Emerging roles of MT-MMPs in embryonic development

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Abstract
Membrane-type matrix metalloproteinases (MT-MMPs) are cell membrane-tethered proteinases that belong to the family of the MMPs. Apart from their roles in degradation of the extracellular milieu, MT-MMPs are able to activate through proteolytic processing at the cell surface distinct molecules such as receptors, growth factors, cytokines, adhesion molecules, and other pericellular proteins. Although most of the information regarding these enzymes comes from cancer studies, our current knowledge about their contribution in distinct developmental processes occurring in the embryo is limited. In this review, we want to summarize the involvement of MT-MMPs in distinct processes during embryonic morphogenesis, including cell migration and proliferation, epithelial-mesenchymal transition, cell polarity and branching, axon growth and navigation, synapse formation, and angiogenesis. We also considered information about MT-MMP functions from studies assessed in pathological conditions and compared these data with those relevant for embryonic development.

KEYWORDS
angiogenesis, axon guidance, cell migration, cell polarity, embryonic development, epithelial-mesenchymal transition, metalloproteinase, synaptogenesis
INTRODUCTION

The extracellular matrix (ECM) is a dynamic structure that supplies mechanical support to the cells, and it is continuously changing and remodeling in order to ensure tissue homeostasis. ECM comprises both the basement membrane and the interstitial matrix, and it is composed of distinct macromolecules, including fibrillar collagens, glycoproteins, proteoglycans, and polysaccharides. Apart from serving as structural scaffold, ECM contributes to complex and diverse cellular functions during embryogenesis, wound healing or cancer progression. These physiological and pathological processes are regulated by matrix-cleaving proteases that are able to remodel ECM by local release or cleavage of distinct matrix components as well as to activate receptors and growth factors, adhesion molecules, cytokines and other pericellular proteins through proteolytic processing at the cell surface. Among the major proteases that participate in these processes to allow tissue remodeling and cellular invasion are Matrix Metalloproteinases (MMPs). MMPs belong to the Metzincin superfamily of proteases found in all organisms, which includes other Zn<sup>2+</sup>-dependent enzymes such as A disintegrin and metalloproteinase (ADAMs), serralysins, and astacins. All members are characterized by the presence of a zinc ion in their catalytic domain that is required for their proteolytic functions. A subgroup within MMP family are the Membrane-type matrix metalloproteinases (MT-MMPs), anchored to the cell membrane by either a type I transmembrane domain (MT1-MMP/MMP-14, MT2-MMP/MMP-15, MT3-MMP/MMP-16, and MT5-MMP/MMP-24) or a glycosylphosphatidylinositol (GPI)-anchor (MT4-MMP/MMP-17 and MT6-MMP/MMP-25). Within membrane bound MMPs, are also included MMP-23A and MMP-23B, which localize as transmembrane type II proteinases via an N-terminal signal anchor.

Several MT-MMPs are expressed in normal tissues playing a role in homeostasis, but most of them are induced in diseases or inflamed tissues mediating repairing or remodeling processes. Although most of our current information regarding these enzymes comes from cancer studies, the first MMP identified, collagenase, was found in tadpole tail resorption during amphibian metamorphosis. Moreover, this collagenolytic activity was found in other tissues such as gills and gut that relies heavily on ECM remodeling during embryogenesis. To date, other members of these endopeptidases have been involved in tail resorption during frog metamorphosis including not only secreted MMPs (MMP-2, MMP-9, MMP-11, MMP-13, and MMP-18) but also metalloproteinases anchored to the cell membrane such as MT1-MMP and MT3-MMP. This is a notable event in developmental biology that emphasizes the relevance of MMPs in tissue remodeling during embryogenesis. Indeed, MMP expression profiles have been reported in the embryo, suggesting the existence of unknown functions for these enzymes during development. However, our current knowledge about the contribution of MMPs in distinct developmental processes occurring in the embryo is limited, in part due to the lack of embryonic lethal phenotypes caused by MMP deficiency.

In this review, we want to summarize the involvement of MT-MMPs, a subgroup of MMPs anchored to the cell membrane, in distinct processes during embryonic morphogenesis, including cell migration and proliferation, epithelial-mesenchymal transition, cell polarity and branching, axon navigation, synapse formation, and angiogenesis. We also considered information about MT-MMP functions from studies assessed in pathological conditions and compared these data with those relevant for development.

1.1 Domain structure of mammalian MMPs

The large number of members in the vertebrate MMP family makes possible different mechanisms of redundancy and compensation that complicate the study of their individual physiological functions. For that reason, Drosophila is an excellent model system to address MMP function as only two encoded MMP genes have been identified: Dm1-MMP that is a secreted enzyme and, Dm2-MMP, which is tethered to the plasma membrane through a GPI anchor. Despite sharing the same kind of anchor, it is not clear if Dm2-MMP is the mammalian orthologue gene of GPI-type MMPs. In Caenorhabditis elegans, there are six known MMPs while 25 MMP members have been reported in the zebrafish genome (six of them are MT-MMP orthologs anchored to the plasma membrane while the remaining ones are non-tethered enzymes). In contrast, in mammals, the MMP family is the most heterogeneous and comprises 28 members, 24 of which are found in humans. All MMPs with no difference have a basic core structure consisting of three conserved domains: the prodomain, the catalytic, and the hemopexin domains (Figure 1). The prodomain, with a length of 80 amino acids and a consensus sequence with unpaired cysteines, keeps the enzyme in latent state. At the C-terminal of the prodomain, all members of the MMP family show the catalytic domain, which also has a conserved sequence (“HEXXHGXGXXH”). Three histidine (H) and the glutamate (E) of this sequence allow holding a zinc ion in the active site of the catalytic domain, which is fully required for MMP proteolytic activity.
activity (Figure 1). This is linked by the hinge region to the hemopexin domain, which participates in substrate recognition and degradation\textsuperscript{10-14} (Figure 1).

Even though there are various type of classifications, mammalian MMP family members are divided into two categories: secreted MMPs to the extracellular milieu and membrane tethered to the cell surface MMPs, named membrane-type MMPs (MT-MMPs) (Figure 1). Secreted MMPs are classified according to their domain's organization into four groups:
archetypal MMPs (MMP-1, MMP-3, MMP-8, MMP-10, MMP12, MMP-13, MMP-19, MMP-20, and MMP-27), which display the basic multidomain organization as previously described; gelatinases (MMP-2 and MMP-9), that conserve the structure of the archetypal MMPs, but further integrate three fibronectin type II repeats in their catalytic domain for the binding of gelatin and denatured collagen; furin-activable MMPs (MMP-11, MMP-21, and MMP-28) characterized by the presence of a furin recognition sequence (“RX[K/R] R”) located between the prodomain and the catalytic domain that allow proenzyme activation; and matrilysins (MMP-7 and MMP-26), an exception since they lack the hinge region and hemopexin domain (Figure 1).10,12,14

**FIGURE 2** Legend on next page.
On the other hand, MT-MMP family is composed of six enzymes classified based on their linkage to the plasma membrane in transmembrane type I MMPs including MT1-, MT2-, MT3-, and MT5-MMP, which are tethered to the membrane surface through a transmembrane domain joined to a short cytoplasmic tail and, MT-MMPs anchored by a GPI motif, comprising MT4-, and MT6-MMP\(^{10,11,14-16}\) (Figure 1). This GPI anchor confers typical mechanisms of regulation and biosynthesis to this group.\(^{15,16}\) Both MT-MMP groups show a stem region downstream of their hemopexin domain which, in the case of the GPI-anchored MMPs, it is crucial for the establishment of homophilic interactions (Figures 1 and 2B).\(^{11,15}\) A unique feature of type I MT-MMPs is the insertion of a short sequence called MT-loop in the catalytic domain (Figure 1).\(^{10,11}\) Finally, a distinct transmembrane MMP subgroup named transmembrane type II includes MMP-23A and MMP-23B which have the transmembrane domain at the N-terminal\(^{17}\) (Figure 1). This group differs from the other membrane-anchored MMPs in the absence of the hinge region and the hemopexin domain which are both replaced by the immunoglobulin and cysteine array domains.\(^{12,17}\) Since MMPs are involved in multiple cellular processes, the list of their substrates is very extensive and heterogeneous. Indeed, thanks to the development of degradomic approaches, it has been possible to predict a wide range of ECM components, secreted and membrane-bound molecules as well as numerous intracellular substrates that can be processed by MMPs (for a more detailed information, see References 12, 13, 18) (Table 1). Among membrane-tethered MMPs, only MT1-MMP has been demonstrated to cleave intracellular proteins such as apoptotic regulators, cytoskeletal proteins, signal transducers, or transcriptional and translational regulators, although the relevance of its intracellular substrate processing during embryonic development is still unknown.\(^{12,13,18}\)

### 1.2 | Regulation of MT-MMP catalytic activity

Uncontrolled proteolytic function of matrix metalloproteinases can enhance cell invasion and tissue damage disrupting tissue homeostasis. In fact, MT-MMPs are involved in a variety of pathological processes, such as tumor progression and metastasis, fibrosis, or chronic inflammation. Therefore, there is an exhaustive control over their proteolytic activity. The first level of regulation comprises a tuned and individually control of MT-MMP gene expression. MT-MMP transcriptional activation can be induced or repressed depending on the kind of stimuli interacting with the cis-element binding sites located at the MMP promoter.\(^{11,19}\) However, MT-MMP catalytic activity is also tightly regulated post-transcriptionally at several points: biosynthesis and intracellular trafficking, proenzyme activation, endocytosis and recycling, cell surface degradation and shedding, endogenous inhibition, dimerization, and post-translational modifications (Figure 2).

