

Membrane-type matrix metalloproteinases: Their functions and regulations



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Abstract

Membrane-type matrix metalloproteinases (MT-MMPs) form a subgroup of the matrix metalloproteinase (MMP) family, and there are 6 MT-MMPs in humans. MT-MMPs are further sub-classified into type I transmembrane-type (MT1-, MT2-, MT3- and MT5-MMPs) and glycosylphosphatidylinositol (GPI)-anchored type (MT4- and MT6-MMPs). In either case MT-MMPs are tethered to the plasma membrane, and this cell surface expression provides those enzymes with unique functionalities affecting various cellular behaviours. Among the 6 MT-MMPs, MT1-MMP is the most investigated enzyme and many of its roles and regulations have been revealed to date, but the potential roles and regulatory mechanisms of other MT-MMPs are gradually getting clearer as well. Further investigations of MT-MMPs are likely to reveal novel pathophysiological mechanisms and potential therapeutic strategies for different diseases in the future.

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Introduction

Membrane-type matrix metalloproteinases (MT-MMPs) form a subgroup within the matrix metalloproteinase (MMP) family, and there are 6 enzymes in humans. The first MT-MMP to be discovered was MT1-MMP (MMP-14) and it was characterized as a cell surface proMMP-2 activator [1]. Following this, MT2-MMP (MMP-15) [2], MT3-MMP (MMP-16) [3], and MT4-MMP (MMP-17) [4] were discovered in a short period of time, and later MT5-MMP (MMP-24) [5,6], and MT6-MMP (MMP-26) [7,8] were further discovered. Among those 6 MT-MMPs, MT4-MMP and MT6-MMP are tethered to the plasma membrane through a glycosylphosphatidylinositol (GPI)-anchor, while the other MT-MMPs are tethered through transmembrane domain. The function of MT1-MMP has been characterized extensively since its discovery while the functions of other MT-MMPs are still not clearly understood. In this mini-review, the known character, biological functions and regulatory mechanism of each MT-MMPs are discussed.

Characters of Mt-Mmps

Domain structures

MT-MMPs share a common domain structure consisting of a signal peptide, a pro-domain, a catalytic domain, a hinge (linker-1), a hemopexin-like (Hpx) domain, and a stalk region (linker-2) (Fig. 1). Transmembrane-type MT-MMPs including MT1, MT2, MT3, MT5-MMPs have a transmembrane (TM) domain and a short cytoplasmic (CP) domain following a linker-2, and GPI-anchored type MT-MMPs including MT4-MMP and MT6-MMP have a short hydrophobic sequence following a linker-2 that functions as a GPI-anchoring signal peptide (Fig. 1). All MT-MMPs have a basic amino acid motif of RX(K/R)R at the C-terminus of their prodomain, which is recognized and cleaved for activation by proprotein convertases (PCs) such as furin during secretion. Thus all MT-MMPs are expressed as active enzymes on the cell surface. MT6-MMP has an unpaired cysteine, Cys-532, at its stalk region (linker-2), and this mediates disulfide

