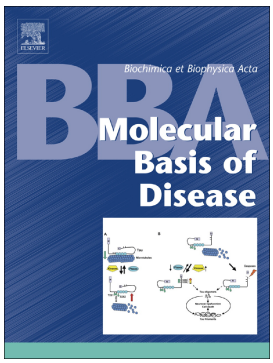

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Role of proteases in dysfunctional placental vascular remodelling in preeclampsia

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Running title: Placental proteases and angiogenesis in preeclampsia.

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Abstract

Preeclampsia is a syndrome characterised by vascular dysfunction, impaired angiogenesis, and hypertension during pregnancy. Even when the precise pathophysiology of preeclampsia remains elusive, impaired vascular remodelling and placental angiogenesis in the placental villi and defective trophoblast invasion of the uterus are proposed as crucial mechanisms in this syndrome. Reduced trophoblast invasion leads to reduced uteroplacental blood flow and oxygen availability and increased oxidative stress. These phenomena trigger the release of soluble factors into the maternal and foetoplacental circulation that are responsible of the clinical features of preeclampsia. New blood vessels generation as well as vascular remodelling are mechanisms that require expression and activity of different proteases, including matrix metalloproteases, a-disintegrin and metalloproteases, and a-disintegrin and metalloprotease with thrombospondin motifs. These proteases exert proteolysis of the extracellular matrix. Additionally, cathepsins, a family of proteolytic enzymes, are primarily located in lysosomes but are also released by cells to the extracellular space. This review focuses on the role that these proteases play in the regulation of the uterine trophoblast invasion and the placental vascular remodelling associated with preeclampsia.

Keywords: proteases; angiogenesis; vascular remodelling; placenta; preeclampsia

1. Introduction

Preeclampsia is a pregnancy-specific syndrome characterised by the new onset of hypertension and either proteinuria or end-organ dysfunction after 20 weeks of gestation in a previously normotensive woman. In the absence of proteinuria, preeclampsia is diagnosed by the new onset of hypertension associated with thrombocytopenia, impaired liver functions renal insufficiency, pulmonary oedema, or the onset cerebral or visual disturbances [1, 2]. Preeclampsia also associates with endothelial dysfunction in both the maternal [3] and foetoplacental circulation [4-9]. This syndrome is a leading cause of maternal morbidity and mortality, with a worldwide prevalence of 2-10% [8, 10], and associates with ~76.000 maternal and ~500.000 foetus and newborn deaths worldwide per year [10]. Preeclampsia also associates with long-term adverse outcomes for the mother and the offspring [11]. Even though the exact pathophysiology of preeclampsia is unknown the impaired vascular remodelling of the uterine spiral arteries by the trophoblast is a crucial phenomenon in this syndrome [10, 12]. Impaired vascular remodelling reduced the maternal blood flow to the intervillous space triggering the release of soluble factors into the maternal circulation, including the soluble fms–like tyrosine kinase (sFlt-1) and soluble endoglin (sEng) [13]. These processes predispose to maternal hypertension, maternal multisystemic damage/malfunction and foetal syndromes such us foetal growth restriction.

The whole process of trophoblast invasion trough the maternal decidua to reach the uterine spiral arteries demands a strict interplay and communication between foetal trophoblasts and maternal cells (Figure 1). In this regard, the invasive capacity of trophoblast requires the expression and activity of different extracellular proteases, such as, matrix metalloproteases (MMPs) [14], a-disintegrin and metalloproteases (ADAMs) [15], a-disintegrin and metalloprotease with thrombospondin motifs (ADAMTSs) [16], and

cathepsins [17]. However, invasive control mechanisms are also required in this process. Invasive trophoblast and decidua cells express protease inhibitors such as tissue inhibitors of metalloproteinases (TIMPs) [18] and cystatins (cathepsin inhibitors) [17]. It is reported that the reversion-induced-cysteine-rich-protein with Kazal motifs (RECK), a plasma membrane-anchored glycoprotein that inhibits MMPs and ADAMs activity, may control the invasiveness of trophoblasts playing a role in preeclampsia [12]. In this review, we highlight the role of proteases and its regulation associated with trophoblast invasion and uterine spiral arteries remodelling involved in the development of preeclampsia.

2. Preeclampsia

2.1 Generalities

Preeclampsia is defined as hypertension (≥140 systolic/90 diastolic mmHg) and proteinuria (≥300 mg/24 h) [8]. In the absence of proteinuria, preeclampsia is recognised when the hypertension evidenced in the pregnant women is accompanied by end-organ dysfunction [8]. Preeclampsia associate whit stillbirth, foetal growth restriction, and preterm delivery [8]. Preeclampsia affects mainly nulliparous woman and is usually reversed after delivery of the placenta [10]. Currently, the only effective treatment for preeclampsia is the premature delivery/termination of pregnancy [10]. The main risk factors for preeclampsia are extreme age groups, obesity, nulliparity, family history of preeclampsia, multiple pregnancy, preeclampsia in a previous pregnancy, chronic hypertension, chronic renal disease, antiphospholipid syndrome, diabetes mellitus, hydatidiform mole, and the expression of the molecular variant of angiotensinogen (Met235Thr), *T235 angiotensinogen* gene [10]. Preeclampsia also associates with higher risk for cardiovascular disease later in life in both the mother and the newborn [11].

Mothers who developed preeclampsia show two-fold increased risk of stroke and eight-fold higher risk of death due to ischemic heart disease, particularly in early-onset preeclampsia (i.e., diagnosed at <34 weeks of gestation) [19].

