Aims RhoA and Rac1 activation plays a key role in endothelial dysfunction. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a major receptor for oxidized low-density lipoprotein (ox-LDL) in endothelial cells (ECs). Membrane type 1 matrix metalloproteinase (MT1-MMP) has been shown to be involved in atherogenesis. This study was conducted to investigate the role of the LOX-1-MT1-MMP axis in RhoA and Rac1 activation in response to ox-LDL in ECs.

Methods and results Ox-LDL induced rapid RhoA and Rac1 activation as well as MT1-MMP activity in cultured human aortic ECs. Inhibition of LOX-1 prevented ox-LDL-dependent RhoA and Rac1 activation. Knockdown of MT1-MMP by small interfering RNA prevented ox-LDL-induced RhoA and Rac1 activation, indicating that MT1-MMP is upstream of RhoA and Rac1. Fluorescent immunostaining revealed the colocalization of LOX-1 and MT1-MMP, and the formation of a complex of LOX-1 with MT1-MMP was detected by immunoprecipitation. Blockade of LOX-1 or MT1-MMP prevented RhoA-dependent endothelial NO synthase protein downregulation and cell invasion, Rac1-mediated NADPH oxidase activity, and reactive oxygen species generation.

Conclusion The present study provides evidence that the LOX-1-MT1-MMP axis plays a crucial role in RhoA and Rac1 activation signalling pathways in ox-LDL stimulation, suggesting that this axis may be a promising target for treating endothelial dysfunction.

1. Introduction

Endothelial dysfunction is crucial for the initiation and development of atherosclerosis.1,2 Oxidized low-density lipoprotein (ox-LDL) impairs endothelial function, giving rise to reactive oxygen species (ROS) generation, and reduced nitric oxide (NO) production.1,2 It is widely acknowledged that ox-LDL downregulates the expression of endothelial nitric oxide synthase (eNOS), which is associated with the activation of small GTP-binding protein, RhoA.3 Earlier, we reported that lysophosphatidylcholine, a phospholipid compound of ox-LDL, activates RhoA within 1 min in cultured human aortic endothelial cells (HAECs).4 These observations indicate that rapid RhoA activation plays a pivotal role in the downregulation of eNOS induced by ox-LDL. Ox-LDL-triggered ROS generation is predominately mediated via NADPH oxidase in the vasculature, and small GTP-binding protein Rac1 is a component of NADPH oxidase,5,6 indicating that Rac1 is critical for ox-LDL-stimulated ROS generation via NADPH oxidase in ECs. Lectin-like ox-LDL receptor-1 (LOX-1) with a type II membrane protein structure has been identified as a major endothelial receptor for ox-LDL in endothelial cells (ECs).5 Thus, an ox-LDL-LOX-1 axis appears to be crucial for the pathogenesis of endothelial dysfunction in coronary artery disease. However, there is no report indicating that LOX-1 forms a complex with other molecule(s).

Degradation of the vascular extracellular matrix by secreted and membrane type matrix metalloproteinases...
(secreted MMPs and MT-MMPs) plays an important role in smooth muscle cell migration and invasion and plaque instability, which in turn, contribute to the pathogenesis of coronary artery disease, especially acute coronary syndromes. Ox-LDL has been shown to increase the expression and activities of MMPs including secreted MMPs and MT-MMPs. MT-MMPs possess transmembrane and cytoplasmic domains in addition to extracellular domains. The activities of secreted MMPs and MT-MMPs are modulated by the tissue inhibitor of metalloproteinases (TIMPs). MT-MMPs are inhibited by TIMP-2, whereas TIMP-1 and TIMP-2 inhibit secreted MMPs. Membrane type 1 MMP (MT1-MMP) participates in skeletal development, cell growth, and angiogenesis. MT1-MMP also has been shown to play a role in cell migration and invasion mediated via the Rho family of GTPases such as Rho, Rac, and Cdc42.

In the present study, we hypothesized that MT1-MMP plays an integral role in ox-LDL-triggered RhoA and Rac1 signalling pathways and that MT1-MMP and LOX-1 may form a complex.

2. Methods

The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.1 Materials

The sources of most of the conventional reagents were described previously. Recombinant human TIMP-1 and TIMP-2 were obtained from DAIICHI Fine Chemical Co., Ltd (Toyama, Japan). Neutralizing antibody to LOX-1, TS92, was used for the inhibition of LOX-1. Diphenyleneiodonium (DPI), a selective NADPH oxidase inhibitor, was from Sigma-Aldrich Co. (St Louis, MO, USA) and C3 exoenzyme, a Rho inhibitor, from Upstate Biotechnology (Lake Placid, NY, USA).

