Placental membrane-type metalloproteinases (MT-MMPs): Key players in pregnancy

Alejandro Majali-Martínez, Ursula Hiden, Nassim Ghaffari-Tabrizi-Wizsy, Uwe Lang, Gernot Desoye, and Martina Dieber-Rotheneder

Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria; Institute of Pathophysiology and Immunology, Medical University of Graz, Graz, Austria; Institute of Pathology, Medical University of Graz, Graz, Austria

ABSTRACT
Membrane-type matrix metalloproteinases (MT-MMPs) are a sub-family of zinc-dependent endopeptidases involved in the degradation of the extracellular matrix. Although MT-MMPs have been mainly characterized in tumor biology, they also play a relevant role during pregnancy. Placental MT-MMPs are required for cytotrophoblast migration and invasion of the uterine wall and in the remodeling of the spiral arteries. They are involved in the fusion of cytotrophoblasts to form the syncytiotrophoblast as well as in angiogenesis. All these processes are crucial for establishing and maintaining a successful pregnancy and, thus, MT-MMP activity has to be tightly regulated in time and space. Indeed, a deregulation of MT-MMP expression has been linked with pregnancy complications such as preeclampsia (PE), fetal growth restriction (FGR), gestational diabetes mellitus (GDM) and was also found in maternal obesity. Here we review what is currently known about MT-MMPs in the placenta, with a focus on their general features, their localization and their involvement in pregnancy disorders.

MT-MMPs: General features

MT-MMP structure

All members of the MMP family share a similar structure differing mainly in their domain organization. These
domains include a pro-domain, a catalytic domain, a hinge region and a hemopexin domain.\textsuperscript{11}

MT-MMPs, which are inserted in the membrane, can be further classified into 2 groups: i) MT-MMPs that are anchored by a transmembrane domain followed by a cytoplasmic domain (MT1-, MT2-, MT3- and MT5-MMP), and ii) MT-MMPs that are anchored by a glycosylphosphatidylinositol (GPI)-anchor and which lack a cytoplasmic domain (MT4- and MT6-MMP).\textsuperscript{12} Figure 1 shows the domain assembly of MT-MMPs.

MT-MMPs can function both as monomers or homodimers.\textsuperscript{13,14} Interestingly, homodimerization has been shown to play an important role in the regulation of MT-MMP activity, e.g. in MMP-2 activation mediated by MT1- and MT2-MMP (section 2.3.).

Although ECM degradation by the catalytic domain is the primary function of MMPs, other functions have been attributed to the rest of the MT-MMP domains. For instance, the hemopexin domain is involved in protein-protein interactions, allowing MT1-MMP oligomerization.\textsuperscript{15} The cytoplasmic domain can interact with other intracellular proteins, transducing information from the extracellular environment.\textsuperscript{16} Furthermore, it is thought to regulate MT-MMP intracellular trafficking, degradation and surface distribution.\textsuperscript{10} The GPI-anchor in MT4- and MT6-MMP is involved in signal transduction as well as in lipid raft localization, and confers their sensitivity to phospholipases.\textsuperscript{17}

**MT-MMP activation**

MMPs are secreted as inactive zymogens with the protease inhibiting pro-domain located at the C-terminus. The pro-domain contains a cysteine residue that interacts with a zinc ion in the catalytic domain. Disruption of this interaction is required for MMP activation. This occurs in a 2-step process. First, the pro-domain is cleaved in a sequence-specific manner. Then, the intermediate MMP product as well as other proteases can completely remove the pro-domain, resulting in a fully-activated MMP.\textsuperscript{18} Additionally, MMP activation can also result from a conformational change of the pro-domain, or by activation by reactive oxygen species, which interact with the free cysteine in the pro-domain.\textsuperscript{19}

In contrast to the general activation process of MMPs, MT-MMPs and MMP11, 21 and 28 contain a recognition sequence for Golgi-associated pro-protein convertases, i.e. RXRXXR, with R=Arg, K=Lys and X=non basic amino acid, and are thus activated in the Golgi network prior to secretion.\textsuperscript{19-21} MT-MMP activation might also occur in 2 steps, since MT1-MMP activation requires a first cleavage in the pro-domain prior to the second cleavage mediated by pro-protein convertases.\textsuperscript{22} Once in the plasma membrane, active-MT1-MMP can undergo autocatalysis generating an inactive cleavage product, which regulates the pool of active-MT1-MMP present on the cell surface by inhibiting active-MT1-MMP endocytosis.\textsuperscript{23,24} The membrane anchored MT1-MMP can also be cleaved and shed. Non-autocatalytical shedding results in a soluble active MT1-MMP.\textsuperscript{25} This shedding of an active MT1-MMP species produces a soluble enzyme and allows MT1-MMP action beyond the surface of the MT1-MMP producing cells.\textsuperscript{26}

**MT-MMP activity**

MT-MMPs accept a wide array of substrates (Table 1). They degrade various ECM components, including those found in the uterine wall and the spiral arteries, such as fibronectin, vitronectin, collagen IV and laminin.\textsuperscript{27} Furthermore, MT-MMPs can cleave pro-forms of cytokines including TGF-β and TNF-α, resulting in their activation.\textsuperscript{28-30} In addition to their regulation of the immune response, these cytokines also modulate some placental functions. TGF-β has been shown to inhibit trophoblast invasion.\textsuperscript{31} TNF-α was able to induce MT1- and MT2-MMP expression in the human first trimester trophoblast cell line ACH-3P and in placental explants from the first trimester.\textsuperscript{32} However, several studies have also shown that TNF-α limits trophoblast invasion by increased secretion of plasminogen activator inhibitor (PAI1). PAI1 is the principal inhibitor of plasminogen activator (tPA) and urokinase (uPA), both activators of fibrinolysis.\textsuperscript{33,34} This mechanism would reduce ECM degradation independent of the MMP/TIMP system.