2.2 Pathophysiological mechanisms of preeclampsia

Preeclampsia shows with impaired transformation of the maternal spiral arteries into high capacitance vessels [10]. This phenomenon has been explained by an impaired invasion of a subgroup of trophoblast-derived cells referred as extravillous trophoblast cells (EVTs) through the maternal decidua to reach the maternal vascular bed [10]. Thus, the subsequent uterine spiral arteries remodelling from the high-resistance, small-diameter vessels into high-capacitance, low-resistance vessels are affected [20]. Under this condition, the maternal blood flow to the placenta is reduced leading to lower oxygen availability, the latter being only a hypothesis rather than evidence-based knowledge. However, the high-resistance, non-remodelled vessels also associated with pulsatile high perfusion blood pressure into the intervillous space, generating a vicious cycle of ischemia and reperfusion in this placental region [21, 22] and shear stress in the trophoblast layer [23]. Also, this phenomenon has been associated with insufficient placental oxygenation, oxidative stress, and release of cell fragments, microparticles, and extracellular vesicles into the maternal circulation [24, 25] triggering molecular events identified as clinical features of preeclampsia [20], systemic inflammatory response, and endothelial dysfunction [10, 21, 25].

Endothelial dysfunction corresponds to an imbalance between the generation, release, and response to vasodilators and vasoconstrictors by the endothelium with consequences in the vascular response to these agents [26]. These alterations are also

described in the maternal [3, 19] and foetoplacental circulation [4, 9] in preeclampsia. Increased release of the soluble form of the endothelial growth factor receptor type 1 (fms–like tyrosine kinase, sFlt-1) and the soluble endoglin (sEng) [27, 28] from the placenta into the maternal circulation is reported in this syndrome. sFlt-1 acts as a decoy/sequester receptor for the vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) [14], both of which are proangiogenic. sFlt-1 prevents VEGF receptor type 2 (VEGFR2) activation in the maternal and foetoplacental tissues [27]. sEng act a decoy/sequester receptor for transforming growth factor ß1 (TGF-ß1) and TGF-ß3 which exerts pro-migratory and pro-angiogenic stimuli in endothelial cells [28]. Thus, the most accepted hypothesis of the aetiology of this syndrome is the inefficient remodelling of the uterine arteries by the inefficient invasion and differentiation of EVTs plays a critical role [10, 20]. EVTs invasion and subsequent differentiation to an endothelial-like phenotype depend on the activity of different extracellular proteases generated by these cells and the maternal tissue, allowing invasion of maternal decidua to reach and modify the maternal spiral arteries [20, 29]. However, the balance between the expression and activity of these proteases is impaired in preeclampsia [14, 15, 29].

3. Proteases and placenta vasculature in preeclampsia

3.1 Matrix metalloproteinases (MMPs)

MMPs belong to a superfamily of zinc-based proteinases, also known as the metzincins that catalyse the proteolytic degradation of specific proteins [30]. MMPs are involved in modifying and remodelling the extracellular matrix in the angiogenesis process, tissue remodelling, embryonic and placental development, cell migration, and morphogenesis [30]. MMPs are classified as secreted MMPs (MMPs 1 to 13 and 18 to 20),

or membrane-type MMPs (or MT-MMPs), which are integrated or associated to the plasma membrane (MMPs 14 to 17) [30]. The membrane localisation of MT-MMPs restricts their proteolytic activities to the cellular boundaries compared to the widespread proteolysis of the secreted MMPs [31]. The function of MMPs is controlled by their expression, secretion, and catalytic activity [30]. The catalytic activity of MMPs could be inhibited by TIMPs (TIMPs 1 to 4), although their inhibitory action is differential [32]. RECK, another MMPs inhibitor, which unlike TIMPs is anchored to the cell surface [18, 33]. RECK inhibits MMP-2, MMP-9, and MT1-MMP (also referred as MMP-14) [31, 33] as well as ADAM-10 and ADAM-17 [31, 34, 35] (see Figure 1).

MMPs are regulated in early pregnancy suggesting a pivotal role in the trophoblast invasion and spiral arteries remodelling process during placenta formation [36]. Trophoblast cells express almost all members of the human MMPs gene family and the inhibitors TIMPs and RECK [12, 36]. Secreted MMP-2, MMP-9, and MMP-14 are required for EVTs invasion [20, 29] as suggested by lost and gain function in *in vitro* assays in trophoblast primary cultures and cell lines [37]. The expression of MMP-14 by primary cultures of trophoblast cells remains stable during the early pregnancy [38, 39]. However, the exact changes in the MMP-2 and MMP-9 expression by trophoblast during the first trimester is still controversial. Some studies described that either MMP-9 [40-43] or MMP-2 [37, 38, 44] are the main gelatinases at the more active stage of trophoblast invasion. However, MMP-9 increases at the end of the first trimester being predominant over MMP-2 until the end of pregnancy [38, 39, 45]. In first trimester villous tissue explants, the level of secreted proMMP2 is ~10 fold that pro-MMP-9, which diminishes in the second trimester suggesting the involvement of stromal cells (for example, decidual, mesenchymal, and

immune cells) in the total abundance of pro-MMP-2 in the first trimester villous tissue [45, 46]. Thus, MMP-2 may plays a major role during implantation and MMP-9 during trophoblast invasion [46]. On the contrary, expression of the MMPs inhibitors TIMP2 and TIMP1 by trophoblast cells, which preferentially bind to MMP2 and MMP9, respectively [27], is reduced in early but induced later in pregnancy, explaining the high MMP activity at the first stages of trophoblast invasion [24].