2.2 Preparation of ECs

HAECs were cultured according to the suppliers’ instructions (Clonetics Inc., Walkersville, MD, USA and Sanko Junyaku Co., Ltd, Tokyo, Japan) and used for all experiments after 5–10 passages.

2.3 Preparation of ox-LDL

Human LDL was isolated from the serum of fasting normolipidaemic volunteers by sequential ultracentrifugation and ox-LDL was prepared by incubating native LDL for 24 h at 4°C in phosphate-buffered saline (PBS) containing 5 μmol/L CuSO4, and then extensively dialyzed against PBS and sterilized by filtration as described previously.

2.4 Western blotting

The expression of RhoA, Rac1, eNOS, and α-tubulin was determined by western blotting. For immunoblotting, we used mouse monoclonal antibodies to RhoA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Rac1 (Upstate Biotechnology) diluted 1:500, and to eNOS (Transduction Laboratories, Lexington, KY, USA) diluted 1:1000 and α-tubulin (Santa Cruz Biotechnology) diluted 1:500. The signals from immunoreactive bands were visualized by...
2.5 GTP/GDP exchange of RhoA and Rac1

GTP-bound active forms of RhoA and Rac1 were determined by pull-down assay as described previously. Extracts of HAECs were incubated at 4 °C for 45 min with glutathione-Sepharose 4B beads coupled with glutathione-S-transferase (GST)-rhotekin fusion protein for determination of RhoA activity or GST-p21-activated kinase for determination of Rac1 activity. Bound RhoA and Rac1 were semi-quantitatively detected by western blotting.

2.6 Measurement of MT1-MMP activity of membrane fractions

To evaluate MT1-MMP activity, we prepared membrane fractions of HAECs as described previously. Briefly, cells were lysed with a hypotonic buffer, then sonicated and centrifuged at 15,000 g for 10 min. The separated membrane fractions were assessed by the commercially available fluorescent assay kit (SensoLyte 520 MMP-14 assay kit, AnaSpec, San Jose, CA, USA) according to the manufacturer’s instructions.

2.7 Small interfering RNA

MT1-MMP expression was silenced by siRNA (sense strand for MT1-MMP) (RNAi Co., Ltd, Tokyo, Japan). HAECs were transfected with double-strand siRNA in serum-free medium mixed with oligofectamine (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Four hours after transfection, HAECs were incubated in a medium containing 2% foetal bovine serum (FBS) for 48 h. Alternatively, cells were treated with an irrelevant siRNA (sense strand) as a negative control.

2.8 Generation of anti-human LOX-1 monoclonal antibodies

Anti-human LOX-1 monoclonal antibodies were generated by immunizing Balb/c mice subcutaneously with the recombinant protein of the extracellular domain of LOX-1, LOX-1(61–273). The splenocytes from the immunized mice were fused with myeloma cell line, P3U1, and the resultant hybridomas were screened using enzyme-linked immunosorbent assay to the antigen. Among the positive clones, purified monoclonal antibody from clone #1-1 was used for western blotting, and another antibody from #10-1 was used for immunocytochemistry.

2.9 Fluorescent immunostaining

A mouse monoclonal antibody available to LOX-1 (termed #10-1) was used for immunostaining. HAECs cultured on chamber slides were fixed in 10% formalin for 10 min, and then incubated with the antibodies to LOX-1 and MT1-MMP (Chemicon International, Inc., Temecula, CA, USA) at room temperature for 60 min. After washing, anti-mouse Alexa 488 and anti-rabbit Alexa 594 (Molecular
Probes, Eugene, OR, USA) were reacted for 60 min. Stained cells were stored in the dark until they were analyzed by a confocal microscope (Olympus, Tokyo, Japan).