Besides ECM degradation and cytokine activation, MT-MMPs can also inactivate cytokines,\textsuperscript{35} activate other MMPs such as MMP2, 8, 9 and 13\textsuperscript{30,36} as well as other
proteases such as ADAM9 (a disintegrin and metalloprotease 9)\(^\text{12}\) and ADAMTS4 (ADAM with thrombospondin motifs 4).\(^\text{37}\)

Pro-MMP2 activation by MT1-MMP has been thoroughly studied, revealing a complex mechanism where TIMP2 (tissue inhibitor of MMPs 2), a natural MMP inhibitor, is also required. Once located on the membrane, MT1-MMP forms a homodimer. One MT1-MMP of this dimer binds TIMP2, which will subsequently bind pro-MMP2.\(^\text{23}\) Then, the other MT1-MMP molecule of the dimer can cleave pro-MMP2 and produce active MMP2. This mechanism is dependent on TIMP2 concentration and occurs only at basal TIMP2 levels. High TIMP2 levels inhibit both, MMP2 and MT1-MMP.\(^\text{38}\)

Interestingly, MT2-MMP can activate pro-MMP2 without interaction with TIMP2. This mechanism also involves an MT2-MMP dimer, of which one MT2-MMP molecule binds pro-MMP2 directly, followed by activation by the other MT2-MMP molecule.\(^\text{39}\) Contradictory data regarding MMP2 activation have been reported for the other 3 MT-MMP members.\(^\text{17}\)

**MT-MMP regulation**

It has been hypothesized that the different members of the MMP family originate from gene duplication, as they are widely spread across several chromosomes.\(^\text{21}\) However, promoter architecture highly differs among distinct MMPs, which allows discriminative regulation at the level of gene expression.

Based on basic promoter conformation, i.e., the distribution of the cis-elements along the promoter, MMPs are classified into 3 different groups: i) MMPs containing a TATA box at -30 bp and a proximal binding site for the transcription factor activator protein-1 (AP-1); ii) MMPs containing a TATA box but no proximal AP-1 binding site; and iii) MMPs lacking both, TATA box and AP-1 binding site.\(^\text{40,41}\)

MT-MMPs belong to the third group, i.e., they lack a TATA-box and an AP-1 binding site, with the exception of MT2-MMP, which contains a TATA box. Thus, lack of common MMP regulatory elements in the promoter regions of MT-MMPs might allow their regulation different from other MMPs. Moreover, the basic promoter conformation represents only one determining aspect of the regulation of MMP expression. The majority of the regulatory elements in MMP promoters are non-canonical, which might also explain the finely tuned regulation with a different set of MMPs being activated in various cell types and at different developmental stages.

Finally, MMP expression is also regulated by cytokines, hormones and growth factors, adding a new level of complexity. Several interleukins such as IL-1, IL-6 and IL-15 have been shown to promote trophoblast invasion, whereas IL-10 inhibits this process.\(^\text{4}\) The role of placental hormones, e.g. human chorionic gonadotropin, in this regard remains still contradictory.\(^\text{42}\) We previously reported that insulin-like growth factors (IGFs) as well as insulin increase the expression of MT1-MMP in isolated first trimester trophoblast.\(^\text{43}\) Whether this entails invasion promotion remains to be studied.

Inhibition of MT-MMP activity could be considered as the last level of regulation of ECM degradation. Plasma proteins such as \(\alpha_2\)-macroglobulin are thought to be broad-range inhibitors of endopeptidases, including MMPs. However, \(\alpha_2\)-macroglobulin action has been mainly found in tissue fluids. Thus, its relevance for MT-MMP inhibition remains unclear.\(^\text{44}\)

By contrast, tissue inhibitors of MMPs (TIMPs) are proteins specifically targeting MMPs. Four members of the TIMP family have been described, which bind to the catalytic domain of MMPs in a 1:1 molar ratio. The zinc ion in the MMP catalytic domain is then chelated, which

---

Table 1. Major substrates of MT-MMP (adapted from ref. 18 with additional data from refs. 10,12,16,17,36,50. *Contradictory data showing that MT4-MMP and MT6-MMP are able/not able to activate pro-MMP2.\(^\text{17}\)

| MT-MMPs | ECM substrates | Non-ECM substrates |
|---------|----------------|-------------------|
| MT1-MMP (MMP14) | Aggrecan, collagen I, II, dermanan sulfate proteoglycan, entactin, fibrin, fibrillrin, fibrinogen, fibronectin, gelatin, laminin-1, -2/4, -5, lumican, nidogen, perlecan, tenascin, vitronectin | \(\alpha_2\)-Macroglobulin, \(\alpha_1\)-Pl, \(\alpha_2\)-integrin, \(\beta\)-glycan \(\beta\)-like 1, ADAM9, ApoE, CD44, CXCL12, death-receptor 6, EMPRIN, factor XII, ICAM-1, IL-8, K55-1, LR1, MCP-3, myelin-inhibitory protein, progranulin, pro-MMP2, pro-MMP8, pro-MMP13, pro-TGF-\(\beta\), pro-TNF-\(\alpha\), RANKL, syndecan, tissue transglutaminase |
| MT2-MMP (MMP15) | Aggrecan, collagen I, IV, entactin, gelatin, fibrin, fibronectin, laminin-1, nidogen, perlecan, tenascin | \(\alpha_2\)-Macroglobulin, \(\alpha_1\)-Ptdlns, casein, pro-MMP2, pro-MMP13, syndecan, tissue transglutaminase |
| MT3-MMP (MMP16) | Aggrecan, collagen III, fibrinogen, gelatin, laminin-1, vitronectin | ADAMTS4, K55-1, pro-TNF-\(\alpha\), pro-MMP2* |
| MT4-MMP (MMP17) | Fibrin, fibrinogen, gelatin | pro-MMP2, pro-MMP13, tissue transglutaminase |
| MT5-MMP (MMP24) | Chondroitin sulfate proteoglycan, dermatan sulfate proteoglycan, gelatin, fibronectin, plasminogen | pro-MMP2, pro-MMP9, urokinase plasminogen activator receptor, vimentin |
| MT6-MMP (MMP25) | Chondroitin sulfate proteoglycan, collagen IV, dermatan sulfate proteoglycan, fibrinogen, fibrinogen, fibronectin, gelatin, laminin | pro-MMP2*, pro-MMP9, urokinase plasminogen activator receptor, vimentin |

---
results in MMP inhibition.\textsuperscript{45} TIMPs are broad-range MMP inhibitors affecting MMPs in general, but with different preference and efficiency. For instance, TIMP1 is a weak inhibitor of MT1-, MT2-, MT3- and MT5-MMP.\textsuperscript{46} Furthermore, different TIMP members can fulfill different functions: As mentioned above, low levels of TIMP2 are even required for MMP2 activation by MT1-MMP. TIMP4 also interacts with MT1-MMP and MMP2, but this interaction results in inhibition of both MMPs.\textsuperscript{47,48} Thus, the balance between the different TIMP members can also fine tune MMP activity.