#### 1.2.1 | Biosynthesis and intracellular trafficking of MT-MMPs

MT-MMP regulation relies on their biosynthesis and intracellular trafficking, which finally lead to their precise localization on the cell surface. The biosynthetic...
| MT-MMP     | Substrates                                    | Relevant biological context                                                                 | References |
|------------|-----------------------------------------------|---------------------------------------------------------------------------------------------|------------|
| MT1-MMP    | Type I, II, III, IV collagen; Gelatin, Fibronectin, Vitronectin, Laminin-1, −2/4, and −5 | Endochondral ossification and angiogenesis. Chondrocyte proliferation. Cell proliferation and morphogenesis in renal, lung and submandibular gland development. | 84,94,150,267,282,283,284 |
|            | Perlecan, Aggrecan                             | Proliferation and migration of renal tubule cells. Basement membrane remodeling during kidney development. | 150,284    |
|            | Fibrinogen/fibrin                             | Tumor cell invasion and growth.                                                               | 115,178    |
|            | Syndecan-1                                    | Pro-migratory effect during tumor progression.                                                | 89         |
|            | Betaglycan                                    | In vitro inhibition of TGF-β-induced tumor angiogenesis.                                      | 285        |
|            | ICAM-1                                        | ICAM-1 shedding under conditions of oxidative stress. Monocyte transmigration.                | 91,92      |
|            | β1-integrin                                    | Skeletal stem cell commitment via MT1-MMP/β1-integrin/YAP/TAZ signaling pathway during osteogenesis. | 83         |
|            | Pro-αv-integrin                               | Tumor cell adhesion and migration.                                                            | 90,286,287 |
|            | E-cadherin, N-cadherin                        | Ischemia-induced cadherin disruption in NRK cells.                                            | 288        |
|            | Pro-MMP-2                                     | Tumor cell invasion and metastasis. Lympathic vessel sprouting. Retinal axon growth.          | 116,215,238,289,290,291 |
|            | Pro-MMP-8                                     | Proteolytic MMP-8 activation during corneal wound healing.                                   | 292        |
|            | Pro-MMP-13                                    | Indirect activation of pro-MMP-9 through the activation of pro-MMP-13 in osteoarthritic condrocytes | 293,294,295 |
|            | MT1-MMP                                       | Tumor invasion a metastasis.                                                                 | 39         |
|            | ADAM9                                         | Negative modulation of ADAM9 activity to maintain FGFR2 signaling during osteogenesis.        | 296        |
|            | EphA2                                         | Cancer cell migration and invasion. Malignant transformation in human ovarian tumors.          | 253,297    |
|            | Pro-TGF-β                                     | Osteoblast survival during trans-differentiation into osteocytes by p44/p42-MAPK-dependent pathway. | 298,299    |
|            | Pro-TNFα                                      | Inflammation                                                                                 | 284        |
|            | HB-EGF                                        | Proliferation and tumor growth by stimulating the EGFR signaling pathway.                     | 300,301    |
|            | DDR1                                          | Regulation of cell proliferation and apoptosis by the collagen/DDR1 axis in breast carcinoma.  | 302,303    |
|            | VEGFR1                                        | Inhibition of corneal angiogenesis by reducing VEGF-A165.                                     | 187        |
|            | LYVE-1                                        | Suppression of lymphatic vascular outgrowth.                                                  | 212        |
|            | PTK7                                          | Cancer cell invasion and PCP-dependent convergent extension during embryogenesis.            | 131        |
|            | TSP-1                                         | Inflammatory intussusceptive angiogenesis via TSP1/nitric oxide pathway.                      | 191,255    |
|            | C3b                                           | Tumor cell immune resistance and survival of metastatic cells by suppressing the complement cascade | 304        |
|            | Dll1                                           | Inhibition of Dll1-induced Notch signaling in hematopoietic progenitor cells to maintain B-cell development in bone marrow. | 305        |
|            | CD44                                          | Tumor cell migration and invasion.                                                            | 63,97,306  |
|            | MCP-3                                         | Inflammation and immune responses.                                                            | 307        |
|            | APP                                           | Pro-amyloidogenic effect via MMP-2 by regulating APP processing.                             | 308,309,310 |
|            | LRP1                                          | Tissue remodeling by migrating tumor cells. Vascular smooth muscle differentiation via PDGFB-PDGFR-β axis in vessel wall architecture. | 205,311    |
|            | gC1qR                                         | gC1qR cleavage in breast carcinoma cells.                                                    | 312        |
|            | Semaphorin 4D                                 | Endothelial cell chemotaxis in vitro and tumor-induced angiogenesis in vivo.                 | 193        |
|            | Endoglin                                       | Tumor angiogenesis.                                                                          | 188        |
|            | KiSS-1                                         | Anti-metastatic effect during cancer progression.                                             | 313        |
|            | α1PI                                          | Not determined.                                                                              | 282        |
|            | αTGF                                         | Tumor cell adhesion and migration.                                                            | 314,315    |
|            | ApoE5                                         | Cell proliferation in cultured cells.                                                        | 316        |
|            | MICA                                          | Tumor cell sensitivity to natural killer cell cytotoxicity.                                  | 317        |
|            | IL8, SLP1, CTGF, DR6                           | Not determined.                                                                              | 318        |
|            | EMMPRIN                                        | In vitro cleavage in tumor cell lines.                                                       | 319        |

(Continues)
| MT-MMP | Substrates | Relevant biological context | References |
|--------|------------|-----------------------------|------------|
| MT1-MMP | Galectin-1, DJ1, Hsp90α, pentraxin 3, progranulin, Cyr61, peptidyl-prolyl cis-trans isomerase A, dickkopf-1 | Not determined. | 320 |
| | NC1 (type IV collagen) Fibronectin, fibrin, tenasin, nidogen, perlecain, aggrecan, laminin-1 E-cadherin Pro-MMP-2 LRP | Proliferation and branching of the submandibular gland. Tumor growth. VEGF-mediated angiogenesis. Epithelial cell proliferation. Tumor growth by promoting cancer cell invasion and adhesion. Tissue remodeling by migrating tumor cells. | 162, 202, 282, 321, 322, 106, 323, 324, 325, 311 |
| MT2-MMP | MT2-MMP NC1 (type IV collagen) Fibronectin, fibrin, tenasin, nidogen, perlecain, aggrecan, laminin-1 | Proliferation and branching of the submandibular gland. | 162 |
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pathway is slightly different in the two main groups of MT-MMPs (Figure 2). The signal peptide located in the N-terminal of both groups of membrane-type MMP is necessary for driving them to the endoplasmic reticulum to start its biosynthesis. Furin-dependent activation of transmembrane type I MMPs occurs in the trans-Golgi (Figure 2). The intracellular trafficking has been studied in MT1-MMP and MT3-MMP, where Golgi-derived vesicles containing the proteinase are intracellularly transported to the plasma membrane.20 MT1-MMP-containing vesicles are driven to the cell membrane by a kinesin-dependent mechanism along actin filaments and microtubules. At the cell surface, KIF5B and KIF3A/KIF3B kinesins drive the release of the enzyme to allow their surface exposure21,22 (Figure 2A). Moreover, MT1-MMP has been shown to traffic from intracellular compartments to the plasma membrane via Rab8-vesicles enabling its delivery in tumor cell protrusions and promoting its proinvasive activity.23

Regarding GPI-type MMPs, the hydrophobic tail of the zymogen at the C-terminal is replaced with a GPI precursor by a GPI transamidase in the endoplasmic reticulum. Subsequently, furin recognizes and cleaves a conserved sequence at the C-terminal of the prodomain, resulting in the mature form of the MT-MMP at the trans-Golgi (Figure 2B). It is still unclear where the transamidase cleavage site is, but possibly corresponds to residue 573 and 549 in MT4- and MT6-MMP, respectively.10,15,16 The biosynthetic pathway continues on the Golgi, where the GPI anchor is modified to generate the mature form. Some of these modifications, such as glycosylation and the change of unsaturated fatty acid chain into saturated ones, are essential for the proper anchoring of GPI-type MT-MMPs to the plasma membrane giving them unique characteristics and a specific localization to specialized lipid microdomains.10,11,15,16 In contrast to transmembrane MT-MMPs, the mechanisms underlying post-Golgi sorting and subsequent surface delivery of these endopeptidases remain unclear to date16 (Figure 2B).

1.2.2 | Proenzyme activation

As all MMPs are synthesized as zymogens, a key step in their regulation is their conversion into mature proteinases.10,11,14-16 The immature enzyme is maintained due to the interaction between the cysteine residue within the prodomain and the catalytic zinc ion through a thiol bond preventing substrate interaction (Figure 2).

Zymogen activation involves both proteolytic and non-proteolytic mechanisms.10,11,13,14,18 Intracellular proteolytic activation of the zymogen comprises the cleavage of the thiol bond resulting in the disruption of the cysteine-zinc ion interaction and the removal of the prodomain (Figure 2). Thus, the catalytic domain is exposed to bind and process distinct substrates. This proteolytic activation is driven by furin for membrane-tethered and furin-activable MMPs.10,11,13,14,18 Also, MMPs can be proteolytically activated by another MMP such as MT1-MMP that is responsible for MMP-2 activation, or by different proteases like serin protease that is required for MMP-3 activation and trypsin that has been reported to activate MMP-9 in vitro.13,18

MMPs can also be activated in both physiological and pathological settings without the proteolytic processing of the prodomain. This is the case of some secreted MMPs such as MMP-1, MMP-2, MMP-7, MMP-8, and MMP-9 that can undergo post-translational modifications induced by oxidants (reactive oxygen-nitrogen species).24,25 This results in the disruption of the interaction between the cysteine and the catalytic zinc ion and therefore, the MMP zymogen being directly activated by oxidative stress. In addition, metalloproteinases can also interact with non-substrates macromolecules which produces a distortion in the active site leading to allosteric activation. Both proteolytic-independent mechanisms can be followed by an autocatalytic degradation of the prodomain to achieve the fully active MMP.13,18

1.2.3 | Endocytosis and recycling

Endocytosis is the proposed mechanism for controlling the amount of the enzyme exposed to the cell surface. Both, transmembrane- and GPI-type MMPs, follow different endocytic pathways to recycle them back to the cell membrane or to target them for lysosomal degradation26 (Figure 2).

In the case of transmembrane MMPs, their endocytosis follows the classic clathrin and dynamin-dependent pathway for being efficiently internalized (Figure 2A). The consensus sequence in their cytoplasmic tail has a motif consisting of two leucine and one tyrosine (LLY573), that interacts with the clathrin adaptor protein 2 (AP2) allowing their endocytosis via clathrin-coated vesicles27-31 (Figure 2A). Additionally, an alternative endocytic clathrin-independent pathway has been demonstrated for MT1-MMP via caveolae in endothelial and tumor cells (Figure 2A).27,30,32 During angiogenesis, this caveolae-mediated endocytosis regulates MT1-MMP association with caveolin and distinct integrins for the proper enzyme localization and tubulogenic process.52

Internalized MT1-MMP also accumulates in invadosomes, which are actin-enriched protrusions of the plasma membrane involved in ECM remodeling31
(Figure 2A). Invadosomes are present in cancer cells where focal degradation of the ECM is necessary for tumor dissemination. The recruitment and the release of metalloproteinases, specifically MT1-MMP, confers proteolytic activity to invadosomes. The regulated MT1-MMP delivery to invadosomes requires a coordinated and complex machinery dependent on endosomal transport along microtubules. It has been described that invadosomes maturation is strongly regulated by the protrudin pathway, which is essential for the translocation of MT1-MMP-containing late endosomes toward immature invadosomes. Subsequently, the exocytosis of the secretory vesicles containing MT1-MMP is driven by SNARE protein family, SNAP-23, Syntaxin-4, and VAMP-7, (Figure 2A). In fact, VAMP-7 colocalize with MT1-MMP at focal sites of degradation and it is known that delivery of MT1-MMP to invadopodia during ECM remodeling relies on VAMP-7 function, possibly through the formation of molecular complex among VAMP-7, Syntaxin-4, and SNAP-23.

