Crucial Role of Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) in RhoA/Rac1-Dependent Signaling Pathways in Thrombin-Stimulated Endothelial Cells

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Aim: Thrombin induces vascular responses including the promotion of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) protein expression, which is modulated by small GTPases RhoA and Rac1, Ca²⁺ signaling and reactive oxygen species (ROS). Recent studies have shown that membrane type 1 matrix metalloproteinase (MT1-MMP) functions not only as a protease but also as a signaling molecule. In this study, we hypothesized that MT1-MMP may mediate RhoA and Rac1 activation and their downstream events in thrombin-stimulated endothelial cells.

Methods: We used cultured human aortic endothelial cells (HAECs). MT1-MMP was silenced by small interfering RNA (siRNA). RhoA was inhibited by C3 exoenzyme, whereas adenovirus-mediated gene transfection of dominant negative RhoA and Rac1 was used for the inhibition of RhoA and Rac1. RhoA and Rac1 activation was determined by pull-down assays. Intracellular Ca²⁺ concentrations ([Ca²⁺]i) were fluorescently measured by fura-2 assay. NADPH oxidase activity was determined by lucigenin-enhanced chemiluminescence.

Results: Inhibition of RhoA attenuated thrombin-triggered [Ca²⁺]i increase and TF and PAI-1 expression in HAECs, whereas thrombin-triggered ROS generation and TF and PAI-1 expression were blocked by inhibition of Rac1. Silencing of MT1-MMP attenuated thrombin-triggered RhoA and Rac1 activation, resulting in the attenuation of downstream events including Ca²⁺ signaling, NADPH oxidase activity, ROS generation, and TF and PAI-1 expression.

Conclusions: The present study shows that MT1-MMP mediates the RhoA/Ca²⁺ and Rac1/NADPH oxidase-dependent signaling pathways in thrombin-induced vascular responses.


Key words: Thrombin, Endothelial dysfunction, NADPH oxidase, Calcium, Molecular expression

Introduction

Thrombin induces vascular responses, including impaired nitric oxide production, increased permeability, Ca²⁺ signaling and thrombogenicity in endothelial cells as well as the migration and proliferation of smooth muscle cells. In terms of endothelial dysfunction, the activation of small GTPases RhoA and Rac1 is involved in Ca²⁺ signaling and redox-sensitive signaling-dependent molecular expression such as tis-
sue factor (TF) and plasminogen activator inhibitor-1 (PAI-1)⁵⁻⁸.

We have reported that thrombin-induced geranylgeranylation, the first step in small GTPase activation, plays an essential role in the full activation of unprocessed RhoA in endothelial cells and that statin rapidly blocks thrombin-triggered RhoA activation signaling pathways and its downstream events⁹. We have also demonstrated that RhoA-dependent Ca²⁺ signaling plays a role in monocyte adhesion-induced TF and PAI-1 molecular expression, which is also blocked by statin⁶, ¹⁰, ¹³. In addition, it is well acknowledged that reactive oxygen species (ROS) generation contributes to endothelial dysfunction-related molecular expression¹².

It has been shown that membrane type 1 matrix metalloproteinase (MT1-MMP) functions not only as a protease but also as a signaling molecule¹³, ¹⁴. Recently, we have shown that MT1-MMP plays an integral role in oxidized low-density lipoprotein (LDL)-triggered RhoA and Rac1 activation and their downstream events in endothelial cells¹⁵.

In the present study, we examined the role of MT1-MMP in thrombin-triggered RhoA and Rac1 activation signaling and intracellular Ca²⁺ concentrations ([Ca²⁺]ᵢ) as well as their downstream events, including NADPH oxidase activity, ROS generation and TF and PAI-1 expression.

**Methods**

**Materials**

The sources of most conventional reagents were described previously⁴⁻¹⁰, ¹⁶. Thrombin, a Ca²⁺ chelator BAPTA-AM, an inhibitor of NADPH oxidase diphenyl iodonium (DPI), and an antioxidant N-acetyl cysteine (NAC) were purchased from Sigma-Aldrich Co. (St. Louis, MO). A RhoA inhibitor C3 exoenzyme was obtained from Upstate Biotechnology (Lake Placid, NY), a GGTase 1 inhibitor GGTI-286 from Calbiochem (San Diego, CA) and protease-activated receptor 1 (PAR1) agonist peptide (TRAP) from TOCRIS bioscience (Ellisville, MO).

