Cancer and Metastasis Reviews 9: 369–379, 1990.

Mechanisms of trophoblast invasiveness and their control: the role of proteases and protease inhibitors[1]

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Key words: choriocarcinoma, collagenase, decidua, invasion, laminin, metalloproteases, plasminogen activator, TGFβ, TIMP, trophoblast

Summary

Implantation and subsequent placental development in many species including the human are dependent on trophoblast invasion of the uterine epithelium, the underlying basement membrane, connective tissue and blood vessels. However, trophoblast invasion *in situ* is strictly controlled by the microenvironment provided by the pregnant uterus.

Key mechanisms underlying various steps in trophoblast invasion of basement membrane and stroma are similar to those identified in the case of invasive tumor cells: (a) attachment to basement membrane by binding to laminin and possibly other basement membrane components; (b) detachment from the basement membrane matrix prior to its penetration, a process that requires the presence of complex-type oligosaccharides on the cell surface; (c) breakdown of basement membrane components by trophoblast-derived metalloproteases (type IV and interstitial collagenase) and serine proteases (plasminogen activator). Type IV collagenase activity is stimulated by binding to laminin, a molecule also secreted by the trophoblast. Activation of trophoblast-derived metalloproteases appears to be plasmin-dependent. Plasmin results from the cleavage of plasminogen by trophoblast-derived plasminogen activator.

Control of trophoblast invasion *in situ* is mediated by decidua-derived transforming growth factor β (TGFβ) which in turn induces tissue inhibitor of metalloproteases (TIMP) both in the decidua and the trophoblast. We suggest that this control of trophoblast invasiveness is regulated both spatially as well as temporally during gestation. A preprogrammed decline in trophoblast invasiveness with increasing gestational age remains an additional possibility. The nature of the loss of control of trophoblast invasiveness in choriocarcinoma remains to be identified. Refractoriness to TGFβ action remains to strong possibility.

Introduction

The human placenta is an invasive organ analogous to a locally invasive tumor. This invasion is exercised by the trophoblast cells of the placenta. However, trophoblast invasion of the uterus is under strict control during normal pregnancy. The degree of placental invasion has been employed as a basis for classifying placentas in various mammalian orders [1]. Epitheliochorial type placentas (e.g. of ungulates inclusive of ruminants) are non-invasive, so that the chorionic trophoblast remain is in close apposition with an intact uterine epithelium. Endotheliochorial type placentas (e.g. of carnivores) exhibit erosion of the uterine epithelium and the subepithelial connective tissue, so that the trophoblast comes in contact with maternal blood vessels. In the more invasive hemochorial type placentas (e.g.

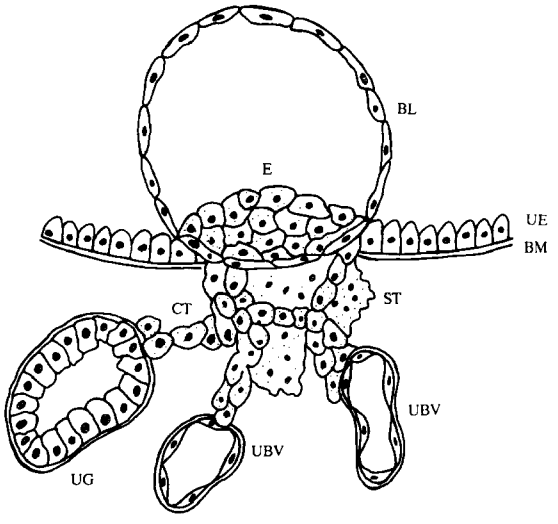

Fig. 1. Diagram illustrating trophoblast invasiveness during implantation and placental development. BL = blastocyst; E = embryo; UE = uterine epithelium; BM = basement membrane; CT = cytotrophoblast; ST = syncytiotrophoblast; UBV = uterine blood vessels; UG = uterine gland.

of certain rodents and anthropoids), the trophoblast erodes maternal blood vessels, so that it becomes directly exposed to the maternal blood in the placental sinusoids. In the present paper we shall discuss the mechanisms of trophoblast invasiveness and their control in hemochorial placentas of humans and mice. We shall show that these mechanisms which allow trophoblast invasion of the uterus are very similar to those used by invasive tumor cells and that the control of invasion *in situ* is primarily provided by the microenvironment of the pregnant uterus.