The mature GPI-anchored MMPs are internalized through the CLIC/GEEC pathway (Figure 2B). This dynamic endocytic route involves the formation of tubular-shaped plasma membrane invaginations named clathrin independent carriers (CLICs) that internalize these metalloproteinases becoming mature GPI early endosomal compartments (GEEC) and it has been demonstrated for MT4-MMP (Figure 2B). The signaling cascade that triggers this pathway involves the small GTPase of the Rho family, Cdc42, and the transcription factors Arf1 and GBF1 that regulates Cdc42 activity.

1.2.4 | Cell surface degradation and shedding

Shedding is another alternative followed by MT-MMPs to control their pericellular proteolytic activity and the amount of proteinase once they are tethered to the cell surface. Shedding active MT-MMPs may either involve the release of the extracellular portion to the pericellular milieu or the removal of the enzyme from the cell surface in order to inactivate it. Several studies confirmed that MT1-MMP shedding is autocatalytic-dependent, involving the cleavage of a peptide bond in the hinge region followed by a second cleavage in the active site. The result of this autocatalytic shedding is the formation of two inactive MT1-MMP species: a membrane-tethered species and a shed soluble catalytic domain fragment (Figure 2A). Moreover, MT1-MMP shedding can also follow non-autocatalytic mechanisms by a cleavage in the stem region which releases the whole active ectodomain (Figure 2A). Similarly, MT5-MMP is also shed through a cleavage in the stem region which results in a major species or in the hinge region releasing smaller MT5-MMP fragments.

In contrast to transmembrane-type, shedding of GPI-anchored MMPs is not totally clear (Figure 2B). It is thought that MT4- and MT6-MMP shedding are MMP-independent because none of them are affected in the presence of tissue inhibitors of metalloproteinases (TIMPs). Therefore, it is possible that their shedding is dependent on phospholipase C (PLC) as demonstrated for MT4-MMP and in a similar manner to the membrane dipeptidase, a GPI protein which is also released in a PLC-dependent manner. Moreover, MT6-MMP is shed through a cytokine-dependent mechanism releasing soluble MT6-MMP species, which play significant roles in tissue repair and host defense during innate immune response.

1.2.5 | Interactions with endogenous inhibitors

Once MT-MMPs are exposed on the cell surface, their enzymatic activity is regulated by endogenous inhibitors, TIMPs (Figure 2). There are four mammalian TIMPs (TIMP-1, 2, 3, and 4), that inhibit MT-MMPs by binding their N-terminal domain with the catalytic zinc ion of the enzyme. Except for TIMP-3, the remaining TIMPs are found as soluble forms. Independently of their inhibitory activities, TIMPs play various biological activities in the embryo. For instance, TIMP-2 regulates kidney morphogenesis by promoting cell growth on mesenchymal cells. Similarly, TIMP-3 null mice display alveolar defects and defective mammary gland branching.

Transmembrane type I MMPs are inhibited by all TIMPs except for TIMP-1 that displays poor inhibitory activity against these enzymes. Kinetic studies demonstrate that TIMP binding affinity is different and highly selective for the distinct MT-MMPs. For instance, TIMP-3 has a higher affinity binding to MT3-MMP while TIMP-2 is a strong inhibitor of MT1-MMP. In addition, the membrane-bound glycoprotein, reversion-inducing cysteine-rich protein with Kazal motifs (RECK) has been described as a MT1-MMP inhibitor and it also interacts with MMP-2 and MMP-9 (Figure 2A). During development, it plays a vital role as a regulator of tissue morphogenesis as RECK deficient embryos showed vascular defects that lead to premature death. Moreover, RECK is also involved in tumor angiogenesis by inhibiting MMP activity which leads to the suppression of the formation of new blood vessels. Interestingly, the proteoglycan Testican-3 and it is
splicing variant gene, N-tes, have been described to negatively affect MT1- and MT3-MMP proteolytic activity.48

Regarding GPI-anchored MMPs, MT4- and MT6-MMP are inhibited by all TIMPs, being TIMP-1 the most effective inhibitor15,16 (Figure 2B). Additionally, other molecules present in the plasma membrane can interfere with their proteolytic activity such as Clusterin (also named apolipoprotein J), that forms a complex with MT6-MMP through the hemopexin domain49 (Figure 2B).

1.2.6 | Dimerization

Oligomerization is important for stabilization, subcellular distribution, and regulation of the amount of MT-MMP and its turnover.29,50-52 Besides, a relationship between metalloproteinase dimerization and a greater proteolytic activity in tumor cells is plausible.29,51

Among transmembrane MT-MMPs, only MT1-MMP can establish homophilic complexes.29,51,52 MT1-MMP dimers are set up by interactions between the hemopexin and/or the transmembrane domains to fix the enzyme to the cell surface and to facilitate its proteolytic activity over the collagen triple helix29,52 (Figure 2A). This dimerization involves cytoskeleton rearrangement, which is regulated by GTPases, Cdc42 and Rac1,11,29 and it can be enhanced by certain chemokines in a PI3K and actin-dependent manner in endothelial cells.32 Besides, MT1-MMP oligomerization via hemopexin domains facilitates the activation of pro-MMP-2 through the formation of a MT1-MMP, TIMP-2, and pro-MMP-2 ternary complex. Firstly, MT1-MMP forms a homodimer interaction via hemopexin domain in which, one molecule is inhibited due to its interaction with TIMP-2. In parallel, TIMP-2 interacts via C-terminal with the hemopexin domain of pro-MMP-2. Following, the molecule of MT1-MMP in the homodimer that is free from TIMP-2 interaction, cleaves the prodomain of pro-MMP-2 leading to its autocatalysis. Finally, MMP-2 is released to the extracellular space as a mature enzyme.3,11,14,21,53

Both GPI-anchored proteins, MT6- and MT4-MMP, are found in homodimer forms at the cell membrane maintained via a disulfide bond between the cysteine residues of the stem region16,26 (Figure 2B).

1.2.7 | Post-translational modifications

MMPs can undergo post-translational modifications, which consist in adding different chemical groups to the residues within the protein chain that influence their enzymatic activity. One of these modifications includes MT1-MMP phosphorylation, specifically the Tyr573 residue located in the cytoplasmic tail, which has been linked to promote endothelial and tumor cell migration.54,55 In fact, Tyr573 phosphorylation represents a tightly regulatory mechanism of carcinoma cell behavior by inducing cell detachment and invasion and thereby, promoting metastasis.56 Moreover, Tyr573 interacts with p130Cas to regulate Rac1 activity in the signaling pathway that underlies the control of macrophage migration and fusion.55 MT1-MMP can also be palmitoylated in its cytoplasmic domain, in particular the Cys574. This post-translational modification regulates cell migration as well as clathrin-dependent endocytosis since culture cells transfected with palmitoylation-defective mutant MT1-MMP constructs impaired cell motility and the ability of MT1-MMP internalization through the clathrin-mediated pathway.57

In addition, glycosylation of the MT1-MMP hinge region has been described as an autocatalytic regulatory mechanism.58,59 Indeed, an incomplete glycosylated MT1-MMP increases its own proteolysis in cancer cells.59 Since glycosylation affects MT1-MMP activity, several in vitro studies have tried to assess the potential of glycosylated MT1-MMP to activate MMP-2. However, it is controversial if this posttranslational modification impairs the interaction between MT1-MMP and TIMP-2 and consequently, MMP-2 activation.58,59 On the other hand, GPI-anchored MT-MMPs can also be post-translationally modified. In this regard, N-glycosylation is essential for MT4-MMP stability by inducing dimerization while MT6-MMP is O-glycosylated.16,26

2 | MT-MMPS PARTICIPATE DURING CELL MIGRATION, PROLIFERATION AND POLARITY

2.1 | Cell migration

Several scenarios lead to cells remodeling their polarity through modifications of cell-cell and cell-matrix adhesions in order to initiate cell migration. This is a fundamental cellular process that enables major tissue rearrangements and organ morphogenesis in the embryo. These processes involve precise trafficking and distribution of MT-MMPs between the apical and basal cell surfaces for extracellular matrix degradation and remodeling.50 As a result, targeted localization of MT-MMPs is found at specific cell sites. This enables pericellular proteolysis at polarized cell structures such as the leading edge of migrating cells, focal adhesion and collagen attachment sites, podosomes, and lamellipodia (and invadopodia in cancerous cells).22,23,31,61-64 Regarding cell migration little is known about the roles of transmembrane MMPs in the embryo. Previous data reported the
expression of MT1-MMP in distinct organs and systems of
the developing mouse embryo.65-70 Notably, a recent study
developed a dynamic expression pattern for MT1-MMP in
the mouse embryo, particularly in the developing cardiovas-
cular and nervous systems and the limbs.71 These results
support that MT1-MMP may participate in distinct morpho-
genetic processes during embryogenesis that requires cell
migration. Indeed, MT1-MMP expression has been reported
to support cell migration.69 Supporting these data, MT1-MMP as well as MT2-, MT3-, MT4- and
MT5-MMP are all expressed in the subventricular zone, the
rostral migratory stream, and the olfactory bulb at postnatal
stages and persists in the adulthood72 (Table 2). In fact, neuroblast migration is reduced by MMP inhibitors and the
migrating olfactory stem cells show MT1-MMP-enriched
lamellipodia72-74 (Table 2).

Interestingly, both MT1- and MT2-MMP are
expressed by trophoblasts and localize in podosomes.75,76
Through the endothelin receptor type B localized in
trophoblast, endothelin-1 down-regulates the expression
levels of both metalloproteinases, which restrains tropho-
blast migration in the embryo77 (Table 2). During Xenopus and chicken embryogenesis, MT3-MMP expres-
sion is developmentally controlled and predominantly
found in the neural tube and the limb buds where ECM
remodeling and cell migration are crucial.78,79 In line
with these data, this enzyme is also expressed by the
developing corneal epithelial cells where it may regulate
their cellular migration through the association with
CD44, which is a cell surface hyaluronic acid receptor
with affinity to osteopontin, collagen, and fibronectin80
(Table 2). Since MT3-MMP has been reported to degrade
collagen III-enriched environment, it has been involved
in cell migration during skeletal development (Table 1).