2.10 Immunoprecipitation

HAECs were extracted in a RIPA buffer (Sigma –Aldrich), and the lysates were centrifuged at 10,000 \( g \) at 4°C. The supernatant was precleared and reacted with anti-MT1-MMP (Chemicon) and anti-LOX-1 antibody (R&D Systems Inc., Minneapolis, MN, USA) at a concentration of 1.0 \( \mu \)g/mL, respectively. Immunoprecipitated protein was resolved by sodium dodecyl sulfate –polyacrylamide gel electrophoresis, followed by western blotting of LOX-1 and MT1-MMP, respectively. The positive controls of endothelial cell lysate were loaded. We used the primary antibody against LOX-1 (termed #1-1) and MT1-MMP (Chemicon), and anti-mouse or anti-rabbit IgG True blot (eBioscience, Inc., San Diego, CA, USA), which detects native antibody but not the denatured 55 kDa heavy chain and 23 kDa light chains of the immunoprecipitating antibody, as the secondary antibody.

2.11 Adenovirus gene transfer

HAECs were infected with adenoviruses encoding a dominant negative form of N19RhoA, N17Rac or LacZ at a multiplicity of infection of approximately 50 as described previously. \(^{22,25}\) This procedure resulted in the expression of LacZ as a marker gene in nearly 100% of the transfected cells. After transfection, cells were washed three times with PBS and incubated for 48 h in medium containing 2% FBS, followed by the experiments.

2.12 EC invasion assay

EC invasion was assayed by a commercially available kit (BD BioCoatTM Angiogenesis System: Endothelial Cell Invasion, BD Biosciences, Bedford, MA, USA) according to the manufacturer’s instructions. Briefly, HAECs were pretreated with N19RhoA or MT1-MMP siRNA as described above and then stimulated with 25 \( \mu \)g/mL ox-LDL for 12 h. Invasive cells were fluorescently labelled with Calcein-AM and the fluorescence intensity was quantitatively determined by Image J 1.34 (National Institutes of Health, Bethesda, MD, USA).

2.13 Measurement of intracellular ROS generation

Detection of intracellular ROS generation was performed by a previously established method using the ROS-sensitive fluorescent probe 2′,7′-dichlorodihydrofluorescein-diacetate (H2DCF-DA) (Molecular Probes).\(^5\) HAECs were incubated in 2% FBS-containing medium and 10 \( \mu \)mol/L H2DCF-DA for 10 min. Ox-LDL (25 \( \mu \)g/mL) was added to the cells and incubated for up to 60 min at 37°C. The fluorescence was measured using a fluorescent microscope (Olympus) at an excitation and emission wavelength of 475 nm and 525 nm, respectively. DCF fluorescence intensity was quantitatively determined by Image J 1.34. For each photograph, the cellular and background fluorescence values were obtained by tracing the

![Figure 4](https://academic.oup.com/cardiovascres/article-abstract/84/1/127/282112)
of endothelial cell lysate is shown in lane 3.

did not contain the LOX-1 band (right panel, lane 1). Each control immunoblot (right panel, lane 2), whereas control antibody-associated immunoprecipitates experiment, LOX-1 (70 kDa) was detected from MT1-MMP immunoprecipitates 1), whereas 64 kDa band was recognized in control cell lysate. In a reversed isotype-matched control antibody did not show 64-kDa band (left panel, lane

band recognized by immunoblotting with anti-MT1-MMP antibody was detected MT1-MMP as determined by immunoprecipitation. In the left panel, 64 kDa band of the LOX-1-immunoprecipitates (lane 2). Immunoprecipitates made using an band recognized by immunoblotting with anti-MT1-MMP antibody was detected MT1-MMP as determined by immunoprecipitation. In the left panel, 64 kDa

shape of cells. Results were displayed in a ratiometric fashion normalized for the control condition.

2.14 Measurement of NADPH oxidase activity
Since ox-LDL increases NADPH oxidase activity, the enzymatic activities of NADPH oxidase of homogenates of the cells were assessed by lucigenin-enhanced chemiluminescence (L-CL) as previously described.26 The assay solution contained 50 mmol/L HEPES (pH 7.4), 1.0 mmol/L EDTA, 6.5 mmol/L MgCl2, 5.0 μmol/L lucigenin as an electron acceptor and 1 mmol/L NADPH as a substrate. After preincubation at 37 °C for 10 min, the reaction was started by adding 50 μg of lucigenin. Final volume of the reaction solution was 1.0 mL. Photon emission was continuously recorded for 15 min with a CL reader (ALOKA, BLR-201, Tokyo, Japan). The chemiluminescent signals observed in the absence of homogenates were subtracted from the chemiluminescence signals of the samples. The chemiluminescence signal was corrected for the protein concentration of each cell homogenate and expressed as counts per minute (cpm) per milligram protein for a 15-min period. In some experiments, the homogenates were preincubated with 10 μmol/L DPI for 20 min before L-CL measurement.