Trophoblast invasion *In vivo*

During implantation, prior to the development of the placenta, the outer cells of the blastocyst (destined to become placental trophoblast) breach the uterine epithelium and penetrate the basement membrane and the underlying connective tissue. Subsequently, during placental development, uterine glands and blood vessels are invaded (Fig. 1). Trophoblast invasion continues until about the mid-second week of gestation in the mouse. In humans, it continues until about the fourth month of gestation [2].

In a series of *in vivo* experiments, Denker investigated the role of degradative enzymes in the process of implantation in the rabbit and to a smaller extent in the cat [3]. He combined morphological, histochemical and biochemical approaches to show that blastocyst-associated proteases and glycosidases were necessary for trophoblast attachment to, penetration, as well as lysis of the uterine mucosa. The proteases included endopeptidases, thiol proteases, and serine proteases. Implantation was prevented by intrauterine application of trasylol (aprotinin), but not epsilon amino caproic acid (EACA), both chemical inhibitors of serine proteases.

In another series of experiments, Kirby [4–7] tested the *in vivo* invasiveness of the mouse trophoblast in orthotopic and ectopic sites. This invasion was histologically evaluated following transplantation to a variety of sites such as underneath the kidney capsule, the testes, the spleen, the liver, the brain, as well as the cycling non-pregnant and pseudopregnant uteri. Results revealed that the extent and duration of invasion were greater in extrauterine sites and non-pregnant uteri than in pseudopregnant or pregnant decidualized uteri. These findings suggested that trophoblast cells were inherently invasive and that the invasion was controlled by the decidua. The possibility that circulating pregnancy hormones played a major role in controlling invasion was excluded since invasiveness of trophoblast in extrauterine sites remained uninfluenced by pregnancy. In humans, a higher degree of trophoblast invasion is associated with ectopic pregnancy and placenta accreta, conditions which are associated with poor decidual response [8]. These findings are consistent with the hypothesis of decidual control of invasion. While chemical [7], physical [8, 9] as well as immunological [8] processes have been proposed for the putative antiinvasive role of the decidua, the precise mechanisms remained to be identified. Studies in this area are highly relevant for an understanding of uteroplacental homeostasis. A strictly controlled and balanced degree of trophoblast invasion of the

uterus is essential during normal pregnancy. Poor invasiveness would result in inadequate feto-maternal exchange, whereas excessive invasiveness may result in pathological destruction of the uterus.

Mechanisms of trophoblast invasiveness: *In vitro* assays

Mechanisms of cellular invasiveness have been extensively analyzed *in vitro* using a variety of invasive tumor lines. Penetration of one or more basement membranes and the underlying connective tissue stroma is a prerequisite for local invasion as well as for metastasis by tumor cells. In pregnancy as well, erosion of the basement membrane of the uterine epithelium is essential for implantation. Invasion of the underlying stroma and uterine blood vessels, inclusive of their basement membranes, is also required for establishment of feto-maternal exchange in the hemochorial placenta. Thus, the ability to penetrate basement membranes serves as a reliable marker for cellular invasiveness.

A variety of *in vitro* invasion assays for cancer cells have been designed. These have employed numerous natural basement membrane-containing substrates [10–12] as well as artificially-reconstituted basement membranes [13]. Most basement membranes are composed of collagen type IV, laminin, fibronectin, heparan sulphate proteoglycan, entactin and nidogen [12]. It has been shown that invasive tumor cells must first attach to the basement membrane via a class of laminin receptors [12] which does not belong to the integrin family. More recently, integrin-related receptors for laminin and collagen type IV have also been implicated in this attachment [14]. Laminin binding may also be important for trophoblast cell attachment to the basement membrane, since trophoblast cells attach better on laminin-coated substrates [15]. Moreover, invasive tumor cells [12] as well as trophoblast cells [16] have been shown to produce laminin, which may facilitate this interaction.