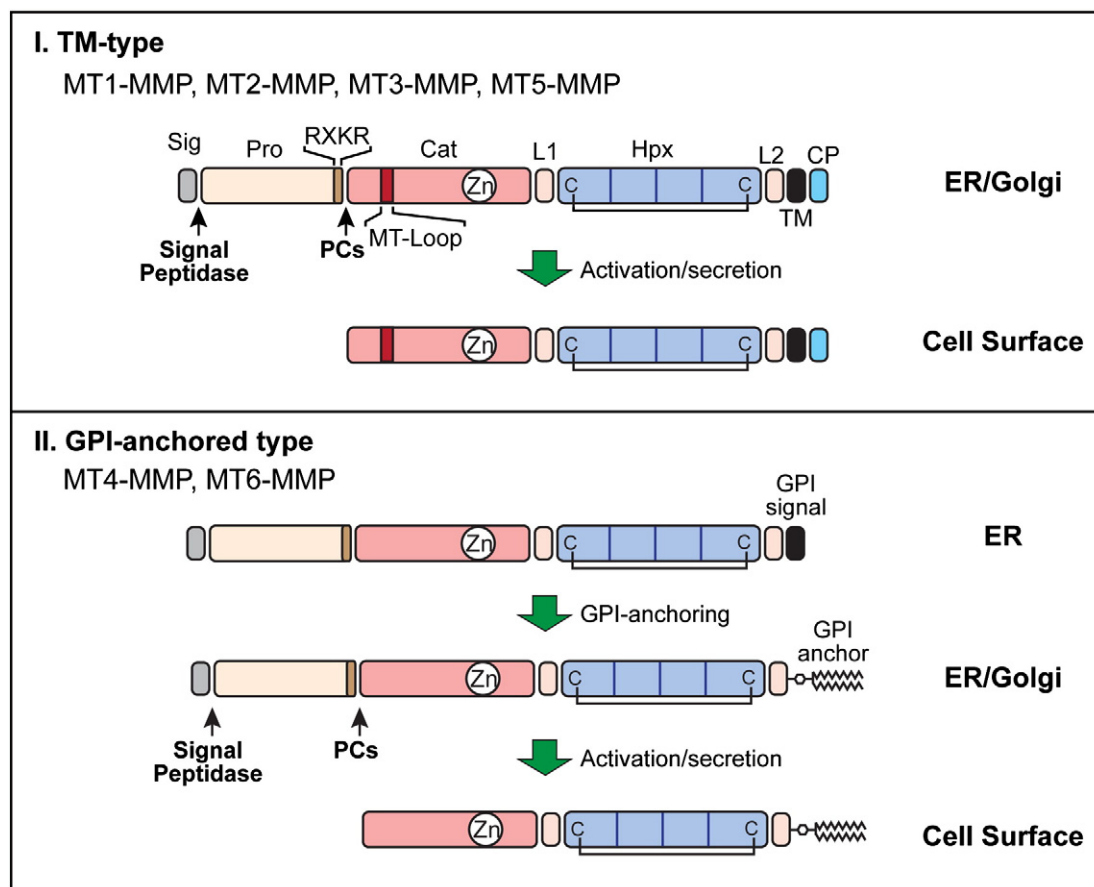


Fig. 1. Domain structure and processing of MT-MMPs. TM-type MT-MMPs including MT1-MMP, MT2-MMP, MT3-MMP and MT5-MMP share the same domain structures. They are synthesized as pre-pro enzymes and processing of the signal peptide and prodomain occurs before they are secreted to the cell surface. They have a basic amino acids motif of RXKR at the C-terminus of the prodomain which is recognized by proprotein convertases (PCs). They also harbour an 8-amino acid loop insertion in the catalytic domain named the MT-Loop. This loop is unique to TM-type MT-MMPs amongst all the MMPs. GPI-anchored MT-MMPs including MT4-MMP and MT6-MMP share the same domain structures. They are synthesized as pre-pro enzymes harbouring hydrophobic amino acids stretch at their C-terminus. This hydrophobic sequence acts as a GPI-anchoring signal peptide. This sequence is cleaved and replaced by a de novo-synthesized GPI-anchor by transamidase in the ER. Processing of signal peptide and propeptide occurs before secretion to the cell surface as for TM-type MT-MMPs. Sig, signal peptide; Pro, prodomain; Cat, catalytic domain; L1, hinge or linker-1; Hpx, hemopexin-like domain; L2, stalk or linker-2; TM, transmembrane domain; CP, cytoplasmic domain; PCs, proprotein convertases; C, cysteine; GPI signal, GPI signal peptide; ER, endoplasmic reticulum.

bond-mediated homo-dimer formation on the cell surface [9]. TM-type MT-MMPs have an insertion of 8–9 amino acids in the catalytic domain, named the MT-Loop (Fig. 1). This is unique to TM-type MT-MMPs and does not exist in any other enzymes of the MMP family.

Substrates

The reported substrates of MT-MMPs are listed in Table 1. Many of these substrates were identified by incubating recombinant soluble MT-MMP proteins with potential substrate in a test tube, whereas others were identified in cell culture systems and by proteomics approaches. While some of the substrates

were confirmed to be physiological substrates, some of them may not be. Among the 6 MT-MMPs, MT1-MMP has the widest substrate specificity, especially against extracellular matrix (ECM) components. Collagens are the most abundant ECM component and act as a major structural component and barrier matrix in tissues. MT1-MMP degrades fibrillar collagens including types I, II, and III, but it does not degrade type IV collagen, a major component of basement membranes [10]. MT2-MMP was also reported to degrade collagen I, but its specific activity was shown to be 1/100 of MT1-MMP [11], and thus it is not considered as a major collagenolytic enzyme. MT3-MMP was shown to degrade type III collagen, but it cannot degrade type I collagen [12]. Other

Table 1. Substrates of MT-MMPs.

MT1-MMP	proMMP-2 [1], proMMP-13 [44], ADAM9 [33,34], gelatin [10], Collagen types I, II, III [10], fibronectin [10], vitronectin [10], laminins-1 [10], -2/4 [76] and -5 [180], fibrin/fibrinogen [13], α 1PI [10], perlecan [181], CD44 [61], ICAM-1 [65], tTG [182], LRP1 [183], syndecan 1 [63], α v-integrin [184], C3b [185], EMMPRIN [186], ApoE [187], MICA [188], betaglycan [189], IL-8 [190], SLP1 [190], CTGF [190], DR6 [190], DJ-1 [191], galectin-1 [191], Hsp90 α [191], pentraxin 3 [191], progranulin [191], Cyr61 [191], peptidyl-prolyl <i>cis-trans</i> isomerase A [191], dickkopf-1 [191], KiSS-1 [192], Dll1 [27]
MT2-MMP	proMMP-2 [15], fibronectin [181], laminin-1 [181], nidogen [181], perlecan [181], collagen I [11], NC1 (coll-IV) [81], fibrin [14]
MT3-MMP	proMMP-2 [3], gelatin [12], collagen type III [12], laminin-1 [12], fibronectin [12], vitronectin [12], fibrin [14], α 1PI [12], NgR1 [88], KiSS-1 [192]
MT4-MMP	gelatin [193], fibrin/fibrinogen [90], proTNF [90], ADAMTS4 [16]
MT5-MMP	proMMP-2 [5,6], gelatin [100], fibronectin [100], laminin-1 [100], CSPG [100], DSPG [100], KiSS-1 [192], N-cadherin [102]
MT6-MMP	gelatin [194], collagen IV [194], fibronectin [194], fibrinogen/fibrin [194], MBP isoforms [195], vimentin [106], cystatin C [106], galectin-1 [106], IGFBP-7 [106], SPARC [106]

MT-MMPs do not degrade fibrillar collagen. Therefore, MT1-MMP is thought to be the membrane-anchored collagenase. MT1-MMP, MT2-MMP, and MT3-MMP have been shown to degrade fibrin and promote cellular invasion into fibrin matrices [13,14]. Several MT-MMPs have been shown to activate proMMP-2 on the cell surface, including MT1-MMP [1], MT2-MMP [15], MT3-MMP [3] and MT5-MMP [5,6], but MT4-MMP and MT6-MMP do not activate proMMP-2 on the cell surface. MT4-MMP was reported to process the C-terminal domain of ADAMTS4, converting it to more an active aggrecanase [16]. MT5-MMP has been shown to shed N-cadherin in neural stem cells [17].

Biological functions of Mt-Mmps

MT1-MMP

MT1-MMP is one of the most extensively investigated enzymes in the MMP family. It was originally discovered and characterized as a cell surface proMMP-2 activator expressed in invasive cancer cells [1], but many other cell types also express the enzyme including mesenchymal stem cells [18], fibroblasts, osteoblasts [19], osteoclasts [20], chondrocytes [21], epithelial cells [22], endothelial cells [13], adipocytes [23], myeloid cells [24], neuronal cells [25], T-cells [26], and B-cells [27]. The phenotype of MT1-MMP null mice includes defects in skeletal development, soft tissue fibrosis, angiogenesis, submandibular gland development and lung development [28–31]. It was thought that many of those phenotypes arise from a lack of cellular collagenase activity [28,32].

One of the phenotypes of MT1-MMP null mice is craniofacial abnormality, and it was found that this is not due to a lack of cellular collagenolytic activities of MT1-MMP, but rather due to increased activity of ADAM9, causing a loss of FGFR2 signalling since ADAM9 inactivates FGFR2 by shedding its ectodomain [33]. It was found that MT1-MMP inactivates ADAM9 by degrading it on the cell surface, playing a crucial role in maintaining FGFR2 signaling [33,34].