2.15 Densitometric analysis and statistical analyses
After scanning blots into a computer (EPSON GT5500 ART, Tokyo, Japan), the optical densities of individual immunoblots were analyzed using the NIH IMAGE Program software as described previously.18,20 Statistical analyses were performed using ANOVA with Scheffe’s post hoc test if appropriate. A value of P < 0.05 was considered significant.

3. Results
3.1 Role of LOX-1 in ox-LDL-induced RhoA and Rac1 signalling
We determined the effect of ox-LDL on RhoA and Rac1 activation in cultured HAECs. Pull-down assays revealed the time-course of GTP-loading of RhoA and Rac1 in response to 25 μg/mL ox-LDL in HAECs (Figure 1A and B). Ox-LDL increased the GTP-loading of RhoA within 5 min after adding ox-LDL and returned to control after 15 min of ox-LDL stimulation. The GTP-loading of Rac1 was induced 5 min and peaked 15 min after exposure to ox-LDL. The relatively high levels of GTP-loading of Rac1 persisted for at least up to 120 min after ox-LDL stimulation. Next, we examined the effect of inhibition of LOX-1 on the ox-LDL-induced RhoA and Rac1 activation. Pretreatment of HAECs with TS92 (10 μg/mL) for an hour prevented ox-LDL-induced RhoA and Rac1 activation 5 and 15 min after exposure to ox-LDL, respectively (Figure 1C and D). This indicated an integral role of LOX-1 in ox-LDL-induced RhoA and Rac1 activation.

3.2 Effect of TIMPs on RhoA and Rac1 activation induced by ox-LDL
To clarify the relation between secreted MMPs or MT-MMPs and LOX-1-mediated RhoA and Rac1 activation, HAECs were pretreated with 40 nmol/L TIMP-1 and TIMP-2 for 60 min before adding ox-LDL. Figure 2A and B show that TIMP-2, but not TIMP-1, inhibited the GTP-loading of RhoA and Rac1 caused by ox-LDL. TIMP-1 or TIMP-2 alone did not alter the basal levels of RhoA and Rac1 in untreated HAECs (data not shown). These findings suggest that MT-MMPs are upstream of ox-LDL-triggered RhoA and Rac1 activation.

3.3 MT1-MMP activity
Since MT1-MMP is one of the well-characterized MT-MMPs, we first measured the activity of MT1-MMP in response to ox-LDL stimulation within 30 min. Ox-LDL induced a significant increase in the activity of MT1-MMP after 15 and 30 min of stimulation (Figure 3).

3.4 Role of MT1-MMP in ox-LDL-induced RhoA and Rac1 signalling
Then, we examined the role of MT1-MMP in ox-LDL-induced RhoA and Rac1 activation through LOX-1. HAECs were transfected with siRNA to MT1-MMP before exposure to ox-LDL. In the transfected cells, knockdown of MT1-MMP prevented the ox-LDL-triggered activation of RhoA and Rac1 as determined by pull-down assays (Figure 4A and B). Figure 4C shows the effect of the transfection of MT1-MMP siRNA on the MT1-MMP protein level as determined by western blotting, indicating the approximately 70% reduction in the MT1-MMP level by treatment with the siRNA.
3.5 Molecular interaction of LOX-1 and MT1-MMP

To determine the distribution of LOX-1 and MT1-MMP in cultured HAECs, we performed immunostaining. Figure 5A shows the expression of LOX-1 and MT1-MMP, as well as the merged image, respectively, indicating that LOX-1 was partially colocalized with MT1-MMP in HAECs.

To clarify the interaction of LOX-1 with MT1-MMP, we first performed the immunoprecipitation using an initial antibody to LOX-1. Although little or no expression was detected by normal mouse IgG as a negative control antibody (Figure 5B, left panel, lane 1), the band of 64 kDa of MT1-MMP was detected in the LOX-1-associated immunoprecipitates (Figure 5B, left panel, lane 2). Counter experiment using anti-MT1-MMP antibody revealed that the band of LOX-1 (Mr 70 kDa) was recognized in the MT1-MMP-immunoprecipitates (Figure 5B, right panel, lane 2).