MT1-MMP is also expressed in embryonic connective tis-
sue cells such as osteoblasts, osteoclasts, and peri-
chondrial, muscle, and tendon fibroblasts.67,71,81,82
During osteogenesis, differentiation of skeletal stem cells
is regulated by MT1-MMP/β1 integrin/YAP/TAZ signal-
ing axis83 (Table 2). In fact, Mt1-mmp−/− mice display
several craniofacial abnormalities as well as skeletal tis-
ue disorders84,85 (Table 2). Moreover, double deficient
mice for MT1- and MT3-MMP result in severe embryonic
defects in bone formation, which ultimately lead to early
death69 (Table 2). MT4-MMP, is expressed during limb
vascularization possibly promoting angioblasts migration
at early stages of mouse development.86 Additionally, this
GPI-anchored MMP is found in various structures of the
embryonic brain such as the olfactory bulb, cerebral cor-
tex, and hippocampus pointing out a possible involve-
ment in neural migration and proliferation during CNS
development.86 Moreover, Dm2-MMP plays an important
role in Drosophila heart development since it is expressed
in cardioblasts where it regulates collective cell migration
that contributes to cardiac lumenogenesis.87

Processing and trafficking of adhesion molecules by
transmembrane MMPs is also pivotal for cell fate and
motility in cancer cells. In that regard, it is known that
vesicular colocalization of MT1-MMP with β1- or
αvβ3-integrins at cell-cell contacts and motile structures
modulates endothelial cell migration.88 Also, localized
proteolytic activity of MT1-MMP cleaves other adhesion
molecules such as laminin, syndecan-1 and ICAM-1 to
promote cell migration (Table 1).89-92 Thus, cleavage of
laminin-5 by MT1-MMP releases an intermediary that
through EFGR signaling induces epithelial cell migra-
ation93,94 (Table 1). Remarkably, several studies have
suggested novel functions for this enzyme regulating
motility and trafficking of distinct immune cells.95,96
MT1-MMP is expressed in human monocyte-derived
immature and mature dendritic cells and participates in
their migration by processing different substrates such as
ICAM-191 or αv-integrin.90 Also, CD44H is processed by
MT1-MMP into its active form (CD44) (Table 1), releas-
ing a fragment that induces a migratory response.63,97,98
Independently of its proteolytic functions, MT1-MMP
also promotes macrophage migration by its association
with p130Cas and regulating Rac1 activity.55 Among the
GPI-anchored MMPs, MT4-MMP is implicated in mono-
cyte migration by cleaving eM-integrin increasing patrol-
ning monocyte crawling and thereby, their recruitment to
inflamed tissues99 (Table 1). In addition, MT6-MMP is
involved in monocyte and neutrophil cell migration by
processing vimentin100 (Table 1).

2.2 | Cell proliferation

Several studies have reported the involvement of MT-
MMPs in cell proliferation, although most of them are
focused on the context of tumor cells.11,52,101,102 For
instance, it has been shown that the proteolytic
processing of N-cadherin by MT5-MMP is necessary for
neural stem cell proliferation at postnatal stages103
(Table 2). Polared distribution of adhesive molecules
determines adult neural stem cell quiescence in the
epithelial stem niche of the subependymal zone: the api-
cal domain interacts with ependymocytes through
E-cadherin104 while the basal process adheres to the vas-
culature by α6β1-integrin-laminin interaction.105 Hence,
MT5-MMP-mediated cleavage of N-cadherin is necessary
for the exit from quiescence of neural stem cells and their
activation and proliferation.103 Similarly, MT2-MMP con-
trols epithelial cell proliferation rate by cleaving
E-cadherin in the intestinal niche106 (Table 1). Thus,
polarized localization of MT2-MMP at the apical junctions
| MT-MMP  | Cellular process   | Biological functions                                                                                                                                                                                                 | References |
|---------|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| MT1-MMP | Cell migration     | Neuroblast migration in postnatal brains.                                                                                                                                                                               | 72,73      |
|         |                    | Trophoblast migration controlled by endothelin receptor type B/endothelin-1 activation.                                                                                                                                 | 75,76      |
|         |                    | Skeletal and connective tissue disorders.                                                                                                                                                                                 |            |
|         |                    | Osteogenesis and differentiation of skeletal stem cells through the MT1-MMP/β1-integrin/Rho GTPase signaling.                                                                                                           | 67,81,82,83,84,85 |
|         |                    | Formation of the cardiac valves by inducing mesenchymal cell migration through degradation of type IV collagen in the developing endocardial cushions.                                                      | 139,140    |
|         |                    | Neural crest cell migration possible through the regulation of cadherin levels. Neural crest cell delamination independently of its catalytic activity.                                                                 | 134,135    |
|         | Cell polarity      | Acquisition of cell polarity during zebrafish gastrulation cell movements.                                                                                                                                             | 125        |
|         |                    | MT1-MMP internalization regulated by the planar cell polarity protein VANGL2 during gastrulation.                                                                                                                     | 126        |
|         | Tissue branching   | Ureteric bud branching morphogenesis regulated by proteolytic degradation of the kidney basement membrane.                                                                                                             | 150,151,152,153 |
|         |                    | Mammary gland morphogenesis mediated by collagen type I and laminin degradation. Epithelial cell branching regulation.                                                                                                    | 157,158,159,160 |
|         |                    | Submandibular gland branching morphogenesis.                                                                                                                                                                               | 66         |
|         | Angiogenesis and vessel wall formation | Lung vascularization and alveolar septum formation in postnatal development.                                                                                                                                              | 66,180,181 |
|         |                    | Secondary ossification. FGF2-induced retinal neovascularization.                                                                                                                                                       | 84,85      |
|         | Axon growth        | Postnatal retinal axon growth through MMP-2 activation.                                                                                                                                                               | 238        |
|         |                    | Neurite outgrowth through L1-ANT1/2-GAPDH-MT1-MMP complex in cerebellar neurons.                                                                                                                                       | 241        |
|         |                    | Nerve sprouting induced by MT1-MMP and MMP-2 via pro-NGF conversion and TrkA signaling in sympathetic neurons.                                                                                                       | 242        |
|         | Synaptogenesis      | Navigation of spinal cord pioneer axons.                                                                                                                                                                                 | 243        |
|         |                    | Retinal neurogenesis and axon growth.                                                                                                                                                                                    | 240        |
| MT2-MMP | Cell migration     | Endocardial cushion development mediated by Snail1-induced mesenchyme cell migration.                                                                                                                                   | 141        |
|         | Cell proliferation | Epithelial cell proliferation mediated by E-cadherin cleavage and interaction with ZO-1 at the apical cell junction during intestinal remodeling.                                                                       | 106        |
|         | Tissue branching   | Submandibular gland branching through the proteolytic release of collagen IV NC1 fragments and via PI3K/Akt signaling pathway.                                                                                       | 162        |
of epithelial cells allows the interaction with zonula occludens protein-1 (ZO-1) through its cytosolic domain as well as the proteolytic processing of E-cadherin. As a result, MT2-MMP-mediated cleavage disrupts apical E-cadherin-mediated epithelial cell quiescence promoting their proliferation during intestine morphogenesis\(^\text{106}\) (Table 2). In line with this data, MT1-MMP also participates in the maintenance of the hematopoietic stem cell niche by promoting the transcription of chemokines/ cytokines via HIF-1 signaling during the postnatal hematopoiesis.\(^\text{107}\)

Regarding the role of GPI-anchored MT-MMPs in cell proliferation during development, MT4-MMP deficiency was shown to result in a transient boost of vascular smooth muscle cell (VSMC) proliferation in the neonatal aorta by not yet defined mechanisms, suggesting a negative role of MT4-MMP in regulating VSCM proliferation.\(^\text{108}\) Moreover, in Drosophila, the GPI-anchored Dm2-MMP is involved in follicle stem cell proliferation, which is orchestrated by Wingless (Wg)/Dally-like protein (Dlp) signaling pathway.\(^\text{109}\) Herein, Dlp is a substrate for Dm2-MMP so that, in wild-type ovaries Dlp processed by the enzyme is not able to interact with Wg and therefore follicle stem cell proliferation is inhibited. However, Dm2-MMP null ovaries show an increase rate of stem cell proliferation as consequence of the interaction between Wg and Dlp that results in an enhancement of Wg signaling.\(^\text{109}\) Also in the morphogenetic events that lead to tissue elongation, flattening, expansion, and proliferation in the Drosophila limb development, Dm1-/ Dm2-MMP proteases act degrading ECM.\(^\text{110}\) These processes may be regulated by the expression of Ubx, a homeotic gene belonging to the HOX family of transcription factors. Ubx regulates apical and basal matrix remodeling during epithelial wing morphogenesis by repressing genes encoding basal matrix metalloproteases (Dm1- and Dm2-Mmp) and inducing Timp in the halteres.\(^\text{111}\)

In a distinct context, high MT1-MMP expression in gastric carcinoma cells contributes to cell migration and

| MT-MMP | Cellular process         | Biological functions                                                                 | References |
|--------|--------------------------|--------------------------------------------------------------------------------------|------------|
| MT3-MMP | Cell migration           | Corneal epithelial cell migration mediated by CD44v6 receptor shedding.              | 80         |
|        |                          | Neural crest cell migration possibly regulated by N-cadherin and laminin cleavage.   | 136        |
|        | Cell migration and proliferation | Palatogenesis and bone formation defects. Impairment of the mesenchymal cell migration and proliferation caused by the loss of collagenolytic abilities in the double MT1−/MT3-MMP mutant mice. | 65         |
|        | Synaptogenesis            | Excitatory synapse development by the proteolytic cleavage of Nogo-66 receptor (NgR1) in cortical neurons. | 277        |
| MT4-MMP | Cell migration           | Neural crest cell migration in zebrafish.                                           | 137        |
|        | Angiogenesis              | Vascular wall maturation by inducing VSCMs migration and differentiation via osteopontin proteolysis and JNK signaling pathway. | 108        |
| MT5-MMP | Cell proliferation        | Neural stem cell proliferation mediated by N-cadherin cleavage.                    | 103        |
|        | Axon growth               | Axon extension of dorsal root ganglia neurons through proteolysis of proteoglicans. | 223        |
|        | Synaptogenesis            | Synapse regulation by binding to PSD, ABP, and GRIP to target AMPA receptors to synapses. Axon growth and synapse remodeling through proteolytic cleavage of N-cadherin. | 246        |
| MT6-MMP | Axon growth               | Sensory neuron axon guidance mediated by remodeling extracellular environment of the axonal pathway. | 233,247    |

Note: Summary of MT-MMP functions described during vertebrate development demonstrates their participation in cellular processes essential for the growing embryo such as cell polarity, proliferation and migration, angiogenesis, tissue branching, axon growth, and synaptogenesis.
proliferation by regulating vimentin and E-cadherin levels.\textsuperscript{112} Moreover, miRNA-mediated down-regulation of MT1-MMP has an inhibitory effect over cervical cancer cell proliferation and invasion through the inactivation of the TNF pathway\textsuperscript{113} and the regulation of HB-EGF expression levels.\textsuperscript{114,115} Indirectly, MT1-MMP also induces tumor cell migration in type IV collagen environment by activating pro-MMP-2.\textsuperscript{82,116} Additionally, MT3-MMP is an important driver of tumor dissemination by activating pro-MMP-2 and its inhibition leads to impaired cancer progression and cell proliferation rate.\textsuperscript{117-119} Similarly, MT5-MMP also promotes cell migration and proliferation through pro-MMP-2 activation and thereby, supports tumor progression.\textsuperscript{120} Independently of its proteolytic activity, MT4-MMP is involved in breast cancer cell proliferation by promoting EGFR phosphorylation, which lead to the progression of the tumor via EGFR signaling.\textsuperscript{121}