**Preparation of Endothelial Cells**

Human aortic endothelial cells (HAECs) were cultured according to the suppliers’ instructions (Clonetics Inc., Walkersville, MD and Sanko Junyaku Co., Ltd., Tokyo, Japan) and used for all experiments after 5 to 10 passages⁷, ⁹, ¹⁵.

**Western Blotting**

The expression of TF, PAI-1, MT1-MMP, RhoA, Rac1, and β-actin was determined by Western blotting⁶, ⁷, ⁹, ¹⁵. For immunoblotting, we used mouse monoclonal antibodies to human TF (Enzyme Research Laboratories, South Bend, IN) diluted 1:500, human PAI-1 ( Molecular Innovation Inc., Royal Oak, MI) diluted 1:500, RhoA (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:500, Rac1 (Upstate Biotechnology) diluted 1:1000, MT1-MMP (Daichi Fine Chemical Co., Ltd., Takaoka, Japan) diluted 1:500, and β-actin (Santa Cruz Biotechnology) diluted 1:2000. The signals from immunoreactive bands were visualized by an Amersham ECL system (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, U.K.). Each filter was reprobed with β-actin for normalization of each protein expression.

**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 previously⁶, ¹⁵, ¹⁷. 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 hours in medium containing 2% fetal bovine serum (FBS), followed by the experiments.

**siRNA**

MT1-MMP expression was silenced by siRNA 5'-CUGGCAGUUCGGCUAGAUUUC-3' (sense strand for MT1-MMP) (RNAi Co., Ltd., Tokyo, Japan)¹⁵. HAECs were transfected with double-strand siRNA in serum-free medium mixed with oligofectamine (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Four hours after transfection, HAECs were incubated in a medium containing 2% FBS for 48 hours. Alternatively, cells were treated with an irrelevant siRNA 5’-GUACCGCACGUCAUUCGCAUC-3’ (sense strand) as a negative control.

**GTP/GDP Exchange of RhoA and Rac1**

GTP-bound active forms of RhoA and Rac1 were determined by pull-down assay as described previously⁶, ¹⁵, ¹⁸. Extracts of HAECs were incubated at 4°C for 45 min with glutathione-Sepharose 4B beads coupled with glutathione-S-transferase (GST)-rh otoxin fusion protein for determination of RhoA activity or GST-p21-activated kinase (PAK) for determination of Rac1 activity. Bound RhoA and Rac1 were detected by Western blotting.

**Measurement of Intracellular ROS Generation**

Intracellular ROS generation was detected by a
previously-established method using a ROS-sensitive fluorescent probe 2',7'-dichlorodihydro-fluorescein-diacetate (H$_2$DCF-DA) (Molecular Probes, Eugene, OR)\textsuperscript{8, 15, 19}. HAECs were incubated in 2% FBS-containing medium and 10 $\mu$M H$_2$DCF-DA for 10 min. Thrombin (1 U/mL) was added to the cells and incubated for up to 30 min at 37$^\circ$C. The fluorescence was measured using a fluorescent confocal microscope (Olympus, Tokyo, Japan) at excitation and emission wavelengths of 475 nm and 525 nm, respectively. DCF fluorescence intensity was quantitatively determined by ImageJ 1.42q\textsuperscript{8, 15}. For each photograph, the cellular and background fluorescence values were obtained by tracing the shape of cells. Results were displayed in a ratiometric fashion normalized for the control condition.

