Regulation of Trophoblast Invasion: The Role of Matrix Metalloproteinases

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Pregnancy success is determined by a complex progress that includes trophoblast invasion and placentation. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are metal-dependent endopeptidases capable of degrading extracellular matrix, and appear to play a critical role in trophoblast invasion. This article reviews in detail the role of MMPs, TIMPs, and their regulators in the mechanism of trophoblast invasion in early human pregnancy.

[Rev Obstet Gynecol. *2012;5(3/4):e137-e143 doi: 10.3909/riog0196]*

KEY WORDS

Trophoblast invasion • Placenta • Matrix metalloproteinases • Tissue inhibitors of metalloproteinases

Invasion of trophoblast cells into the endometrial stroma and inner third of the myometrium is an essential process for the development of the definitive maternal-fetal circulation and for pregnancy success in humans. Between 8 and 18 weeks of gestation, some invading extravillous cytotrophoblast (EVCT) cells depart from the anchoring villi and migrate into the maternal decidua, reaching all the way through to the inner third of the myometrium; these are known as interstitial EVCT cells.[1] Other invading trophoblasts penetrate the maternal spiral arteries and migrate proximally against the direction of blood flow forming intravascular islands and mosaic vessels as they replace the maternal vascular endothelium with a pseudo-endothelium of fetal origin; these are known as endovascular EVCT cells.[2] This vascular remodeling of the 120 to 140 spiral arteries that supply a given placenta, which occurs in early pregnancy, is critical to establish the definitive uteroplacental circulation, which needs to dilate enormously to

accommodate one-fifth of the cardiac output at term.

Trophoblast implantation and malignant tumors use the same biochemical mediators to facilitate invasion. These include the expression of proteases such as matrix metalloproteinases (MMPs) that degrade the extracellular matrix, telomerase activity, and immunosuppressive environmental conditions. As such, trophoblast has been termed a *pseudomalignant* tissue. However, there are significant differences between these two model systems. Trophoblast cell invasion is initiated immediately after embryo implantation and is precisely regulated by a series of integrated adhesion and signaling events. It is controlled both temporally and spatially, occurs only during the first and early second trimesters of pregnancy, and does not generally go beyond the myometrium; tumor invasion, on the other hand, is uncontrolled and does not respect tissue boundaries.[3-5] Temporal control results in a peak of trophoblast penetration into the maternal tissues of the uterus at around week 12 of gestation and declines thereafter. Spatial control restricts the depth of trophoblast invasion to the decidua and the inner third of the myometrium.[6]

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Dysregulation of the finely controlled process of trophoblast invasion can lead to a wide spectrum of pregnancy abnormalities.[7-10] Excessively shallow invasion has been implicated in fetal intrauterine growth restriction (IUGR) and preeclampsia. Preeclampsia, one of the most common pregnancy complications, is characterized by disturbed and inadequate remodeling of the maternal spiral arteries by invading trophoblast cells, thus reducing blood flow to the intervillous space. Insufficient conversion of the spiral arteries into low-resistance, high-capacity vessels in early pregnancy leads to systemic hypertension and fetal hypoxia in later pregnancy as the fetus and placenta outgrow their blood supply, features often observed in preeclampsia. In contrast, excessive invasion can result in abnormally deep uteroplacental infiltration leading to placenta accreta, increta, or percreta (depending on the depth of invasion) and even choriocarcinoma. Proper trophoblast invasion is therefore of paramount importance for maternal health and adequate growth and development of the fetus.

The precise molecular mechanisms that regulate trophoblast invasion during gestation and its relationship to fetoplacental development are largely unknown, but several proteinases, cytokines, and growth factors appear to be involved. MMPs are metal-dependent endopeptidases capable of degrading extracellular matrix. MMPs and their regulators, including tissue inhibitors of metalloproteinase (TIMPs), appear to play a critical role in mediating trophoblast invasion.[6-9] This article reviews in detail the role of the MMPs, TIMPs, and their regulators in the mechanism of trophoblast invasion at the maternal-fetal interface.