Many investigators have studied mechanisms of proMMP-2 activation by MT1-MMP, and the currently accepted model involves TIMP-2 and homo-dimerization of MT1-MMP (Fig. 2). In this model MT1-MMP on the cell surface forms a homo-dimer complex through its Hpx [35] and TM domains [36]. One of the MT1-MMP molecules in the dimer complex is inhibited by TIMP-2 that binds to the catalytic site of MT1-MMP thorough the N-terminal domain of the inhibitor [37]. ProMMP-2 Hpx domain has affinity for the exposed C-terminal domain of TIMP-2, thus they form a (MT1-MMP)₂-TIMP-2-proMMP-2 ternary complex [38] (Fig. 2). TIMP-2-free MT1-MMP in the complex then activates proMMP-2 by cleaving the middle of the pro-domain of proMMP-2 at Asn37-Leu, and this triggers auto-catalytic activation of the intermediate MMP-2 molecule [39]. The MT-Loop of MT1-MMP was shown to be involved in this activation process, potentially mediating interaction of proMMP-2 with MT1-MMP during activation [40]. Activated MMP-2 can be released or remain bound to TIMP-2, but there is also another TIMP-2 bound to the membrane in a different mode, and this TIMP-2 inhibits activated MMP-2 [41]. Since inhibition of MMP-2 results in MMP-2 molecules stabilized in media and body fluids, active MMP-2 species are easily detected when media or body fluids are analysed by zymography. The significance of MT1-MMP-dependent proMMP-2 activation is in their substrate specificities. While MT1-MMP cannot degrade type IV collagen, a major component of basement membrane [10], activated MMP-2 can degrade it [42]. It has been shown that this MT1-MMP/MMP-2-mediated basement membrane degradation indeed plays a role in growth of epithelial cancers *in vivo* [43].

MT1-MMP has also been shown to activate proMMP-13 on the cell surface [44]. TIMP-2 is not involved in this activation, but the hemopexin domain of MMP-13 is essential [45]. However, the detailed mechanism is not clearly understood.

MT1-MMP is a cellular migration/invasion promoter and involved in cancer cell invasion [1], metastasis [46] and growth [47], angiogenesis [13], epithelial morphogenesis [22], skeletal development [28], wound healing [48], inflammation [49], atherosclerosis

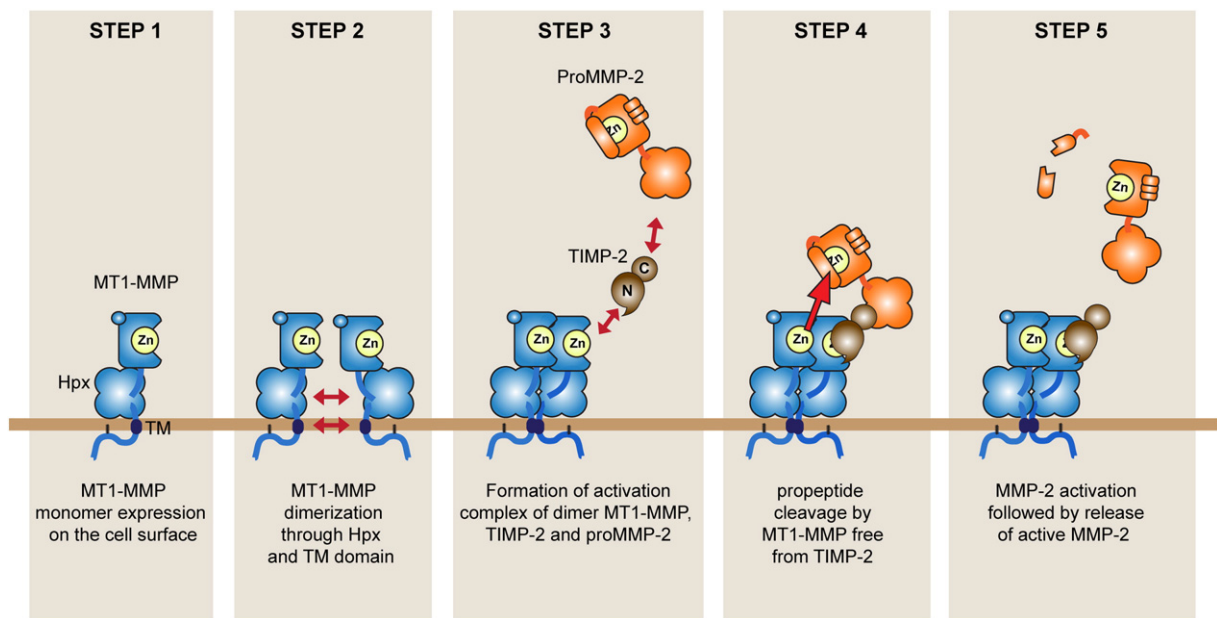


Fig. 2. Current model of activation of proMMP-2 by MT1-MMP. STEP 1: Secretion of MT1-MMP monomer to the cell surface. STEP 2: dimerization of MT1-MMP through Hpx and TM domains. STEP 3: Formation of $(\text{MT1-MMP})_2$ -TIMP-2-proMMP-2 complex. N-terminal domain of TIMP-2 has an inhibitory site and the C-terminal domain has affinity for the Hpx domain of MMP-2. STEP 4: proteolytic cleavage of prodomain of proMMP-2 by MT1-MMP free from TIMP-2 in the complex. STEP 5: MMP-2 activation followed by release of active MMP-2 from the cell surface. Hpx, hemopexin-like domain; TM, transmembrane domain.

[50], obesity [23], rheumatoid arthritis [51,52] and so on, and it promotes cell migration/invasion by different means [53]. One of the ways in which it does this is by directly degrading the pericellular matrix, especially fibrillar collagens. Fibrillar collagens, including type I collagen, is a major barrier during cellular migration, and it is resistant to many proteinases at neutral pH except collagenases that belong to MMP family, including MMP-1, MMP-2, MMP-8, MMP-13 and MT1-MMP [54]. Among them, MT1-MMP is the only pericellular collagenase that promotes cellular invasion in 3D-collagenous matrices [55,56]. This cell invasion promoting activity requires its membrane-anchored nature, as soluble mutant MT1-MMP cannot promote cellular invasion [55].

