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Vascular endothelial growth factor, epidermal growth factor and fibroblast growth factor-4 and -10 stimulate trophoblast plasminogen activator system and metalloproteinase-9

E.Y.Anteby[1,3], C.Greenfield[1], S.Natanson-Yaron[1], D.Goldman-Wohl[1], Y.Hamani[1], V.Khudyak[1], I.Ariel[2] and S.Yagel[1]

[1]Department of Obstetrics and Gynecology and[2]Department of Human Pathology, Hadassah University Hospital, Mt Scopus, Jerusalem, Israel

[3]To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Hadassah University Hospital, Mt Scopus, P.O.B. 24035, Jerusalem 91240, Israel. E-mail: anteby@cc.huji.ac.il

Trophoblast invasion, accompanied by degradation of extracellular matrix, is crucial to normal pregnancy development, whereas shallow placental invasion and implantation likely plays a role in the subsequent development of pre-eclampsia. The growth factors vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF) are placental growth factors that activate degradation of extracellular matrix. We determined the effect of VEGF, EGF, FGF-2, FGF-4 and FGF-10 on the plasminogen activator system of first trimester cytotrophoblasts cultured *in vitro*. We studied the activity of urokinase plasminogen activator (uPA), its inhibitor plasminogen activator inhibitor-1 (PAI-1), and 92 kDa gelatinase-B (matrix metalloproteinase-9, MMP-9), using protein gel and reversed gel zymography. The expression pattern of FGF-4 and FGF-10 in human placental sections was determined by immunohistochemistry. FGF-4 was expressed in first trimester villi stroma, primarily in endothelial cells. FGF-10 expression was localized to first trimester extravillous trophoblasts. VEGF, EGF, FGF-4 and FGF-10, but not FGF-2, stimulate the activity of trophoblast uPA, PAI-1 and MMP-9. These results support the hypothesis that specific growth factors modulate the invasive potential of trophoblasts, and therefore may play an important role in early placental development. Our findings may contribute to the understanding of the pathophysiology of diseases associated with shallow placentation, such as pre-eclampsia.

Key words: epidermal growth factor/fibroblast growth factor/placenta/trophoblast/vascular endothelial growth factor

Introduction

Trophoblast invasion and migration into the decidua and maternal vessels is an essential part of placental development. Abnormal trophoblast invasion has been associated with significant maternal and fetal morbidity and mortality resulting from pre-eclampsia and intrauterine growth restriction (IUGR) (Brosens *et al*., 1967, 1988). The plasminogen activator (PA) system induces extracellular matrix (ECM) remodelling by converting the abundant extracellular zymogen plasminogen into the active protease plasmin. This system thus facilitates cell attachment, migration and invasiveness. The key components of the plasminogen activator system include the two PA, the urokinase type (uPA) and the tissue-type (tPA), the two PA inhibitors PAI-1 and PAI-2, and cell surface uPA receptor. Of the two PA, uPA plays the most important role in generating extracellular proteolysis for ECM degradation, cellular migration and invasiveness (Littlefield, 1991). Several lines of evidence support the role of the plasminogen activator system in implantation. Queenan *et al.* (1987) showed that human trophoblasts synthesize and secrete uPA. Floridon *et al*. (1999) using immunohistochemistry found that the expression of uPA and its receptor is localized to the syncytiotrophoblast layer and to endovascular and perivascular trophoblasts within maternal vessels. This distribution further emphasizes the role of the plasminogen activator system, in particular uPA, in ECM degradation and subsequent trophoblast invasion during early stages of placentation (Hu *et al*., 1999). In addition, the role of plasminogen activator in trophoblast implantation has been shown in mice (Strickland *et al*., 1976). While the PA system is believed to play an important role in ECM degradation during trophoblast implantation (Blasi *et al*., 1987), the regulation of the trophoblast PA system has not been studied.

The activity of the plasminogen activator system in various tissues is under hormonal and cytokine control. Because angiogenesis requires proteolysis, several angiogenic factors have been shown to promote activity of the plasminogen activator system. Kroon et al. showed that administration of FGF to human microvascular endothelial cells, grown in hypoxic conditions, stimulated the expression of uPA and its receptor (Kroon *et al*., 2001). Mandriota and Pepper (1997) studied the effect of FGF and vascular endothelial growth factor (VEGF) on bovine microvascular endothelial cells. They demonstrated that VEGF-induced angiogenesis and PA expression are dependent on endogenous FGF. Kasza and Koj (2002)found that EGF enhances uPA, tPA and PAI-1 expression in astrocytoma