Subsequent invasion of the basement membrane depends on two simultaneous events: migration of the invasive cell and breakdown of the various basement membrane components [12]. Ability to detach and migrate has been positively correlated with the presence of sialylated β1–6 branched complex-type ASn-linked oligosaccharides on certain cancer cells with invasive phenotype [17]. These molecules may reduce adhesive interaction between the cell (e.g. the cell surface integrins) and the matrix. A similar correlation has also been documented for first trimester human trophoblast cells [18]. Culturing the cells in the presence of swainsonine (an inhibitor of Golgi α mannosidase II) which inhibits the complex branching of terminal sugars blocked the detachment ability of tumor and first trimester trophoblast cells. Consequently their ability to invade the basement membrane was also prevented [17, 18].

The invasive ability of cancer cells *in vitro* has been correlated with the production and activation of numerous enzymes capable of lysing basement membrane components: metalloproteases capable of degrading collagen [11, 13, 19, 20]; serine proteases, e.g. plasminogen activators and plasmin, the latter being capable of activating procollagenases and degrading laminin [11, 13, 19]; cysteine proteases capable of cleaving laminin, fibronectin and to some extent collagen [21]; and heparan sulphate endoglycosidases capable of degrading heparan sulphate proteoglycans [22]. We shall show that most or all of these mechanisms are also employed by the trophoblast during invasion of the basement membrane and the underlying stroma.

Matrix degradation by the peri-implantation trophoblast

Investigations on the mechanisms of invasion by the peri-implantation trophoblast (derived from the outer cells of the blastocyst, see Fig. 1) have been limited by the difficulty in obtaining sufficient amount of embryonic material. Proteolytic activity has been demonstrated in guinea pig and rat blastocysts [23]. Glass *et al.* [24] observed that *in vitro* trophoblast outgrowths from peri-implantation murine blastocysts were able to invade and degrade

extracellular matrices (ECM). These investigators found that this degradation was not prevented by pregnancy- associated hormones. It was also unaffected by the addition of EACA, indicating that plasmin-independent enzymes were sufficient for this degradation. Nevertheless, plasminogen-activators have been shown to be produced by trophoblast outgrowths from blastocysts of similar age *in vitro* [25]. As well, fibrinolytic activity by similar trophoblast outgrowths could be prevented by nitrophenol-p-guanidino benzoate (NPGB), another inhibitor of plasminogen activation [26]. Using zymographic procedures, preimplantation mouse embryos have recently been shown to secrete functional ECM-degrading metalloproteases including collagenase and stromelysin which were inhibited by tissue inhibitor of metalloproteases (TIMP) [27]. In the same study, using polymerase chain reaction (PCR) technique, transcripts for both metalloproteases and TIMP were detected.