In addition to making a path for migration, it has been shown that MT1-MMP stimulates epidermal growth factor (EGF) receptor-dependent cell motility and growth. MT1-MMP and/or MMP-2 cleave $\gamma 2$ chain of laminin 5, a basement membrane component, releasing an EGF-like fragment, resulting in EGF receptor-dependent cell motility [57–59]. MT1-MMP was also shown to cleave off the N-terminal heparin-binding domain of Heparin-binding EGF-like growth factor (HB-EGF), converting HB-EGF to a heparin-independent growth factor, promoting cancer invasiveness [60].

MT1-MMP also promotes cell migration by processing cell adhesion molecules. The hyaluronan receptor, CD44, is shed by MT1-MMP, and this shedding

promotes cellular migration and invasion [61]. The mechanism by which CD44 shedding triggers migration is not known, but CD44 shedding by ADAM enzymes including ADAM10 and ADAM17 has also been shown to promote cellular motility [62]. MT1-MMP also sheds the transmembrane heparan sulfate proteoglycan, Syndecan 1, and this shedding plays a role in cell motility of HT1080 human fibrosarcoma cells [63]. Finally, MT1-MMP was shown to process αV integrin, converting it to the mature form, and promoting cell migration on a vitronectin substrate [64]. In monocyte MT1-MMP interacts with intracellular adhesion molecule-1 (ICAM-1) in cytoplasmic domain of ICAM-1 dependent manner and sheds ectodomain of ICAM-1, and this interaction was shown to be essential for efficient endothelial transmigration [65].

MT1-MMP was shown to be a major enzyme that promotes angiogenesis [13,66,67]. Since MT1-MMP null mice develop vascular structures normally [28], it is considered that the enzyme is dispensable in vasculogenesis. The role of MT1-MMP during angiogenesis is likely to be promoting invasion of endothelial cells by degrading pericellular ECM components [13,66,67]. It was found that MT1-MMP is expressed selectively at the tip of growing vessels where ECM degradation is needed [68]. In addition to the direct roles in endothelial cells, MT1-MMP may modulate tumor-induced angiogenesis in or around the cancer mass. MT1-MMP expression in cancer cells stimulates production of VEGF-A

[69,70]. It was shown that this upregulation is attributed to complex formation of MT1-MMP-VEGFR2-Src, resulting in activation of Akt and mTOR [71].

MT1-MMP also promotes cellular migration in catalytic activity-independent manner through its cytoplasmic domain. The cytoplasmic domain was shown to interact with FIH-1 (Factor inhibiting HIF-1) where Arg576 is a crucial amino acid for this interaction [72]. This interaction with FIH-1 was shown to be important for maintaining high HIF-1 α transcription factor activity in macrophages under normoxia to generate ATP via glycolytic pathways [72]. The cytoplasmic domain phosphorylated at Tyr573 was shown to interact with p130Cas, which regulates Rac1 signalling [24,73]. This interaction was shown to play a role in osteoclast formation [24]. The cytoplasmic domain has also been shown to interact with p27RF-Rho (LAMTOR1) that regulates RhoA activation. p27RF-Rho co-localizes with MT1-MMP and may contribute to spatial activation of RhoA-dependent actin polymerisation to promote cellular invasion [74].

It was also reported that MT1-MMP modulates inflammatory responses of macrophages in a protease-independent fashion in tandem with its trafficking to the nuclear compartment, where it triggers the expression and activation of a phosphoinositide 3-kinase d (PI3Kd)/Akt/GSK3b signaling cascade. In turn, MT1-MMP-dependent PI3Kd activation regulates the immunoregulatory Mi-2/NuRD nucleosome remodeling complex that is responsible for controlling macrophage immune response [75].

MT1-MMP also regulates Notch signalling by cleaving the Notch ligand Delta-like 1 (Dll1) on the cell surface of bone marrow stromal cells. This event negatively regulates Notch signalling to maintain normal B-cell development in bone marrow [27].

MT1-MMP plays a role in myoblast fusion during the myogenic program [76]. MT1-MMP expressed in myoblasts degrades fibronectin, a possible inhibitory factor for myogenic cell fusion, and laminins 2/4, a component of basement membranes, in the stage-specific manner, facilitating the myoblast fusion process [76].

MT2-MMP

Like MT1-MMP, MT2-MMP also activates proMMP-2 on the cell surface [15]. However, TIMP-2 is not required for this activation process [11]. It is not clear how proMMP-2 is attracted to the cell surface-expressed MT2-MMP, but Hpx domain of proMMP-2 is required for the activation [11], like activation by MT1-MMP.

MT2-MMP has been implicated in angiogenesis within fibrin matrices [77]. Overexpression of MT2-MMP was also shown to promote cell invasion into 3D-fibrin matrices as well [14]. Although MT2-MMP is capable of degrading type I collagen, its specific

activity was shown to be 1/100 of MT1-MMP [11]. Thus MT2-MMP is unable to promote cellular invasion into type I collagen gel in an efficient manner [14,55]. On the other hand, it was shown that MT2-MMP could promote cell invasion into basement membrane matrices [55,78,79]. As many invasive cells express MT2-MMP with MT1-MMP and/or MT3-MMP, these enzymes may cooperatively work to promote the invasion [78–80]. Like MT1-MMP [43], the ability of MT2-MMP to promote basement membrane invasion may be attributed to its ability to activate proMMP-2 [15].

Recently MT2-MMP was found to cleave the NC1 domain of collagen IV and release bioactive NC1 fragment [81]. This MT2-MMP-dependent release of NC1 was found to be critical for integrating collagen IV synthesis and proteolysis with epithelial proliferation during branching morphogenesis [81].

MT3-MMP

MT3-MMP also activates proMMP-2 on the cell surface though to a lesser extent [3,82]. This activation does not require TIMP-2, but chondroitin sulfate (CS) proteoglycans mediate interaction of proMMP-2 with MT3-MMP [83]. CS binds to Hpx domain of proMMP-2, thus Hpx domain of MMP-2 is essential for MT3-MMP-dependent activation [83]. It was also shown that MT3-MMP-dependent activation of proMMP-2 can be enhanced in the presence of TIMP-2 or TIMP-3, and it was concluded that TIMP-2 and TIMP-3 mediate ternary complex formation of MT3-MMP-TIMP-proMMP-2 [84]. TIMP-3 was shown to interact with Hpx domain of MMP-2 although it has 16-fold weaker affinity than TIMP-2 [85].