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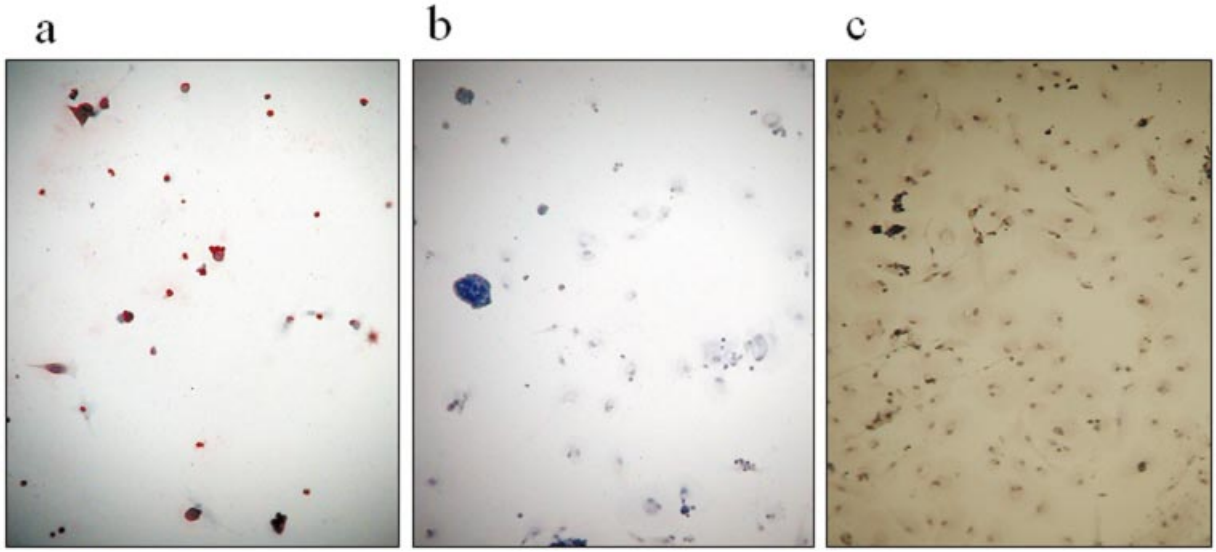

Figure 1. Immunohistochemical analysis of isolated placental cells after 96 h in culture. (**a**) Cells stain positive for mouse anti-human cytokeratin 7 (1:100; Dako A/S, Denmark). (**b**) Cells stain negative for mouse anti-human CD68 (1:50; Dako Corp., USA). (**c**) Cells stain negative for mouse anti-human vimentin (1:100; Dako A/S, Denmark). Magnification: ×100.

Table 1 The level of βhCG in trophoblast conditioned media cultured in the presence or absence of VEGF, EGF, FGF-2, FGF-4 and FGF-10. Results are mean (± SE).

Factor	Concentration/βHCG		
VEGF	10 ng/ml		Control
	1810 (± 56)		1755 (± 78)
EGF	10 ng/ml	100 ng/ml	Control
	1745 (± 78)	1650 (± 212)	1986 (± 113)
FGF-2	100 ng/ml		Control
	1091 (± 146)		1113 (± 123)
FGF-4	10 ng/ml	50 ng/ml	Control
	851 (± 340)	838 (± 116)	941 (± 80)
FGF-10	500 ng/ml	1000 ng/ml	Control
	1290 (± 226)	1087 (± 145)	1170 (± 85)

cells. These findings support the role of VEGF, FGF and EGF in promoting proteolytic activity during angiogenesis.

VEGF, EGF and FGF are major growth factors of the placenta (Morrish *et al*., 1987; Hamai *et al*., 1998; Amemiya *et al*., 1994; Reynolds and Redmer, 2001). The expression of VEGF, EGF, FGF and their receptors in trophoblasts together with the marked proteolytic activity in the placental bed suggests an interaction between these two systems. We therefore hypothesized that VEGF, EGF and FGF modulate placental ECM degradation by regulating the activity of components of the plasminogen activator system. To test this hypothesis, we studied the effect of VEGF, EGF and FGF on the activity of trophoblast uPA and PAI-1. Because the PA system plays an important role in regulating metalloproteinase activity, we also determined the effect of these cytokines on the activity of trophoblast metalloproteinase-9 (MMP-9).

Materials and methods

Preparation and culture of cytotrophoblasts

First trimester placental cells from legal interruptions of pregnancy were studied in accordance with the protocol for the study of human subjects approved by our institutional board. Cytotrophoblasts were isolated and purified as described by Kliman *et al.* (1986), with some modifications (Shimonovitz *et al*., 1994; Yagel *et al*., 1994). Briefly, the villous tissue was rinsed with phosphate-buffered saline (PBS), then subjected to digestion in HEPES buffered saline solution (HBSS) containing 0.125% trypsin type XII-S (Sigma, USA) and 0.2 mg/ml DNase 1 (DN-25; Sigma). The dissociated cells were resuspended in media containing 17% newborn calf serum (NBCS) (Beth Haemek, Israel). After centrifuging, the pellet was applied to a 15–70% Percoll (Pharmacia, Sweden) gradient. Following centrifugation at 1200 *g* for 30 min at room temperature, the middle bands (35–55%) were collected and the cells washed with HBSS and Dulbeco's modified Eagle's medium (DMEM-F12, 1:1). The cell suspension was then incubated for 20 min on plastic dishes in a 5% CO_2, 37°C incubator to minimize contamination by macrophages. Previous studies using immunohistochemistry with specific anti-macrophage antibodies (CD 68) revealed that a 5% contamination with macrophages is reduced to 0.5% by allowing the macrophages to attach to the bottom of the culture well (Yagel *et al*., 1994). The cells were then diluted to a concentration of 1×10^6 cells/ml with serum-free DMEM–F-12.