Preeclampsia associates with reduced expression of MMPs. The expression of MMP-2, MMP-8, MMP-9 and MMP-11 are downregulated in placental bed biopsies (protein extracts and tissue immunostaining) from patients diagnosed with mild pre-eclampsia compared with normal pregnancies [14, 47, 48]. In some of the placentas from patients with this type of preeclampsia, no changes in the expression of MMP-2 compared with placentas from normal pregnancies were seen [48]. In isolated cytotrophoblast from normal pregnancies and from mild preeclampsia the main secreted MMP corresponded to MMP-9; however, MMP-9 abundance is lower in mild preeclampsia. Moreover, this metalloproteinase is mainly present in its inactive form (proMMP-9) [49]. As in severe cases of this syndrome, MMP9-null mouse embryos exhibit deficiencies in trophoblast differentiation and invasion along with intrauterine growth restriction or embryonic death. However, pregnant MMP9-null mice bearing null embryos exhibited clinical features of severe preeclampsia including VEGF dysregulation and proteinuria accompanied by pre-existing elevated blood pressure and kidney pathology [50].

On the other hand, the placental expression of MMP-14 [12, 51, 52] is unaltered in preeclampsia compared with normal pregnancies. However, MMP-14 activity may result reduced in preeclampsia since higher expression of RECK in the plasma membrane of the syncytiotrophoblast colocalize whit MMP-14 which associates whit reduced *in situ*

gelatinase activity [12]. Moreover, primary cultures of cytotrophoblast from preeclampsia express reduced level of MMP-1, MMP-2, MMP-7, MMP-9, and MMP-12 that is concomitant to reduced invasiveness [53]. Table 1 summarises the available information regarding the expression of proteases and their function in preeclampsia.

Maternal DNA polymorphism in MMP-2 (MMP-2 C-735T) [54] and MMP-8 (MMP-8 C-799T) [55, 56] which relates to decreased MMP-2 and MMP-8 expression, respectively, are described as risk factors for preeclampsia [54-56]. Moreover, the MMP-7 A-181G polymorphism, which is not associated to preeclampsia [56], shows a synergistic effect increasing the risk of preeclampsia in subjects with MMP-2 C-735T polymorphism [56]. A recent systematic review and meta-analysis provided the first comprehensive synopsis of case-control studies unveiling the association of MMPs and TIMPs gene polymorphisms with disorders that influence fertility and pregnancy complications, including preeclampsia [57]. Besides the potential diagnostic and clinical advantage of these observations, further studies are required to unveil the effect on trophoblast and placental vascular remodelling in preeclampsia.

Preeclampsia associates with high expression of TIMP-1 and TIMP-3 in placental biopsies [14]. Moreover, the expression of TIMP-1 and TIMP-2 is elevated in HUVECs endothelial cells [58]. Cultured human decidual endothelial cells from normal and preeclamptic pregnancies, produces similar levels of MMP-9 and TIMP-1 levels however, MMP1 secretion was markedly reduced in preeclampsia derived decidual endothelial cells. The lower MMP-1 may be involved in inhibition of endovascular invasion by cytotrophoblasts [59]. This phenomenon might further decrease the activity of the already reduced level of MMP-2 and MMP-9 contributing to the impaired invasion of the uterine vasculature in this syndrome. Trophoblasts express RECK and the expression of this

molecule is increased in preeclampsia [12]. The latter also associates with reduced MMPs gelatinolytic activity and increased level of the MMPs substrate fibronectin in preeclampsia compared with normal pregnancies [12]. Loss and gain of function approaches in the first trimester trophoblast cell line HTR8/SVneo suggest that lower RECK protein abundance associated with higher but RECK overexpression with reduced MMPs-dependent activity, migration and invasion [12]. Thus, preeclampsia-associated lower cytotrophoblast migration and invasion associated with reduced activity of MMP-2, MMP-9, and MMP-14, may result from overexpression of RECK [12].

In contrast to placenta and trophoblast cells where the expression and activity of different MMPs is reduced, the expression of MMP-2 and MMP-9 in the maternal blood serum is increased [36, 46]. Since MMPs mediate the release of VEGF from the ECM reservoir [30, 31] the higher levels of total VEGF detected in the maternal blood in preeclampsia compared with normal pregnancies [60, 61] could be explained by higher MMPs expression. However, due to the increased levels of sFlt-1 seeing in preeclampsia, the bioavailability of free-VEGF (active) is lower compared with normal pregnancies [60, 61]. Other studies show that MMP-14 is involved in the proteolytic shedding of the integral membrane protein endoglin to generate sEng and its subsequent release to the peripheral circulation [51]. sEng inhibits TGF-β signalling, contributing to the endothelial dysfunction seen in preeclampsia, which is more likely to occur in early-onset than late-onset preeclampsia [27, 28]. Recently it has been proposed that endoglin is cleaved to sEng into the maternal circulation by an MMP-14 dependent shedding in lipid rafts enriched membranes at the apical membrane of the syncytiotrophoblast from preeclampsia [52]. Moreover, exosomes released into the maternal circulation from these lipid rafts-enriched membranes contain the TGF-β receptor 1 and TGF-β receptor 2 in addition to MMP-14 and

endoglin. Thus, a protein complex formed by both of the TGF-β receptors, endoglin, and sEng in the exosomes could block the pro-angiogenic and vascular effect of TGF-β1 in the circulation of women with preeclampsia [52]. Other soluble factors, chemokines, and cytokines at the foetal-maternal interface also regulate the trophoblast motility. For instance, the endogenous purine nucleoside adenosine [8], TGF-β1 [62], and angiotensin II (Ang II) [63], whose concentration or expression is increased in preeclampsia, restrain trophoblast motility by reducing MMPs activity due to increased TIMPs expression [64]. Also, MMP-2 and MMP-9 seems epigenetically silenced in villous samples from preeclampsia since higher levels of the histone 3 trimethylation at lysine 9 and lysine 27 at the promoters of coding genes for MMP-2 and MMP-9 was found [64]. Thus, preeclampsia shows with reduced MMPs activity, which is crucial for placental vascularisation involving proangiogenic factors and cytokines and because of a higher expression of TIMPs, and potentially RECK, in the human placenta (Figure1).