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Role of MMPs and TIMPs in Implantation

MMPs, also called matrixins, are a family of at least 17 zinc-dependent endopeptidases, which are important proteases in many biological processes (Table 1). The various members of the MMP family degrade different components of the extracellular matrix, including collagenases (MMP-1, MMP-4, MMP-8), stromelysins (MMP-3, MMP-10, MMP-11), and gelatinases (MMP-2, MMP-9). The evolving literature suggests that MMPs and their regulators control many aspects of reproductive function, including follicular development, ovulation, menstruation, implantation, and parturition.

The regulation of MMP activity at the maternal-fetal interface appears to be critical for successful implantation and placentation. Trophoblast cells constitutively produce MMPs and are thus invasive by nature.[10] Interestingly, according to numerous studies using animal models, most MMP subtypes are expressed not only by invading trophoblast cells, but also by endometrial stromal cells and natural killer (NK) cells within the maternal tissues of the uterus (with the noted exception of MMP-20 and MMP-25, which are expressed only in EVCT cells).[11] Indeed, studies looking systematically at MMP messenger RNA (mRNA) and protein expression throughout gestation suggest that decidual stromal cells have higher levels of MMP expression than do trophoblast cells, and the susceptibility of the decidua to invasion seems to be increased in presence of cytotrophoblast cells.[12] Regional differences in MMP expression have also been demonstrated. For example, expression of MMP-2 and -9 has been localized most strongly to the placental bed in early pregnancy—primarily to

TABLE 1

Classification of Matrix Metalloproteinases

Subfamily	MMP	Other Names	MW	Substrates
Gelatinases	MMP-2	Gelatinase A, 72 kDa gelatinase	73,882	Col IV, V, VII, X, gelatin, fibronectin, elastine
	MMP-9	Gelatinase B, 92 kDa gelatinase	78,427	Col IV, V, gelatin
Collagenases	MMP-1	Interstitial collagenase, fibroblast collagenase	54,007	Col I, II, III VII, X, MMP-5, entactin
	MMP-8	Neutrophil collagenase, PMNL collagenase	53,412	Col I, III
	MMP-13	Collagenase-3	53,819	Col I
Stromelysins	MMP-3	Stromelysin-1, transin-1	53,977	Col III, IV, IX, X, gelatin, laminin, fibronectin, elastine, casein
	MMP-7	PUMP-1, matrilysin	29,677	Casein, fibronectin, gelatin
	MMP-10	Stromelysin-2, transin-2	54,151	Col II, IV, V, fibronectin, gelatin
	MMP-11	Stromelysin-3	54,595	Col IV
	MMP-12	Metalloelastase	54,000	Elastine, fibronectin
Membrane Bound	MMP-14	MT1-MMP, MP-X1	65,883	MMP-2
	MMP-15	MT2-MMP	75,807	MMP-2
	MMP-16	MT3-MMP	69,158	MMP-2
	MMP-17	MT4-MMP		

Col, collagen; MMP, matrix metalloproteinases; MT, membrane type; MW, molecular weight; PMNL, polymorphonuclear leucocyte; PUMP, punctuated metalloproteinase.

EVCT cells at 6 to 8 weeks of gestation—and these proteins appear to regulate trophoblast invasion.[13] As pregnancy progresses, trophoblast expression of pro-MMP-3 and active MMP-13 and MMP-23 is downregulated, whereas the proforms of MMP-8, MMP-19 and MMP-23, active forms of MMP-9, MMP-10, MMP-12, MMP-15, MMP-16, MMP-26, and MMP-28, and both pro- and active forms of MMP-14 are increased.[14] Differential MMP expression has also been demonstrated before and after labor.[15,16] Moreover, aberrant MMP expression has been implicated in pregnancy abnormalities, including IUGR and preeclampsia.[17,18]

MMP activity in any given tissue is a function of MMP gene expression, mRNA translation, and the action of various regulators of MMP action. MMP regulators, such as TIMPs, exert their affect either directly by binding to MMPs or indirectly by activating nuclear transcription factors that control the expression of select MMP genes. Appropriate trophoblast invasion and vascularization requires a functional synergism between MMPs and their regulating factors. For example, trophoblast invasion can be increased by either upregulating MMP expression or downregulating TIMP expression.[19]