Preliminary experiments were performed to determine the optimal period of trophoblast culture before ligands were applied. The maximal baseline activity, and growth factor-induced activity, of uPA, PAI-1 and gelatinase was seen after 96 h in culture. We verified that the cultured cells were trophoblasts using immunohistochemistry with specific antibodies to cytokeratin, CD68 and vimentin. As shown in Figure 1, cytotrophoblasts grown in serum-free media fail to aggregate or fuse and thus remain in a mononuclear state (Ringler and Strauss, 1990).

The cells were grown for 96 h, and then cultured for 8–24 h in the presence or absence of VEGF (10 ng/ml), EGF (10 and 100 ng/ml), FGF-2 (100 ng/ml), FGF-4 (10 and 50 ng/ml) and FGF-10 (500 and 1000 ng/ml). Zymography was used to assess trophoblast u-PA as well as gelatinase; and reversed zymography was used to evaluate trophoblast PAI-1 activity. To specify the effects of VEGF we used anti-VEGF antibodies (Sigma). To control for trophoblast differentiation or proliferation in response to growth factor stimulation, we analysed β-hCG levels in the medium. Media β-hCG levels did not differ between the groups (Table I). Recombinant human VEGF, FGF-4 and FGF-10 were obtained from R&D Systems (USA); and recombinant human EGF and FGF-2 were purchased from Sigma. All experiments were performed four times.

Zymography and reversed zymography

Zymography of secreted proteins from the various experiments was carried out by electrophoresis in 10% sodium dodecyl sulphate polyacrylamide (SDS–PAGE) gels impregnated with 3 mg/ml gelatin to demonstrate the presence of

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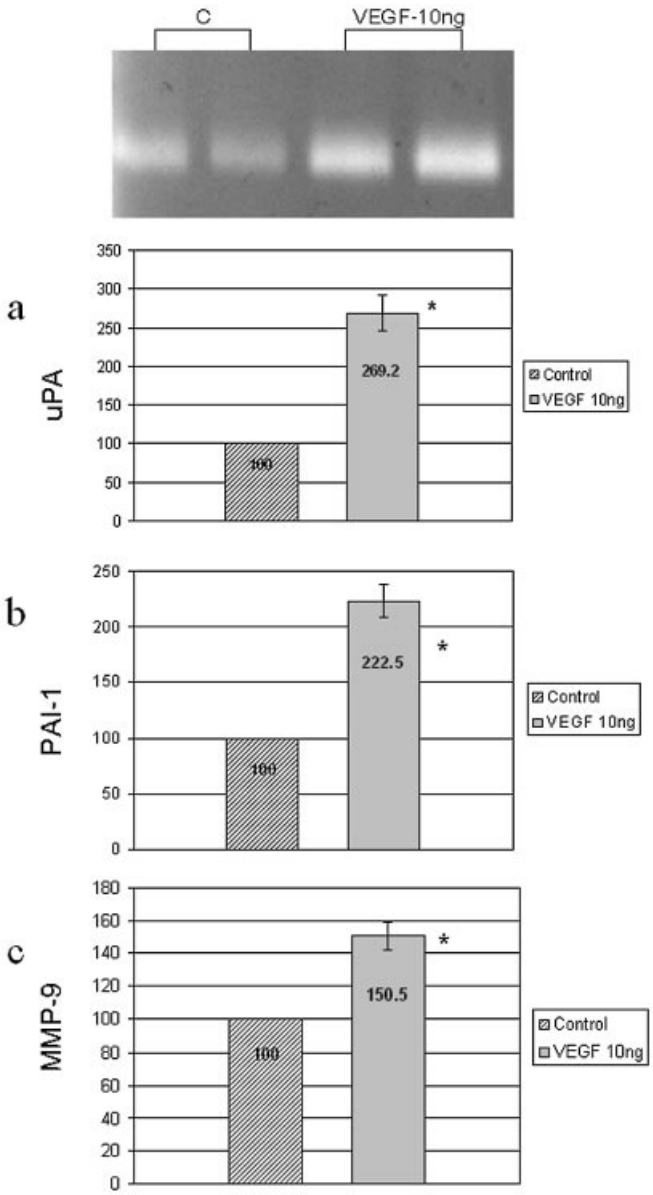

Figure 2. The effect of VEGF (10 ng/ml of VEGF for 8 h) on the trophoblast PA system and gelatinase activity. Densitometry of four zymographic studies is presented as mean ± SE. Results are standardized relative to control where control is considered 100. (**a**) The effect of VEGF on trophoblast uPA activity. The results of a representative gel are shown above the densitometric results. (**b**) The effect of VEGF on trophoblast PAI-1 activity. (**c**) The effect of VEGF on trophoblast MMP-9 activity. *Statistically significant at $P < 0.05$ (*t*-test).