Figure 6  Role of the LOX-1/MT1-MMP axis in RhoA-dependent eNOS protein expression. (A, B) Effect of inhibition of RhoA by C3 exoenzyme and N19RhoA on ox-LDL-induced eNOS downregulation. HAECs were treated with 0.25 μg/mL of C3 exoenzyme overnight or infected with adenoviruses encoding N19RhoA or LacZ and then stimulated with 25 μg/mL ox-LDL for 18 h, followed by western blotting. (C) Effect of inhibition of LOX-1 on ox-LDL-induced eNOS downregulation. Cells were treated with 10 μg/mL TS92 for 1 h and then stimulated with 25 μg/mL ox-LDL for 18 h, followed by western blotting. (D, E) Effects of TIMP-2 and knockdown of MT1-MMP on ox-LDL-induced eNOS downregulation. Immunoblots are from an experiment representative of four similar experiments. Quantitative results of eNOS expression were normalized by α-tubulin levels. Bars are mean ± SD of quantitative densitometric analyses from three separate experiments. *P < 0.05 vs. control; †P < 0.05 vs. ox-LDL.
results strongly suggested that LOX-1 forms a complex with MT1-MMP. Exposure to ox-LDL did not change the level of formation of LOX-1 and MT1-MMP (data not shown).

3.6 Role of MT1-MMP-RhoA axis in ox-LDL-induced downregulation of eNOS protein expression

First, we confirmed that ox-LDL-induced downregulation of eNOS protein expression is mediated via RhoA activation in our culture system. Inhibition of RhoA by C3 exoenzyme (Figure 6A) and dominant-negative RhoA (Figure 6B) reversed ox-LDL-induced decrease of eNOS protein expression. Treatment of HAECs with TS92 for LOX-1 inhibition reversed the ox-LDL-induced eNOS protein downregulation after 18 h of incubation (Figure 6C). In addition, TIMP-2, but not TIMP-1, prevented ox-LDL-induced downregulation of eNOS protein expression (Figure 6D). Ox-LDL-induced eNOS protein downregulation was also reversed in the MT1-MMP-silenced cells, whereas scrambled siRNA had no effect (Figure 6E).

3.7 Role of RhoA-MT1-MMP axis in cell invasion

To further clarify the role of the MT1-MMP/RhoA axis in RhoA-mediated events, we performed an endothelial invasion assay. Ox-LDL-induced EC invasion was attenuated by N19RhoA, indicating the RhoA-dependent mechanism (Figure 7A). Pretreatment of HAECs with silencing of MT1-MMP by siRNA suppressed the oxidized LDL-triggered EC invasion (Figure 7B).

3.8 Role of LOX-1-MT1-MMP axis in NADPH oxidase-dependent ROS generation

The levels of intracellular ROS generation were increased 1 h after exposure to ox-LDL (Figure 8A and B), which was prevented by TS92 (Figure 8A and C) as well as by DPI and N17Rac (Figure 8A, E, and I). These indicated that ox-LDL-induced ROS generation occurs via Rac1/NADPH oxidase through LOX-1. Silencing of MT1-MMP by siRNA significantly blocked ox-LDL-triggered ROS generation and NADPH oxidase activity (Figures 8B and C).

4. Discussion

In this study we demonstrated the crucial role of MT1-MMP in LOX-1-initiated RhoA- and Rac1-dependent signalling pathways in ox-LDL stimulation in ECs. Another important finding of the present study is that LOX-1 forms a complex with MT1-MMP. Here, we propose the concept of the regulation of ox-LDL-induced endothelial dysfunction by the LOX-1-MT1-MMP axis.

For the first time we show that knockdown of MT1-MMP markedly attenuated rapid RhoA and Rac1 activation caused by ox-LDL via LOX-1. Meriane et al. have shown that GM6001, a broad spectrum of MMP inhibitor, blocked sphingosine-1-phosphate-induced RhoA activation within 1 min in bone marrow-derived stromal cells, suggesting cooperation between MMP-mediated signalling events and RhoA signalling pathway. Our experiments demonstrated that GM6001 (data not shown) and TIMP-2, but not TIMP-1, prevented ox-LDL-triggered rapid RhoA and Rac1 activation in HAECs, suggesting that extracellular domain of MT1-MMP may play a role in the signal transduction of ox-LDL-exposed cells via LOX-1. In fact, the increase in MT1-MMP activity was induced by ox-LDL within 15 min. However, the detailed mechanism(s) remain(s) to be clarified.

