prepared using a FLAG tag at the C-terminus that was purified from culture medium of human embryonic kidney (HEK)-293 cells stably expressing PCSK9 using ANTI-FLAG® M2 affinity gel (Sigma) and FLAG peptide (Sigma) as described in our previous studies6,54. The following antibodies were used: a rabbit anti-FLAG® M2 polyclonal antibody, 772B, HL-1, a mouse mononuclear anti-the linker sequence between ligand binding repeat (LR) 4 and LR5 of LDLR antibody7,54, 3145, a rabbit anti-LDLR polyclonal anti-serum directed against the C-terminal 14 amino acid residues of LDLR5,34, 15A6, a mouse anti-PCSK9 monoclonal antibody53; mouse anti-MT1-MMP monoclonal antibodies (Millipore, clone LEM-2/15B, MAB3329); a rabbit anti-MT1-MMP monoclonal antibody, 2 (Abcam, ab51074); a rabbit anti-MT2-MMP polyclonal antibody (ThermoFisher, PA5-13184); a rabbit anti-LRP1 polyclonal antibody (Novus Biologicals, NB1P-40726); a mouse anti-calnexin monoclonal antibody (BD Biosciences, 610352); a mouse anti-actin monoclonal antibody (BD Biosciences, 612657); a mouse anti-transferrin receptor monoclonal antibody (BD Biosciences, 612125); DyLight® 800 and DyLight® 680 conjugated rabbit anti-hemagglutinin epitope (HA) antibody (Rockland Immunocytogenics, 600-445-384 and 600-444-384); a mouse anti-Myc monoclonal antibody 9E10 that was purified from culture medium from hybridoma cells obtained from the American Type Culture Collection (CRL-1729) using Protein G Sepharose Fast Flow (GE Healthcare).

Animal. C57Bl/6 and apoliprotein E−/− mice were purchased from The Jackson Laboratory, bar Harbor, Maine. The animal facility at the University of Alberta. Thelox strategy was used to selectively inactivate MT1-MMP in mouse liver. The vector, in which exon 2 and exon 4 of mouse MT1-MMP gene were flanked by LoXP sites, was purchased from EUCOMM. MT1-MMPlox/lox® mice were generated in the Clara Christie Centre for Mouse Genomics at the University of Calgary and the embryonic stem cells that have 50% C57 background. The homozygous MT1-MMPlox/lox® was crossed to the FLPo mice (The Jackson Laboratory) to remove the FRT-flanked selection marker (Fig. S3a). The resulting MT1-MMPlox/lox® mice were then backcrossed with C57BL/6 for 6 times and then crossed with transgenic mice expressing Cre recombinase under the control of the albumin promoter (The Jackson Laboratory) to produce mice with a homozygous MT1-MMP null allele (MT1−/−). Genotyping was performed by PCR using AccuStart™ II mouse genotyping kit (Quanta Biosciences, Beverly, MA) and three primers that were designed with Primer3 and synthesized by Integrated DNA Technologies (IDT®, Coralville, CA) (Supplementary Table 1).

Mice were housed cage free with free access to H2O in a climate-controlled facility (22 °C, 43% humidity) with a 12 light/dark cycle. After weaning, mice were fed chow diet ad libitum containing 20% protein, 5% fat, and 48.7% carbohydrates (LabDiet, PICO Laboratory Rodent Diet 20, high density, 4.31 kcal per gm). For the Western diet experiment, mice were fed the Western Diet containing 1.5% cholesterol from TestDiet or Research Diet Inc (kcal from fat 40%, protein 16%, and carbohydrate 44%). All animal procedures were approved by the University of Alberta’s Animal Care and Use Committee (protocol number AUP00000456) and were conducted in accordance with guidelines of the Canadian Council on Animal Care.

Site-directed mutagenesis, cell culture and transfection. Plasmid pCR3.1 containing cDNA of the full-length MT1-MMP with a HA-tag between Asp115 and Glu116 (pCR3.1-MT1-MMP, a kind gift from Dr. Weiss, University of Calgary) was used to generate the mutant form of MT1-MMP. Mutagenesis was performed using the QuickChange Lightning site-directed mutagenesis kit (Agilent Technologies) according to the manufacturer’s instructions53–58. The sequences of the oligonucleotides containing the residues to be mutated were synthesized by IDT® and listed in Supplementary Table 1. The presence of the desired mutation and the integrity of each construct were verified by DNA sequencing.