gelatinolytic metalloproteinase, 92 kDa gelatinase-B (matrix metalloproteinase-9, MMP-9) in conditioned media. Gels impregnated with casein (3 mg/ml) and plasminogen (5 mg/ml) were used to demonstrate the presence of plasminogen activator (uPA). Reversed zymography in 10% SDS–PAGE gels impregnated with casein and plasminogen was performed to examine PAI-1 activity in the condition media. (Mishkin and Abramovitz, 1995) Briefly, following electrophoresis and subsequent washes with 2.5% Triton X-100 (0.05%), the gels were gently shaken in 0.1 mol/l Tris–HCl, pH 8.1 for 10 min. The gels were then incubated with 0.4 IU/ml urokinase plasminogen activator (uPA; CalBiochem, USA) for 8 h at 37°C on a shaker. Following incubation activation, the gels were stained with Coomassie Blue and destained. We used 0.25% Coomassie Brilliant Blue R-250 (Bio-Rad Labs, USA) in 30% isopropanol and 10% glacial acetic acid. Destaining was performed with 30% isopropanol and 10% acetic acid. PAI-1 yielded a dark band resulting from inhibition of uPA degradation. The molecular weights of the gelatinase and uPA were estimated by comparing its electrophoretic migration with that of protein standards; PAI was compared to human PAI standard (American Diagnostica Inc., USA). The activity was quantified by computerized image analysis with two-dimensional scanning densitometry (Bio-Imaging System 202D). The densitometric results with gels of representative experiments are presented.

Statistical analysis

Each experiment was standardized relative to control where control is considered 100. Results are presented as mean ± SE. We used the one sample *t*-test to compare the effect of ligands versus control. The average results of each parameter are compared to a value of 100. For comparison of different doses, the Mann–Whitney non-parametric test was applied. $P < 0.05$ was considered significant.

Immunohistochemistry

Immunohisochemistry was performed using the Histostain-Plus kit (Zymed laboratories Inc., USA). Briefly, frozen placental tissue/cell sections were fixed in ice-cold acetone for 10 min and quenched with 3% hydrogen peroxidase to eliminate endogenous peroxidase activity. The slides were washed, blocked and incubated at room temperature with primary antibodies using the dilutions stated below.

Primary antibodies used: mouse anti-human cytokeratin 7, and anti-human vimentin (1:100, Dako A/S, Denmark), monoclonal mouse anti-human CD68 (1:50, Dako Corp., USA), goat anti-human FGF-4 (1:10) and FGF-10 (1:5, R&D Systems Inc.). Secondary antibodies used: Universal Immunoperoxidase polymer anti-goat (Histofine-Nichirei Corp., Japan) and EnVision, Peroxidase, mouse (Dako Corp., USA). The slides were then developed with a substrate-chromagen solution of aminoethyl carbazole (Sigma, USA).

Results

VEGF stimulates trophoblast uPA, PAI-1 and MMP-9 activity

We studied the effect of VEGF on the trophoblast PA system and MMP-9 activity. We exposed the cells to VEGF after 96 h in culture. Time-course and dose–response experiments showed that exposure of cells to 50 ng/ml of VEGF for 8 h yielded the maximal response. The effect of VEGF on trophoblast uPA, PAI-I and MMP-9, studied by zymography and presented by densitometry, are shown in Figure 2. VEGF enhanced uPA activity by a magnitude of 169%, PAI-1 activity by a magnitude of 122%, and MMP-9 activity by a magnitude of 50% over baseline levels (Figure 2A, B and C respectively). The results of a representative gel that shows uPA activity are shown in Figure 2A. The change in uPA, PAI-I and MMP-9 activities was statistically significant at $P < 0.05$. To confirm the effect of VEGF, we used anti-VEGF antibodies with VEGF. The antibodies attenuated the effect of VEGF on uPA, PAI-I and MMP-9 (results not shown).

EGF stimulates trophoblast uPA, PAI-1 and MMP-9 activity

We studied the effect of EGF (10 and 100 ng/ml for 8 h) on the trophoblast PA system and MMP-9 activity (Figure 3). Exposure to 10 ng/ml of EGF enhanced uPA activity by a magnitude of 78%, PAI-1 activity by a magnitude of 83%, and MMP-9 activity by a magnitude of 79% over baseline levels (Figure 3A, B and C respectively). The results of a representative gel that shows PAI-I activity are shown in Figure 3B. The change in uPA, PAI-I and MMP-9 activities in response to 10 and 50 ng/ml of EGF was statistically significant at $P < 0.05$.

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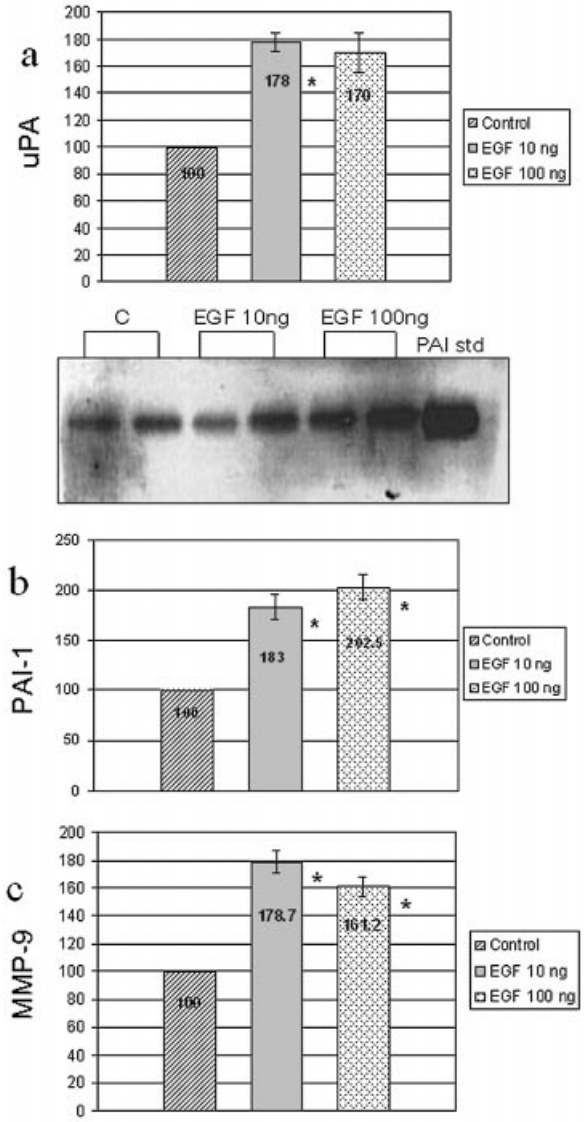