Matrix degradation by the human placental cytotrophoblast

Fisher *et al.* [28] observed morphological evidence of ECM degradation by cytotrophoblast outgrowths from explants of chorionic villi isolated from first trimester but not second trimester placentas. These findings were confirmed by the same authors in a more recent study using short-term cultures of fractionated enzyme-dispersed cytotrophoblast cells from first, second and third trimester human placentas [29]. They reported that isolated first trimester but not second or third trimester human trophoblasts were able to degrade an ECM produced by a teratocarcinoma cell line. Degradation was assessed by the ability of the cell to form circular areas clear of matrix and by the spontaneous release of ^{3}H-labeled matrix components. They also showed, using substrate gels copolymerized with gelatin, that cell extracts from first trimester trophoblasts presented a more complex pattern of gelatin-degrading enzymes than second or third trimester trophoblasts. Since all the enzymes could be inhibited by 1,10-phenanthroline and not by other protease inhibitors, it indicated that they were metalloproteases. These studies suggested that the invasive ability of human trophoblast is temporally regulated during pregnancy. Whether this regulation is preprogrammed or environmentally controlled remained undetermined because of the short-term nature of the trophoblast cultures employed. Surprisingly, human choriocarcinoma cell lines BeWo and JAR exhibited no ECM-degrading ability in this study. These findings are in contrast with those of Kliman and Feinberg [30] who showed that term trophoblast cells are highly capable of degrading ECM (Matrigel). They placed first trimester and term trophoblasts on a thickness gradient of Matrigel to examine trophoblast-ECM interactions and to evaluate invasiveness at the light and electron microscopic levels. They showed that the thickness of the ECM affected the morphology of trophoblast cells as well as their degrading ability. In areas where the Matrigel layer was thicker, trophoblasts from either first trimester or term placentas failed to degrade matrix, whereas in thinner areas Matrigel degradation was high. This degrading behaviour of first trimester and term trophoblasts was similar to that exhibited by JEG-3 choriocarcinoma cell line when cultured on a similar substrate.

Trophoblast invasion of intact basement membrane and stroma

A stringent testing of the ability of human trophoblast cells to invade a naturally- occurring basement membrane and its underlying stroma has recently become feasible due to two methodological advances: (i) development of an amnion invasion assay by Mignatti *et al.* [31], modified from the original Liotta assay [32], and (ii) development of a methodology for maintaining pure cytotrophoblasts in long-term cultures by Yagel *et al.* [33] in our laboratory. The amnion invasion assay measures the extent of invasiveness as the percentage of radioactivity retained within the epithelium-free amnion at a specific interval after seeding the basement membrane surface of the amnion with ^{125}I-dUR-labeled invasive cells. This percentage is equivalent to the proportion of cells at various

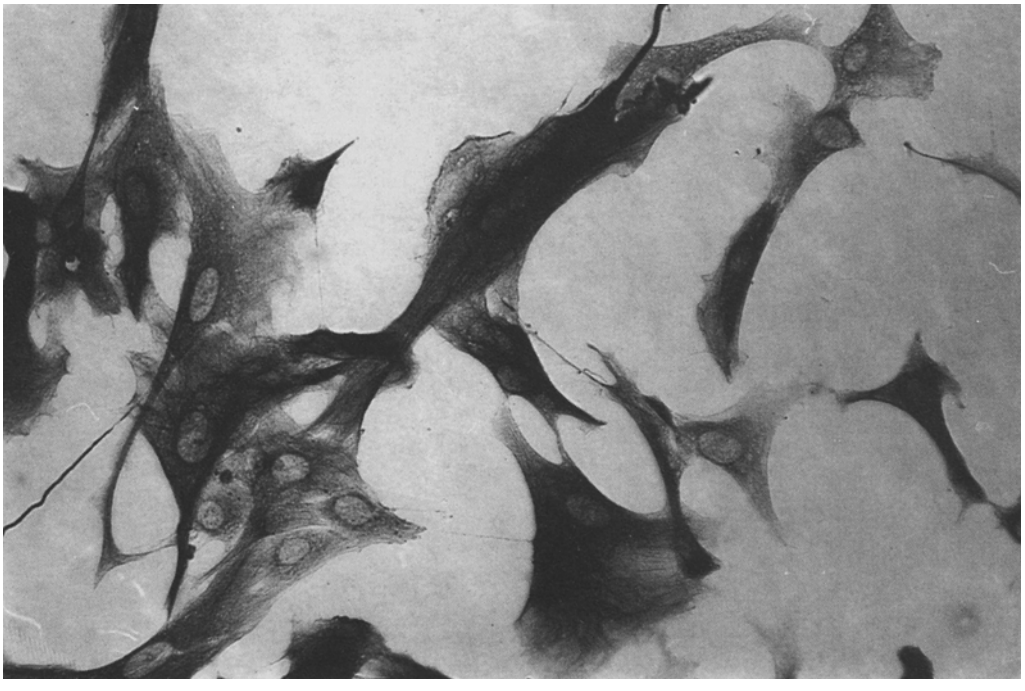

Fig. 2. Long-term culture of first trimester human trophoblast cells stained with a monoclonal antibody to cytokeratin. Note cytokeratin filaments in all cells. (× 270)