3.2 a-Disintegrin and metalloproteases (ADAMs)

ADAMs are members of a superfamily of zinc-based transmembrane proteinases involved in the proteolytic shedding and activation/inactivation of surface proteins including growth factors, cytokines, receptors and their ligands [65, 66]. Thus, ADAMs regulate different signalling pathways further than extracellular matrix breakdown for which these proteases are also referred as ‘sheddases’ [66]. To date, 21 different ADAMs have been described in the human genome but only half of these proteinases exhibit catalytic function [65]. ADAM-10, ADAM-12, and ADAM-17 have been studied regarding their involvement to control trophoblast cell signalling, differentiation, and invasion.

Moreover, ADAM-10 and ADAM-17 are shown as essential in Notch signalling for regulation of EVTs invasion and differentiation [67, 68] and angiogenesis [31]. Notch signalling is a highly conserved cell signalling pathway that regulates almost every cell process (stemness, fate decision, differentiation, survival, proliferation, migration, and invasion) and tissue morphogenesis [31, 67, 68]. Immunostaining analyses of 2nd trimester basal plate biopsies from human placentas revealed a step-wise modulation of Notch receptors/ligands expression during human trophoblast invasion [68]. Inhibition of Notch signalling reduced the invasion of cultured human trophoblast and the expression of markers of endothelial-like differentiation [68].

In placenta tissue extracts from late preterm or term pregnancies complicated by preeclampsia, the mRNA level of Notch receptors (Notch2 and Notch3), Notch ligands (*Delta-like*-3, *Delta-like*-4, Jagged-1 and Jagged-2) as well as Notch gene targets (Hairy/enhancer-of-split related with YRPW motif protein 1 and 2, *HEY1* and *HEY2* respectively) were downregulated compared with normal pregnancies. The latter results suggest that the Notch signalling pathway is reduced in preeclampsia [68, 69]. This possibility is supported by the reduced abundance of the Notch intracellular domain of Notch2 (NICD2) and Notch3 (NICD3), i.e. the active forms of these receptors [69]. This phenomenon associated with a lack of EVTs invasion and EVTs-dependent vascular remodelling suggesting a role of Notch in the pathogenesis of preeclampsia [68].

RECK also regulates Notch signalling via ADAM-10 and ADAM-17 by repressing or activating these proteinases. In cells that express Notch receptors (referred as Notch signal-receiving cells), RECK inhibits the ADAMs–dependent cleavage of the Notch receptor required for Notch activation [34]. However, RECK also protects ADAM-10–dependent shedding of Notch ligand from the ligand-expressing cells (referred as Notch

signal-sending cells) thus enhancing the receptor activation in the Notch signal-receiving cell [35, 70]. It is suggested that deregulated expression of RECK could associate with deregulation of Notch signalling as seen in preeclampsia [12, 31] (Figure 1).

The ADAM-17 expression is induced in placentas from preeclampsia as well as in primary trophoblast from normal pregnancies cultured under hypoxia which associates to increased levels of bioactive pro-apoptotic cytokine tumour necrosis factor α (TNFα) considered an essential component of placental dysfunction in preeclampsia [71] (Table 1). ADAM-12 causes ECM degradation associated with invasive phenotypes in early gestation placentas [72]. Suppression of ADAM-12 expression inhibits trophoblasts invasion while its over-expression promotes migration and invasion [72]. A role for ADAM-12s, a truncated form of this enzyme, as an inducer of trophoblast invasion and EVTs outgrowth through a mechanism that requires its catalytic activity is reported [72, 73].

The expression level of ADAM-10 (pro and active forms) is increased in placentas from women with preeclampsia which correlated with the increased release of sFlt-1 from human placental explants [74]. Also, ADAM-10 expression was inversely correlated to the level of hydrogen sulphide (H_2S) [75], which acts as anti-oxidative stress and reduces inflammation [76]. The H_2S reduced the expression of ADAM-10 and sFlt-1 release in primary cultured trophoblasts and placental explants-derived cells [74]. Moreover, ADAM-10 knockdown reduced the release of sFlt-1 in placental explants-derived cells [74]. It was suggested that a deregulated biosynthesis of H_2S may contribute to an excessive Flt-1 shedding in preeclampsia [74] (Table 1).

3.3 a-Disintegrin and metalloprotease with thrombospondin motifs (ADAMTSs)

ADAMTSs are proteins belonging to the metzincin protease superfamily with at least 20 members [16]. Nineteen members of this family have been identified. They are structurally and evolutionarily related to ADAMTs and more distant from MMPs [16]. ADAMTSs contain an angiogenesis inhibitor thrombospondin (TSP) domain, which corresponds to the ECM adhesion glycoprotein secreted from thrombocytes [16]. ADAMTSs proteins are sub-grouped by their known substrates, i.e. aggrecanases or proteoglycanases, procollagen N-propeptidases, cartilage oligomeric matrix protein-cleaving enzymes, von Willebrand factor proteinase, and a currently called 'orphan' sub-group whose physiological substrates have yet to be identified [65]. ADAMTSs modulate the female reproductive system and are involved in the physiology of pregnancy, angiogenesis, tissue remodelling, and tumour cell invasion and metastasis [16]. ADAMTSs are expressed in the trophoblast and associate with their invasiveness capacity of maternal tissues and spiral arteries remodelling. ADAMTS-12, a cartilage oligomeric matrix protein-cleaving enzyme, is needed for trophoblast cell adhesion to ECM substrate and for cell invasion via an avβ3 integrin-dependent mechanism that is independent of its proteolytic activity [77]. A recent study using the serum of patients with preeclampsia shows reduced level of ADAMTS-12 compared with the level detected in serum from women with normal pregnancies [78] (Table 1). Also, no differences between the sub-groups of early versus late onset preeclampsia in the level of this protein was found [78]. However, no significant variations were detected for ADAMTS-16 and ADAMTS-18, members of the 'orphan' sub-group, between preeclampsia and normal pregnancies [78]. These findings suggest that reduced invasiveness in preeclampsia may involve ADAMTS-12 indistinctly in early and late onset preeclampsia, conditions where the placental vasculature is dissimilarly altered [8].