Figure 3. The effect of EGF (10 and 100 ng/ml for 8 h) on the trophoblast PA system and MMP-9 activity. Densitometry of the experiments performed is presented as mean ± SE. Results are standardized relative to control where control is considered 100. (**a**) The effect of EGF on trophoblast uPA activity. (**b**) The effect of EGF on trophoblast PAI-1 activity. The results of a representative gel are shown above the densitometric results. (**c**) The effect of EGF on trophoblast MMP-9 activity. *Statistically significant at $P < 0.05$ (*t*-test).

FGF-2 attenuates trophoblast uPA, PAI-1 and MMP-9 activity

We studied the effect of FGF-2 (100 ng/ml for 8 h) on the trophoblast PA system and MMP-9 activity (Figure 4). FGF-2 decreased uPA activity by a magnitude of 30%, PAI-1 activity by a magnitude of 27%, and MMP-9 activity by a magnitude of 17% in comparison to baseline levels (Figure 4A, B and C respectively). These results were statistically significant at $P < 0.05$.

FGF-4 and FGF-10 stimulate trophoblast uPA, PAI-1 and MMP-9 activity

We used immunohistochemistry to localize FGF-4 and FGF-10 expression in first trimester placentas. We found that FGF-4 was expressed in the stroma of the villi, specifically in endothelial cells (Figure 5A). FGF-10 expression was localized to extravillous trophoblasts invading the decidua (Figure 5B).

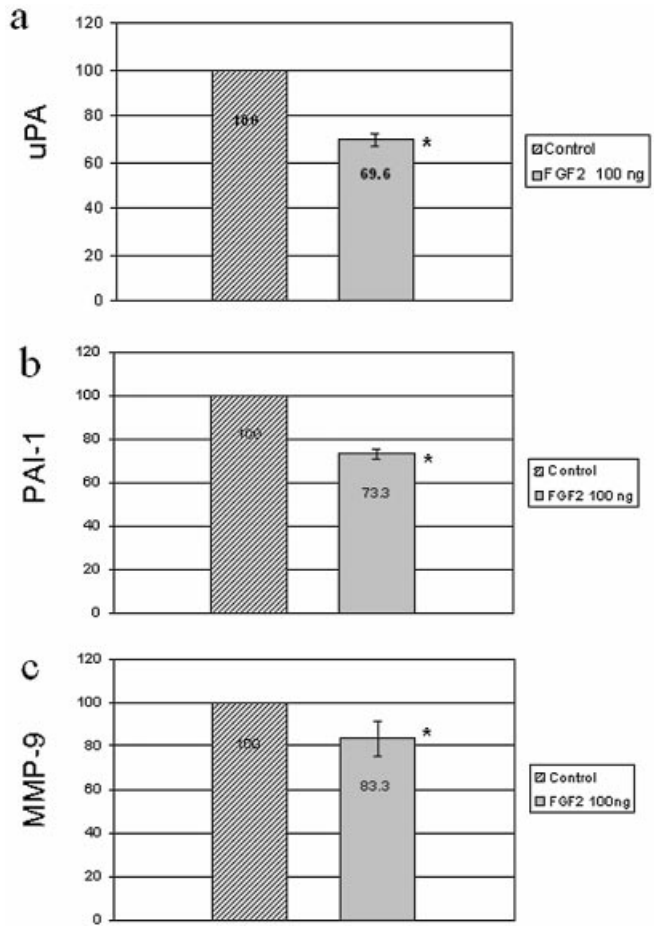

Figure 4. The effect of FGF-2 (100 ng/ml for 8 h) on the trophoblast PA system and gelatinase activity. Densitometry of four zymographic studies is presented as mean ± SE. Results are standardized relative to control where control is considered 100. (**a**) The effect of FGF-2 on trophoblast uPA activity. (**b**) The effect of FGF-2 on trophoblast PAI-1 activity. (**c**) The effect of FGF-2 on trophoblast MMP-9 activity. *Statistically significant at $P < 0.05$ (*t*-test).

We studied the effect of FGF-4 (10 and 50 ng/ml for 8 h) on the trophoblast PA system and MMP-9 activity. Exposure to 10 ng/ml of FGF-4 enhanced uPA activity by a magnitude of 72% and PAI-1 activity by a magnitude of 65%. MMP-9 activity rose by a magnitude of 43% over baseline levels (Figure 6A, B and C respectively). The change in uPA, PAI-I and MMP-9 activities in response to 10 ng/ml of FGF-4 was statistically significant at $P < 0.05$.