stages of migration through the basement membrane and the underlying connective tissue stroma. Long-term culture of trophoblast cells was achieved by plating fragments of chorionic villi retrieved from first trimester human placentas without enzymatic treatment [33, 34]. Cytotrophoblast cells migrating out of these explants were maintained by several passages. Cultures had a finite life span of up to 14 passages with a doubling time of 48–96 hours. These cells were mostly cytotrophoblasts, with a small incidence of syncytia. All cells were positive for the epithelial cell marker cytokeratin [34], as shown in Fig. 2. These cells also expressed cytoplasmic hCG, surface Trop-1 and Trop-2 antigens (trophoblast markers) but not cytoplasmic vimentin (mesenchymal cell marker) or surface 63D3 antigen (macrophage marker) [33]. Their functional integrity was documented by hCG production *in vitro* [33] which was further stimulated in the presence of IL-1 [35]. Progesterone production by these cells was enhanced in the presence of exogenous hCG. Proliferation of these trophoblast cells was reduced in the presence of an anti-hCG antibody indicating that hCG acted as an auto-stimulating agent within a defined concentration [33].

We tested these trophoblast cell cultures (2–4 passages) in the amnion invasion assay outlined above to determine whether the invasive ability of normal trophoblast cells is genetically determined, independent of the uterine microenvironment and if so, whether they share some of the molecular mechanisms of invasion identified in metastatic malignant cells as stated earlier. We compared their invasive ability with a variety of invasive tumor cells. These included B16F10 murine melanoma, JAR choriocarcinoma [16], C3L5 murine mammary adenocarcinoma, MeWo human melanomas, and NIH 3T3 murine fibroblast lines rendered tumorigenic by transfection with anti-sense TIMP RNA [36]. Non-tumorigenic 3T3 cells [36] as well as tumorigenic but non-invasive C10 murine mammary adenocarcinoma cells [16] served as controls.

Three approaches were employed to identify mechanisms of invasion by trophoblast and tumor cells: (i) by evaluating invasion-blocking effects of

protease inhibitors or antibodies (e.g. 1,10-phenanthroline, a chemical inhibitor of metalloproteases; trasylol and EACA, inhibitors of serine proteases; human recombinant TIMP; anti-plasminogen antibody); (ii) by assessing the invasion-stimulating ability of a metalloprotease activator, mersalyl; (iii) by measuring protease (collagenase and plasminogen activator (PA)) and TIMP levels in the media. Selected results of the first two approaches are summarized in Table 1. This table shows that the invasion by trophoblast cells and invasive tumor cells was affected similarly. Inhibition of both metalloproteases and serine proteases blocked invasion, indicating that both groups of proteases were required. Mersalyl was found to enhance invasion. This effect was blocked by metalloprotease inhibitor 1,10-phenanthroline, but not by serine protease inhibitor trasylol, indicating that the activation of procollagenase by mersalyl follows a plasmin-independent pathway. We suggest that trophoblast cells as well as tumor cells listed in Table 1 normally depend on a plasmin-dependent pathway of collagenase activation, and that Type IV collagenase is the key molecule essential for basement membrane degradation. This suggestion is based on the following evidence: (i) trophoblast cells produced significant levels of collagenase [16] as well as plasminogen activator [18] *in vitro*; (ii) the collagenase production was drastically reduced in serum-free (plasminogen-deficient) media; and (iii) the production in serum-containing medium was blocked in the presence of trasylol[37].