### 2.3 Gastrulation and cell polarity

Increased cadherin levels and adhesion at the cell surface seems to be dependent on glypican 4, a Wnt co-receptor anchored to the membrane by a GPI linkage that is required for the correct establishment of planar cell polarity (PCP) during frog gastrulation.\textsuperscript{122-124} Since there is a strong genetic interaction between zebrafish MT1-MMP and glypican 4, we can speculate that PCP proteins regulate ECM assembly and remodeling possibly by ensuring matrix metalloproteinase localization during developmental events, such as formation of the three embryonic layers during gastrulation\textsuperscript{125} (Table 2). Gastrulation requires cell movements involving ECM proteolytic degradation and remodeling to enable proper PCP establishment. Both canonical and non-canonical Wnt pathways have been implicated in the ECM reorganization during this process and in that regard, it is known that MT1-MMP activity coordinates with non-canonical Wnt signaling cascade through colocalization with its key regulator VANGL2\textsuperscript{122,125} (Table 2). Vanl2 mutant embryos show increased MMP activity that possibly facilitates the formation of cell protrusions.\textsuperscript{122,126} Moreover, inhibition of MT1-MMP/MMP-2 rescues cell adhesion in VANGL2 knockout cells, and deregulation of both VANGL2 and MT1-MMP alters cell polarity leading to abnormal cell protrusion formation.\textsuperscript{125,127-130} VANGL2 regulates MT1-MMP negatively by controlling \(\alpha\beta3\)-integrin levels and activating MMP-2.\textsuperscript{127,130} In addition, evidence suggests that there are further links between MT1-MMP function and the Wnt/PCP signaling pathway through the transmembrane receptor PTK7, which is an essential regulator of both the canonical and non-canonical Wnt pathways, as well as a proteolytic target of MT1-MMP required for redistribution of polarized cell membranes upon cleavage\textsuperscript{131} (Table 1). In this regard, a defect in PTK7 cleavage may explain the inappropriate ciliary organization in mouse ependymal cells, where ciliary defects associated to disrupted polarity are shown upon MT1-MMP loss, suggesting that MT1-MMP may be essential for planar polarization of ependymal cells.\textsuperscript{74} Furthermore, Wnt signaling pathway may contribute to ensure proper trafficking of MT1-MMP to specific sites for polarized structure formation in several contexts, in a process required to enable distribution between the apical and basal cell surfaces.\textsuperscript{34,60}

Epithelial polarization is also essential to allow cells to organize in sheets and tubules. In this sense, the proper localization of MT1-MMP required for tubulogenesis and lumen formation is mediated by the receptor tyrosine kinase for collagen, discoidin domain receptor 1 (DDR1), which regulates the apicobasal distribution of this metalloproteinase in epithelial cells.\textsuperscript{132} (Table 1).

### 2.4 Epithelial-mesenchymal transition (EMT)

One of the paramount developmental processes, which include cell proliferation and migration as well as changes in cell polarity, consists in the transition of epithelial into mesenchymal cells. The neural crest cells (NCCs) are a multipotent progenitor population, which accomplish the EMT that enables them to delaminate from the dorsal folds of the neural tube and subsequently migrate to various destinations to form differentiated tissues.\textsuperscript{133} MT-MMPs play an important role during EMT in different tissues and species. As a matter of fact, MT1-MMP is expressed in both premigratory and migrating NCCs in Xenopus embryos and it is involved in their transition and migration independently of MMP-2 activation. MT1-MMP promotes neural crest EMT and migration possibly by regulating N-cadherin and E-cadherin levels and ECM degradation\textsuperscript{134} (Figure 3A; Table 2). Thus, down-regulation of MT1-MMP levels leads to an increase of N-cadherin expression and a reduced NCC migration.\textsuperscript{134} Supporting these data, the enzyme is also reported in the migrating NCCs along the neural tube in the mouse embryo.\textsuperscript{71} Additionally, MT1-MMP is required for basal positioning of NCC nuclei prior to epithelial delamination during the formation of the chicken neural tube. This novel function is proposed to be relevant for the regulation of EMT during development independently of its catalytic activity, suggesting that it may play an important role in the accomplishment of epithelial basal cell extrusions through mechanisms yet to be
explored\textsuperscript{135} (Table 2). Similarly, MT3-MMP is also expressed in cranial NCCs enhancing their migration probably through the degradation of N-cadherin and laminin during chick development. Thus, embryos lacking MT3-MMP displayed defects in the NCC migratory routes\textsuperscript{136} (Figure 3A; Table 2). Among the GPI-anchored
MMPs, MT4-MMP expression has been reported in NCCs in the mouse embryo and its ortholog mmp17b has been demonstrated to be necessary for the proper NCC migration in zebrafish embryos\textsuperscript{86,137} (Figure 3A; Table 2). Moreover, this study suggests a functional interaction of mmp17b and the MMP inhibitor RECK during NCC development.

Heart morphogenesis is also considered as an EMT-like process, which comprises several events such as endocardium and endocardial cushion formation.\textsuperscript{138} In fact, MT1-MMP as well as MMP-2 have been described to promote mesenchymal cell migration by degrading type IV collagen of the developing endocardial cushions leading to the formation of the cardiac valves\textsuperscript{139,140} (Table 2). This is in line with the reported expression of MT1-MMP in the mouse embryonic heart where the enzyme was found in the primitive atrium, the ventricle, the cardiac outflow tract, and the endothelial cells of the endocardium.\textsuperscript{71} Interestingly, this expression pattern persists along heart morphogenesis supporting the requirement of this transmembrane MMP during cardiac development.\textsuperscript{71} Similarly, MT2-MMP, through a direct transactivation by Snail1, has been also involved in endocardial cushion development.\textsuperscript{141} Thus, Snail1-mediated mesenchyme cell migration requires MT2-MMP activity, which is sufficient to rescue migratory phenotypes observed in targeted Snail1 knockdown during the formation of heart valves structures.\textsuperscript{141} (Table 2).

During mesenchymal transmigration in invasion programs, Snail1 and Snail2 regulate the activity of different metalloproteinases such as MT1-, MT2- and MT4-MMP.\textsuperscript{141-144} In this context, the acquisition of an invasiveness phenotype by cancer cells triggered by Snail inducing MT1- and MT2-MMP expression provide to these cells with the ability to migrate by remodeling the underlying basement membrane.\textsuperscript{142,143} Similarly, E-cadherin is also processed by MT2-MMP in cancer cells to trigger EMT in invasion programs.\textsuperscript{145}

For instance, MT1-MMP localizes in the proximal part of the embryonic midgut where ECM remodeling activity is essential for intestine development.\textsuperscript{71} According to this, previous studies have described a correlation between MT1-MMP expression and villi morphogenesis during chicken embryonic development.\textsuperscript{148}

In addition, renal system development comprises multiple steps of branching and ECM degradation to achieve a mature collecting system. In this regard, MT1-MMP expression is found in different embryonic renal structures such as the urogenital tract, the bulbourethral gland mesenchyme, and the metanephric mesenchyme.\textsuperscript{67,149-151} Moreover, migrating ureteric tip cells express MT1-MMP to remove ECM barriers and to allow tip cell branching, suggesting a role for MT1-MMP as a pericellular protease essential for the developing renal system\textsuperscript{152,153} (Table 2). In fact, defective MT1-MMP functioning has been associated with an excessive accumulation of basement membrane components such as laminin, collagen, and perlecain which ultimately leads to defective branching pattern and impaired cell proliferation\textsuperscript{150} (Tables 1 and 2). The excessive accumulation of collagen IV in the underlying basement membrane of the tubular epithelium has implicated MT2-MMP as a required regulator of renal branching morphogenesis, even though its function in this context is yet unclear.\textsuperscript{150} The enzyme expression has been also reported in the pronephros of the chicken embryo supporting a possible role of MT2-MMP in this process.\textsuperscript{154} MT4-MMP is localized in the collecting ducts of the embryonic kidney although further studies are needed to elucidate how the enzyme participates in the developing kidney.\textsuperscript{155}

MT1-MMP has been reported to modulate cell proliferation within mammary gland morphogenesis since its expression is found in the branching ductal tree as well as in the mammary stroma.\textsuperscript{147,156} Moreover, epithelial end bud cells localize their MT1-MMP at lamellipodia of the tip cells to degrade local type I collagen and laminins of the surrounding mesenchyme promoting cell proliferation and branching\textsuperscript{147,157,158} (Table 2). For instance, stroma-cell specific targeting of MT1-MMP resulted in the complete suppression of branching morphogenesis and collagen accumulation indicating that mammary gland development relies on stroma cell functions.\textsuperscript{147,159} Moreover, in vitro studies in which Mt1-mmp\textsuperscript{−/−} mammary epithelial organoids were embedded in 3D type I collagen hydrogels showed defects in the branching duct pattern.\textsuperscript{147} Interestingly, MT1-MMP is a key player in the invasive response during breast carcinoma, although its mechanisms of action in the mammary gland during tumorigenesis are quite different from those followed by normal mammary epithelial cells. Thus, while the normal mammary gland development depends on
MT1-MMP in the stroma, tissue invasion depends on MT1-MMP expressed in breast epithelial carcinoma cells themselves. 159

Additionally, MT1-MMP exerts non proteolytic functions to regulate epithelial branching morphogenesis. Thus, MT1-MMP hemopexin domain acts together with CD44 during the patterning of the developing mammary tissue to promote cell rearrangement and motility by activating Rho kinase signaling. 160 Similarly, since it has been described the relevance of integrin function in branching morphogenesis, 161 the interaction between the MT1-MMP transmembrane/cytoplasmic domain with β1-integrins has been implicated in mammary branching by promoting epithelial cell invasion independently of its catalytic activity 158 (Table 2). MT2-MMP is also expressed in the mammary epithelium during all developmental stages except for the lactating period when ECM degradation is not required 156 and therefore, both enzymes may play complementary or redundant roles during mammary gland development (Table 2). Moreover, according to previous actions described for MT2-MMP in cancer cells, 115 it is possible that its action is based on providing the epithelial cells that do not have collagenolytic activity with proliferative ability to degrade mammary basement barriers. Regarding MT3-MMP, is found in the stroma of the mammary gland, 156 although there is not a direct evidence for a similar role in branching morphogenesis.