We then analysed the effect of FGF-10 (500 and 1000 ng/ml for 8 h) on the trophoblast PA system and MMP-9 activity. Exposure to 1000 ng/ml of FGF-10 yielded the maximal effect. It enhanced uPA activity by a magnitude of 110%, PAI-1 activity by a magnitude of 57% and MMP-9 activity by a magnitude of 111% over baseline levels (Figure 7A, B and C respectively). The results of a representative gel showing the effect of FGF-10 on trophoblast MMP-9 activity are shown in Figure 7C. Results of exposure to FGF-2 are shown in the same gel. The change in uPA, PAI-I and MMP-9 activity in response to 500 ng/ml versus 1000 ng/ml of FGF-10 was statistically significant at $P < 0.05$. The difference in MMP-9 activity between the two doses was also statistically significant at $P < 0.05$.

Discussion

Cytotrophoblasts, the placental specialized epithelial cells, invade the decidual interstitium, the inner third of the myometrium, and finally

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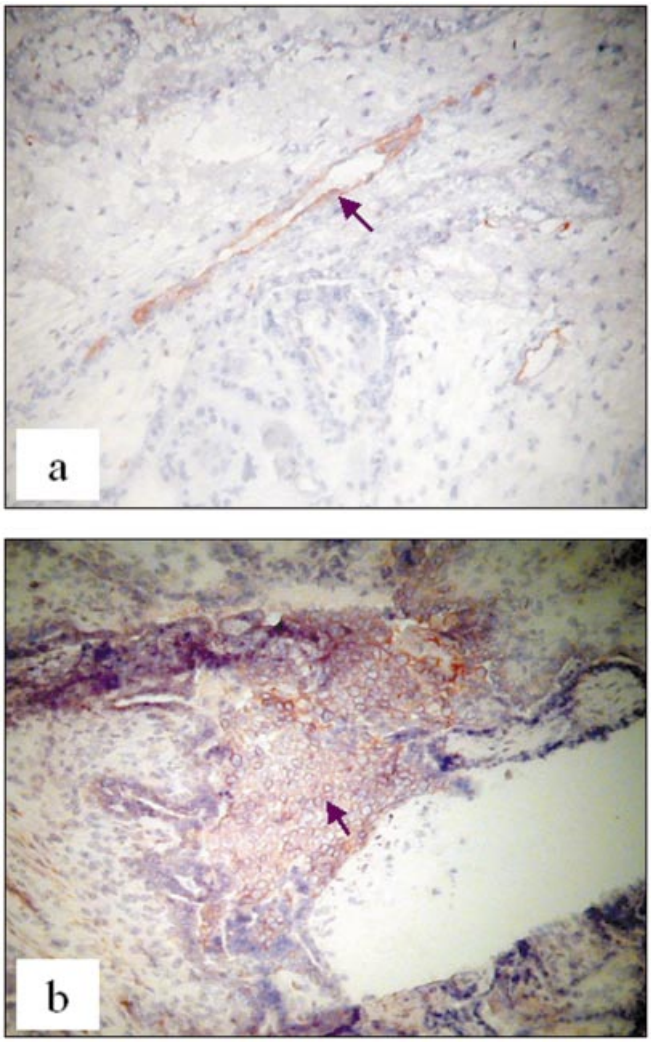

Figure 5. Immunohistochemical staining of FGF-4 (**a**) and FGF-10 (**b**) expression in first trimester placentas. FGF-4 is expressed in the stroma of the villi, specifically in endothelial cells. FGF-10 expression is localized to extravillous trophoblasts invading the decidua. Magnification: ×100.