**Placental MT-MMPs and their relevance during pregnancy**

Table 2 summarizes the cellular and tissue distribution of MT-MMPs in human placenta and decidua in the course of pregnancy.

**MT-MMPs during the first trimester of pregnancy**

The placenta is a fast developing and growing villous organ. Specialized placental cells, the cytotrophoblasts, undertake various processes essential for placental function and pregnancy success. Three different cytotrophoblast subpopulations can be distinguished: the villous cytotrophoblasts (VTs), which can fuse and form the syncytiotrophoblast (ST), a syncytium that represents the classical placental barrier, transporting maternal nutrients to the fetal circulation and producing pregnancy hormones. Finally, the extravillous cytotrophoblasts (EVTs), which migrate from tips of some specialized placental villi, invade the maternal decidua and anchor the placenta in the uterus. This process predominantly takes place in the first trimester of pregnancy.\textsuperscript{4} Some EVT also reach the uterine spiral arteries and remodel them into wide, low resistance vessels, allowing an adequate blood supply to the fetus.\textsuperscript{49} All these processes, i.e. trophoblast fusion, invasion and the remodeling of the spiral arteries, require ECM degradation, pointing out once more the relevance of MMPs in pregnancy. However, due to their involvement in the process of trophoblast invasion, MMP expression has been mainly characterized in first trimester trophoblasts.

Because of their membrane anchor, MT-MMPs can be located to specific membrane regions, where they

| MT-MMPs | Cell type/Tissue | Trimester | References |
|---------|-----------------|-----------|------------|
| MT1-MMP (MMP14) | Feto-placental endothelial cells | 1\textsuperscript{st} | 59 |
| Syncytiotrophoblast (ST) | 1\textsuperscript{st} | 58,59 |
| Villous cytotrophoblasts (VTs) | 1\textsuperscript{st} | 43,52,58,59,92 |
| Extravillous cytotrophoblasts (EVTs) | 1\textsuperscript{st} | 53,56,58,60 |
| Cell columns | 1\textsuperscript{st} | 57,60 |
| Perivascular EVTs | 1\textsuperscript{st} | 57,60 |
| Decidua | 1\textsuperscript{st} | 60,64,65,91 |
| Decidua | 2\textsuperscript{nd} | 64 |
| Decidua | 3\textsuperscript{rd} | 59,62 |
| Feto-placental endothelial cells | 3\textsuperscript{rd} | 59,62,66 |
| ST | 3\textsuperscript{rd} | 59,62 |
| VTs | 3\textsuperscript{rd} | 64 |
| Decidua | 3\textsuperscript{rd} | 69 |
| Amniochorion | 3\textsuperscript{rd} | 43 |
| MT2-MMP (MMP15) | EVTs | 1\textsuperscript{st} | 53,54,60,61 |
| Decidua | 1\textsuperscript{st} | 64,65,91 |
| Decidua | 2\textsuperscript{nd} | 64 |
| Decidua | 3\textsuperscript{rd} | 3,67 |
| ST | 3\textsuperscript{rd} | 64 |
| Decidua | 3\textsuperscript{rd} | 70 |
| Amniochorion | 3\textsuperscript{rd} | 43 |
| MT3-MMP (MMP16) | Decidua | 1\textsuperscript{st} | 64,65,91 |
| Decidua | 2\textsuperscript{nd} | 64 |
| Decidua | 3\textsuperscript{rd} | 3,68 |
| ST | 3\textsuperscript{rd} | 64 |
| MT4-MMP (MMP17) | Decidua | 1\textsuperscript{st} | 64 |
| Decidua | 2\textsuperscript{nd} | 64 |
| Feto-placental endothelial cells | 3\textsuperscript{rd} | 68 |
| Decidua | 3\textsuperscript{rd} | 64 |
| Decidua | 3\textsuperscript{rd} | 70 |
| MT5-MMP (MMP24) | Decidua | 1\textsuperscript{st} | 64,65,91 |
| Decidua | 2\textsuperscript{nd} | 64 |
| Decidua | 3\textsuperscript{rd} | 93 |
| Decidua | 3\textsuperscript{rd} | 64 |
| Amniochorion | 3\textsuperscript{rd} | 70 |
| MT6-MMP (MMP25) | ST | 3\textsuperscript{rd} | 68 |
| Amniochorion | 3\textsuperscript{rd} | 70 |
play an active role in pericellular proteolysis, a feature required in the spatially directed process of trophoblast invasion. Indeed, in first trimester trophoblasts MT1-MMP is localized in invadopodia, a structure at the cellular edge of ECM degradation and invasion progression. MT1-MMP expression in cytotrophoblasts is maintained through the first trimester of pregnancy. This suggests that, together with its role as pro-MMP2 activator, MT1-MMP may be involved in trophoblast invasion. In fact, MT1-MMP expression has been observed in EVTs and in perivascular EVTs, i.e. EVTs migrating into the spiral arteries. Blocking of MT1-MMP with neutralizing antibodies reduced migration of a first trimester trophoblast cell line.

Among the other members of the MT-MMP family, only MT2-MMP is expressed in isolated first trimester trophoblasts at levels similar to MT1-MMP. MT2-MMP is predominantly expressed in EVTs, with weaker expression in VTs. Thus, a role of MT2-MMP in trophoblast invasion is also likely.