The murine knockout of the procollagen N-propeptidase ADAMTS-3 was developed to evaluate the role of this protein in embryonic lymphangiogenesis and placental angiogenesis [79]. Lack of ADAMTS-3 caused massive lymphedema due to the absence of lymphatics development an abnormal blood vessel structure in the placenta and impaired liver development, a response likely due to the reduced activation of vascular endothelial growth factor C (VEGF-C). VEGF-C associates with lymphangiogenesis acting via its receptor VEGFR-3 on lymphatic endothelium promoting the cell survival, growth, and migration [79]. Since preeclampsia associates with impaired lymphangiogenesis it is proposed that ADAMTS-3 was playing a role in this disease in this experimental model.

The mRNA expression of the von Willebrand factor proteinase ADAMTS-13 in the placenta, its protein abundance and proteolytic activity are higher at the first trimester of pregnancy but lower at term in normal pregnancies [80]. Moreover, recombinant ADAMTS-13 protease stimulated the proliferation, migration, invasion, and network formation of the trophoblast [80]. In preeclampsia, ADAMTS-13 expression in the placental villous tissue is reduced, a phenomenon likely resulting from placental ischemia [80]. These results suggest a role of ADAMTS-13 protease in the pathogenesis of pregnancy-associated vascular remodelling in preeclampsia [80]. A recent study shows that protein level of the proteoglycanases ADAMTS-1, ADAMTS-4, and ADAMTS-12, as well as ADAMTS-13 were lower in maternal and umbilical cord blood but higher, except ADAMTS-13, in placental tissue from preeclampsia compared with normal pregnancies [81]. While these results provide insights for the role of ADAMTS in the pathogenesis of preeclampsia, large-scale studies are required to establish a conclusion on the role of ADAMTSs in the underlying mechanisms of preeclampsia.

3.5 Cathepsins

Cathepsins are a family of proteolytic enzymes with either serine, aspartic or cysteine protease activities [82]. They are synthesised as inactive proenzymes, glycosylated and delivered to the lysosome via mannose-6 phosphate receptors. Within the lysosome, the cathepsins are cleaved and activated following proteolysis [82]. These enzymes are regulated by several mechanisms, including zymogen processing, endogenous inhibitors (e.g. cystatins), pH, among others [82]. Under physiological conditions, the lysosomes release their content to the extracellular compartment [83] where a slightly alkaline extracellular pH (pHo ~7.2) predominates. Under this environmental condition most of the cathepsins are active, although they are less efficient, and their lifetime is limited due to unfolding-induced inactivation [84]. Cathepsins at the extracellular space mediate degradation of extracellular proteins modulating different cellular processes including inflammation and angiogenesis [85].

The cathepsin family members are expressed in human placental tissues with a tight temporal and spatial regulation [86]. Trophoblasts require proteases for their correct implantation [87], and in cases of recurrent spontaneous miscarriages, an elevation in the level of cathepsins B and H are seen in the placenta tissue and maternal serum [88]. Thus, deregulation of proteolytic activity may trigger the early termination of pregnancy. The role of cathepsins in the pathogenesis of preeclampsia is still under debate. A higher level of cathepsin B but lower level of cathepsin L in the maternal blood is described in preeclampsia [86, 89] (Table 1). Moreover, it has been proposed that the level of cathepsin B in combination with PlGF and bone morphogenetic protein (BMP) in maternal plasma could be potential predictors of late-onset preeclampsia [90]. Cathepsin B is expressed in macrophages, a cell-type enriched in lysosomes [91]. Thus, higher cathepsin B expression

could be consequence of the inflammatory processes associated with preeclampsia. Cathepsin L expression is confined to extravillous cytotrophoblast columns suggesting that this enzyme may be involved in implantation [86] (Figure 1). Cathepsin D is expressed in endo-lysosomes compartments at implantation sites of giant trophoblast cells [92]. Cystatin C, an inhibitor of cathepsins B and L, is likely synthesised in the decidua stroma controlling the activities of both proteinases at the implantation site [93]. Several studies show that cystatin C level is elevated in individuals with preeclampsia compared with normal pregnancies [94, 95], and this phenomenon is proposed as an early biomarker for preeclampsia by itself and in combination with other markers [96].

Lysosome biogenesis is transcriptionally coordinated by the transcription factor EB (TFEB) [97]. TFEB also controls the secretion of lysosomes and their components to the extracellular compartment, including cathepsins [97]. TFEB is expressed at a low level in the embryo but a higher level in the labyrinthine trophoblast cells of the mouse placenta suggesting a role during vascular remodelling of mouse placenta vessels [98]. Some studies show that deregulation in TFEB is present in pathological conditions coursing with abnormal placenta function and structure. A microarray analysis performed in placentas from preeclampsia show reduced TFEB transcript level compared with placentas from normal pregnancies. Unexpectedly, the level of some of the TFEB target genes, such as *GBA* (for acid β-glucosidase), were upregulated in preeclampsia [99]. The authors proposed that increased *GBA* expression might relate to placentation through increased vascularisation as a result of downregulation of TFEB expression [71]. Thus, TFEB may be a potential drug target for many disorders since its capacity to control lysosomal-related processes [100]. Whether these drugs are useful for treating vascularisation abnormalities in preeclampsia is not yet addressed.