Urokinase-type plasminogen activator (uPA) is a major product of first trimester cytotrophoblast [18]. uPA production has also been reported for term placental cytotrophoblast [38]. First trimester cytotrophoblast cell lines (mentioned earlier in our study) as well as term placental cytotrophoblasts express saturable binding sites (receptors) for single chain (biologically active form) uPA (scuPA) (MacCrae, K., Cines, D., Graham, C.H. and Lala, P.K., unpublished), the number of binding sites being higher on first trimester cells. These sites remain normally saturated by endogenous uPA in both cases. The high number of uPA binding sites on first trimester trophoblast cells correlates with their high invasive ability. Interestingly, human cytotrophoblasts also express membrane-associated and cytoplasmic plasminogen activator inhibitor (PAI) types 1 and 2 [39]. It has been suggested that PAI type 1 may serve as a marker for the invasive trophoblast [39], however, functional relevance of this marker to trophoblast invasiveness remains unknown. PAI type 2 is predominantly located in the syncytiotrophoblast [39]. It is possible that the membrane-associated form of PAI type 2 is responsible for an additional type of uPA binding. Jensen *et al.* [40] reported that uPA binding by the microvillous plasma membrane of the (non-invasive) syncytiotrophoblast was due to PAI-2, pos-

Table 1. Amnion invasion index under various conditions

	Invasion index (normalized to 100 for controls)		
	Trophoblast	JAR choriocarcinoma	MeWo melanoma
Control*	100 ± 5.0	100 ± 5.0	100 ± 17.0
1,10-Phenanthroline	19.5 ± 1.3	15 ± 3.0	29 ± 6.0
TIMP (human recombinant)	9.3 ± 0.5	21.9 ± 1.0	21.8 ± 2.0
Anti-plasminogen ab	4.2 ± 0.1	7.8 ± 0.2	ND
Mersalyl	165 ± 6.0	ND	151 ± 19.0
Trasylol	6.3 ± 0.1	10.9 ± 0.3	18 ± 1.0
EACA	11.3 ± 0.1	14.1 ± 1.0	ND
Mersalyl + Trasylol	142 ± 5.0	ND	138 ± 17.0
Mersalyl + 1,10-Phenanthroline	13 ± 1.5	ND	ND

* Indices before normalization were 4.0 ± 0.2, 3.2 ± 0.15 and 3.4 ± 0.6, respectively, for trophoblast, choriocarcinoma and MeWo cells in controls.

sibly anchored to the cytoskeleton. This binding, which involved the catalytic site of uPA, was blocked by antibodies against PAI-2. This form of uPA binding may result in down regulation of invasiveness by quenching uPA activity.

Laminin, a component of the basement membrane, is produced by metastatic cancer cells [12] as well as by first trimester trophoblast cells [16]. When bound to normal as well as malignant trophoblast cells it stimulates type IV collagenase activity. However, this stimulation appears to be higher for malignant trophoblast cells [41].

In summary, first trimester human cytotrophoblast cells are inherently invasive and they utilize similar mechanisms for invasion as invasive cancer cells. Some of these properties are also noted in term placental trophoblast, although to a lesser extent.

Control of trophoblast invasion

Since the experiments of Kirby [4–7], very few studies have been conducted to elucidate the precise mechanisms by which trophoblast invasion is regulated *in situ*. Findings of greater trophoblast invasion associated with little or no decidua have led to the suggestion that this tissue plays a major invasion-controlling role. However, this evidence has only been circumstantial.