MMPs are inhibited by TIMPs, which are composed of a family of four endogenously expressed extracellular proteins (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) that act as specific protease inhibitors.[20] Typically, TIMPs inhibit MMPs once they are activated by binding to the highly conserved zinc-binding site of active MMPs. Among the TIMP family, TIMP-1 preferentially inhibits MMP-9.[21] In some cases, however, MMPs form complexes with TIMPs while they are still in their latent form. For example, the complex of pro-MMP-2 and TIMP-2 serves to promote the activation of pro-MMP-2 at the cell surface by the membrane-bound MMP, MMP-14.[22] A decrease in TIMP-2 expression leads to a reduction in MMP-2/TIMP-2/MMP-14 complex

formation and an inhibition of trophoblast invasion.[13] By regulating pro-MMP activation, extracellular matrix turnover, cell proliferation, apoptosis, and angiogenesis through both MMP-dependent and MMP-independent pathways, TIMPs serve important roles in numerous physiological processes including embryo implantation, reproductive tissue remodeling, and wound healing.[23] Differential expression of TIMPs has also been shown in various cancer cells,[23] suggesting that these proteins may also be important in cancer invasion and metastasis. Reverse transcriptase polymerase chain reaction analysis has detected mRNA expression of all four TIMPs in first trimester cytotrophoblast cells as well as endometrial stromal cells, uterine NK cells, and myofibroblasts.[14]

Trophoblast invasion in the first trimester is regulated both temporally and spatially.

Regulation of MMP and TIMP Expression

Trophoblast invasion in the first trimester is regulated both temporally and spatially. This appears to be mediated both in an autocrine fashion by trophoblastic factors and in a paracrine fashion by uterine factors. A number of factors have been shown to regulate the synthesis, activation, and/or secretion of MMPs and TIMPs at the maternal-fetal interface, including a variety of cytokines, chemokines, growth factors, hormones, and oxygen tension.[24,25]

Urokinase Plasminogen Activator System

The urokinase plasminogen activator (uPA) system—which includes uPA, uPA receptor (uPAR), and two major uPA inhibitors (PAI-1 and PAI-2)—has a broad spectrum of substrates and is involved primarily in tissue remodeling. On binding to uPAR on the cell surface, pro-uPA is cleaved to uPA through a mechanism that likely involves redox and extracellular signal-regulated kinase (ERK) signal transduction pathways,[26] which then binds plasminogen and converts it into the active protease, plasmin. Plasmin, in turn, acts both directly as a proteolytic enzyme for various components of the extracellular matrix and indirectly by converting pro-MMPs to active MMPs. Plasminogen activator inhibitor-1 (PAI-1) acts to inhibit the activation of plasminogen to plasmin, and prevents pro-MMPs being converted to active MMPs by binding directly to the uPA/uPAR complex. Both uPA and uPAR are expressed by trophoblast cells and appear to promote localized matrix proteolysis as trophoblast invasion commences; this activity is activated by MMPs and inhibited by PAI-1, both of which are produced locally.[27] Mice deficient in PAI-1 exhibit a reduction in both maternal and fetal vascularization within the placenta and increased trophoblast cell density.[28] Abnormalities in PAI-1 expression have also been documented in patients with recurrent pregnancy loss, early preeclampsia, and HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelet count).[29-31] Mammalian target of rapamycin, a member of the phosphoinositide kinase-related kinase family, has also been shown to regulate uPA expression and activity in trophoblast cells, likely through the Janus kinase-signal transducer and activator of transcription second messenger pathway.[32]

Cytokines and Growth Factors

The molecular mechanisms that control trophoblast invasion are incompletely understood, but likely involve cytokines and growth factors. In addition to TIMPs, the expression of MMP genes is transcriptionally also regulated by a variety of extracellular agonists, including epidermal growth factor (EGF), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), angiogenic factors, transforming growth factor-β, and interleukins (ILs). For example, EGF induces the expression of critical invasion-promoting genes such as MMP-2 and uPA by stimulating the express of small hairpin microRNAs.[33] ERKs and p53 pathways have also been shown to play important roles in EGF-mediated regulation of trophoblast growth and migration.[34-36]