6. Concluding remarks

Shallow EVTs invasion and incomplete remodelling of the spiral arteries is a primary event in preeclampsia. This phenomenon leads to poor placental perfusion and subsequent widespread of maternal endothelial dysfunction and adverse outcomes for the newborn and mother. The proteases MMPs, ADAMs, ADAMTSs, and cathepsins expressed by both invasive EVTs as well as maternal decidua cells are crucial for a correct EVTs invasiveness and placental vascular remodelling. It is intriguing that preeclampsia associates with downregulated expression and reduced activity of these proteases in the foetoplacental unit. Equally, they are involved in the abnormally elevated release of the anti-angiogenic factor sEng and sFlt1 to the maternal and foetal circulation. These findings may show a crossroad on how the activity of the same proteases is diminished in the trophoblast reducing their invasive capacity. A potential explanation for this phenomenon may arise from the differential distribution of these molecules and their inhibitors TIMPs and RECK. Placental distribution and abnormal expression level of cathepsins in placentas from preeclampsia suggest a critical role for these proteases in normal placentation as well as in the aetiology of this syndrome. Evaluation of the plasma level of MMPs, ADAMs, ADAMTSs and cathepsins is proposed as a tool to predict preeclampsia in pregnant women with a high risk of developing this syndrome. However, the relevance of serum MMPs and the other proteases in mediating the increase in the blood pressure and other features of preeclampsia remains unclear.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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Disclosures

None.

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Figure 1. Metalloproteases involvement in decidua-extracellular matrix remodelling by the trophoblast in preeclampsia. Preeclampsia associates with defective invasion of the trophoblast through the decidua and the subsequently altered remodelling of the maternal spiral arteries. This phenomenon may in part be explained by reduced (red ⇩) or increased (green ⇧) expression and activity of matrix metalloproteases (MMPs), a-disintegrin and metalloproteases (ADAMs), a-disintegrin and metalloprotease with thrombospondin motifs (ADAMTSs), and cathepsins in the human placenta. Increased expression and activity of the metalloproteases inhibitors (TIMP-1, TIMP-2, RECK, and Cystatin C) is also described in the placenta from this syndrome. The inhibitory actions (segmented red arrows) of RECK is on MMPs (MMP-2, MMP-14) and ADAMs (ADAM-10, ADAM-17), TIMPs inhibition is on MMPs (MMP-2, MMP-9, MMP-14), and cystatin C inhibition on cathepsins (cathepsin L, cathepsin B). TIMP-1, tissue inhibitor of metalloproteinase 1; TIMP-2, tissue inhibitor of metalloproteinase 2; RECK, reversion-induced-cysteine-rich-protein with Kazal motifs. Composed from data in references quoted in the body text and Table 1.

Table 1. Proteases expression and function in preeclampsia.

Proteases	Effect of preeclampsia	Cell/tissue	Studied phenomena	Type of preeclampsia	Potential consequence in preeclampsia	References
MMPs						
MMP-1	Decreased (protein)	Cytotrophoblast; syncytiotrophoblast	Trophoblast invasion Cell survival	Severe	Reduced trophoblast invasion; increased apoptosis of decidua cells	[101]
MMP-2	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	Mild and severe	Reduced trophoblast invasion in response to miR-106b	[102]
MMP-2	Decreased (mRNA)	Placental villous tissue	Trophoblast invasion	Late-onset	Reduced trophoblast invasion; potential lower MMP-2 mRNA expression due to increased H3K9me3 on its promoter	[64]
MMP-2	Decreased (mRNA)	Maternal superficial tissue of the placenta	Trophoblast invasion Cell survival	Severe	Reduced trophoblast invasion; increased apoptosis; reduced level of the MMP-2 expression inducer Stat3	[103]
MMP-2	Decreased (protein)	Cytotrophoblast	Trophoblast invasion	Early-onset	Reduced trophoblast invasion; prostasin-dependent suppression of MMP-2 release	[104]
MMP-2	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	Severe	Reduced trophoblast invasion; increased AP-2α–dependent suppression of MMP-2 expression	[105]
MMP-2	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	*ns*	Reduced trophoblast invasion	[102]

MMP-2	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	*ns*	Reduced trophoblast invasion; increased MMPs inhibitor TIMP-1 expression; increased metastasis suppressor gene KiSS-1 expression	[106]
MMP-3	Decreased (protein)	Placental tissue	Trophoblast invasion	Severe early-onset	Reduced trophoblast invasion; increased LIF signalling	[107]
MMP-7	Decreased (protein)	Placental tissue	Trophoblast invasion	Severe early-onset	Reduced trophoblast invasion; increased LIF signalling	[107]
MMP-8	Decreased (protein)	Placental villous tissue	Trophoblast invasion	Mild	Reduced trophoblast invasion; increased TIMP-1 and TIMP-3	[14]
MMP-9	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	Mild and severe	Reduced trophoblast invasion; reduced Rac1 activity	[108]
MMP-9	Decreased (mRNA)	Placental villous tissue	Trophoblast invasion	Late-onset	Reduced trophoblast invasion; potential lower MMP-9 mRNA expression due to increased H3K9me3 and H3K9/27me3 on its promoter	[64]
MMP-9	Decreased (protein)	Cytotrophoblast	Trophoblast invasion	Early-onset	Reduced trophoblast invasion; higher prostasin-dependent suppression of MMP-9 release	[104]
MMP-9	Decreased (protein)	Syncytiotrophoblast	Trophoblast invasion	Mild and severe	Reduced trophoblast invasion; reduced KLF-8 expression	[109]