**Measurement of Intracellular Ca$^{2+}$ Concentrations ([Ca$^{2+}$])**

[Ca$^{2+}$]$_i$ were measured in response to thrombin in the absence or presence of external Ca$^{2+}$ as described previously\textsuperscript{7, 8, 10, 11, 16}. Briefly, endothelial cells were grown to confluence in a 35 mm glass bottom dishes (MarTek Co., Ashland, MA). After the medium was discarded, the cells were washed twice with prewarmed Tyrode’s solution containing 0.2% bovine serum albumin. The cells were incubated with 5 $\mu$M fura-2/acetoxyethyl ester (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 37$^\circ$C for 30 min and washed with the same Tyrode solution. The dish was mounted on an inverted fluorescence microscope (Nikon eclipse TE2000, Nikon, Tokyo, Japan), and then fixed with a flow-through chamber (0.2 mL volume). Fura-2-loaded cells were superfused continuously (4 mL/min) with prewarmed (37$^\circ$C) Tyrode’s solution with or without agents. In some experiments, Ca$^{2+}$-free Tyrode’s solution was used. The cells were exposed to alternating excitation wavelengths at 340 and 380 nm, and the fluorescence image was monitored by a cooled digital CCD camera (ORCA-R2; HAMAMATSU Photonics, Hamamatsu, Japan). Data were recorded and processed using an Aqua Cosmos (HAMA-MATSU Photonics, Hamamatsu, Japan). Data were recorded and processed using an Aqua Cosmos (HAMA-MATSU Photonics, Hamamatsu, Japan). Data were recorded and processed using an Aqua Cosmos (HAMA-MATSU Photonics, Hamamatsu, Japan). Data were recorded and processed using an Aqua Cosmos (HAMA-MATSU Photonics, Hamamatsu, Japan). Data were recorded and processed using an Aqua Cosmos (HAMA-MATSU Photonics, Hamamatsu, Japan).

**Measurement of NADPH Oxidase Activity**

The enzymatic activities of NADPH oxidase of homogenates of the cells were assessed by lucigenin-enhanced chemiluminescence as previously...
described\textsuperscript{15, 20}. The assay solution contained 50 mM HEPES (pH 7.4), 1.0 mM EDTA, 6.5 mM MgCl\textsubscript{2}, 5.0 μM lucigenin as an electron acceptor and 1 mM NADPH as a substrate. After preincubation at 37°C for 10 min, the reaction was started by adding 50 μg/mL C3 exoenzyme overnight or infected with adenoviruses encoding N19RhoA or LacZ and then stimulated with 1 U/mL thrombin for 18 hours, followed by Western blotting. TF and PAI-1 expression was normalized by β-actin. Representative immunoblots are shown at the top. Bars represent the mean ± SD of 4 independent experiments. *p<0.05 vs control. C. Effect of Ca\textsuperscript{2+} chelation on TF and PAI-1 expression in thrombin-stimulated HAECs. HAECs were pretreated with or without 10 mM BAPTA-AM for 30 min before adding 1 U/mL thrombin, followed by Western blotting after 18 hours of incubation. Representative immunoblots from 3 similar experiments are shown. Bars represent the mean ± SD of 3 independent experiments. *p<0.05 vs control and **p<0.05 vs thrombin.

Densitometric Analysis and Statistical Analyses
After scanning blots into a computer (CanoScan 8400F, Canon, Tokyo, Japan), the optical densities of individual immunoblots were analyzed using NIH IMAGE Program software as described previously\textsuperscript{8, 9, 15}. Statistical analyses were performed using ANOVA with Scheffe’s post hoc test if appropriate. A value of p<0.05 was considered significant.

Results
Effect of Silencing of MT1-MMP on TF and PAI-1 Expression
We first determined the effect of knockdown of MT1-MMP on TF and PAI-1 molecular expression.
HAECs were pretreated with MT1-MMP siRNA or scrambled siRNA and then incubated with 1 U/mL thrombin for 18 hours, followed by Western blotting. Fig. 1 shows that the silencing of MT1-MMP by siRNA markedly suppressed thrombin-induced TF and PAI-1 expression. This indicates that MT1-MMP mediates thrombin-induced TF and PAI-1 protein expression in HAECs.

**Role of RhoA and Ca\(^{2+}\) in Thrombin-Induced Protein Expression of TF and PAI-1**

We next examined the effect of inhibiting RhoA by C3 exoenzyme or N19RhoA on the expression of TF and PAI-1. HAECs were pretreated with 5 μg/mL C3 exoenzyme overnight or infected with adenoviruses encoding N19RhoA or LacZ and then stimulated with 1 U/mL thrombin for 18 hours. Fig. 2A and 2B show that inhibition of RhoA markedly attenuated thrombin-triggered TF and PAI-1 expression in cultured HAECs. Then, we examined the effect of Ca\(^{2+}\) chelation by BAPTA-AM on TF and PAI-1 expression in response to thrombin, indicating that thrombin-evoked TF and PAI-1 expression was blocked by Ca\(^{2+}\) chelation (Fig. 2C).

