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Involvement of ERK1/2 Pathway in TGF-β1-Induced VEGF Secretion in Normal Human Cytotrophoblast Cells

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ABSTRACT Transforming growth factor-β1 (TGF-β1) plays a pivotal role in the angiogenesis during the development of placenta, but the intracellular signaling mechanism by which TGF-β1 stimulates this process remains poorly understood. In this article, we demonstrated that exposure of normal human cytotrophoblast cells to TGF-β1 stimulated the secretion of the *VEGF* gene encoding vascular endothelial growth factor, which is a key factor in angiogenesis. Meanwhile, treatment of normal human cytotrophoblast cells with TGF-β1-induced expression of HIF-1a, the regulated subunit of hypoxia-inducible factor 1, a known transactivator of the *VEGF* gene. Our data indicated that TGF-β1 induced extracellular signal-regulated kinase (ERK) 1/2 phosphorylation in normal human cytotrophoblast cells. Moreover, treating cells with PD98059, an inhibitor of ERK1/2 signaling, inhibited TGF-β1 stimulation of VEGF secretion and HIF-1a protein expression. These data indicated that in normal human cytotrophoblast cells, TGF-β1 induced HIF-1a-mediated VEGF secretion, and TGF-β1-stimulated-ERK1/2 activation may be involved in this process. *Mol. Reprod. Dev. 68: 198–204, 2004.*

Key Words: TGF-β1; VEGF; HIF-1; ERK1/2; normal human cytotrophoblast cells

INTRODUCTION

The placenta plays a vital function as gas, nutrient, and waste exchange barrier between the maternal circulation and the developing fetus (Tse et al., 2002). The human placenta is a highly invasive tumor-like structure in which a subpopulation of placental trophoblast cells known as the cytotrophoblast invades the uterine deciduas and its vasculature to establish adequate fetal–maternal exchange of molecules (Lala et al., 2002). Recent studies have suggested that cytokines and growth factors such as TGF-β1 play important roles in placental vasculature (Chung et al., 2000), but it is poorly understood how these are involved in this process.

Transforming growth factor-β1 (TGF-β1) is a member of the large family of polypeptide factors that participates in a wide array of biological activities such as development and wound repair, as well as pathological processes (Massague, 1990; Miyazono et al., 2000; Rodriguez-Barbero et al., 2002). Biological actions of TGF-β are mediated by two transmembrane Ser/Thr kinases, type I and type II TGF-β receptors, which are co-expressed by most cells (Ehrlich et al., 2001). TGF-β1 has been classified as an indirect angiogenic factor, because it stimulates angiogenesis by facilitating capillary formation in vivo (Pepper, 1997). Recent studies have clearly showed that TGF-β1 could regulate the expression of vascular endothelial growth factor (VEGF) (Benckert et al., 2003). VEGF has been regarded as a mitogen for endothelial cells and a potent inducer of angiogenesis. It is a homodimeric glycoprotein of two 23-kDa subunits exhibiting sequence homology with platelet-derived growth factor (PDGF). At present, five isoforms of human VEGF containing 121, 145, 165, 189, and 206 amino have been identified by analyses of genomic and cDNA clones (Wang et al., 2003). It has been found that VEGF is important for normal cell growth, development, differentiation, wound healing, and reproduction (Ferrara, 2002).

TGF-β1 induced increase in VEGF mRNA expression, which appears to be primarily a transcriptionally mediated event, and stimulated VEGF protein production in MC3t3-E1 osteoblastic cells (Chua et al., 2000). In addition, TGF-β1 has been demonstrated to induce VEGF expression in both fibroblasts and epithelial cells (Pertovaara et al., 1994). Studies have indicated that the trophoblast cell line (HTR-8/Svneo) shows a time-dependent and a dose-dependent increase in VEGF mRNA production when cultured in the presence of TGF-β1, and TGF-β1 may regulate the production of VEGF in early gestational trophoblasts and serve to modulate placental vascular permeability and

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angiogenesis that are necessary for embryo implantation and placentation (Chung et al., 2000).