The present study indicates that MT1-MMP is upstream of ox-LDL-stimulated RhoA and Rac1 activation. It is generally accepted that MT1-MMP is an MMP-2 activator. This raises the issue whether or not inhibition of MMP-2 might prevent ox-LDL-triggered RhoA and Rac1 activation in our experimental system. We found that TIMP-1, which inhibits MMP-2 but not MT-MMPs, did not suppress the ox-LDL-dependent RhoA and Rac1 activation. These results suggest that MMP-2 is not involved in ox-LDL-triggered RhoA and Rac1 signalling pathways in ECs.
Fluorescent immunostaining revealed that LOX-1 was colocalized with MT1-MMP to some degree in cultured HAECs. The notable finding of the present study by immunoprecipitation is that LOX-1 formed a complex with MT1-MMP in HAECs. To the best of our knowledge, the present study is the first to show the formation of a complex of LOX-1 with another molecule in ECs. It would be of importance to determine whether LOX-1 and MT1-MMP forms a complex in vivo.

Our experiments suggested that LOX-1 also formed a complex with MT1-MMP in the aortae of Watanabe heritable hyperlipidaemic rabbits (data not shown). The issue should be extensively investigated in terms of the physiopathology.

Figure 8  (A) HAECs were stimulated with 25 μg/mL ox-LDL for 60 min with or without pretreatment using 10 μg/mL TS92 for 1 h or 10 μmol/L DPI for 30 min. Cells were also infected with adenoviruses encoding N17Rac or LacZ (g, h, i). Then, intracellular ROS formation was assessed by H2DCF-DA oxidation-based fluorescence. Photomicrographs are from an experiment representative of six independent experiments. Bars represent mean ± SD of six independent experiments. (B, C) Effect of inhibition of MT1-MMP on ox-LDL-induced ROS generation and NADPH oxidase activity. (B) HAECs were stimulated with 25 μg/mL ox-LDL for 60 min with MT1-MMP siRNA or scrambled siRNA. Intracellular ROS formation was then assessed by H2DCF-DA oxidation-based fluorescence. Photomicrographs are from an experiment representative of six independent experiments. Bars are mean ± SD of quantitative analyses from six separate experiments. (C) Endothelial NADPH oxidase activity was measured with or without silencing of MT1-MMP 15 min after adding ox-LDL. Treatment of endothelial homogenates with DPI abolished the enzymatic activity of NADPH oxidase. Data are the mean ± SD from five separate experiments. *P < 0.01 vs. control; †P < 0.01 vs. ox-LDL.
LOX-1-MT1-MMP axis in ox-LDL stimulation

of vascular remodelling. We also found that ox-LDL did not change the levels of a complex of LOX-1 and MT1-MMP, although ox-LDL increased the protein expression of LOX-1 and MT1-MMP after 18 h of incubation (data not shown). We further attempted to determine whether MT1-MMP modulates LOX-1 in cultured HAECs. Our data indicated that silencing of MT1-MMP did not alter the uptake of Dil-labelled ox-LDL (data not shown). This would suggest that there is no change in LOX-1 function because of MT1-MMP knockdown at least insofar as ox-LDL uptake is concerned. These findings of the present study might open new avenues in the understanding of signal transduction and phsiopathy of ox-LDL-LOX-1-dependent endothelial dysfunction.

MT1-MMP reportedly plays a role in angiotensin-mediated vascular remodelling. It has also been shown that MT1-MMP is involved in NO-induced endothelium migration and tube formation. Various agonists related to vascular remodelling such as angiotensin II, endothelin, and thrombin regulate the cascade of Rac1/NADPH oxidase/ROS generation, which modulates nitric oxide synthesis including eNOS protein expression. Here, we show that the silencing of MT1-MMP inhibited the RhoA-dependent eNOS protein expression. Thus, it would be of great interest to investigate the role of MT1-MMP in the agonist-triggered NADPH oxidase-dependent ROS generation and NO synthesis other than ox-LDL stimulation.

Recent studies of bone marrow transplantation of MT1-MMP−/− mice have demonstrated that macrophage-derived MT1-MMP plays a role in the pathogenesis of plaque stability. Since LOX-1 is also expressed by plaque macrophages, it will be of interest to investigate the involvement of the LOX-1-MT1-MMP axis in macrophages.

In summary, the present study provides new insights into the regulation by the LOX-1-MT1-MMP axis of ox-LDL-mediated RhoA and Rac1 activation and their downstream signalling pathways in ECs, and suggests that this axis may be a promising target for treating endothelial dysfunction in coronary artery disease.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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