**Role of RhoA in thrombin-triggered Ca\(^{2+}\) Signaling**

Effect of inhibition of RhoA by C3 exoenzyme on thrombin-triggered [Ca\(^{2+}\)]\(_i\) increase was examined. Fig. 3A and 3B show that inhibition of RhoA suppressed thrombin-triggered [Ca\(^{2+}\)]\(_i\) increase in the presence or absence of external Ca\(^{2+}\), indicating that RhoA is involved in intracellular Ca\(^{2+}\) mobilization and Ca\(^{2+}\) influx from the plasma membrane in thrombin-stimulated HAECs, as previously described in stimulation with a phospholipid component of oxidized LDL lysophosphatidylcholine or monocyte attachment\(^7, 10\). In addition, an inhibitor of GGTase I, which is an enzyme responsible for the first activation step geranylgeranylation of RhoA, suppressed thrombin-triggered [Ca\(^{2+}\)]\(_i\) (data not shown). Taken together, these findings suggested that RhoA-dependent Ca\(^{2+}\) signaling plays a role in TF and PAI-1 expression in thrombin-stimulated HAECs.

**Role of Rac1 in Thrombin-Triggered ROS Generation and TF and PAI-1 Expression**

Fig. 4A shows thrombin-induced TF and PAI-1 expression in cells pretreated with a relatively specific inhibitor of NADPH oxidase DPI or an antioxidant NAC, indicating the prevention of thrombin-triggered...
TF and PAI-1 expression by both agents. Next, we treated HAECS with N17Rac to determine the role of Rac1 in thrombin-induced ROS generation and TF and PAI-1 expression. ROS generation was determined 30 min after adding thrombin to HAECS. 

**Fig. 4B** shows photomicrographs of intracellular ROS generation, indicating that inhibition of Rac1 suppressed ROS generation in HAECS in response to thrombin. In addition, inhibition of Rac1 prevented thrombin-triggered TF and PAI-1 expression in HAECS stimulated for 18 hours (**Fig. 4C**). These results suggest that Rac1 plays a key role in the regulation of thrombin-induced ROS generation and TF and PAI-1 protein expression.

**Role of MT1-MMP in Thrombin-Induced RhoA and Rac1 Activation**

We then focused on the role of MT1-MMP in thrombin-stimulated RhoA and Rac1 activation. MT1-MMP expression was silenced by siRNA before thrombin stimulation. **Fig. 5A** shows the protein expression of MT1-MMP in HAECS treated with
MT1-MMP siRNA or scrambled siRNA as determined by Western blotting, indicating approximately more than 60% reduction in MT1-MMP protein levels by siRNA. In transfected cells, knockdown of MT1-MMP markedly attenuated thrombin-triggered activation of RhoA and Rac1 as determined by pull-down assays (Fig. 5B, 5C). These results suggest that MT1-MMP plays an integral role in RhoA and Rac1 activation in thrombin-stimulated HAECs.

**Role of MT1-MMP in Ca\(^{2+}\) Signaling, NADPH Oxidase Activity, and ROS Generation**

To further clarify the role of MT1-MMP in the downstream events of RhoA and Rac1 activation caused by thrombin stimulation, we examined the effect of knockdown of MT1-MMP by siRNA on thrombin-triggered [Ca\(^{2+}\)]\(_i\) increase and ROS generation. Fig. 6A shows that silencing of MT1-MMP suppressed [Ca\(^{2+}\)]\(_i\) increase after adding 1 U/mL thrombin in the presence or absence of external Ca\(^{2+}\). Knockdown of MT1-MMP reduced thrombin-triggered [Ca\(^{2+}\)]\(_i\) increase by 30 ± 10% in the presence of external Ca\(^{2+}\), whereas thrombin-triggered [Ca\(^{2+}\)]\(_i\) increase was decreased by 23 ± 7% in the absence of external Ca\(^{2+}\). These findings indicated that silencing of MT1-MMP suppressed both intracellular Ca\(^{2+}\) mobilization and Ca\(^{2+}\) influx caused by thrombin. Fig. 6B shows that knockdown of MT1-MMP attenuated thrombin-induced NADPH oxidase activity. In addition, Fig. 6C shows that silencing of MT1-MMP by siRNA attenuated ROS generation in thrombin-stimulated HAECs as determined by H\(_2\)DCF-DA fluorescence.