Placental growth and development are characterized by intense vascular remodeling and angiogenesis. This coordinated process requires the expression of vascular endothelial growth factor (VEGF) and other proangiogenic growth factors such as placental growth factor by invasive EVCT cells.[37] Attenuation of proangiogenic signaling, for example, by placental secretion of excessive amounts of soluble receptor such as the soluble VEGF receptor-1—also known as soluble Fms-related tyrosine kinase-1 (sFlt-1)—is well documented in preeclampsia. Over-expression of sFlt-1 in animal models results in a syndrome that closely mimics preeclampsia,

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including gestational hypertension, proteinuria, and glomerular endotheliosis.[38] In contrast, women with placenta previa and invasive placentation (placenta accreta, increta, or percreta) have lower circulating levels of VEGF, which has been attributed to a decrease in MMP-2 and MMP-9 production by invading trophoblast cells.[39] Recent studies have identified an additional angiogenic factor in placenta, known as endocrine gland-derived VEGF (EG-VEGF), which is expressed in high levels in early pregnancy, but whose expression falls after 11 weeks. Interference in EG-VEGF signaling by altering expression of EG-VEGF receptors (PKR1, PKR2) in the placenta suggests that EG-VEGF inhibits the ability of EVCT cells to invade and form tube-like structures by decreasing MMP-2 and MMP-9 production.[39]

Preimplantation factor (PIF) is a peptide that is secreted from human embryos and placental tissues, and is present in the maternal circulation. PIF appears to modulate local immunity, promote the expression of adhesion molecule expression within the maternal decidua, and enhance trophoblast invasion.[39] Exactly how PIF functions at a molecular and cellular level remains unclear.

A number of other cytokines and growth factors, many of which are produced by uterine NK cells, may be involved in regulating MMP-3 and MMP-9 expression and trophoblast invasion.[40] For example, TNF-α decreases villous cytotrophoblast cell proliferation, and increases apoptosis and expression of pro-MMP-9, uPA, and PAI-1 by EVCT cells. Moreover, the combination of TNF-α and IFN-γ acts synergistically to further promote EVCT apoptosis and inhibit EVCT proliferation by affecting the production of both pro-MMP-2 and uPA.[34] Similarly, TNF-α inhibits the invasion of trophoblast-like cells (JEG-3) into maternal endothelial cellular networks in vitro, a process that involves the inhibition of MMP-2.[9] The synergy between TNF-α and IFN-γ suggests that the local cytokine milieu may be critical in determining the spatial and temporal changes that affect EVCT invasion.

Other inflammatory mediators also appear to be important in mediating trophoblast invasion. Lipopolysaccharide increases IL-8 and IL-6 production, and decreases EVCT cell invasion through activation of mitogen-activated protein kinase/MEK signaling pathways.[41] Treatment of extravillous trophoblast JEG-3 cells with IL-11 leads to an increase in migration and invasion across extracellular matrices, which is mediated through activation of intracellular STAT3[42,43] and possibly by altering the expression of glucose-related protein or the isomerase enzyme, GRP78.[44,45] A similar effect is seen with IL-6, although this appears to be mediated at least in part by activation of select integrin isoforms.[46] IL-10, a key immunosuppressant molecule, is increased in early pregnancy and remains elevated until the onset of labor and appears to induce the expression of a number of MMPs at a transcription level.[4,47]

Insulin-Like Growth Factor

During implantation, endometrial stromal fibroblasts transform morphologically and biochemically into secretory cells and begin to express decidual proteins such as insulin-like growth factor (IGF) and IGF binding protein-1.[48,49] IGF-II has been shown to promote placental trophoblast invasion, proliferation, and maturation via a complex interaction with the IGF-II receptor, uPA, and plasminogen, which is further enhanced by low oxygen tension.[49]

Hormones

Human chorionic gonadotropin (hCG) is critical to early pregnancy success because it regulates the initial stages of implantation, decidualization, and placental development.[50] It appears to act in part by regulating the MMP/TIMP system at the fetal-maternal interface. Indeed, hCG has been shown to increase MMP-2 activity[51] and repress TIMP-1, TIMP-2, and TIMP-3 expression in decidualized endometrial stromal cells,[52] although these effects may wane with increasing gestational age.