We have recently shown that first trimester human decidual cells produce molecules that block trophoblast invasion [42, 43]. In these studies, first trimester human trophoblast cells from early and late passage cultures were examined for their ability to invade an epithelium-free human amniotic membrane *in vitro* under various conditions. Early passage (three to four) trophoblast cells exhibited a high degree of invasion when assayed in culture medium containing 10% fetal calf serum. However, addition of conditioned medium from first trimester human decidual cell cultures almost completely abrogated this invasion. This suppression was prevented by addition of neutralizing antibody against anti-TGFβ and was mimicked by addition of porcine $TGF\beta_1$. Furthermore, a neutralizing antibody to TIMP also relieved the suppressive effect of decidual supernatant. In addition, both these antibodies provided minor stimulation of trophoblast invasion. Addition of decidual supernatant to tenth passage trophoblast cells also blocked their invasiveness. However, anti-TGFβ antibody in this instance produced only a minor relief of the anti-invasive effect of decidual supernatant. Furthermore, these trophoblast cells assayed in the presence of $TGF\beta_1$ alone exhibited an incomplete reduction in their invasiveness, indicating a reduced sensitivity to TGFβ. Some of these results, summarized in Table 2 indicate that: (a) first trimester decidua produces molecules which can control trophoblast invasion; (b) TGFβ and TIMP are key members of this family of molecules, and (c) trophoblast sensitivity to anti-invasive action of TGFβ may decrease after long-term culture. Since addition of anti-TIMP or anti-TGFβ resulted in stimulation of invasiveness beyond control levels, it appears that trophoblasts themselves produce TIMP and TGFβ, and/or that these molecules were initially present in the culture medium. We are currently investigating these possibilities. Moreover, we found that presence of $TGF\beta_1$ reduced collagenase type IV activity in trophoblast culture media whereas presence of anti-$TGF\beta_1$ antibody stimulated it [43]. Preliminary studies suggest that the anti-invasive effect of TGFβ is due to induction of TIMP in trophoblast and decidual cells [42]. Experiments showed a complete abrogation of decidual TIMP production when anti-TGFβ antibody was added to decidual cultures. Similarly, the presence of this antibody in trophoblast cultures resulted in a decrease in TIMP level. TIMP production in response to TGFβ has been previously shown in fibroblasts [44]. Thus, an increase in TIMP level in response to TGFβ may explain the reduction of collagenase activity in trophoblast cultures exposed to exogenous $TGF\beta_1$ [43].

Another mechanism by which TGFβ may block trophoblast invasion is by induction of differentiation. TGFβ has been shown to induce differentiation of certain epithelial cells [45–47]. The non-invasive, non-proliferative and terminally-differentiated syncytiotrophoblast of the placental cho-

rionic villi arise from the fusion and differentiation of mononuclear cytotrophoblast cells [2]. Invasive trophoblast belongs to the latter category. We have recently shown that $TGF\beta_1$ is anti-proliferative to trophoblast and that it induces differentiation of cytotrophoblast into syncytia [34]. In the later stage of pregnancy, syncytiotrophoblast makes up the large proportion of the trophoblast, thus possibly accounting for the lack of invasion by the villous trophoblast of the placenta in the second and third trimesters of pregnancy.

Whether trophoblast cells themselves can regulate their own invasiveness in an autocrine manner remains unclear. This may occur by TGFβ production. We have also suggested that trophoblast-derived hCG may regulate invasion by down-regulating collagenase production [37].

Figure 3 presents a model for the mechanisms of trophoblast invasion and its control. Metalloproteases and serine proteases seem to be the key enzymes allowing invasion; the action of serine proteases (plasmin derived from plasminogen) results in an activation of metalloproteases. Binding of trophoblast-derived or matrix-derived laminin via laminin receptors stimulates metalloprotease activity. Trophoblast-secreted PA activates plasminogen. PA also remains in an active form bound to PA receptors on the cell membrane. However, PA activity may be quenched by free or membrane-associated PAI-2. The key control of invasion is provided by the decidual tissue. It produces TGFβ which in turn induces TIMP production in an autocrine manner in decidual cells themselves and in a paracrine manner in trophoblast cells. Thus, primary control of trophoblast invasiveness by the decidua is provided via TGFβ. TIMP acts as the final mediator in down-regulating metalloprotease activity and thus blocking trophoblast invasion. Trophoblast-derived hCG may provide an additional negative signal for metalloprotease production. In addition, certain cells in the decidua, e.g. macrophages, decidual cells and fibroblasts may secrete PAI which can inactivate PA. Whether trophoblast cells themselves, particularly in late gestation, are capable of producing TGFβ for an autocrine type negative regulation of invasiveness remains to be determined. It also remains to be tested whether uncontrolled invasiveness of choriocarcinoma *in situ* results from a loss of sensitivity to TGFβ.