All cells were cultured at 37°C in a 5% CO2 humidified incubator. cDNA and plasmid DNA were introduced into cells using Lipofectamine RNAiMAX and X-tremeGENE® vectors (Roche Applied Science) or Lipofectamine™ 3000 (HepG2 cells), respectively, according to the manufacturer’s instructions59. Scrambled and predesignsed siRNAs against MT1-MMP were purchased from Dharmacon (Table 1). The presence of the resulting siRNA was confirmed by qRT-PCR using Lipofectamine™ 3000 (HepG2 cells) and primers specific to the gene of interest.

Immunoblot and immunoprecipitation analysis. Whole-cell lysate was prepared from cells 48 h after transfection59. Culture medium was removed from plates and cells were washed with 1 ml of PBS. The cells were scraped, collected in 1 ml of PBS and then centrifuged at 4°C for 5 min (1500 x g). The supernatant was discarded. Cells were lysed in buffer A [1% Triton, 150 mM NaCl, 50 mM HEPES, pH 7.4] containing 1× Complete EDTA-free protease inhibitors for 30 min on ice. After centrifugation for 15 min at 20,000 x g at 4°C, the supernatant was collected as whole-cell lysate. Protein concentrations were determined by the BCA protein assay.

Snap frozen liver tissue samples stored at −80°C were thawed and homogenized using PowerGen 500 Homogenizer (Fisher Scientific) in a buffer (250 mM Sucrose, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1% triton X-100, and protease inhibitors). Homogenized tissue was then incubated on ice for 30 min with intermittent vortex in every 10 min. After centrifugation for 15 min at 20,000 × g at 4°C, the supernatant was collected and protein concentrations were determined by the BCA protein assay. Same amount of total proteins from tissue homogenate or whole-cell lysate subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE, 8%) and transferred to membrane by electroblotting (8000004, GE Healthcare) by electroblotting. Immunoblotting was performed using specific antibodies as indicated. Antibody binding was detected using IRDye®680 or IRDye®800-labeled goat anti-mouse or anti-rabbit IgG (Li-Cor). The signals were detected on a Licor Odyssey Imaging System (Li-Cor). All image figures were adjusted in Adobe Photoshop 2020 and then made in Adobe Illustrator 2020. The color of most images was discarded in Photoshop. Contrast and exposure were adjusted equally across the whole image in Photoshop for better visualization. The sizes of images were adjusted in Illustrator.

Immunoprecipitation and western blotting. Cells were cultured in DMEM containing 5% newborn calf lipoprotein-poor serum (NCLPPS) for 16 h to increase expression of LDLR and then lysed in insulin buffer A containing 1× Complete EDTA-free protease inhibitors. Same amount of total proteins was applied to a monoclonal anti-LDLR antibody (HL-1), a monoclonal anti-MT1-MMP antibody (Clone LEM-2/15B, MAB3329), a monoclonal anti-Myc antibody (9E10) and protein G beads to immunoprecipitate LDLR or MT1-MMP. Immunoprecipitated samples were washed three times with lysis buffer A. The immunoprecipitated proteins were then eluted from the beads by addition of 2 × SDS-PAGE sample buffer (100 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 0.04% bromophenol blue) containing 5% β-mercaptoethanol. The eluted samples and whole-cell lysate were subjected to SDS-PAGE (8–20%) and immunoblotting.
Folch method
Liver samples were homogenized in 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, and 1 mM EDTA by a glass/Te
plating and protein concentrations were measured by the BCA assay. Same amount of culture medium containing 5% NCLPPS for 16 h, the cells were cultured in DMEM containing 5% NCLPPS for 16 h. Cell surface proteins were then biotinylated with EZ-Link Sulfo-NHS-LC-Biotin (Pierce) in PBS (0.5 mg per ml, pH 8.0) for 15 min at 4 °C. After washing with 100 mM glycine, the cells were lysed in 150 μl of lysis buffer A. A total of 50 μl of cell lysate was saved and ~100 μl of the lysate (same amount of total proteins) were added to 60 μl of 5% TCA (in 0.1% SDS) contacting 1× protease inhibitors. The lysate was then transferred to a 96-well black plate for the measurement of fluorescence using a SYNERGY plate reader (Exitation: 520 nm; Emission: 580 nm). The concentrations of total proteins in each well were measured using the BCA protein assay. LDL uptake was calculated by normalization of the fluorescence units to the amount of total proteins in the same well. The results obtained in the presence of excess LDL revealed the nonspecific binding. Specific binding was calculated by subtraction of nonspecific binding from the total counts measured in the absence of unlabeled LDL.