As MT3-MMP cannot degrade collagen I [12], it does not promote collagen invasion [14,55], but does promote invasion into fibrin matrices [14,86]. Contrary to these reports, MT3-MMP has also been reported to cleave collagen matrix and this activity plays a major role in mesenchymal cell proliferation during embryogenesis [87]. Further study may be necessary to resolve this discrepancy.

MT3-MMP null mice do not show any physiological deficit and are fertile, but exhibit retarded growth of the skeleton especially in the cranium and long bones. Interestingly mice lacking both MT1-MMP and MT3-MMP display increased perinatal death and more severe skeletal defects than MT1-MMP null mice. This suggests that MT3-MMP and MT1-MMP act together during development [87].

In aggressive melanoma, MT3-MMP is highly expressed. However, cells with high MT3-MMP expression were shown to exhibit limited invasiveness to collagen matrices while they invaded well into fibrin matrices [86]. It was shown that MT3-MMP decreased MT1-MMP levels on the cell surface by degrading MT1-MMP and thereby inhibiting MT1-MMP-dependent collagen invasion [86]. These findings indicate that

MT3-MMP is a matrix-dependent modifier of invasive tumor cell functions during melanoma progression.

MT3-MMP also cleaves Nogo-66 receptor 1 (NgR1), a GPI-anchored receptor for myelin-associated inhibitors that restricts plasticity and axonal regrowth in the central nervous system [88].

MT4-MMP

MT4-MMP does not activate proMMP-2 nor degrade many ECM components [89,90]. MT4-MMP-null mice also showed no apparent abnormal developmental phenotypes [91]. However, it was found that MT4-MMP plays a role in water homeostasis [92]. The null mice have decreased intake of water and daily urine output. As MT4-MMP is expressed in the hypothalamus, which regulates thirst, it has been proposed that MT4-MMP may play a role in regulating thirst [92].

MT4-MMP was shown to mediate C-terminal processing of ADAMTS-4, one of the aggrecanases that are thought to play a role in arthritis, increasing its proteolytic activity [16]. Aggrecan release from cartilage induced by intra articular injection of IL-1 was inhibited in MT4-MMP-null mice, and incubating recombinant MT4-MMP with cartilage *in vitro* induced aggrecan release from the cartilage [93]. Although the exact role of MT4-MMP is still not clear, MT4-MMP may play a role in arthritis development.

MT4-MMP has been also shown to play a role in progression of breast cancer. High level of MT4-MMP protein has been detected in breast cancer cells, but not in normal epithelial cells. Ectopic expression of MT4-MMP in breast cancer cells significantly enhanced their ability to grow and metastasize to the lung when they are injected into RAG-1 immuno-deficient mice [94]. This tumor-promoting activity of MT4-MMP is thought to be mediated by inducing tumor blood vessel enlargement [95] and inducing an angiogenic switch [96] in an MT4-MMP proteolytic activity dependent manner. In addition, it has been reported that MT4-MMP associates with EGFR and promotes its signaling upon ligation to TGF- α and EGF [97]. Interestingly this effect was shown to be independent from the proteolytic activity of MT4-MMP [97]. However, the detailed mechanisms of how MT4-MMP exerts these effects are not known at present.

MT5-MMP

MT5-MMP is mainly expressed in neuronal cells of both central and peripheral nervous systems, although its presence has also been detected in inflammatory cells [5,6,98,99]. MT5-MMP was shown to activate proMMP-2 on the cell surface [5,6], but the role of MT5-MMP as a proMMP-2 activator *in vivo* is not known. Proteoglycans are major ECM components in brain and have been shown to inhibit neurite growth. MT5-MMP was shown to degrade proteoglycans and to promote neurite outgrowth [98,100]. However, in

MT5-MMP null mice, there were no apparent histological defects in the nervous system [101]. On the other hand, it was found that null mice lack mechanical allodynia and A β -fiber sprouting after sciatic nerve injury, suggesting that the enzyme plays a role in neuronal plasticity [101]. It was also reported that MT5-MMP mediates peripheral thermal nociception and inflammatory hyperalgesia by shedding N-cadherin [17]. Additionally, MT5-MMP-dependent N-cadherin shedding was shown to play a role in regulating adult neural stem cell function quiescence [102].

MT6-MMP

MT6-MMP is expressed exclusively in leucocytes and some cancer cells [7,8]. It has been reported to activate proMMP-2 on the cell surface [8], but its activity was not very high or not detected on the cell surface [103,104] although its isolated recombinant catalytic domain could effectively activate proMMP-2 *in vitro* by directly cleaving the Asn109-Tyr bond [104]. It was later found that full length MT6-MMP cannot activate proMMP-2 very well, but the enzyme lacking its Hpx domain does activate proMMP-2 in an efficient manner on the cell surface [105]. Since full-length enzyme does not activate proMMP-2 in an efficient manner, the role of MT6-MMP in proMMP-2 activation *in vivo* is questionable. MT6-MMP was shown to process extracellular vimentin that acts as a chemoattractant for monocytes [106]. Interestingly, this product is a potent stimulus for phagocytosis while intact vimentin is not. Thus, it was suggested that MT6-MMP might be playing a role in resolving inflammation by promoting phagocytic removal of neutrophils [106].

Regulation of Mt-Mmps

Gene and protein expression

Despite the characteristic tissue expression pattern of each MT-MMP, all MT-MMP genes are highly expressed in different cancers. However, detailed mechanisms of how activation of these genes occurs in specific tissues at specific times and how transformation of epithelial cells activates these MT-MMP genes are not clearly understood.

MT1-MMP is upregulated by phorbol ester in HT1080 cells [107], concanavalin A in fibroblasts [44], MDA-MB231 cells [108] and HT1080 cells [109], and by culturing cells within a 3D collagen lattice in fibroblasts [110,111], endothelial cells, [112], and epithelial cells [22]. Although there are reports showing that the inflammatory cytokine TNF α upregulates MT1-MMP in fibroblasts [113] and endothelial cells [114], observations are not consistent [51]. The promoter

region of MT1-MMP contains overlapping SP-1 and EGR1 binding sites, and it was shown that collagen-stimulated MT1-MMP activation was mediated by Egr-1 [112]. In normal epithelial cells, transformation with *v-src* highly upregulated the MT1-MMP gene [115], and this *v-src*-induced upregulation was mediated by SP-1 [116]. Other transcription binding sites include CARG Box, Nkx-2, AP4, E-box, Lyf1, and *c-myc* within 2 kb upstream the translation start site [112]. There is one NFkB binding site at -2.16 kb, but it lacks Ap-1 binding sites [112]. Relatively distant NFkB binding sites and the lack of an AP-1 site may be a reason why TNF α can stimulate MT1-MMP expression in some cells but not others. HIF (Hypoxia-inducible factor)-2 α binding sites were found at -125 bp, and it was shown to cooperate with SP-1 to activate MT1-MMP gene expression in von Hippel Lindau renal cell carcinoma [117,118].