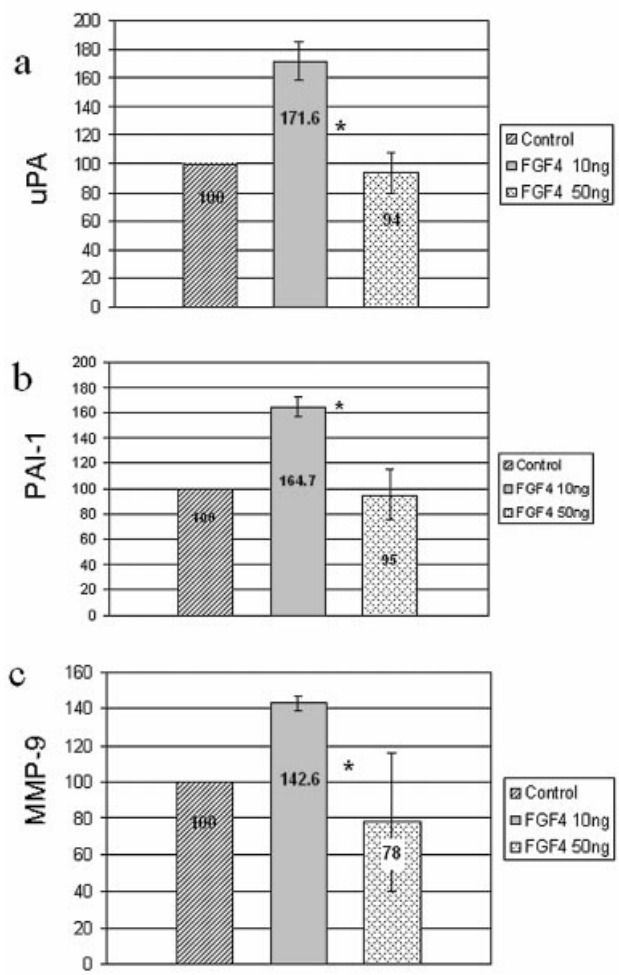

Figure 6. The effect of FGF-4 (10 and 50 ng/ml for 8 h) on the trophoblast PA system and gelatinase activity. Densitometry of four zymographic studies is presented as mean ± SE. Results are standardized relative to control where control is considered 100. (**a**) The effect of FGF-4 on trophoblast uPA activity. (**b**) The effect of FGF-4 on trophoblast PAI-1 activity. (**c**) The effect of FGF-4 on trophoblast MMP-9 activity. *Statistically significant at $P < 0.05$ (*t*-test).

the uterine blood vessels. This invasion plays a critical role in establishing and maintaining adequate blood supply to the developing conceptus (Brosens *et al*., 1967; Brosens, 1988). The ability of trophoblasts to attach to, degrade, and invade maternal tissue seems to be mediated and controlled by proteases and their inhibitors (Strickland *et al*., 1976). Proteolysis may thus be one of the regulatory processes involved in the highly controlled human implantation. Because trophoblasts are able to implant in various locations, the mechanisms that regulate implantation are considered primarily a function of trophoblast activity. We found that VEGF, EGF, FGF-4 and FGF-10 enhance trophoblast uPA, PAI-I and MMP-9 activity. These findings may contribute to the understanding of the regulation of ECM degradation during trophoblast implantation.

Plasminogen activators (PA) are highly specific serine proteases that cleave the inactive zymogen plasminogen to the potent general protease plasmin. PA-dependent ECM degradation involves not only direct degradation of non-collagenous components by plasmin, but also degradation of collagen via activation of collagenases (Littlefield, 1991). Indeed, stimulation of uPA and PAI-I activity resulted in enhancement of MMP-9 activity. The PA system thus provides localized extracellular proteolysis for degradation of matrix proteins and subsequent trophoblast migration or invasion. Local changes of VEGF, EGF or FGF content at the feto-maternal interface may therefore be relevant to modulation of proteolytic activity during normal or pathological placental implantation.

VEGF is widely distributed in human placenta and decidua, the latter being the primary site of VEGF production in pregnant uteri (Ahmed *et al*., 1995; Clark *et al*., 1996). Expression of VEGF isoforms and their receptors in the materno-fetal interface has been recently localized (Vuorela *et al*., 1997; Zhou *et al*., 2002). Zhou *et al*. (2002) showed that VEGF-A is expressed in villous stromal cells and in the syncytiotrophoblast. VEGF-A and VEGF-C are expressed in the villous columns with the expression of the former being markedly up-regulated on cytotrophoblasts in the distal region of the column. VEGF receptors 1 and 3 (VEGFR-1, VEGFR-3) are expressed on invasive cytotrophoblasts in early gestation. Again, anti-VEGFR-1 reactivity was up-regulated in the first cell layers of the column. Similar findings were found by other investigators (Ahmed *et al*., 1995; Clark *et al*., 1996; Dunk and Ahmed, 2001). This distribution suggests an autocrine and paracrine effect that culminates in activation of VEGF receptors on cytotrophoblasts that are in close contact with the maternal ECM. Indeed, binding of VEGF to cytotrophoblasts is

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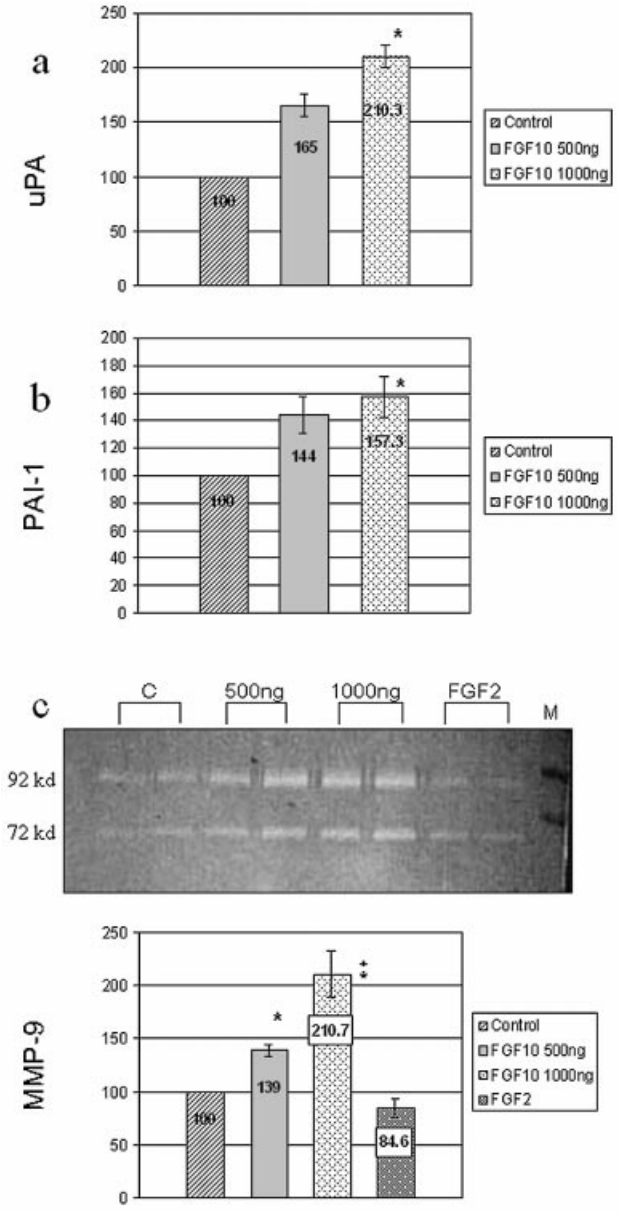