Biotinylation of cell surface proteins
Huh7.5 cells were seeded in a 6-well plate in 2 ml of culture medium at the density of 2.5 × 10⁵ cells per ml. 24 h later, cells were transfected with scrambled or MT1-MMP siRNA. 24 h after transfection, cells were cultured in DMEM containing 5% NCLPPS for 16 h. Cell surface proteins were then biotinylated with EZ-Link Sulfo-NHS-LC-Biotin (Pierce) in PBS (0.5 mg per ml, pH 8.0) for 15 min at 4 °C. After washing with 100 mM glycine, the cells were lysed in 150 μl of lysis buffer A. A total of 50 μl of cell lysate was saved and ~100 μl of the lysate (same amount of total proteins) were added to 60 μl of 5% TCA (in 0.1% SDS) contacting 1× protease inhibitors. The lysate was then transferred to a 96-well black plate for the measurement of fluorescence using a SYNERGY plate reader (Exitation: 520 nm; Emission: 580 nm). The concentrations of total proteins in each well were measured using the BCA protein assay. LDL uptake was calculated by normalization of the fluorescence units to the amount of total proteins in the same well. The results obtained in the presence of excess LDL revealed the nonspecific binding. Specific binding was calculated by subtraction of nonspecific binding from the total counts measured in the absence of unlabeled LDL.

Immunofluorescence
Huh7.5 or HepG2 cells seeded onto coverslips (1.0 × 10⁵ cells per ml) were transfected with or without MT1-MMP cDNA as indicated69,70. 36 h later, cells were cultured in DMEM containing 5% NCLPPS for 16 h, fixed with 3% paraformaldehyde and then permeabilized with cold methanol for 10 min at −20 °C. The cells were then incubated with anti-human LDL receptor (Abcam) and a rabbit anti-MT1-MMP (Abcam) (1:100) or anti-HA antibody. Antibody binding was detected using Alexa Fluor 488 goat anti-rabbit or mouse IgG and Alexa Fluor 568 goat anti-mouse or rabbit IgG. Nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI, Thermofisher). After washing, coverslips were mounted on the slides with ProLong Diamond Antifade Mountant (ThermoFisher). Localization of LDLR and MT1-MMP were determined using a Leica SP5 laser scanning confocal microscope (filters: 461 nm for DAPI, 519 nm for Fluor 488, and 543 nm for Fluor 568).

Immunohistochemistry
All liver sectioning and staining were performed by the HistoCore facility in Alberta Diabetes Institute at the University of Alberta. Briefly, a portion of livers was fixed in 10% formalin and embedded in paraffin, followed by a section at 5 μm thickness onto Histobond slides. The slides were then subjected to hematoxylin and eosin (H&E) or Trichrome staining. Cryosectioned liver tissues were used for Oil Red O staining. The resulting slides were imaged using ZEISS Axio Observer A1 in triplicate. Relative stained area was then quantified with ImageJ software (1.52s, National institute of Health) using color segmentation and threshold analysis.

Extraction of lipids
Lipids were extracted from the liver and the aorta using the Folch method85. Liver samples were homogenized in 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, and 1 mM EDTA by a glass/Teflon homogenizer for 20 s. Lipids were extracted from 4 mg of liver homogenate with chloroform/methanol (2:1). The aorta was cut into small pieces and homogenized in 250 μl of 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, and 1 mM EDTA by a glass/Teflon homogenizer. Samples were sonicated 3 × 10 s on ice and the volume was completed to 1 ml with PBS. Homogenized samples were transferred into a glass tube with 4 ml of chloroform/ methanol (2:1) and incubated for 1 h with vigorous agitation. The extraction from liver or aorta homogenate was spun at 10,000 × g for 10 min, the bottom phase was transferred to a new glass tube and dried under nitrogen. Lipids were dissolved in 1 ml or 500 μl of chloroform with 2% Triton X-100 for the liver and the aorta homogenate, respectively. The samples were dried under nitrogen and redissolved in 1 ml or 250 μl of distilled H2O for the liver and the aorta samples respectively. TG and total cholesterol were then measured using commercial kits from Roche Diagnostics and Wako Diagnostics, respectively. For the aorta samples, 30 μl of lipid extract was loaded per well on a 96-well plate together with a set of known cholesterol standards for the measurement of total cholesterol and free cholesterol using commercial kits from Cell Biolabs. Cholesterol ester was then determined by deducting the values of free cholesterol from that of total cholesterol.