However, besides trophoblast invasion, also other functions of placental development may involve MT-MMPs in the first trimester of pregnancy. MT1-MMP is produced in feto-placental endothelial cells forming the first placental vessels. In fact, MT1-MMP is a key player in matrix degradation during angiogenesis, and blocking of MT1-MMP reduces the network formation potential of human feto-placental endothelial cells isolated from full term placentas. This suggests a role of MT1-MMP in neovascularisation or vascular development of the early placenta.

In addition to its location in EVT and endothelial cells with both cell types performing directed migration during trophoblast invasion and angiogenesis, respectively, MT1-MMP is also expressed in VTs and ST. Here, MT1-MMP has been observed to promote VT proliferation as well as their fusion to ST in vitro.

All MT-MMP members, except MT6-MMP, are expressed in first trimester decidua. MT1- and MT4-MMP are expressed in EVTs reaching the decidua, but also in decidual fibroblasts and uterine natural killer cells, 2 cell types controlling the depth of trophoblast invasion. MT2-MMP is predominantly produced in cytotrophoblasts, whereas MT3- and MT5-MMP are mainly located in decidual stromal cells.

When MT1-, MT2-, MT3- and MT5-MMP expression was studied in the decidual secretory endometrium, in decidua parietalis and in decidua basalis, all 4 MT-MMPs were detected in all parts of the decidua, as well as in the syncytiotrophoblast and EVTs reaching the decidua basalis.

### Placental MT-MMPs during the second trimester of pregnancy

For obvious reasons, sampling during the second trimester of pregnancy is mainly based on non-invasive techniques such as serum screening and ultrasound. Therefore, in this period placental MT-MMPs remain uncharacterized. All MT-MMP members except MT6-MMP were detected in second trimester decidua.

### Placental MT-MMPs during the third trimester of pregnancy

All MT-MMP members are present in the third trimester placenta. This is especially surprising for MT6-MMP, which is not expressed in the placenta during the first trimester of pregnancy. In the third trimester, MT1-MMP is expressed in several placental cell types including the syncytiotrophoblast, the underlining VTs and the endothelium of the feto-placental vessels. As mentioned above, in vitro experiments suggest a role of MT1-MMP in proliferation and fusion of VTs as well as in feto-placental angiogenesis. MT2-, MT3-, MT5- and MT6-MMP have been localized in the syncytiotrophoblast, with MT2-, MT3- and MT5-MMP being also present in VTs. By contrast, MT4-MMP expression was predominantly observed in fetal vessels.

Similar to their expression in the first and the second trimester of pregnancy, all MT-MMP members except for MT6-MMP are expressed in the decidua at term. MT-MMPs have also been characterized in fetal membranes after delivery. With the only exception of MT3-MMP, all MT-MMPs are expressed in the amnionchorion.

### Changes in placental MT-MMP expression during pregnancy

Another aspect regarding placental MT-MMPs that has been insufficiently documented is the dynamic in their expression during the course of pregnancy. In a microarray study several genes involved in different biological processes such as cell proliferation, differentiation and angiogenesis were differentially regulated in first and third trimester human placentas, with most genes involved in these processes, including MT1-MMP but not MT2-MMP, being higher expressed in first trimester placentas.

In another microarray study we have previously analyzed MT-MMP gene expression in cytotrophoblasts from first trimester placenta (FT) and showed that only MT1- and MT2-MMP could be detected in FT. Here we present novel RT-PCR data comparing MT-MMP
expression between cytotrophoblasts isolated from first trimester placenta (FT) and third trimester placenta (TT) by tissue digestion, Percoll gradient centrifugation and immunopurification72,73 (Fig. 2). Of all MT-MMPs, only MT1-MMP and MT2-MMP were expressed in both FT and TT, with MT2-MMP expression being higher in FT (Fig. 2). This expression change underlines the functional difference of FT and TT, since in contrast to FT, TT cannot fulfill endovascular remodeling both in vivo and in vitro.74 Interestingly, we were not able to detect MT3- and MT5-MMP in TT, despite its previously reported presence3 (Table 2). This may in part be due to differences between mRNA and protein, or due to differences in the trophoblast subpopulations investigated. While freshly isolated trophoblasts mainly resemble cytotrophoblast cells, Zhu et al. investigated whole placental tissue and identified the syncytiotrophoblast as major MT3- and MT5-MMP producing site.

Placental MT-MMPs in pregnancy complications

It is well established that severe pregnancy complications associated with shallow or impaired trophoblast invasion and spiral artery remodeling, such as preeclampsia (PE) and fetal growth restriction (FGR), have their origin in early pregnancy.49 However, PE and FGR clinically manifest only during the second trimester.75 Therefore, the relationship of MMPs with PE and FGR has been mainly analyzed in third trimester placentas from preterm and term pregnancies.

Investigating chorionic villous biopsies provides the possibility to study placental tissue in the period of potential functional failure. Huismann et al.76 found no differences in MMP2 and MMP9 activity in first trimester placenta from pregnancy subsequently developing PE or FGR, but the study was limited by the small sample size of the groups.

Also from the studies investigating placenta from third trimester, the majority of analyses focused on MMP2 and MMP9.77,78 Few publications have studied the role of MT-MMPs in FGR. We have previously shown a decrease in MT1-MMP mRNA and protein levels in FGR placentas when compared to gestational age matched healthy controls.59 This change in FGR may be limited to MT1-MMP, because MT2-, MT3- and MT5-MMP are unaltered in FGR.3 However, more data are required to draw firm conclusions.