Depletion of a zinc finger repressor, Snail1, in fibroblasts abrogates MT1-MMP-dependent collagen invasion by fibroblasts [80]. On the other hand, ectopic expression of Snail1 in MCF7, a well-differentiated breast cancer cell line that displays minimal invasive activity, induced MT1-MMP- and MT2-MMP-dependent basement membrane invasion, initiation of angiogenesis and intravasation to vascular networks by inducing expression of these enzymes [79]. SNAIL-1 has been shown to cause epithelial to mesenchymal transition (EMT) by down regulating E-cadherin [119], thus expression of these enzymes may accompany Snail1-induced EMT during progression of cancer.

The MT2-MMP gene is expressed in squamous cell carcinoma HSC-4, fibrosarcoma HT1080, bladder carcinoma UMK-1, cervical cancer HeLaS3 [120], and breast cancer MDA-MB231 [78]. MT2-MMP expression was found to be upregulated by hypoxia in pancreatic cancer cells (PANC1), non-small cell lung carcinoma cell (A-549), and cervical cancer cells (HeLa), and it was found to be mediated by binding of HIF-1 α to one of the two HREs (hypoxia-responsive elements) in its promoter region [121]. As describe above, Snail1 can induce expression of MT2-MMP in MCF7 cells [79].

MT3-MMP is expressed in various cell types including neuronal cells [88], glioblastoma U-87MG cells, fibrosarcoma HT1080 cells, bladder cancer cells T24 [120], melanoma cells WM853 [86], endothelial cells [77], stromal fibroblasts [122], smooth muscle cells [82]. In human macrophages, expression of MT3-MMP was shown to be upregulated by oxidized low-density lipoprotein (Ox-LDL), TNF α , and macrophage colony-stimulating factor (M-CSF) [123].

MT4-MMP was originally isolated from a breast cancer cDNA library, and various breast cancer cells were shown to express the enzyme [4]. It was also shown to be expressed in prostate cancer, oral cancer, fibroblasts, colon cancer, leukemia [124] and chondrosarcoma cells JJ012-TS4 [16]. MT4-MMP

was also found to be expressed in eosinophils, lymphocytes, and monocytes, and it is upregulated by TNF α in eosinophils [125].

MT5-MMP expression is restricted to brain [5,6], kidney [6,126] and pancreas [126].

Expression of MT6-MMP was also shown to be restricted to leucocytes [7,8]. However, MT6-MMP expression can be induced in the cornea upon infection with *P. aeruginosa* [127].

Cell surface localization

One of the advantages of MT-MMPs over soluble enzymes is their membrane-bound nature. Cells can localize the enzyme to particular areas of the plasma membrane, so as to allow polarized proteolytic actions across the cell surface. Therefore, it is crucial for cells to regulate the localization of these enzymes. Among the 6 MT-MMPs, regulation of MT1-MMP in this aspect has been investigated the most.

MT1-MMP is the only MMP that can directly promote cellular invasion in collagen-rich matrices [55,56]. It has been shown that MT1-MMP is localized in membrane structures that represent leading edge of cells including lamellipodia [20,35], filopodia [128] and invadopodia [129,130]. Localization of MT1-MMP to lamellipodia was shown to be due to association with CD44, a hyaluronan receptor, through its Hpx domain [131,132]. This allows MT1-MMP to indirectly associate with cytoskeletal F-actin through CD44 [131]. Invadopodia are a membrane structures that extends toward the ECM and contain proteolytic enzymes capable of degrading the ECM. Originally a serine proteinase, seprase, was identified as a invadopodia-localized proteinase [133], but later, it was found that MT1-MMP is the major enzyme that express ECM-degrading activity in invadopodia structures [129]. MT1-MMP is now established as one of the essential components of invadopodia [130,134]. It was proposed that localization of MT1-MMP at invadopodia is cytoplasmic domain-dependent [129] and it was also later reported that the cytoplasmic domain of MT1-MMP is necessary to localize the enzyme to invadopodia-like structures driven by N-WASP [135]. It was also reported that MT1-MMP localizes to focal adhesions due to association of the MT1-MMP cytoplasmic domain with a focal adhesion kinase (FAK)-p130Cas [136]. On the other hand, it has been shown that the cytoplasmic domain of MT1-MMP is dispensable for degradation of the underlying matrix [36,47,137] and localization to focal adhesion sites [138]. Thus, requirement for the cytoplasmic domain for localization in invadopodia or focal adhesions needs to be re-evaluated. It was shown that 8 amino acids loop structure, MT-Loop, located in the catalytic domain is necessary to degrade underlying matrices in an efficient manner and concluded that the MT-Loop acts as an interface for molecular interactions that allow localization of the enzyme to focal adhesion sites

where matrix degradation takes place [138]. MT1-MMP was also shown to localize at podosomes, invadopodia-related membrane structures found in endothelial cells, macrophages, dendritic cells and *src*-transformed fibroblasts [134,139–142].

Cell surface expression of MT1-MMP was shown to be dependent on expression of ADAM12 proteinase [143]. When ADAM12 was knocked down, the cell surface level of MT1-MMP was significantly decreased without change in mRNA levels while over expressing ADAM12 increased the cell surface levels and activity of MT1-MMP [143]. It was found that formation of ternary complex of ADAM12- α v β 3 integrin-MT1-MMP on the cell surface is crucial for degradation of the underlying matrix, and that the proteolytic activity of ADAM12 is not necessary to regulate MT1-MMP [143].

Vesicle transport is a major step regulating MT1-MMP secretion. It was reported that transport of MT1-MMP-containing vesicles towards a collagen matrix was triggered by binding of β 1 integrin with the collagen matrix in a Rab8 GTPase-dependent manner in invasive breast cancer cells, MDA-MB231 [144]. In macrophage, MT1-MMP secretion to podosomes was found to be regulated by a set of Rab GTPases, including Rab5a, Rab8a and Rab14 [145]. It was also reported that MT1-MMP-containing vesicle transport to podosomes is mediated by KIF5B and KIF3A/KIF3B kinesin molecules in primary macrophages [140]. It is not clear if these kinesin molecules are also responsible for MT1-MMP secretion to other membrane structures.