Plasma lipid, ALT, and sLDLR analysis
Blood samples were collected into heparin-coated tubes (BD Biosciences) from tail or saphenous veins of mice (age of 8–12 weeks) on a chow or the Western-Type diet. The blood was centrifuged at 3000 × g for 20 min for plasma isolation. 10 or 20 μl of plasma from each mouse were subjected to ALT or total cholesterol measurement using the ALT colorimetric assay kit and the Cholesterol E kit (Wako Diagnostics), respectively, according to the manufacturer’s instructions. Plasma HDL and non-HDL were separated using the HDL and LDL/VLDL Cholesterol Assay kit. 5 μl of plasma were mixed with 5 μl of the Precipitation Reagent, centrifuged at 2000 × g for 20 min. The supernatant (HDL fraction) was transferred to a new tube. The pellet was dissolved in 10 μl of PBS. Cholesterol content in each fraction was measured using the Cholesterol E kit. Lipoprotein profiles were analyzed by the Lipidome Core Facility at the University of Alberta. Briefly, 5 μl of plasma from each mouse in the same experiment group was pooled and subjected to Fast Protein Liquid Chromatography (FPLC) with a Superose 6 column. Immunohistochemistry was measured by both western blot and ELISA. Cells were transfected with siRNA or plasmid vectors as described above. 24 h later, cells were switched to serum free medium for 16 h. After collection, culture medium was subjected to trichloroacetic acid (TCA) precipitation (15% final TCA concentration) overnight at 4 °C. After a centrifugation at 20,000 × g for 20 min at 4 °C, the pellets were washed once in cold acetone (~20 °C), dried and redissolubilized in 9 M urea. Protein concentrations were measured using the BCA assay. Equal amount of total proteins was analyzed by immunoblotting. sLDLR levels in culture medium (1:5 diluted in the Reagent Diluent) and plasma from each mouse (1:20 diluted in the Reagent Diluent) were assessed using the Human or Mouse LDLR DuoSet ELISA assay kit in accordance to manufacturer’s protocol (R&D Systems). Briefly, standards and diluted samples were added to each well in a 96-well plate that was coated with the Capture Antibody and blocked in Reagent Diluent. After incubating for 2 h at room temperature, the plate was washed and then incubated with the Detection Antibody, followed by Streptavidin-HRP, Substrate Solution and Stop Solution. The optical density of each well was measured using a SYNERGY plate reader at 540 nm. Plasma sample from Ldlr−/− mice was used as a negative control for mouse plasma samples. Specific levels were calculated by subtraction of counts of plasma samples of Ldlr−/− mice from that of MT1EKO and MT1EKO mice.

Human plasma sLDLR and lipoprotein cholesterol
148 subjects were recruited for the collection of overnight fasting blood samples at the Taishan Medical University. Participants were non-obese healthy adults, without pre-existing health problems. All patient information was obtained based on the questionnaire. Inclusion criteria were healthy adults (20+ years) without pre-existing health problems. No exclusion was applied. All participants did not report health problems. EDTA-plasma was isolated, aliquoted and stored at −80 °C. Plasma triglycerides (TG), LDL, HDL total cholesterol were measured in the clinical laboratory of the Taishan Medical University. Plasma levels of sLDLR and PCSK9 were determined using the human LDLR and PCSK9 DuoSet ELISA assay kit according to manufacturer’s instruction (R&D system), respectively. All participants provided written informed consent and the procedure was approved by the Research Ethics Committee in Institute of Atherosclerosis at Taishan Medical University (Shandong First Medical University).

Plasma lipoprotein profile was assessed by gel filtration. Briefly, 500 μl of pooled normal fasting human heparin-plasma (Innovative Research Inc. Novi, MI) or pooled fasting mouse plasma (C57BL/6J) was applied to a size exclusion (Superose 6) column (HR 16/60 FPLC purification column, GE Life Sciences) and then eluted with 1× PBS. The eluate was collected in 1 ml fractions. The levels of total cholesterol and sLDLR in each fraction were measured using the Cholesterol E kit (Wako Diagnostics) and the Human or Mouse LDLR DuoSet ELISA assay kit (R&D system), respectively.