MT2-MMP is mostly expressed by epithelial cells and its proteolytic activity is required for cell proliferation during submandibular gland morphogenesis. 162 In this context, NC1 fragments obtained from MT2-MMP-mediated collagen IV proteolysis (Table 1) increases cell proliferation and end buds branching via PI3K/Akt signaling pathway in the submandibular gland 162 (Table 2). Indeed, in submandibular gland explants treated with MT2-MMP siRNA intracellular collagen IV accumulation reached high levels while the release of NC1 domains dropped, which ultimately resulted in a notable impaired branching morphogenesis. 162 Similar to mammary gland branching, mesenchymal cells surrounding the submandibular epithelium express MT1-MMP. 162 In fact, embryos lacking the enzyme showed enlarged gland end buds and subsequently, defective branching 66 (Table 2).

The Drosophila tracheal system has been proven as an excellent tool for the study of MMPs in branching morphogenesis. 163,164 Tracheogenesis starts when epithelial cells differentiate in tracheal cells and migrate to build sac-like structures. Subsequently, these tracheal sacs undergo successive branching programs in order to form the complex network of epithelial tubes characteristic of the fly tracheal system. 164-166 This mechanism is coordinated by BNL/BTL axis signaling. Tracheal cells, which harbors FGF receptor breathless (Btl), respond to brancheless (BNL) gradient provided by the surrounding mesodermal cells. 167 Both Dm1- and Dm2-MMP are essential for this morphogenetic event and are expressed in the air sac primordium. Notably, although knockdown of both MMPs display similar phenotypes in the developing tracheal tube network, their roles are quite different. Secreted Dm1-MMP-dependent proteolysis is required for tracheal cell invasion into myotubes by removing collagen IV barriers that avoid invading branch tip cell migration. 168 Thus, Dm1-MMP fly mutant embryos show severely disrupted trachea elongation supporting that the catalytic domain is essential for tracheal branching. 163 Similarly, the membrane anchored Dm2-MMP has been also involved in ECM remodeling during tracheal development since Drosophila embryos lacking the enzyme showed an aberrant air sac development. 169 Its proteolytic functions over basal lamina components are essential to disc cell migration resulting in a precise disc trachea association and air sac development 169 (Table 2). Indeed, Dm2-MMP is involved in tracheal branching independently of its proteolytic actions. It has been described that Dm2-MMP regulates tip cell specification by modulating FGF signaling during air sac development, which ultimately leads to avoid the acquisition of tip cell phenotype. This lateral inhibition mediated by Dm2-MMP is important to control tracheal branching pattern 170 (Table 2).

4 ROLE OF MT-MMPS DURING ANGIOGENESIS AND LYMPHANGIOGENESIS

4.1 Angiogenesis

Angiogenesis is an essential developmental process for the proper organogenesis and growth of the embryo. After the formation of the primary plexus by vasculogenesis, new blood vessels sprouted from the pre-existing ones in order to supply the nutritional requirements of the growing embryo. 171,172 Angiogenesis also takes place in the adulthood during the menstrual cycle and tissue repair or under pathological circumstances such as cancer progression and in response to an inflammatory insult. Blood vessel formation by sprouting angiogenesis involves a sequence of events in the endothelial cells: cellular activation, degradation, and invasion of the ECM, cell migration into the tissues, proliferation, cell fusion and tubulogenesis, and stabilization of new capillaries. 51,173,174 These regulated steps in angiogenesis are facilitated by MMPs through different mechanisms such as perivascular matrix remodeling, release of angiogenic factors from the ECM or cleavage of angiogenic inhibitors.
In this context, the most studied cell surface-associated MMP is MT1-MMP which has a key function promoting endothelial cell migration.\textsuperscript{175-177} Since MT1-MMP displays fibrinolytic activity, endothelial cells expressing the enzyme can penetrate fibrin-enriched tissues and switch on tubulogenic programs during neovascularization.\textsuperscript{178} In fact, null mice for this metalloproteinase display defective vascular invasion of cartilage, which results in an unsuitable secondary ossification\textsuperscript{84,85} (Table 2). Moreover, these post-natal mice displayed a null FGF-2-induced retinal neovascularization supporting that MT1-MMP is a key player in the angiogenic process\textsuperscript{85} (Table 2). In addition, \textit{in vitro} studies demonstrated that vascular outgrowth of aortic explants isolated from \textit{Mt1-mmp} deficient-mice was impaired due to the inability of the endothelial cells to degrade the surrounding matrix.\textsuperscript{51,179} In the same context, lung endothelial cells deficient for MT1-MMP showed an impaired \textit{in vitro} migration and tubulogenesis ability and consequently, the postnatal alveolar septum formation was disrupted\textsuperscript{66,176,180,181} (Table 2). Next to tubular neovessel formation, endothelial cells also participate in a complex process named lumenogenesis, which results in the formation of intracellular space where the blood can flow through. Since ECM remodeling is necessary for lumen expansion, several studies have supported that MT1-MMP catalytic activity is also essential to control the vascular lumen formation through Cdc42 activation.\textsuperscript{172,182,183}

Despite these observations and the fact that its expression has been reported in the endothelium of a variety of organs during embryogenesis, including the eye, brain and limbs,\textsuperscript{66,71,85,184} the role of MT1-MMP in developmental angiogenesis is poorly understood. For example, MT1-MMP expression in the hyaloid vessels during the eye development has pointed out a possible function of this enzyme in regressing vessels of the postnatal retina similar to that described for MT1-MMP in vascular regression in the aortic ring model.\textsuperscript{71,185} Moreover, the protein also localized in the nerve fiber layer of the mouse retina where it plays role related to vascularization.\textsuperscript{186}

It should be noticed that, although MT1-MMP is generally considered as a pro-angiogenic factor, it may play a dual role by blocking the angiogenic response in some avascular tissues such as the cornea. In this context, VEGFR1 is proteolytically processed by MT1-MMP generating N-terminal VEGFR1 fragments which can still bind VEGF-A\textsubscript{165} reducing its proangiogenic effect and thereby inhibiting corneal angiogenesis\textsuperscript{187} (Table 1). Similarly, MT1-MMP-mediated endoglin cleavage, which results in soluble forms of the receptor, also impairs tumoral angiogenesis by sequestering angiogenic ligands such as TFG-\(\beta\) that inhibits sprouting angiogenesis.\textsuperscript{188} During wound healing, keratinocytes-derived MT1-MMP are also involved in vascular regression by releasing endostatin fragments which have been previously described to harbor anti-angiogenic activity.\textsuperscript{189} In this context, the epidermis-specific \textit{Mt1-mmp} knockout mice showed a significant increase in vascular density because of the reduction in the levels of endostatin fragments.\textsuperscript{189}

Angiogenesis can also occur under pathological circumstances and generally appeared associated with an inflammatory response.\textsuperscript{171,190-192} In fact, upregulation of MT1-MMP expression induced by the pro-inflammatory chemokines CCL2 and CXCL8 triggers the angiogenic response in endothelial cells.\textsuperscript{32} MT1-MMP has been also reported to control the intestinal vasculature expansion by proteolytically processing thrombospondin-1 (TSP1). The resulting C-terminal fragment works as intermediary in the CD47/\(\alpha\)v\(\beta\)3-integrin signaling cascade that ultimately triggers intussusceptive angiogenesis through NO production\textsuperscript{193} (Table 1). Interestingly, the vascular expansion via intussusceptive angiogenesis may also rely on the ability of NO to induce MT1-MMP expression in endothelial cells promoting their migration.\textsuperscript{190} In cancer cells, unregulated MT1-MMP activity enhances cell migration and invasion through the proteolytic processing of semaphorin 4D into soluble forms that lead to the impairment of Sema4D/Plexin B1 signaling pathway.\textsuperscript{102,193,194} Moreover, elastin-derived peptides trigger the angiogenic response and upregulate MT1-MMP expression, which is required to promote cell motility and cell polarity in human microvascular endothelial cells.\textsuperscript{195}

All these data are consistent with the reported expression of MT-MMPs during endometrial angiogenesis, an essential physiological process for a vascularized receptive endometrium in the adult tissue. Remarkably, MT1-, MT2-, MT3-, and MT4-MMP localize in endothelial and perivascular cells of the human endometrium and during menstrual phases associated with high angiogenic activities. In particular, MT2- and MT3-MMP regulate capillary-like tube formation during endometrial neovascularization.\textsuperscript{196-198}

Apart from these data, little is known about MT2- and MT3-MMP function during embryonic angiogenesis and most of the research is focused on cancer studies. This role in tumoral angiogenesis is also supported by the fact that MT1- and MT2-MMP display pro-invasive, angiogenic, and metastatic activities in cancer cells in response to Snail1 induction.\textsuperscript{142} Remarkably, MT2-MMP increases intratumoral microvessel density in human esophageal and lung cancer tissues.\textsuperscript{199,200} One possible mechanism by which MT2-MMP can promote basal membrane degradation during pathological angiogenesis is through the processing of versican, an ECM component of the venular basement membranes. Thus, VEGF-induced
angiogenesis in pathological conditions up-regulates ADAMTS-1 and MT2-MMP in endothelial cells that degrade versican201 (Table 1). Similarly, it has been described that the fibrinolytic activity of MT2-MMP promotes VEGF-induced angiogenesis. Thus, HUVEC cells expressing MT2-MMP were able to degrade fibrin matrices inducing cell migration and invasion.202 The implication of MT3-MMP in angiogenesis was described by Bakhashab et al., who tested that under the pro-angiogenic effect of metformin, a cardioprotective treatment used in type 2 diabetes, MT3-MMP was up-regulated inducing endothelial cell migration and therefore, promoting angiogenesis.203 Regarding MT6-MMP, elevated mRNA levels were observed in acute cutaneous wounds suggesting a contribution in angiogenesis during wound healing.204 Additionally, MT1-MMP has a pivotal role in blood vessel wall development and during vascular remodeling.184,205 The maturation of the newly built vascular network involves the deposition of a new basement membrane and the recruitment of mural cells including pericytes and vascular smooth muscle cells (VSMCs).51,174 The cellular investment of the nascent vasculature is regulated by an interplay between MT1-MMP and the PDGFR-β/PDGF-B signaling pathway.177,184,205 Therefore, MT1-MMP-deficient mice showed aberrant retinal and brain VSMC recruitment as consequence of the defective PDGFR-β signaling.184 Moreover, MT1-MMP expression in VSMCs covering the embryonic arteries suggested that its function in vessel wall remodeling is established during development.67 Indeed, its expression has been reported in umbilical vessels71 where it may play a role in the umbilical vascular wall similar to that described for ADAMTS9.206 Importantly, MT1-MMP provides VSMCs with proteolytic and migratory abilities, during pathological vessel remodeling.207 Hence, MT1-MMP-dependent LDL receptor related protein 1 (LRP1) cleavage regulates VSMC dedifferentiation program inducing the expression of pro-migratory and pro-invasive genes which are determinant for the vascular wall remodeling after injury205 (Table 1).