MMP-9	Decreased (mRNA, protein)	Placental tissue and trophoblast	Trophoblast invasion	Severe	Reduced trophoblast invasion; increased AP-2α–dependent suppression of MMP-9 expression	[105]
MMP-9	Increased (mRNA)	Chorionic villi	Shallow placentation; EVTs invasion	*ns*	Reduced EVTs invasion; increased demethylation of MMP-9 promoter	[110]
MMP-9	Decreased (protein)	Trophoblast, decidua and stromal cells	Trophoblast invasion; placentation	Severe	Reduced trophoblast invasion; reduced placentation	[48]
MMP-9	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	*ns*	Reduced trophoblast invasion	[102]
MMP-9	Decreased (mRNA, protein)	Placental tissue	Trophoblast invasion	*ns*	Reduced trophoblast invasion; increased MMPs inhibitor TIMP-1 expression; increased metastasis suppressor gene KiSS-1 expression	[106]
MMP-9	Decreased (protein)	Placental extract	Trophoblast invasion	*ns*	Reduced trophoblast invasion	[111]
MMP-11	Decreased (protein)	Placental tissue	Trophoblast invasion	Mild	Reduced trophoblast invasion; increased TIMP-1 and TIMP-3	[14]
MMP-14	No change (protein)	Placental tissue	Syncytiotrophoblast-associated gelatinase activity	Severe	Reduced total gelatinase activity in the syncytiotrophoblast	[12]
MMP-14	No change (protein)	Placental tissue	Tissue association of MMP-14 and endoglin	Severe	Endoglin proteolysis by MMP-14	[51]

ADAMs						
ADAM-10	Increased (protein)	Placental tissue, cultured placental cells	Processes Flt-1 to sFlt-1	Early-onset	Increased sFlt-1 release; H_2S–dependent suppression of pro-form and active ADAM-10 expression	[74]
ADAM-10	Increased (protein)	Placental tissue	Processes cMet to sMet	Severe	Downregulation of HGF/cMet signalling	[112]
ADAM-12	Increased (mRNA)	Placental villi	Bioavailability of IGF-I and IGF-II	*ns*	Increased IGF-I and IGF-II due to proteolytic activity against IGFBP-3	[73]
ADAM-17	Increased (protein)	Placental tissue	Processes cMet to sMet	Severe	Downregulation of HGF/cMet signalling	[112]
ADAM-17	Increased (protein)	Placental tissue	Processes proTNFα to sTNFα	Early and late-onset	Increased sTNFα	[71]
ADAMTSs						
ADAMTS-1	Increased (mRNA, protein)	Placental tissue	Anti-angiogenic function; implantation and placentation; continuation of pregnancy; gestational trophoblast disease	Mild	Anti-angiogenic	[81]
ADAMTS-1	Decreased (Protein)	Maternal and cord blood	Expression	Mild	-	[81]
ADAMTS-4	Decreased (Protein)	Maternal and cord blood	Expression	Mild	-	[81]
ADAMTS-4	Increased (mRNA, protein)	Placental tissue	Antiangiogenic function; gestational trophoblast	Mild	Anti-angiogenic	[81]

			disease			
ADAMTS-12	Decreased (protein)	Maternal blood serum; EVTs	Cell invasion	*ns*	-	[77, 78, 113]
ADAMTS-12	Increased (mRNA, protein)	Placental tissue	Trophoblast invasion	Mild	-	[81]
ADAMTS-13	Decreased (mRNA, protein)	Villous cytotrophoblast; syncytiotrophoblast; EVTs; foetal endothelium; stromal cells in the villous core	Trophoblast proliferation; placenta angiogenesis	Severe	Reduced trophoblast proliferation; reduced angiogenesis; hypoxic tissue	[80, 81]
Cathepsins						
Cathepsin B	Increased (mRNA, protein)	Villous and decidual macrophages; syncytiotrophoblast; EVTs; maternal blood serum	Angiogenesis	Early and late onset	Reduced angiogenesis by reducing VEGF mRNA expression	[86, 88, 89]
Cathepsin C	Increased (protein)	Maternal vascular endothelium	Vasoactive peptide synthesis	Late onset	Increases vasoactive peptide synthesis via chymase activation	[114]
Cathepsin D	Decreased (mRNA, protein)	Chorionic villous; maternal blood serum	Trophoblast invasion	Early onset	*ns*	[115, 116]
Cathepsin K	Increased (mRNA)	Chorionic villous	*ns*	Early onset	*ns*	[86]
Cathepsins F, L, and L2	Decreased (mRNA)	Extravillous cytotrophoblast columns	Trophoblast invasion	Early and late onset	*ns*	[86]

Legend for Table 1 in the next page.

Legend for Table 1

MMPs, matrix metalloproteases; ADAMs, a-disintegrin and metalloproteases; ADAMTSs, a-disintegrin and metalloprotease with thrombospondin motifs; miR-106b, micro RNA 106b; H3K9me3, histone trimethylation at lysine 9; H3K9/27me3, histone trimethylation at lysine 9 and 27; Stat3, signal transducer and activator of transcription; AP-2a, activator protein-2a; TIMP-1, tissue inhibitor of metalloproteinase 1; TIMP-3, tissue inhibitor of metalloproteinase 3; KiSS-1, kisspeptins-1; LIF, leukemia inhibitor factor; Rac-1, Ras- related C3 botulin toxin substrate 1; KLF-8, krüppel-like factor 8; EVTs, extravillous trophoblast; Flt-1, fms related tyrosine kinase 1; sFlt-1, soluble Flt-1; H_2S, hydrogen sulfide; cMet, hepatocyte growth factor receptor; sMet, soluble cMet; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor I; IGF-II, insulin-like growth factor II; IGFBP-3, insulin-like growth factor-binding protein 3; proTNFa, transmembrane tumour necrosis factor; sTNFa, soluble TNFa; VEGF, vascular endothelial growth factor. *ns*, not specified.