In PE placental MT6-MMP is down-regulated,68 whereas MT2-MMP67,80 and MT4-MMP68 are up-regulated. Since MT4-MMP is involved in angiogenesis, its up-regulation might be a compensatory mechanism to overcome poor blood supply to the fetus with placental hypervascularisation.68 The role of MT-MMPs in the pathology of PE may involve their function in cleavage and thus activation or inactivation of circulating substrates. For instance, soluble endoglin (sEng), a soluble form of the TGF-β receptor, is increased in PE.81 Both sEng and MT1-MMP have been localized in the ST of third trimester placenta. Indeed, although its expression is not upregulated in pre-eclamptic placentas, MT1-MMP has been reported to cleave endoglin.66

Thus, contrasting the concept that poor placentation may relate to lower MMP expression, some MT-MMP members are up-regulated in PE and FGR during the third trimester of pregnancy. This might represent a compensatory mechanism to overcome poor placentation due to MT-MMP down-regulation in the first trimester of pregnancy.82

Diabetes in pregnancy is associated with both an increased risk for PE83 and FGR, suggesting placental invasion failure, as well as excessive fetal fat accretion and fetal overgrowth. Furthermore, the placenta in diabetes is characterized by hypervascularization.84 Accordingly, MT1-MMP imbalances have been described in diabetes. MT1-MMP is increased in first trimester placentas of Type 1 diabetic women, and upregulation of trophoblast MT1-MMP by insulin, IGF-1, IGF-2 and TNF-α may underlie this observation.43 Gestational diabetes mellitus (GDM) is a glucose intolerance with first clinical manifestation in the second trimester. We showed an up-regulation of MT1-MMP protein levels in third trimester placenta as a result of GDM in lean women. The upregulation of MT1-MMP by insulin and IGF-2 in endothelial cells accounts for the increase.62 In contrast, MT1-MMP is downregulated in GDM placenta of obese women.85 This suggests a complex interplay between diabetes-associated and obesity-associated...
factors to ultimately determine placental MT1-MMP levels in the third trimester of pregnancy.

Indeed, maternal obesity is associated with an increased risk of preterm delivery, PE and fetal death and might also play a role in the regulation of placental MT-MMPs. In a rat model mimicking maternal obesity, trophoblast invasion and blood vessel remodeling is altered. Leptin, a classical hormone upregulated in obesity and involved in placental lipid metabolism, can also modulate placental ECM molecule expression and up-regulates MT1-MMP expression in human EVTs. However, more studies are required to identify the obesity-related factors regulating MT-MMPs.

MT-MMP expression is also altered in pregnancy disorders leading to miscarriage. Decidual MT2- and MT5-MMP expression is increased in first trimester decidua of spontaneous abortions, whereas absence of MT4-MMP in amniochorion has been described in preterm premature rupture of the membranes (pPROM) when compared with term labor placenta.

Conclusion

In the present review we have summarized current knowledge about MT-MMPs with a focus on the human placenta. Their location, functions and involvement in pregnancy complications highlight the crucial role of MT-MMPs for a successful pregnancy. However, it is obvious that MT-MMPs are understudied and more research is required to completely understand their role in placental implantation and trophoblast invasion. Additional studies addressing MT-MMP activity and function in PE and FGR could help in finding diagnostic markers for these pregnancy complications.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The work was supported by funds of the Oesterreichische Nationalbank (Anniversary Fund, project number: 14796), by the Doctorate program MOLIN (FWF, W1241) and the Medical University of Graz.

Authors contributions

AM reviewed the literature and wrote the first draft. UH modified the first draft and critically discussed and reviewed the manuscript. NG, UL, GD and MD critically discussed and reviewed the manuscript.

References

[1] Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harbor Perspect Biol 2011; 3:a005058; PMID:21917992; http://dx.doi.org/10.1101/cshperspect.a005058
[2] Forbes K, Westwood M. Maternal growth factor regulation of human placental development and fetal growth. J Endocrinol 2010; 207(1):1-16; PMID:20817666; http://dx.doi.org/10.1677/JEO-10-0174
[3] Zhu J, Zhong M, Pang Z, Yu Y. Dysregulated expression of matrix metalloproteinases and their inhibitors may participate in the pathogenesis of pre-eclampsia and fetal growth restriction. Early Hum Dev 2014; 90(10):657-64; PMID:25194834; http://dx.doi.org/10.1016/j.earhumdev.2014.08.007
[4] Bischof P, Meisser A, Campana A. Biochemistry and molecular biology of trophoblast invasion. Ann N Y Acad Sci 2001; 943:157-62; PMID:11594536; http://dx.doi.org/10.1111/j.1749-6632.2001.tb03799.x
[5] Seiki M. Membrane-type matrix metalloproteinases. APMIS 1999; 107(1):137-43; PMID:10190290; http://dx.doi.org/10.1111/j.1699-0463.1999.tb01536.x
[6] Cohen M, Meisser A, Bischof P. Metalloproteinases and human placental invasiveness. Placenta 2006; 27(8):783-93; PMID:16249026; http://dx.doi.org/10.1016/j.placenta.2005.08.006
[7] Isaka K, Usuda S, Ito H, Sagawa Y, Nakamura H, Nishi H, Suzuki Y, Li YF, Takayama M. Expression and activity of matrix metalloproteinase 2 and 9 in human trophoblasts. Placenta 2003; 24(1):53-64; PMID:12495660; http://dx.doi.org/10.1053/plac.2002.0867
[8] Seval Y, Akkoyunlu G, Demir R, Asar M. Distribution and regulations of matrix metalloproteinase (MMP)-2 and -9 and their inhibitors (TIMP-1 and TIMP-2) in the human decidua during early pregnancy. Acta Histochem 2004; 106(5):353-62; PMID:15530530; http://dx.doi.org/10.1016/j.acthis.2004.07.005
[9] Huppertz B, Kertschanska S, Demir AY, Frank HG, Kaufmann P. Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. Cell Tissue Res 1998; 291(1):133-48; PMID:9394051; http://dx.doi.org/10.1007/s004410050987
[10] Hernandez-Barrantes S, Bernardo M, Toth M, Fridman R. Regulation of membrane type-matrix metalloproteinases. Semin Cancer Biol 2002; 12(2):131-8; PMID:12027585; http://dx.doi.org/10.1006/scbi.2001.0421
[11] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 2006; 69(3):562-73; PMID:16405877; http://dx.doi.org/10.1016/j.cardiores.2005.12.002
[12] Itoh Y. Membrane-type matrix metalloproteinases: Their functions and regulations. Matrix Biol 2015; 44-46:207-23; PMID:25794647; http://dx.doi.org/10.1016/j.matbio.2015.03.004
[13] Sela-Passwell N, Rosenblum G, Shoham T, Sagi I. Structural and functional bases for allosteric control of MMP activities: can it pave the path for selective inhibition? Biochim Biophys Acta 2010; 1803(1):29-38; PMID:19406173; http://dx.doi.org/10.1016/j.bbamcr.2009.04.010
[14] Sohail A, Marco M, Zhao H, Shi Q, Merriman S, Mobashery S, Fridman R. Characterization of the dimerization interface of membrane type 4 (MT4)-matrix metalloproteinase. J Biol Chem 2011; 286(38):33178-89; PMID:21828052; http://dx.doi.org/10.1074/jbc.M111.253369