In epithelial cells, secretion of proteins is tightly regulated according to their epithelial polarity, and the default secretory pathway for MT1-MMP in inert epithelial cells was found to be towards the apical surface, separating MT1-MMP from the ECM at the basal surface [146]. When cells were stimulated with a morphogen such as hepatocyte growth factor (HGF), MT1-MMP secretion was partially switched over to the basal side, resulting in ECM in extension of epithelial tubule structures into collagen matrix [146]. Exogenous HGF triggers this switchover and tubulogenesis, but endogenous TGF β was also shown to be involved in efficient basal localization of MT1-MMP and tubulogenesis [146].

GPI-anchored MT4- and MT6-MMPs are synthesized with a C-terminal hydrophobic sequence that act as a GPI-signal peptide [103,147] (Fig. 1). In general, a 16–30 amino acid hydrophobic stretch located at the C-terminus of a translated protein acts as a GPI-signal peptide [148]. This GPI-signal peptide is cleaved off in the endoplasmic reticulum lumen, and the ectodomain is transferred to the *de novo* synthesized GPI moiety which contains 2–3 fatty acids [148] (Fig. 1). Thus, the mature GPI-anchored proteins have no transmembrane or intracellular domains. Attachment of the GPI moiety after translation of MT4-MMP and MT6-MMP seems to occur efficiently as around 50% of cellular

MT-MMPs contain the GPI moiety under an over expressing system [103,147]. As GPI-anchored proteins are linked to fatty acids, non-related GPI-anchored proteins preferentially localize to the hydrophobic plasma membrane domain called lipid raft rich in cholesterol and glycosphingolipids [149]. Indeed MT4-MMP was found to co-localize well with a non-related GPI-anchored protein, uPAR [147]. Thus it is possible that GPI-anchoring at least in part determines localization of MT4- and MT6-MMP on the cell surface, and possibly their functions.

Endocytosis and recycling

MT1-MMP is endocytosed from the cell surface in clathrin- and caveolae-dependent manners and its half-life on the cell surface is less than 30 min [143,150–152]. Clathrin-dependent endocytosis is attributed to the interaction of LLY⁵⁷³ in the cytoplasmic domain with adapter protein 2, a component of clathrin-coated pits, and this mode of endocytosis was found to be crucial to promote cell migration [150]. MT1-MMP was found to be palmitoylated at Cys⁵⁷⁴ just downstream of LLY⁵⁷³ in the cytoplasmic domain, and this lipid modification was shown to be essential for MT1-MMP to be endocytosed by the clathrin-dependent pathway and to promote cell migration [152].

In endothelial cells, activation of VEGFR-2 (vascular endothelial growth factor receptor-2) was shown to induce Src family kinase-dependent phosphorylation of caveolin-1, and this triggered interaction of caveolin-1 and MT1-MMP through its cytoplasmic domain [153]. This interaction was shown to play a role in VEGF-induced cell migration of endothelial cells [153]. As caveolin-1 is a component of caveolae, it is possible that this interaction induces caveolae-dependent endocytosis of MT1-MMP in endothelial cells.

Endocytosed MT1-MMP was also found to be recycled back to the cell surface [154,155]. Its recycling kinetics were found to be the same for MT3-MMP, and the C-terminal DKV582 sequence of the MT1-MMP cytoplasmic domain is essential for recycling [155]. It is not known how much impact recycling of MT1-MMP has, as expression levels, proMMP-2 activation levels and the cell migration-promoting effect of the DKV582-deleted mutant are almost identical to the wild-type enzyme [150]. A similar finding of cytoplasmic domain-dependent recycling was also made for MT5-MMP. In MT5-MMP, the sequence of the last three amino acids in the cytoplasmic domain is EWV645 and this sequence was also shown to be essential for recycling of MT5-MMP, which determines cell surface levels of the enzyme [156]. EWV645 acts as a PDZ binding motif, and Mint-3 possessing two type-III PDZ domains was found to interact with EWV645 [156].

Dimerization

MT1-MMP forms homo-dimers through its Hpx domain [35] and transmembrane domain [36]. As described above, homo-dimerization of MT1-MMP facilitates proMMP-2 activation on the cell surface [35]. Dimerization is also found to be crucial for MT1-MMP to degrade collagen on the cell surface [157]. Dimerization was shown to be another mechanism regulating MT1-MMP activity, and it is regulated by cdc42- and/or Rac1-driven actin cytoskeleton which allows it to occur at the leading edge of invading cells where collagen degradation is required [128]. The crystal structure of the Hpx domain and site-directed mutagenesis indicated that it is a symmetric dimer, and that the interface is located on blade 2 and 3 of Hpx domains [158]. Based on the crystal structure, small molecular compounds were identified as dimer inhibitors, and one of the compounds were found to be effective in inhibiting proMMP-2 activation, collagen degradation, and *in vivo* tumor growth [159].

Inhibition

Tissue inhibitors of metalloproteinase (TIMPs) are endogenous MMP inhibitors and there are four TIMPs namely TIMP-1, -2, -3 and -4. They share the same domain structures, having N- and C-terminal globular domains where the N-terminal domain contains the inhibitory site. All TIMPs are capable of inhibiting all soluble MMPs, but TIMP-1 is very weak inhibitor against transmembrane-type MT-MMPs including MT1-, MT2-, MT3- and MT5-MMPs so that physiological concentrations of TIMP-1 cannot inhibit them [160]. On the other hand, GPI-anchored MT-MMPs including MT4- and MT6-MMPs can be inhibited effectively by all TIMPs including TIMP-1 [161].