Conclusions

1. First trimester human trophoblast cells are highly invasive. Their ability to invade natural or reconstituted basement membranes *in vitro* is equivalent to or higher than that of metastatic cancer cells including choriocarcinoma.
2. Mechanisms of trophoblast cell attachment to, detachment from and degradation of basement membranes are analogous to those employed by invasive tumor cells. Plasmin-dependent activation of collagenase, in particular, collagenase Type IV,

Table 2. Trophoblast invasion under different conditions

Experiment number	Normalized invasion index (%)*				
	Trophoblast alone	Trophoblast + decidual supernatant	Trophoblast + DCM + anti-TGFβ	Trophoblast $TGF\beta_1$	Trophoblast + DCM + anti-TIMP
1 (Third passage)	100 ± 29.7	3.2 ± 0.8	139 ± 7.6	2.3 ± 0.1	–
2 (Fourth passage)	100 ± 12.1	43.5 ± 1.2	–	–	144.9 ± 13.9
3 (Tenth passage)	100 ± 10.6	36.8 ± 6.1	57 ± 13.3	73.3 ± 5.2	–

* Invasion index of trophoblast alone normalized to 100.

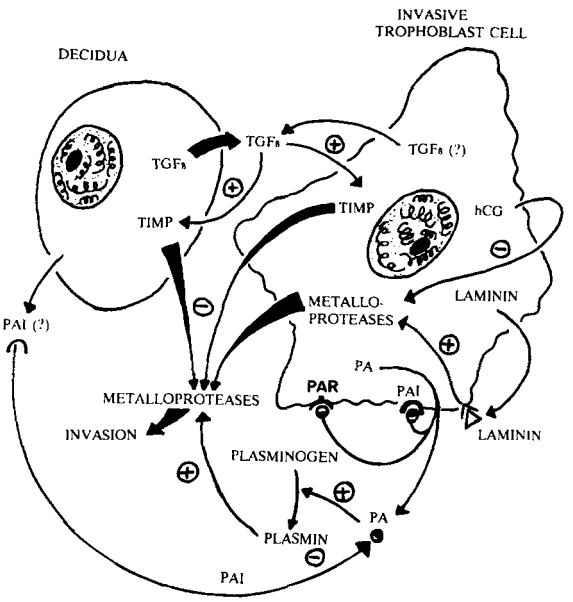

Fig. 3. A model for trophoblast invasion and its control. hCG = human chorionic gonadotropin; PA = plasminogen activator; PAI = plasminogen activator inhibitor; PAR = plasminogen activator receptor; TGFβ = transforming growth factor β; TIMP = tissue inhibitor of metalloproteases.

represents a key mechanism of trophoblast invasion.

3. Unlike tumor cells, trophoblast invasion *in situ* is highly controlled. This control is exercised by two key mediators in the microenvironment provided by the decidua. TGFβ serves as a prime mediator which in turn leads to the induction of TIMP, the final mediator of this control, in both decidua and the trophoblast.

Key unanswered questions

1. Do late gestational trophoblast cells show a major reduction of invasive ability? If so, is this reduction preprogrammed or induced by the microenvironment of the late gestational uterus?
2. Is there an autocrine type negative regulation of trophoblast invasion? If so, is this mediated by TGFβ production by the trophoblast?
3. Which cells in the decidua produce TGFβ?
4. Which cells in the decidua produce TIMP?
5. Are choriocarcinoma cells refractory to the anti-invasive action of decidua-derived TGFβ?

Acknowledgements

We acknowledge the studies conducted by Dr. Simcha Yagel while he was a post-doctoral fellow of the Medical Research Council of Canada in this laboratory. We thank Rachel Zimmerman for the artwork, Mrs. Agnes Bentley for typing and Dr. Elias Gammal for editing the manuscript. Supported by the Medical Research Council of Canada, The National Cancer Institute of Canada and the Cancer Research Society, Inc.

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