Highlights

1- Preeclampsia characterises by adverse maternal and newborn outcome.

2- Placenta MMPs, ADAMs, ADAMTSs, and cathepsins regulate vascular remodelling.

3- MMPs inhibitors TIMPs and RECK activity is higher in placentas from preeclampsia.

4- Deregulation of placental MMPs inhibitors and proteases is critical in preeclampsia.

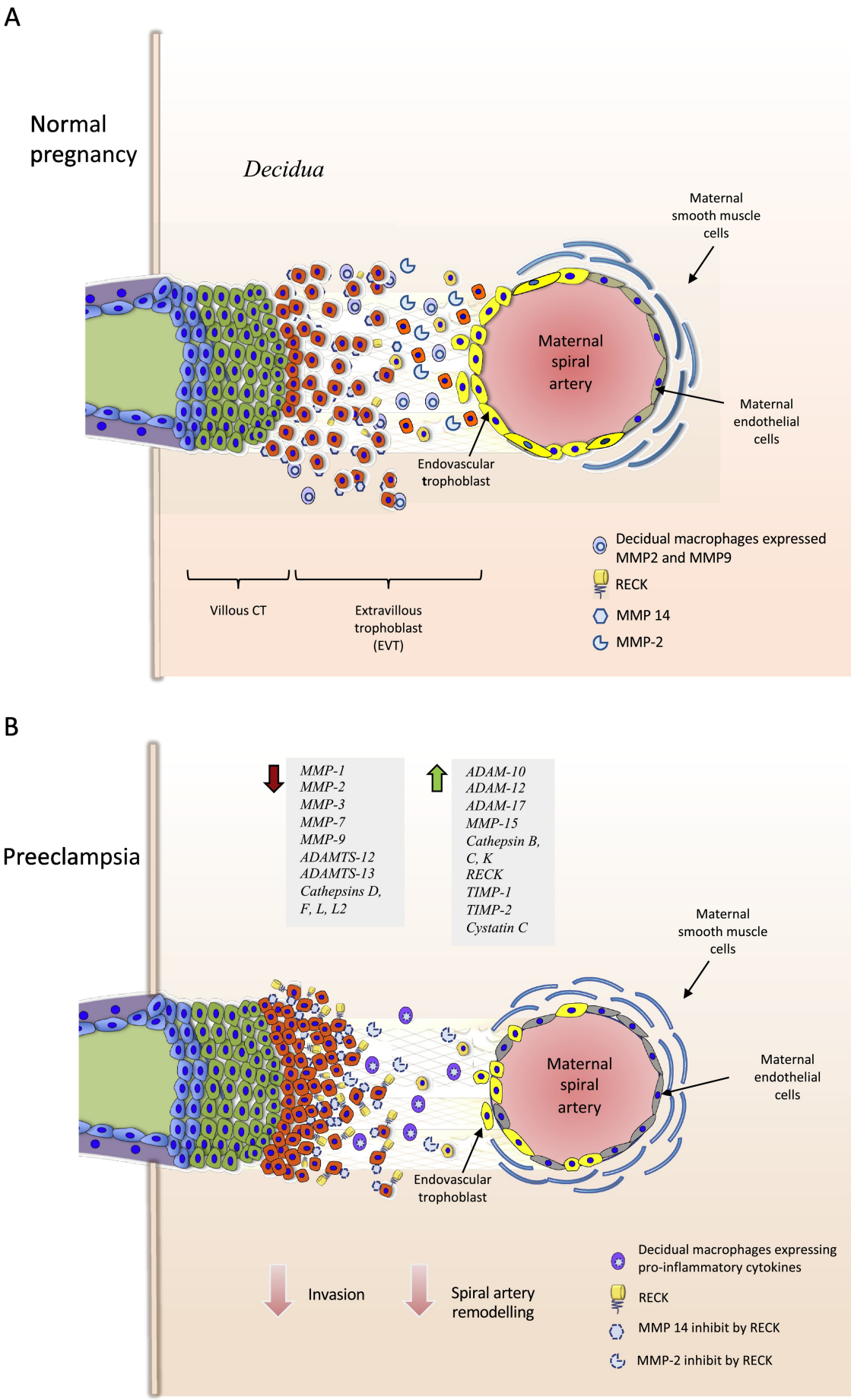
A
Normal pregnancy
Decidua
Maternal smooth muscle cells
Maternal spiral artery
Maternal endothelial cells
Endovascular trophoblast
Villous CT
Extravillous trophoblast (EVT)
Decidual macrophages expressed MMP2 and MMP9
RECK
MMP 14
MMP-2
B
Preeclampsia
MMP-1
MMP-2
MMP-3
MMP-7
MMP-9
ADAMTS-12
ADAMTS-13
Cathepsins D, F, L, L2
ADAM-10
ADAM-12
ADAM-17
MMP-15
Cathepsin B, C, K
RECK
TIMP-1
TIMP-2
Cystatin C
Maternal smooth muscle cells
Maternal spiral artery
Maternal endothelial cells
Endovascular trophoblast
Invasion
Spiral artery remodelling
Decidual macrophages expressing pro-inflammatory cytokines
RECK
MMP 14 inhibit by RECK
MMP-2 inhibit by RECK

Figure 1