**Discussion**

In the present study, we provide evidence that MT1-MMP is involved in thrombin-triggered RhoA and Rac1 activation and their downstream events in cultured endothelial cells, which may imply that MT1-MMP is a membrane modifier of endothelial dysfunction (Fig. 7).

Thrombin has been shown to increase the expression of MT1-MMP (Fig. 8). We also observed that the increase of membrane translocation of MT1-MMP induced by thrombin took place 4 hours after
adding thrombin to HAECs with no significant change up to 1 hour (Fig. 9). This may contribute to MT1-MMP-triggered downstream events including TF and PAI-1 expression although we observed that thrombin-triggered RhoA and Rac1 activation signaling pathways were induced within 1 hour. The issue of the time-course profile among MT1-MMP membrane translocation/expression, RhoA/Rac1 activation, and downstream events, including various molecular expression and Ca$^{2+}$ signaling in thrombin stimulation, needs to be elucidated.

Thrombin plays an important role in coagulation, platelet aggregation and thrombus formation and also exerts vascular responses mediated via a novel family of G-protein-coupled receptors, protease-activated receptors (PARs), which have a unique activation mechanism\(^1\). Thrombin-triggered proteolytic cleavage of the extracellular domain generates a new short peptide sequence that binds to the segment of the receptor, resulting in transmembrane signaling and G protein activation\(^1\). Among four different types of PARs, PAR1 appears to be the primary receptor that

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**Fig. 6.** Role of MT1-MMP in thrombin-induced [Ca$^{2+}$]$_i$ increase and ROS generation. A) Effect of knockdown of MT1-MMP by siRNA on [Ca$^{2+}$]$_i$ in response to thrombin in the presence or absence of external Ca$^{2+}$ in HAECs. HAECs were pretreated with MT1-MMP siRNA and then 1 U/mL thrombin was added. Bars represent the mean ± SD of 40 cells in an experiment representative of 3 separate experiments. *p<0.0001. B) Effect of silencing of MT1-MMP on NADPH oxidase activity in thrombin-stimulated HAECs. NADPH oxidase activity was measured with or without silencing of MT1-MMP 5 min after adding 1 U/mL thrombin in endothelial cells. The homogenates were preincubated with or without 10 μM DPI for 20 min before lucigenin-enhanced chemiluminescence assays. Data are expressed as the mean ± SD of 5 independent experiments. *p<0.0001 and **p<0.0005. C) Effect of silencing of MT1-MMP on thrombin-induced ROS generation. Fluorescence intensity was quantitatively analyzed by ImageJ 1.42q. Photomicrographs are from an experiment representative of 6 separate experiments. Bars represent the mean ± SD of 6 separate experiments. *p<0.0001 and **p<0.0005.
mediates active responses in endothelial cells\textsuperscript{21, 22}. In the present study, in addition to thrombin, we employed the PAR1 agonist peptide (TRAP) which also induced RhoA and Rac1 activation as well as the up-regulation of TF and PAI-1 protein expression in HAECs (data not shown). Thus, it is conceivable that thrombin-induced RhoA and Rac1 activation and their downstream events, including ROS generation and prothrombotic molecular expression, may be mediated via the axis of PAR1 and MT1-MMP. In the present study, we attempted to determine the association of MT1-MMP with PAR1. Although MT1-MMP appeared to co-localize with PAR1, we failed to detect the formation of PAR1 and MT1-MMP as determined by immunoprecipitation. This issue remains to be elucidated by considering the quality of antibodies against PAR1 and MT1-MMP employed as well as the molecular affinity of the two molecules in the cultured endothelial cells used in the present study. An issue other is that we cannot preclude the possibility that thrombin signaling is through a mechanism other than PAR1.