Although of the mechanisms by which MT4-MMP participates in blood vessel formation has been poorly investigated during development, its expression has been reported in the cardiovascular system as well as in the vascularization of the limb, brain, and eye of the mouse embryo.86 In particular, MT4-MMP localizes in the endocardial endothelium of the primitive heart tube as well as in endothelial cells and VSMCs in the dorsal aortic wall at distinct developmental stages.86,108 Moreover, previous studies confirmed MT4-MMP expression in VSMCs in different tissues such as the intestine, lung, stomach, ovary, testis, and uterus in the postnatal mouse.208 Although its function in the endothelium is not clear so far, MT4-MMP has an essential role in the maturation and stabilization of the blood vessel wall in the embryo. Thus, the ortholog in zebrafish embryos, mmp17b, localized in neural crest cells, which are the precursors of VSMCs, and its activity is necessary for their proper migration137 (Table 2). Supporting these data, MT4-MMP-driven osteopontin (OPN) proteolysis regulates blood vessel wall stabilization during aortic wall development. Thus, N-terminal OPN fragments released by MT4-MMP-mediated cleavage modulate, via JNK signaling, the differentiation and positioning of VSMCs for the proper establishment of the arterial vessel wall108 (Tables 1 and 2). This essential function in vessel stabilization is supported by previous studies in MT4-MMP-overexpressing breast carcinoma cells, which established a correlation between the blood vessel architecture and the development of metastasis. Therefore, MT4-MMP proteolytic activity in tumor cells induces pericyte detachment and blood vessel enlargement increasing vascular leakage and consequently favoring the increase of metastatic spread.209 Additionally, tumor-derived MT4-MMP has been shown to contribute to tumor growth and metastasis through its pro-angiogenic effect210 (Table 1). Besides its involvement in mural cell recruitment during blood vessel architecture, the fact that MT4-MMP is expressed in the early embryo suggests additional functions in endothelial cells during angiogenesis.

### 4.2 Lymphangiogenesis

Lymphangiogenesis, or the generation of new lymphatic vessels sprouted from the pre-existing blood vessels, is a dynamic process during embryogenesis that includes proliferation, sprouting, and migration of lymphatic endothelial cells.211 Like blood vessel elongation, ECM remodeling is required to provide a path for lymphatic endothelial cell migration and lymphatic vessel outgrowth. In fact, these cells express some MMPs and different in vitro studies have suggested essential roles of MT-MMPs for lymphangiogenesis. In addition, lymphatic vessel remodeling is mainly regulated by VEGFR3 signaling through VEGF-C and VEGF-D ligands and the endothelial hyaluronan receptor-1 (LYVE-1) located in the lymphatic endothelium.211-213

In the adulthood, lymphangiogenesis is linked to pathological conditions such as inflammation, tissue repair, tumor growth and metastasis and fat metabolism.211,213 Several studies suggest essential roles of MT-MMPs in this process. In this regard, MT1-MMP is involved in corneal inflammatory lymphangiogenesis by inducing VEGF-C and VEGF3 expression and therefore,
promoting the development of new lymphatic vessels. In addition, studies in vitro have demonstrated that MT1-MMP-mediated pro-MMP2 activation promotes lymphatic vessels sprouting.

In contrast to these pro-lymphangiogenic behaviors, this enzyme can also exert anti-lymphangiogenic effects through two independent mechanisms. MT1-MMP was shown to suppress lymphatic vascular outgrowth by proteolytically processing LYVE-1 located on the lymphatic endothelial cell surface and therefore, blocking the LYVE-1-dependent lymphangiogenesis. Moreover, this enzyme negatively regulates VEGF-C production in macrophages through PI3K signaling which lead to impairment of spontaneous lymphangiogenesis.

Since cancer cells use the lymphatic vasculature to be transported for the primary tumor site to distant organ and thus, enhance cancer cell dissemination, most of the studies have focus on how MT-MMPs contribute to lymph node metastasis. Indeed, MT3-MMP which is overexpressed in primary human melanomas, promotes lymphatic invasion by cleaving the cell adhesion molecule L1-CAM involved in transendothelial migration (Table 1). Regarding MT4-MMP, little is known about its role in lymphatic invasion but its expression in human breast cancer lymph nodes as well as metastatic nodes has pointed out a possible function as an effector of breast carcinoma dissemination.

5 | FUNCTION OF MT-MMPS IN AXON GROWTH AND GUIDANCE

Axon guidance relies in the capacity of the growth cones, at the tip of the axon, to respond to guidance cues present in the environment. The growth cones guide the extension of the navigating axons providing sites of attachment and degradation of the ECM where they express metalloproteinases. In this context, MMPs allow the advance of the growth cone by degrading the ECM, consequently facilitating neurite outgrowth, axon extension and synaptic plasticity. On account of this, we can argue that MT-MMPS are key regulators of axon guidance during development, enabling axon growth, and processing guidance cues and their receptors as well as their inhibitors. The molecular processes by which MT-MMPS make this possible remain elusive, possibly due to the fact that these proteinases show considerable redundancy in vertebrates.

In that regard, Drosophila has been proven an amenable tool for the study of the role of MMPs in axonal fasciculation and guidance during development. MMP activity in the developing nervous system is essential for both axon pathfinding and dendritic plasticity in the fly brain. Both MMPs, Dm1- and Dm2-MMP are widely expressed in the CNS. In the fly, these proteinases inhibit the regulated separation/defasciculation of motor axons at defined choice points and are also redundantly required by motor neurons to be guided to their target muscles. Individual motor axons must separate from their original nerves when they reach their appropriate target, a process influenced by the membrane anchored Dm2-MMP and, to a lesser extent, secreted Dm1-MMP, indicating that both endopeptidases can perform similar functions. In fact, loss of Dm2-MMP function shows failed fasciculation of motor neuron axons and splinter off before reaching their targets.

MMPs are expressed dynamically in the developing and adult CNS and after brain damage in vertebrates. They localize on the growth cones of neurites extending in vitro and their activity is involved in axon guidance as well as in neuronal migration, myelination, synaptic plasticity, and neurogenesis. In this context, MT1-MMP and its substrate MMP-2 have been posted as promising axon-outgrowth promoting molecules within the CNS. In fact, neurite outgrowth was reduced in postnatal retinal explants derived from Mt1-mmp deficient pups and after blocking pro-MMP-2 activation by MT1-MMP (Figure 3B). These data revealed that MT1-MMP stimulates retinal axon growth through activation of MMP-2.

Thus, in vivo exposed preparations of the brain treated with broad-range MMP inhibitors resulted in misguidance of retinotectal projections in Xenopus embryos. Also in zebrafish, mmp14a, one paralog of MT1-MMP, is required for retinal neurogenesis and the development of the retinotectal projections (Table 2). In the mouse embryo, a recent study reported that Mt1-mmp localized at distinct points of the visual pathway, including the optic stalk and optic chiasm region as well as in the superficial layers of the superior colliculus that receive direct visual input from the retina, supporting a role for this proteinase in the guidance of visual axons.

Similar effects of MT1-MMP promoting neurite outgrowth from cultured granule cells were described in the mouse cerebellum. In this study, MT1-MMP interacts with the cell adhesion molecule L1, the adenine nucleotide translocator (ANT1 and 2) and GAPDH forming a complex at the cell surface to promote L1-induced neurite outgrowth in cerebellar neurons (Figure 3B; Table 2). In a different model, sympathetic neurons under conditions of hyperactivity by electrical stimulation respond by increasing nerve growth factor (NGF), MMP-2, and MT1-MMP levels. MT1-MMP activates
MMP-2, which in turn cleaves and activates NGF to promote neurite outgrowth via TrkA signaling (Table 2). Notably, it has been shown that actin remodeling drives synaptic-like vesicles release and MT1-MMP activity at specific points regulating axon growth cone microenvironment, and enabling pioneer axons to navigate across the boundary of the zebrafish spinal cord (Table 2). The function of MT1-MMP in the brain has been associated with the control of appetite and body weight. Thus, loss of proteinase activity results in nuclear accumulation and decreased axonal transport of two neuropeptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), in the arcuate nucleus of the hypothalamus, leading to the characteristic phenotype observed in these mutant mice related to insufficient nutrient intake.244

Although there is not a direct evidence for similar functions, MT4-MMP expression in the retinal ganglion cell layer as well as in the dorsal lateral geniculate nucleus, one of the main central targets for retinal axons, makes likely its involvement in steering axons to their final targets.86 Interestingly, this metalloproteinase localizes in the anterior hypothalamus, an important brain center for regulating thirst in the brain, and mutant mice for MT4-MMP display abnormalities in water homeostasis.155 Regarding MT5-MMP, is expressed specifically in the brain40 and the developing dendrites of Purkinje cells, suggesting its involvement in neuronal plasticity and dendrogenesis.245 Moreover, MT5-MMP localized at the growth cone of sensory neurons and it has been involved in axon growth during development223,246 (Figure 3B). These data suggest that pericellular proteolysis of proteoglycans which normally inhibits neurite outgrowth, mediated by MT5-MMP may contribute to axon extension of dorsal root ganglia neurons (Tables 1 and 2). Also in zebrafish, morpholino-mediated knockdown of mmp25b, the orthologous gene of Mt6-mmp, results in larvae that are uncoordinated and insensitive to touch suggesting defects in the development of sensory neurons. Supporting these data, reduced type IV collagen degradation was observed throughout the pathway of trigeminal ganglion axons in the morphant embryos, indicating that this MMP is involved in sensory neuron axon guidance233,247 (Table 2).

It remains to be determined the molecular mechanisms by which these proteases may participate in axon guidance and growth. It seems plausible that their proteolytic activity is not only necessary for the local degradation of the surrounding matrix to ensure the accuracy of axon pathfinding. In fact, numerous evidence supports that MT-MMPs can also cleave and/or activate substrates such as axon guidance and adhesion molecules as well as growth factors, all of which can facilitate axon outgrowth in the developing CNS.220,248 In Drosophila, the ECM molecule Frac is expressed concurrently with axon pathfinding and Dm2-MMP-expressing glia, and its proteolytic cleavage by Dm2-MMP may contribute to the activation of non-canonical BMP signaling pathway in motoneurons.249 Also in the fly, the degree of motor axon fasciculation in the embryo depends on the level of MMP catalytic activity, suggesting that particular MMP substrate(s) plays an instructive role in motor axon guidance. Indeed, several studies suggest that MMP-mediated cleavage is directed toward ectodomains of guidance cues in the environment and their receptors on growth cones in order to activate or terminate their motility. That is the case of the ADAM metalloprotease Kuzbanian, which mediates the proteolytic activation of the Slit/Roundabout receptor complex, essential for the removal of Roundabout receptor from commissural axons to allow them to cross to the contralateral side.250 Kuzbanian also participates in contact-mediated axon repulsion by ephrins and forms a stable complex with ephrin-A2.251 Thus, the membrane anchored ephrin-A2 cleavage by the protease after Eph receptor binding, initiates axon detachment, and retraction.