[15] Lehti K, Lohi J, Juntunen MM, Pei D, Keski-Oja J. Oligomerization through hemopexin and cytoplasmic domains regulates the activity and turnover of membrane-type 1 matrix metalloproteinase. J Biol Chem 2002; 277(10):8440-8; PMID:11779859; http://dx.doi.org/10.1074/jbc.M109128200

[16] Marco M, Fortin C, Fulop T. Membrane-type matrix metalloproteinases: key mediators of leukocyte function. J Leukoc Biol 2013; 94(2):237-46; PMID:23695309; http://dx.doi.org/10.1189/jlb.0612267

[17] Sohail A, Sun Q, Zhao H, Bernardo MM, Cho JA, Fridman R. Characterization of the dimerization interface of membrane type 4 (MT4)-matrix metalloproteinase: structure, function, and biochemistry. Circ Res 2003; 92(8):827-39; PMID:12730128; http://dx.doi.org/10.1161/01.RES.0000071012.80711.3D

[18] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003; 92(8):827-39; PMID:12730128; http://dx.doi.org/10.1161/01.RES.0000071012.80711.3D

[19] Lőfek S, Schilling O, Fränzke CW. Series “matrix metalloproteinases in lung health and disease:” Biological role of matrix metalloproteinases: a critical balance. Eur Respir J 2011; 38(1):191-208; PMID:21177845; http://dx.doi.org/10.1183/09031936.00146510

[20] Yana I, Weiss SJ. Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. Mol Biol Cell 2000; 11(7):2387-401; PMID:10888676; http://dx.doi.org/10.1091/mbc.11.7.2387

[21] Fanjul-Fernandez M, Folgueras AR, Cabrera S, Lopez-Otin C. Matrix metalloproteinases: evolution, gene regulation and functional analysis in mouse models. Biochem Biophys Acta 2010; 1803(1):3-19; PMID:19631700; http://dx.doi.org/10.1016/j.bbamcr.2009.07.004

[22] Golubkov VS, Chekanov AV, Shiyarova SA, Aleshin AE, Ratnikov BI, Gawlik K, Radichev I, Motamedchaboki K, Smith JW, Strongin AY. Proteolysis of the membrane type-1 matrix metalloproteinase prodomain: implications for a two-step proteolytic processing and activation. J Biol Chem 2007; 282(50):36283-91; PMID:17938169; http://dx.doi.org/10.1074/jbc.M706290200

[23] Hernandez-Barrantes S, Toth M, Bernardo MM, Yurkova M, Gervasi DC, Raz Y, Sang QA, Fridman R. Binding of active (57 kDa) membrane type 1 matrix metalloproteinase (MT1-MMP) to tissue inhibitor of metalloproteinase (TIMP)-2 regulates TIMP-1-MMP processing and pro-MMP-2 activation. J Biol Chem 2000; 275(16):12080-9; PMID:10766841; http://dx.doi.org/10.1074/jbc.275.16.12080

[24] Cho JA, Osenkowski P, Zhao H, Kim S, Toth M, Cole K, Aboukameel A, Saliganan A, Schuger L, Bonfil RD, et al. The inactive 44-kDa processed form of membrane type 1 matrix metalloproteinase (MT1-MMP) enhances proteolytic activity via regulation of endocytosis of active MT1-MMP. J Biol Chem 2008; 283(25):17391-405; PMID:18413312; http://dx.doi.org/10.1074/jbc.M708943200

[25] Toth M, Hernandez-Barrantes S, Osenkowski P, Bernardo MM, Gervasi DC, Shimura Y, Meroueh O, Kotra LP, Gálvez BG, Arroyo AG, et al. Complex pattern of membrane type 1 matrix metalloproteinase shedding. Regulation by autocatalytic cells surface inactivation of active enzyme. J Biol Chem 2002; 277(29):26340-50; http://dx.doi.org/10.1074/jbc.M200655200

[26] Osenkowski P, Toth M, Fridman R. Processing, shedding, and endocytosis of membrane type 1-matrix metalloproteinase (MT1-MMP). J Cell Physiol 2004; 200(1-2):10-10; PMID:15137052; http://dx.doi.org/10.1002/jcp.20064

[27] Kemp B, Kertschanska S, Kadyrov M, Rath W, Kaufmann P, Huppertz B. Invasive depth of extravillous trophoblast correlates with cellular phenotype: a comparison of intra- and extrauterine implantation sites. Histoch Chem Biol Cell 2002; 117(5):401-14; PMID:12029487; http://dx.doi.org/10.1007/s00418-002-0396-0

[28] English WR, Puente XS, Freije JM, Knauper V, Amour A, Merryweather A, Lopez-Otin C, Murphy G. Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor-α convertase activity but does not activate pro-MMP2. J Biol Chem 2000; 275(19):14046-55; PMID:10759947; http://dx.doi.org/10.1074/jbc.275.19.14046

[29] Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements J, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, et al. Processing of tumour necrosis factor-α precursor by metalloproteinases. Nature 1994; 370(6490):555-7; PMID:8052310; http://dx.doi.org/10.1038/370555a0

[30] Sounni NE, Paye A, Host L, Noel A. MT-MMPS as Regulators of Vessel Stability Associated with Angiogenesis. Front Pharmaco 2011; 2:111; PMID:21687519; http://dx.doi.org/10.1016/j.fphar.2011.00111

[31] Cheng JC, Chang HM, Leung PC. Transforming growth factor-beta1 inhibits trophoblast cell invasion by inducing Snail-mediated downregulation of vascular endothelial-cadherin protein. J Biol Chem 2013; 288(46):33181-92; PMID:24106276; http://dx.doi.org/10.1074/jbc.M113.488886