Gelatin zymography
Culture medium was collected from cells 48 h after treatment and protein concentrations were measured by the BCA assay. The same amount of culture medium (80 μg of total proteins) or 5 μl of plasma sample from each mouse was used for zymography analysis as described31. Briefly, samples were mixed with 4 × denaturing sample buffer (200 mM Tris-HCl, pH 6.8, 8% SDS, 40% glycerol, 0.08% bromophenol) and subjected to electrophoresis on an 8% SDS gel containing 2 mg/ml of gelatin. After, the gel was washed 3 times with 2.5% Triton X-100 in H2O and then incubated for 16–20 h in a renaturing buffer (30 mM Tris-HCl, pH 7.5, 5 mM CaCl2, 150 mM NaCl and 0.05% NaN3). Gels were stained with Coomassie Blue R250, destained in 40% (v/v) methanol with 10% (v/v) acetic acid.
and then scanned on a Licor Odyssey Imaging System. MMP2 was visualized as a clear band of degraded gelatin on the blue background of Coomassie Blue staining.

**Adenovirus and adeno-associated virus.** Recombinant adenoassociated vectors containing cDNAs for the wild-type and mutant human MT1-MMP were generated using the AdEasy™ Adenoviral Vector System (Agilent Technologies) (8). MT1-MMP cDNA was subcloned into the pSHuttle-CMV vector between the restriction enzyme sites HindIII and EcoRV, followed by in vitro re-lex recombinase. Adenoviruses were packaged and amplified in QBI 293 cells and then purified using CsCl density-gradient ultracentrifugation. After desalting with the EconoPac 10DG column (Bio-Rad), adenoviral particles were injected into mice intravenously (1.0 × 1011 particles per mouse). 72 h later, tissues and blood were collected from euthanized mice. For cultured cells, cells were plated on a 12-well plate at the density of 1.5 × 105 cells per well in 1 ml of culture medium. 24 h later, cells were infected with adenovirus (4 × 109 particles per well). 48 h after infection, the cells were collected for analysis.

AAV carrying cDNA for human MT1-MMP or cre recombinase under the control of a hepatitis-specific thymoxine-binding globulin (TGB) promoter was generated using the AAV-DJ® Helper Free Expression Complete System in accordance with the manufacturer’s instruction (Cell BioLabs, Inc). pAAVTR1G.P, EGFP.WPRE.SGH (Addgene) was cut with NotI and HindIII and a fragment of about 4.8 kb was isolated. cDNA for human MT1-MMP and cre recombinase was amplified from plasmid pCRII-MT1-MMP and adenovirus expressing cre with PCR using forward primer with the NotI site in the beginning and a reverse primer containing the HindIII site at the end, respectively. The PCR fragment was digested with NotI and HindIII, purified and then ligated to NotI-HindIII digested pAAVTR1G.PLEGFP.WPRE.SGH using the Quick ligation kit (NEB). The integrity of the cDNA of MT1-MMP and cre was verified by DNA sequencing. The resulting vector together with pAAV-DJ® was retransfected into QBI 293A cells using polyethyleneimine (PEI, Polysciences, Warrington, PA). 72 h post-transfection, AAV was purified from the cells using OptiPrep™ (Sigma) density-gradient ultracentrifugation as described (22). AAV particles were collected from the 40% density step, diluted in PBS and concentrated with Amicon Ultra-15 Centrifugal Filter Units (Millipore, 100k MWCO). AAV titers were determined using qRT-PCR. Purified AAV particles were injected into mice intravenously (1.0 × 1011 genomic copy gc per mouse). Mice were euthanized at the indicated time after injection. Blood and tissues were collected for analysis.
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Author contributions

D.W.Z. designed and performed the experiments, collected and analyzed data, and supervised and directed this project. A.A., X.X., H.M.G., F.W. and N.Y. performed the experiments, collected and analyzed data. N.Y., Y.X., L.C. and S.Q. collected, processed and analyzed data. A.A., X.X., H.M.G., F.W. and N.Y. performed the experiments, collected and analyzed data. A.A., X.X., H.M.G., F.W. and N.Y. performed the experiments, collected and analyzed data. A.A., X.X., H.M.G., F.W. and N.Y. performed the experiments, collected and analyzed data.

Competing interests

The authors declare no competing interests.

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