In the axis of platelet-derived growth factor (PDGF) and PDGF receptor $\beta$ (PDGFR$\beta$), MT1-MMP plays a crucial role in its signaling pathways and MT1-MMP and PDGFR$\beta$ form a complex in vascular smooth muscle cells\textsuperscript{19}. Similar results have been shown in the axis of hyaluronic acid and CD44\textsuperscript{23}. We have recently shown that MT1-MMP mediates oxidized LDL-induced RhoA and Rac1 activation as well as their downstream events, including eNOS protein down-regulation and ROS generation, and that MT1-MMP forms a complex with lectin-like oxidized LDL receptor-1 (LOX-1), a major receptor for oxidized LDL, as determined by immunofluorescent staining and immunoprecipitation in cultured HAECs\textsuperscript{15}. We also found that the activation signaling pathways via a receptor for advanced glycation end products (RAGE) are mediated via MT1-MMP and that RAGE and MT1-MMP form a complex in cultured smooth muscle cells\textsuperscript{24}. These findings suggest that MT1-MMP plays a critical role in vascular responses and is associated with membrane molecules.

Moreover, it has been shown that the complex formation of MT1-MMP and phosphorylated caveolin-1 takes place in caveolae membrane with involvement of the cytoplasmic domain of MT1-MMP\textsuperscript{25, 26} in human umbilical venous endothelial cells and breast cancer cells, as determined by immunoprecipitation and immunofluorescent studies. The issues of molecular interaction between these two molecules in various cell types in response to agonist stimulation remain to be elucidated.

More recently, we have shown that RhoA and Rac1 activation plays a key role in rapid vascular responses \textit{in vivo}, which is a good target for endothe-
The current study suggests that silencing of MT1-MMP contributes to suppress thrombin-induced endothelial dysfunction via NADPH oxidase-mediated Rac1 activation, including ROS generation and thrombosis-related molecular expression. NADPH oxidase activity and ROS generation in addition to Rac1 activation were attenuated by knockdown of MT1-MMP in this study. This suggests that MT1-MMP plays a central role in oxidant stress, which is crucial for the pathogenesis of cardiovascular disease. We performed additional experiments to see whether the overexpression of the dominant negative mutant of Rac1 affects MT1-MMP. The results showed that the down-regulation of Rac1 significantly increased MT1-MMP expression in the presence of thrombin, while the expression of MT1-MMP tended to increase in response to N17Rac treatment without thrombin stimulation (Fig. 8). The interpretation of the result does not seem to be simple. One possibility is that the knockdown of downstream Rac1 may induce an increase in the expression of upstream MT1-MMP. It seems unlikely that MT1-MMP functions downstream of Rac1 in our experimental situation. Another possibility is that this may be just a redox-sensitive response due to the knockdown of Rac1/NADPH oxidase/ROS generation.

We reported that RhoA-dependent NF-κB phosphorylation and RhoA-related Ca²⁺ signaling mediate TF and PAI-1 expression in monocyte adhesion to endothelial cells and that there is cross talk between Ca²⁺ signaling and Rac1-dependent ROS generation.⁶, ⁸, ¹⁰ In this study, we found that thrombin-triggered TF and PAI expression was abolished by inhibition of MT1-MMP-mediated NADPH oxidase/Rac1/ROS generation and RhoA/Ca²⁺ signaling. Moreover, Fortier et al. have shown that silencing of MT1-MMP attenuates sphingosine-1-phosphate-triggered Ca²⁺...
mobilization in glioblastoma cells. We here show that thrombin-triggered Ca\(^{2+}\) signaling is suppressed by silencing of MT1-MMP in endothelial cells. Together, our results suggest the role of MT1-MMP in agonist-triggered Ca\(^{2+}\) signaling. Since Ca\(^{2+}\) entry via transient receptor potential canonical (TRPC) channels is crucial for thrombin-induced NF-κB phosphorylation in endothelial cells, it will be of interest to investigate the relation between MT1-MMP and TRPC channels in endothelial cells.

It was reported that thrombin-induced matrix invasion of vascular smooth muscle cells was inhibited via direct inhibition of MT1-MMP in the possible beneficial effects of red wine polyphenolic compounds, a green tea extract and cocoa procyanidins. Recently, it has been shown that inhibition of MT1-MMP may be a key to preventing the myocardial remodeling after myocardial infarction. Taken together with our study, these findings suggest that MT1-MMP may play an integral role in the pathogenesis of cardiovascular disease and that its inhibition may be an attractive strategy for prevention of the disease.

In conclusion, we show that MT1-MMP mediates thrombin-triggered RhoA and Rac1 activation signaling pathways, suggesting that this molecule may be a good target for endothelial dysfunction and oxidant stress.

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