Progesterone may also regulate trophoblast invasion, especially the process of vascular remodeling in early pregnancy. This is because progesterone stimulates PAI-1 and inhibits MMP-1, MMP-2, MMP-3, and MMP-9 expression in endometrial stromal cells.[53]

Gonadotropin-releasing hormone (GnRH) has also been shown to regulate MMP-2/MMP-9, TIMP-1, and uPA/PAI protease systems in trophoblast cells during placentation. In this regard, the GnRH-II isoform appears to be more potent than GnRH-I.[54] Both GnRH-I and -II appear to exert their effects on trophoblast invasion by activating protein kinase C and thereby ERK1/2 and c-Jun N-terminal kinase signal transduction cascades.[54]

Oxygen Tension

Higher oxygen tension may be an important factor that initiates primary cytotrophoblast cell differentiation along the invasive pathway.[49] This may explain why EVCT cells invade and remodel the maternal spiral arteries, but not veins. Early placentation (< 10-12 weeks of gestation) occurs in an environment characterized by relatively low concentrations of oxygen (17-19 mm Hg) as compared with the endometrium (39-40 mm Hg).[13] Maternal

hypoxia during the early stages of placentation activates the invasive endovascular trophoblast cell lineage and promotes uterine vascular remodeling in vivo.[54] Under hypoxic conditions, increased expression of pro-MMP-2 and decreased production of TIMP-2 in trophoblast leads to an increase in MMP-2 activity and an environment that favors degradation of type IV collagen, the major structural component of basement membranes.[13] Physiologic hypoxia may, therefore, be a key regulator of placental morphogenesis and function in both normal and pathological pregnancies.

Conclusions

During early pregnancy, controlled trophoblast cell invasion into the maternal endometrium is critical for successful implantation and placentation. Trophoblast cells derived from the trophectoderm, the outermost epithelial cell layer of the blastocyst, trigger attachment and implantation into the maternal endometrium through a complex series of interactions between various regulatory factors and the uterine microenvironment. Members of the MMP/TIMP system are present in high concentrations at the maternal-fetal interface and are important regulators of trophoblast invasion. The role of specific MMP/TIMP proteins may vary as the pregnancy progresses. An improved understanding of the molecular mechanisms that control trophoblast invasion and uterine spiral arteriole remodeling will help to identify the defects in these processes that contribute to such pregnancy complications as IUGR and preeclampsia. ■

This work was supported by a grant from the National Natural Science Foundation of China (No. 81170595) and the Dean's Foundation of Nanfang Hospital, Guangzhou, China (No. 2010Z010).

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MAIN POINTS

- Invasion of trophoblast cells into the endometrial stroma and inner third of the myometrium is an essential process for the development of the definitive maternal-fetal circulation and for pregnancy success in humans.
- Dysregulation of the finely controlled process of trophoblast invasion can lead to a wide spectrum of pregnancy abnormalities. Excessively shallow invasion has been implicated in fetal intrauterine growth restriction and preeclampsia, one of the most common pregnancy complications.
- The regulation of matrix metalloproteinase (MMP) activity at the maternal-fetal interface appears to be critical for successful implantation and placentation. MMP activity in any given tissue is a function of MMP gene expression, messenger RNA translation, and the action of various regulators of MMP action.
- Trophoblast invasion in the first trimester is regulated both temporally and spatially. This appears to be mediated both in an autocrine fashion by trophoblastic factors and in a paracrine fashion by uterine factors.
- Human chorionic gonadotropin is critical to early pregnancy success because it regulates the initial stages of implantation, decidualization, and placental development.

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