Figure 7. The effect of FGF-10 (500 and 1000 ng/ml for 8 h) on the trophoblast PA system and gelatinase activity. Densitometry of four zymographic studies is presented as mean ± SE. Results are standardized relative to control where control is considered 100. (**a**) The effect of FGF-10 on trophoblast uPA activity. (**b**) The effect of FGF-10 on trophoblast PAI-1 activity. (**c**) The effect of FGF-10 on trophoblast MMP-9 activity. The results of a representative gel showing the effect of FGF-10 on trophoblast gelatinase activity are shown above the densitometric results. Results of exposure to FGF-2 are shown in the same gel. *Statistically significant at $P < 0.05$ (*t*-test). +Statistically significant at $P < 0.05$ (Mann–Whitney test).

essential for trophoblast motility and integrin expression (Lash *et al*., 1999; Zhou *et al*., 2002). Using trophoblast cell lines, it has been found that VEGF did not stimulate trophoblast invasion into matrigel or fibrin (Athanassiades *et al*., 1998; Lash *et al*., 1999). This finding suggests that trophoblast invasion is probably regulated by a wide complex of different effectors. Our results demonstrate that VEGF enhances the trophoblast PA system as well as MMP-9 activity. We therefore suggest that VEGF plays an important role in stimulating ECM by cytotrophoblasts. The recent finding that VEGF and VEGFR expression is diminished in severe pre-eclampsia (Zhou *et al*., 2002) provides further support for the importance of the VEGF system in regulating trophoblast invasion during normal and abnormal placental development.

The expression of EGF at the maternal–fetal interface has been studied by several authors: EGF expression has been identified in the decidua (Hofmann *et al*, 1991). EGF expression in trophoblasts, at various stages of differentiation, has been reported by some (Hofmann *et al*., 1991; Amemiya *et al*., 1994), but not by other (Bass *et al*., 1994) investigators. In trophoblasts, EGF supports mature syncytium formation (Morrish *et al*., 1997), and stimulates trophoblast proliferation (Maruo *et al*., 1992) as well as hCG secretion (Amemiya *et al*., 1994). It is thought that ligands for the EGF receptor are produced in a paracrine fashion by maternal decidua or expressed as autocrine effectors by trophoblasts. Our findings that EGF stimulates the trophoblast PA system support the role of these autocrine or paracrine loops. Together, our findings indicate that EGF plays an important role in trophoblast functional differentiation and proteolytic activity. The subsequent effect of EGF on trophoblast invasion into the myometrium may explain the recently reported association between altered EGF receptor expression and the development of pre-eclampsia or IUGR (Faxen *et al*., 1998).

Fibroblast growth factors (FGF) are a family of multifunctional mitogenic polypeptides containing 23 distinct members (Metzger and Krasnow, 1999). The biological activities of FGF are mediated by FGF receptors 1–4 (FGFR-1–4). Using immunohistochemistry, we demonstrated that FGFR-4 is expressed in human trophoblasts (unpublished results). We initially analysed the influence of FGF-2 on the trophoblast PA system. Interestingly, FGF-2 attenuated the activity of the trophoblast PA system and MMP-9. Because FGF-4 and FGF-10 have been implicated in the development of murine and ovine placenta (Tanaka *et al*., 1998; Chen *et al*., 2000), we hypothesized that these ligands could affect the trophoblast PA system and gelatinase. Using immunofluoresence, we found that FGF-4 is expressed in the villi stroma adjacent to fetal blood vessels and FGF-10 is expressed in extravillous trophoblasts. These two cytokines had a marked stimulatory effect on the trophoblast PA system and MMP-9 activity. The doses of FGF-10 used in our experiments were in the upper range of doses that have been shown to give a biological effect (Qiau *et al*., 2001). In addition to its action through the FGFR, FGF-10 can also act through the keratinocyte growth factor receptor that is expressed by human trophoblasts (Matsui *et al*., 1997). Because data regarding human placental FGF-4 and FGF-10 is limited, their role in placental development remains to be determined.

Our analysis of the cytokine effect on the trophoblast PA system and MMP-9 activity suggests a precise regulation by placental cytokines. It appears that VEGF, EGF, FGF-4 and FGF-10 play an important role in regulating trophoblast invasion. Altered expression of these cytokines at the maternal–fetal interface may contribute to abnormal placental development in diseases such as pre-eclampsia and IUGR.

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