[32] Hiden U, Wadsack C, Prutsch N, Gauster M, Weiss U, Frank HG, Schmitz U, Fast-Hirsch C, Hengstschlager M, Pötgens A, et al. The first trimester human trophoblast cell line ACH-3P: a novel tool to study autocrine/paracrine regulatory loops of human trophoblast subpopulations--TNF-α stimulates MMP15 expression. BMC Dev Biol 2007; 7:137; PMID:18093301; http://dx.doi.org/10.1186/1471-213X-7-137

[33] Bauer S, Pollheimer J, Hartmann J, Husslein P, Aplin JD, Knofler M. Tumor necrosis factor-α inhibits trophoblast migration through elevation of plasminogen activator inhibitor-1 in first-trimester villous explant cultures. J Clin Endocrinol Metab 2004; 89(2):812-22; PMID:14764800; http://dx.doi.org/10.1210/jc.2003-031351

[34] Renaud SJ, Postovit LM, Macdonald-Goodfellow SK, McDonald GT, Caldwell JD, Graham CH. Activated macrophages inhibit human cytotoxic trophoblast invasiveness in vitro. Biol Reprod 2005; 73(2):237-43; PMID:15800179; http://dx.doi.org/10.1095/biolreprod.104.038000

[35] Tam EM, Morrison CJ, Wu YI, Stack MS, Overall CM. Membrane protease proenzymes: Isopeptide-coded affinity tag MS identification of undescribed MT1-matrix metalloproteinase substrates. Proc Natl Acad Sci U S A 2004; 101(18):6917-22; PMID:15118097; http://dx.doi.org/10.1073/pnas.0305862101
[36] Folgueras AR, Pendas AM, Sanchez LM, Lopez-Otin C. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. Int J Dev Biol 2004; 48 (5-6):411-24; PMID:15349816; http://dx.doi.org/10.1387/ijdb.041811af

[37] Gao G, Plaa A, Thompson VP, Jin S, Zuo F, Sandu JD. ADAMTS4 (aggrecanase-1) activation on the cell surface involves C-terminal cleavage by glycosylphosphatidylinositol-anchored membrane type 4-matrix metalloproteinase and binding of the activated proteinase to chondroitin sulfate and heparan sulfate on syndecan-1. J Biol Chem 2004; 279(11):10042-51; PMID:14701864; http://dx.doi.org/10.1074/jbc.M312100200

[38] Lu KV, Jong KA, Rajasekaran AK, Cloughesy TF, Mischel PS. Upregulation of tissue inhibitor of metalloproteinases (TIMP)-2 promotes matrix metalloproteinase (MMP)-2 activation and cell invasion in a human glioblastoma cell line. Lab Invest 2004; 84(1):8-20; PMID:14631378; http://dx.doi.org/10.1038/lab.370003

[39] Morrison CJ, Butler GS, Bigg HF, Roberts CR, Soloway PD, Overall CM. Cellular activation of MMP-2 (gelatinase A) by MT2-MMP occurs via a TIMP-2-independent pathway. J Biol Chem 2001; 276(50):47402-10; PMID:11584019; http://dx.doi.org/10.1074/jbc.M106843200

[40] Clark IM, Swingler TE, Sampieri CL, Edwards DR. The regulation of matrix metalloproteinases and their inhibitors. Int J Biochem Cell Biol 2008; 40(6-7):1362-78; PMID:18258475; http://dx.doi.org/10.1016/j.biocel.2007.12.006

[41] Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J Cell Physiol 2007; 211(1):19-26; PMID:17167774; http://dx.doi.org/10.1002/jcp.200948

[42] Bischof P, Meisser A, Campana A. Paracrine and autocrine regulators of trophoblast invasion–a review. Placenta 2000; 21 Suppl A:555-60; PMID:10831123; http://dx.doi.org/10.1016/S0143-722X(00)00521

[43] Hiden U, Glitzner E, Ivanisevic M, Djelmis J, Wadsack C, Lang U, Desoye G. MT1-MMP occurs via a TIMP-2-independent pathway. J Biol Chem 2001; 276(50):47402-10; PMID:11584019; http://dx.doi.org/10.1074/jbc.M106843200

[44] Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci 2002; 115(Pt 19):3719-27; PMID:12235282; http://dx.doi.org/10.1242/jcs.00063

[45] Murphy G. Tissue inhibitors of metalloproteinases. Genome Biol 2011; 12(11):233; PMID:22078297; http://dx.doi.org/10.1186/gb-2011-12-11-233

[46] Arpino V, Brock M, Gill SE. The role of TIMPs in regulation of extracellular matrix proteolysis. Matrix Biol 2015; 44-46:247-54; PMID:25805621; http://dx.doi.org/10.1016/j.matbio.2015.03.005

[47] Hernandez-Barrantes S, Shimura Y, Soloway PD, Sang QA, Fridman R. Differential roles of TIMP-4 and TIMP-2 in pro-MMP-2 activation by MT1-MMP. Biochem Biophys Res Commun 2001; 281(1):126-30; PMID:11178970; http://dx.doi.org/10.1006/bbrc.2001.4323

[48] Bigg HF, Morrison CJ, Butler GS, Bogoyevitch MA, Wang Z, Soloway PD, Overall CM. Tissue inhibitor of metalloproteinases-4 inhibits but does not support the activation of gelatinase A via efficient inhibition of membrane type 1-matrix metalloproteinase. Cancer Res 2001; 61(9):3610-8; PMID:11325829.