As discussed above, TIMP-2 mediates proMMP-2 activation by MT1-MMP by bridging MT1-MMP and proMMP-2. Therefore, the effect of TIMP-2 on MT1-MMP-dependent proMMP-2 activation on the plasma membrane is bi-phasic: at lower concentrations of TIMP-2 the activation is enhanced while at higher concentrations the activation is inhibited. As complex of TIMP-2 and MT1-MMP is readily found in cells, it is often considered that TIMP-2 is a major endogenous inhibitor of MT1-MMP, but neither TIMP-2 null mice nor TIMP-2 deficient cells showed any signs of uncontrolled high MT1-MMP activity [162]. English et al. [163] examined mouse embryonic fibroblasts deficient in each individual TIMP, and found that proMMP-2 activation by MT1-MMP in TIMP-3-deficient mouse embryonic fibroblasts (MEF) was much more robust than that by wild-type MEF, while TIMP-2-deficient mice only showed inhibition of proMMP-2 activation [163]. This indicates that TIMP-3 may play more important role in regulating MT1-MMP activity on the cell surface.

MT1-MMP is also inhibited by other molecules. RECK (Reversion-inducing-cysteine-rich protein with kazal motifs) is an GPI-anchored form of metalloproteinase inhibitor that can inhibit MT1-MMP [164] and ADAM10 [165]. Originally RECK was isolated due to its activity to revert the transformed phenotype of cells expressing mutated K-ras, and later it was found to inhibit MMPs [164,166]. Mice lacking the RECK gene die at around E10.5, showing an abnormal turnover of ECM and mal-development of vasculature [164] due, in part, to poorly regulated MMP activities including MT1-MMP. Testican 3, its variant N-Tes [167] and claudin [168] were also identified as modulators of MT1-MMP activity.

Upon purification of recombinant soluble MT6-MMP, clusterin, a major serum component, was co-purified [169]. It was found that clusterin forms a complex with MT6-MMP and inhibits MT6-MMP activity. Interestingly clusterin is a selective inhibitor for MT6-MMP and it does not inhibit MMP-2 or MT1-MMP. Clusterin was also found to form a complex with soluble MT4-MMP, but it is not known if it inhibits MT4-MMP activity. However, it is possible that this interaction is a common feature of GPI-anchored type MT-MMPs. Because clusterin is an abundant protein in the body fluid in tissues, it may act as a negative regulator of MT6-MMP *in vivo* [169].

Cell surface degradation and Shedding

It has been shown that the 60 kDa active MT1-MMP undergoes further processing to a 44–45 kDa species by MMP-2 or MT1-MMP itself [107,170,171]. This removes the catalytic domain of MT1-MMP making it inactive, and is a mechanism of down regulation. The proteolytic processing of MT1-MMP may be an indication of how active the enzyme is on the cell surface. A high level of 45 kDa form coincides with high proMMP-2 activation whereas no proMMP-2 activation occurs when 45 kDa form is not detected besides full length mature MT1-MMP on the cell surface [107,170]. Thus, MT1-MMP can be “functionally active”, and as a result undergoes further processing, or “functionally inactive” remaining as an intact mature form. This suggests that there may be a step that regulates its functional activity after expression of the mature enzyme on the cell surface, and one of the possible mechanisms to achieve this could be a homo-oligomer complex formation of the enzyme (see above) [172].

Several MT-MMPs have been shown to be shed from the cell surface. Whole ecto-domain shedding of MT1-MMP was detected in ConA-treated MDA-MB231 cells [173] and TPA-treated HT1080 cells [171]. The shedding was metalloproteinase-independent [171]. MT5-MMP was also shown to be shed from the cell surface [5], but it was found that this was mediated by proprotein convertases recognizing a basic motif at the stalk legion [174]. GPI-anchored MT-MMPs, MT4- and

MT6-MMPs can also be shed from the cell surface [147,175]. MT4-MMP was constitutively shed in a metalloproteinase-dependent manner [147], but release of MT6-MMP in neutrophils was triggered by inflammatory cytokines, chemokines or phorbol ester, and more than 85 % of MT6-MMPs can be released by these stimuli, suggesting that soluble MT6-MMP may have biological roles [175]. The shedding was insensitive to any proteinase inhibitors, suggesting that proteinase may not be responsible for the shedding, but rather phospholipase, that hydrolyzes the GPI-anchor moiety [175]. The biological significance of these MT-MMP shedding events is not clear, but most likely they represent a down regulation of the functions of these MT-MMPs.

Concluding remarks

MMPs have been implicated in many physiological events and progression of various diseases including cancer and arthritis, and they are thought to be therapeutic targets. In the 1990s, several MMP inhibitors were developed as a potential drugs for cancer and arthritis, but no efficacy was found in clinical trials [176,177]. It is now understood that a major reason for these failures was the broad-spectrum nature of these inhibitors, and a lack of understanding of the target enzymes in the diseases [176,177]. It is crucial to identify target enzymes and to develop means to inhibit them in a highly selective manner. There are 23 MMPs in humans and 17 of them are soluble MMPs. Many of them are extensively characterized biochemically, but *in vivo* function of those soluble MMPs have been difficult to understand because active enzymes in tissue cannot be easily detected. Since many of them are secreted as inactive zymogens, they need to be activated extracellularly to carry out their function. Although the activation mechanism of these MMPs has been suggested from many biochemical studies, it is still not understood how most MMPs are activated *in vivo*. The only active soluble MMP detected in tissues has been MMP-2, detected by zymography. There is a good correlation between the presence of active MMP-2 and cancer invasion [178]. Therefore, the discovery of MT1-MMP as a proMMP-2 activator [1] was a leaps forward in understanding the roles of MMPs *in vivo*. We now know that MT-MMP subgroup consists of 4 TM-types and two GPI-anchored types. The membrane anchored nature of MT-MMPs separates them from the other soluble MMPs as the cell surface is an interface between the extracellular environment and the intracellular compartment, and MT-MMPs are modifiers of the immediate cellular microenvironment, which effectively modulates cellular functions. Among MT-MMPs, MT1-MMP is the most extensively investigated enzyme, and its role in cancer progression and arthritis development has been especially highlighted. Inhi-

tion of MT1-MMP by a highly selective antibody inhibitor in an animal model of cancer showed a significant effect in abrogating cancer development [179], suggesting that MT1-MMP is indeed a potential therapeutic target for cancer. Besides MT1-MMP, MT2-MMP and MT4-MMP may also be important enzymes involved in disease progression. Their roles in cancer progression as discussed in this review may suggest that these MT-MMPs can also be potential target enzymes. Since we now have technologies able to create highly selective inhibitors, such as phage-display screening of humanized antibody inhibitors, it is now time that these question are addressed. Further investigation of MT-MMPs may provide important novel insights into understanding mechanisms of pathophysiological events and enable development of novel therapies for different diseases in the future.

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