In vertebrates, MMPs are capable of modulating the interactions between guidance cues and their receptors in vitro,224,251 regulating the migratory activity of commissural axons acting through chemoattractive signals. Specific MMP inhibitors potentiate neurite outgrowth mediated by netrin-1 through the regulation of the activity of its receptor DCC in dorsal spinal cord explants.224 Thus, MMP cleaves DCC, possibly to a nonfunctional form of the receptor, and therefore, the blockade of the proteinase enhances responsiveness to netrin-1 on commissural axons. In a distinct context, previous work has shown reduced MT1-MMP expression levels upon loss of the axon guidance molecule SEMA7A in oral squamous carcinoma cells.252 In addition, MT1-MMP has been reported to promote cancer cell migration and invasion by cleaving the axon guidance receptor EphA2.253,254 An intriguing possibility is that, in a similar manner, MT1-MMP mediates axon guidance responses through that activation of the Eph/ephrin signaling pathway via the proteolytic processing of the receptor at the growth cone. Interestingly, following a quantitative proteomic analysis distinct guidance cues such as SEMA3C, SLIT1, SLIT2, and SFRP1 have been identified as putative substrates of MT1-MMP in activated endothelial cells.255 β1-integrin is also an important mediator of axonal outgrowth in the retina and interactions between integrins and MMPs in other physiological processes have been described before.256,257 In this context, it has been proposed that MT1-MMP activates MMP-2 to promote axonal outgrowth in mouse retinal explants. In turn, activated MMP-2 might affect axon outgrowth via a
β1-integrin-dependent pathway (Figure 3B). Another proposed metalloproteinase sensitive target is FGF, since deregulation of FGF signaling produces defects in the extension and targeting of retinal axons similar to that observed upon broad-spectrum MMP inhibition in the developing Xenopus brain.258,259

6 | ROLE OF MT-MMPS IN SYNAPTOSTEGESIS

Neural plasticity allows neural circuits to adapt the assembly of synaptic contacts in order to guarantee synaptic transmission and communication in the brain. However, the molecular mechanisms underlying synaptic plasticity are not fully understood. As mentioned above, MT-MMPS are good candidates to exert relevant roles as regulators of axonal plasticity via synaptic remodeling. MMP function modulates synapse formation and activity-dependent plasticity via local ECM remodeling, allowing the modification of synaptic architecture. Remarkably, membrane-bound MMPs are found at the synaptic junction within axons and sites critical to this process.246

Several studies in Drosophila demonstrate that both MMPs regulate synaptogenesis including in axonal terminals and dendrite reshaping.231,261 In particular, Dm2-MMP mutants display a total block of dendrite reshaping in sensory neurons due to defects in local degradation of basement membranes.261 This suggests that matrix micro-environmental degradation driven by the GPI-anchored Dm2-MMP is an essential mechanism to allow dynamic changes in dendritic arbors during reorganization of neural networks. An excellent model to study synapses due to their accessibility and simplicity is the neuromuscular junction (NMJ) in the peripheral nervous system.235 At the NMJ, TIMP inhibits synaptic MMP function and regulates its proteolytic activity in the extracellular space surrounding synaptic boutons. Failure to inhibit MMP activity leads to delays in muscle peristalsis due to alterations on the BMP signal transduction pathway that modulates NMJ structure and function.262 Also, Dm1- and Dm2-MMP proteolytic activities regulate basal synapse morphogenesis to restrict NMJ development over time through a mechanism that modulates the HSPG Dally-like protein (Dlp) co-receptor to restrict Wingless (Wg) trans-synaptic signaling.263 However, only the secreted Dm1-MMP is required for fast, activity-dependent synapse formation.264 In line with these data, Dm1-MMP levels are increased after acute neuronal stimulation enabling synaptogenesis through a direct interaction with Dlp, which can modulate Dm1-Mmp proteolytic function.263 On the other hand, Dm2-MMP spatially confines Dlp at the synapse, antagonizing the Dlp-Dm1-MMP interaction. Moreover, Dm1-MMP is constitutively increased while activity-dependent Dm1-Mmp augmentation is lost in a Fragile X syndrome model in fly.265 Both defects are prevented when Dlp is suppressed.266 Altogether, these data suggest that both MMPs are differentially required in distinct phases of synapse formation by a reciprocal co-regulation of Dm1- and Dm2-MMP.

Similar to Drosophila, MMP activity is also necessary for NMJ formation in vertebrates.235 Expression of MT1-MMP has been reported during muscle development and knockout mice display altered myogenic differentiation.71,267 Interestingly, a recent study demonstrated that vesicular trafficking and surface insertion of postsynaptic MT1-MMP regulate aneural AChR clustering via focal ECM degradation (Figure 3C). Thus, precise targeting of MT1-MMP to podosome-like structures initiates focal ECM remodeling to allow the recruitment of AChR clusters at the synapse (Table 2). MT1-MMP deficient mice also display a reduction of axonal growth and arborization in the diaphragm muscles, suggesting that a retrograde signaling from the post- to the presynaptic regions is necessary for the proper muscle innervation.235

In mammals, secreted MMPs have been involved in dendritic reshaping and synaptic plasticity both at the pre- and postsynaptic compartments.268-273 In addition, MMP activity appear to be important in the process of synaptic plasticity necessary for memory consolidation. There has been found a significant correlation between learning and memory acquisition processes and expression of MMP-9.274 MMP-9 together with MMP-2 are differentially expressed in the rat hippocampus and cerebellum and both are susceptible to trigger changes in tissue remodeling upon kainate administration.272,275 Also inhibition of MMP catalytic activity affects de-afferentiation-induced sprouting in the adult nervous system.276 MMP-2 inhibition reduces cortical dendritic growth in response to Sema3A in a PKCalpha-dependent pathway implicating NRP-1.271 As both MMP-2 and MMP-9 are substrates of some MT-MMPS, we can speculate on a possible function of membrane-tethered proteinases in synaptogenesis mediated through shedding of secreted MMPs. However, only a few studies have directly associated MT-MMP proteolytic activity with synaptic plasticity. The fact that MT-MMPS localize in dendritic spines supports this potential function in synaptogenesis. In cortical neurons, MT3-MMP loss of function reduces excitatory synapse development. Proteolytic cleavage of Nogo-66 receptors (NgR1) by MT3-MMP generates a soluble ectodomain fragment that accelerates excitatory synapse formation (Figure 3D; Tables 1 and 2).
MT5-MMP is highly expressed in the CNS, particularly in the hippocampus and cerebellum, both brain regions with relevant synaptic plasticity, and its synaptic expression increased in response to traumatic brain injury. Related to a role in synaptogenesis, synaptic expression increased in response to traumatic neurotransmitter receptors (Table 2). Interaction between MT5-MMP and ABP targets MMP-mediated proteolysis to growth cones in developing neurons and to synapses in mature neurons, where it may contribute to axon growth or synapse remodeling through proteolytic cleavage of N-cadherins or other ECM molecules (Figure 3B). In a different context, the synaptic adhesion protein N-cadherin is also a MT5-MMP substrate that participates in pain control and inflammatory hyperalgesia (Table 1). This response is mediated by the interaction between nociceptive neurites and mast cells and requires that MT5-MMP and N-cadherin concentrate at the sites of cell-cell interaction. Further, loss of MT5-MMP in mice enhances nociceptive responses through a hyperinnervation phenotype that increases sensitivity to thermal stimuli. On the other hand, expression levels of MT5-MMP and N-cadherin are modified after damage to the brain circuitry in correlation with a functional recovery of synaptic function. Interestingly, MT5-MMP is also involved in mechanical allodynia and in neural plasticity induced by peripheral nerve injury. There is not a direct evidence for similar functions of GPI-anchored MT-MMPs in synapse formation, but the restricted expression of MT4-MMP during brain development, particularly in the olfactory bulb, cerebral cortex, and hippocampus makes it possible.

7 | CONCLUSIONS

Since only several studies have addressed their function in the developing embryo, understanding the role of MT-MMPs is still an open question today in the field of developmental biology. On the one hand, the complex cell biology of these endopeptidases involves many points of regulation to ensure the precise control of their proteolytic activities. Moreover, as shown for other MMPs, it is reasonable that different biological functions of MT-MMPs depend on other protein domains distinct from the catalytic site. On the other hand, possible redundancy in the proteolytic actions among distinct members of the MT-MMPs may add difficulty to assess functional studies. Regarding their involvement in embryonic development, increasing evidence supports that MT-MMPs participate in different developmental processes. Most of these functions in the embryo require a polarized cell distribution of the enzyme to mediate in angiogenesis, gastrulation, axon guidance, branching morphogenesis, or EMT among other processes. However, how MT-MMPs are targeted to motile cell structures such as the leading edge of migrating cells, lamellipodia, or the growth cone is still an unclear question. Interestingly, MT1-MMP has been demonstrated in distinct subcellular compartments where it is able to cleave several intracellular proteins such as cytoskeletal proteins, apoptotic regulators, signal transducers, or transcriptional and translational regulators that can be also relevant to distinct morphogenetic processes in the embryo. However, further studies are needed to elucidate the importance of intracellular substrate proteolysis and actions for MT-MMPs during embryonic development.

What are the target substrates that MT-MMPs may process to mediate their physiological functions in the embryo? Apart from ECM components, adhesion molecules such as integrins or cadherins are among the main substrates that MT-MMPs cleavage to mediate cell migration, proliferation, and polarity during embryonic development. It is worth mentioning that morphogens, which are essential signaling molecules for embryogenesis, may be good candidates for MT-MMP-mediated proteolysis as it has been reported for the Wnt/PCP signaling pathway during gastrulation. A role of MT-MMPs in contributing to axon growth and guidance has been also suggested, although the mechanisms underlying are poorly understood and may involve not only ECM degradation but also cleavage and activation of adhesion molecules, receptors, and guidance cues such as ephrins, semaphorins and netrins. In addition, MT-MMP-mediated shedding and proteolytic cleavage of ECM components and receptors could stimulate neural circuit remodeling and enhance functional connectivity after brain injury by targeting neurotransmitter receptors to synapses. However, further studies are necessary to understand how MT-MMPs contribute to the formation and maturation of synapses and the refinement of neural circuits during development and in pathological conditions.

AUTHOR CONTRIBUTIONS

Natalia Moracho: Conceptualization; investigation; visualization; writing - original draft; writing-review & editing. Ana I. R. Learte: Conceptualization; investigation; validation; writing - original draft; writing-review & editing. Emma Muñoz-Sáez: Investigation; writing - original draft; writing-review & editing. Miguel A. Marchena: Investigation; validation. María A. Cid: Investigation; validation. Alicia G. Arroyo: Conceptualization;
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