[49] Whitley GS, Cartwright JE. Cellular and molecular regulation of spiral artery remodelling: lessons from the cardiovascular field. Placenta 2010; 31(6):465-74; PMID:20359743; http://dx.doi.org/10.1016/j.placenta.2010.03.002

[50] Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001; 17:463-516; PMID:11687497; http://dx.doi.org/10.1146/annurev.cellbio.17.1.463

[51] Patel A, Dash PR. Formation of atypical podosomes in extravillous trophoblasts regulates extracellular matrix degradation. Eur J Cell Biol 2012; 91(3):171-9; PMID:22284833; http://dx.doi.org/10.1016/j.ejcb.2011.11.006

[52] Xu P, Wang YL, Zhu SJ, Luo SY, Piao YS, Zhuang LZ. Expression of matrix metalloproteinase-2, -9, and -14, tissue inhibitors of metalloproteinase-1, and matrix proteins in human placenta during the first trimester. Biol Reprod 2000; 62(4):988-94; PMID:10727268; http://dx.doi.org/10.1095/bioreprod62.4.988

[53] Tarrade A, Goffin F, Munaut C, Lai-Kuen R, Tricottet V, Foidart JM, Vidaud M, Frankenne F, Evaïn-Brion D. Effect of matrigel on human extravillous trophoblasts differentiation: modulation of protease pattern gene expression. Biol Reprod 2002; 67(5):1628-37; PMID:12390897; http://dx.doi.org/10.1095/bioreprod.1001925

[54] Harris LK, Smith SD, Keogh RJ, Jones RL, Baker PN, Knoxler M, Cartwright JE, Whitley GS, Aplin JD. Trophoblast- and vascular smooth muscle cell-derived MMP-12 mediates elastolysis during uterine spiral artery remodeling. Am J Pathol 2010; 177(4):2103-15; PMID:20802175; http://dx.doi.org/10.2353/ajpath.2010.100182

[55] Onogi A, Naruse K, Sado T, Tsunemi T, Shigetomi H, Noguchi T, Yamada Y, Akasaki M, OI H, Kobayashi H. Hypoxia inhibits invasion of extravillous trophoblast cells through reduction of matrix metalloproteinase (MMP-2) activation in the early first trimester of human pregnancy. Placenta 2011; 32(9):665-70; PMID:21764444; http://dx.doi.org/10.1016/j.placenta.2011.06.023

[56] Francis VA, Abera AB, Matjila M, Millar RP, Katz AA. Kisspeptin regulation of genes involved in cell invasion and angiogenesis in first trimester human trophoblast cells. PloS One 2014; 9(6):e99680; PMID:24923321; http://dx.doi.org/10.1371/journal.pone.0099680

[57] Hurskainen T, Seiki M, Apte SS, Syrakallio-Ylitalo M, Sorsa T, Oikarinen A, Auto-Harmainen H. Production of membrane-type matrix metalloproteinase-1 (MT-MMP-1) in early human placenta. A possible role in placental implantation? J Histochem Cytochem 1998; 46(2):221-9; PMID:9446829; http://dx.doi.org/10.1177/00221554980460221

[58] Bai SX, Wang YL, Qin L, Xiao ZZ, Herva R, Piao YS. Dynamic expression of matrix metalloproteinases (MMP-2, -9 and -14) and the tissue inhibitors of MMPs (TIMP-1, -2 and -3) at the implantation site during tubal pregnancy. Reproduction 2005; 129(1):103-13; PMID:15615902; http://dx.doi.org/10.1530/rep.1.00283

[59] Hiden U, Ghaffari-Tabrizi N, Gauster M, Tam-Amsdorfer C, Cetin I, Dieber-Rotheneder M, Lang U, Desoye G. Membrane-type matrix metalloproteinase 1 regulates trophoblast functions and is reduced in fetal growth restriction. Am J Pathol 2013; 182(5):1563-71; PMID:23470162; http://dx.doi.org/10.1016/j.ajpath.2013.01.011
for chronic stress and inflammatory pathways. Diabetes 2003; 52(12):2951-8; PMID:14633856; http://dx.doi.org/10.2337/diabetes.52.12.2951

[86] Lamminpaa R, Vehvilainen-Julkunen K, Gissler M, Selander T, Heinonen S. Pregnancy outcomes of overweight and obese women aged 35 years or older - A registry-based study in Finland. Obes Res Clin Pract 2015; PMID:26054598.

[87] Hayes EK, Tessier DR, Percival ME, Holloway AC, Petrik JJ, Grusin A, Raha S. Trophoblast invasion and blood vessel remodeling are altered in a rat model of lifelong maternal obesity. Reprod Sci 2014; 21(5):648-57; PMID:24155067; http://dx.doi.org/10.1177/1933719113508815

[88] White V, Gonzalez E, Capobianco E, Pustovrh C, Martinez N, Higa R, Baier M, Jawerbaum A. Leptin modulates nitric oxide production and lipid metabolism in human placenta. Reprod Fertil Dev 2006; 18(4):425-32; PMID:16737635; http://dx.doi.org/10.1071/RD05105

[89] Castellucci M, De Matteis R, Meisser A, Cancell R, Monsurro V, Islami D, Sarzani R, Marzioni D, Cinti S, Bischof P. Leptin modulates extracellular matrix molecules and metalloproteinases: possible implications for trophoblast invasion. Mol Hum Reprod 2000; 6(10):951-8; PMID:11006325; http://dx.doi.org/10.1093/molehr/6.10.951

[90] Wang H, Cheng H, Shao Q, Dong Z, Xie Q, Zhao L, Wang Q, Kong B, Qu X. Leptin-promoted human extravillous trophoblast invasion is MMP14 dependent and requires the cross talk between Notch1 and PI3K/Akt signaling. Biol Reprod 2014; 90(4):78; PMID:24571988; http://dx.doi.org/10.1095/biolreprod.113.114876

[91] Plaisier M, Dennert I, Rost E, Koolwijk P, van Hinsbergh VW, Helmerhorst FM. Decidual vascularization and the expression of angiogenic growth factors and proteases in first trimester spontaneous abortions. Hum Reprod 2009; 24(1):185-97; PMID:18854409; http://dx.doi.org/10.1093/humrep/den296

[92] Xu P, Wang Y, Piao Y, Bai S, Xiao Z, Jia Y, Luo S, Zhuang L. Effects of matrix proteins on the expression of matrix metalloproteinase-2, -9, and -14 and tissue inhibitors of metalloproteinases in human cytotrophoblast cells during the first trimester. Biol Reprod 2001; 65(1):240-6; PMID:11420245; http://dx.doi.org/10.1095/biolreprod65.1.240