Cellular signaling pathways modulate gene expression by altering the activity or expression of specific transcription factors (Fukuda et al., 2002). Hypoxia-inducible factor 1 (HIF-1) is one of the most important factors in the regulation of the *VEGF* gene transcription (Forsythe et al., 1996). HIF-1 is a heterodimer of basic helix-loop-helix transcription factors of the PAS (period ARNT similar) family (Wang et al., 1995). The β-subunits of HIF-1 are constitutive nuclear proteins originally identified as the aryl hydrocarbon receptor nuclear translocator (ARNT), an essential component of the xenobiotic transcriptional response (Wood et al., 1996). The a-subunits are the regulatory components that are affected by cellular oxygen tension. Under normoxic conditions, HIF-1a subunits are degraded by the proteasome (Huang et al., 1998). Recent studies found that HIF-1a is regulated by some growth factors such as IGF-1and IGF-2 (Elazar et al., 1998; Feldser et al., 1999). Furthermore, some data indicated that in human HT-1080 fibrosarcomas, TGF-β1 induced VEGF expression and enhanced HIF-1 DNA binding activities (Shih and Claffey, 2001).

However, the routes that participate in VEGF expression in response to TGF-β1 in normal human cytotrophoblast cells are still unknown. It has been proved that the mitogen-actived protein kinase (MAPK) routes were implicated in signal transduction by TGF-β (Mulder, 2000). Studies show that extracellular signal-regulated kinase (ERK) 1/2 is activated after TGF-β1 addition to proliferating cultures of L(6)E(9) myoblasts (Rodriguez-Barbero et al., 2002). However, to which extent this route may participate in VEGF accumulation by TGF-β1 in normal human cytotrophoblast cells is still unsolved.

To elucidate the molecular mechanism of TGF-β1-regulated VEGF secretion in normal human cytotrophoblast cells, we analyzed VEGF secretion and HIF-1a expression by treating these cells with TGF-β1 and the inhibitor of ERK1/2 signaling.

MATERIALS AND METHODS

Materials

Recombinant human TGF-β1 is provided by PEPROTECH EC Ltd. (London, England). PD98059 is purchased from Sigma (Oakville, Ontario, Canada). The oligonucleotides primers were synthesized from SBS Genetech (Beijing, China). All other reagents used are of the highest quality available.

Isolation and Cultivation of Human Cytotrophoblast Cells

The normal cytotrophoblast cells were isolated and maintained as previously described with some modifications (Knofler et al., 2000; Xu et al., 2000; Wu et al., 2001; Zhang et al., 2002). For 6–8 weeks, human chorionic villi tissues were obtained from patients who underwent therapeutic termination of pregnancy at early gestation. Informed consent was provided by the patients, and the project was approved by the local ethics committee. The time of gestation was defined according to the first day of the last menstrual period and further morphological examination was conducted by means of stereomicroscope. The experiments were repeated more than three times. Tissues from each week were minced separately and digested with 0.25% trypsin (Sigma) at 4°C for 45 min and at 37°C for 10 min, then with 15 IU/ml DNase I (Sigma) at 37°C for 15 min. Trypsinization was stopped by addition of two volumes of FD medium (Ham's F-12/DMEM: 1:1; Gibco-BRL, Gaithersburg, MD). After washing, the dispersed cells were filtered through a nylon sieve to remove the gross villous core residues. The filtered cell suspension (1 the top of a BSA gradient (prepared by sequential addition of 3 ml of 5, 3, 2, and 1% BSA (Sigma) in FD medium to a 15-ml centrifuge tube). The cells were sedimented for 1 hr at unit gravity, and cytotrophoblast cells were collected from the bottom of the tube. The purified cytotrophoblast cells were plated at $0.5–1 \times 10^5$ cells with 1 ml of FD medium supplemented with 10% FBS (Invitrogen, Gaithersburg, MD), 1 mM sodium pyruvate (Sigma), and 2 mM glutamine (Sigma) in fibronectin-coated 24-well dishes. The cells were cultured in 95% air and 5% CO_2 at 37°C and began to attach within 2 hr after plating. The cells spread and showed monolayer epithelial cell morphology after 24 hr. Immunocytochemical studies revealed that more than 99% of the cells exhibited positive staining for cytokeratin and GnRH and were vimentin negative, consistent with their identification as cytotrophoblast cells.

RNA Isolation and Semi-Quantitative RT-PCR

Isolation of total cellular RNA was performed using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. Semi-quantitative PCR was based on Esteve's protocol (Esteve et al., 2002) with some modifications. After removal of contaminating chromosomal DNA with DNAse I treatment, aliquots of 1 μg of total cellular RNA were used for first strand cDNA synthesis in 20 μl of reaction volume using 100 U of SuperscriptTM II reverse transcriptase (Invitrogen). Semi-quantitative PCR was performed in a final volume of 25 μl containing 0.25 mM primers, 2.5 U of Taq polymerase (Takara, Dalian, China), 0.25 mM of each dNTP (Takara) and 1× reaction buffer (Takara). In order to compare the PCR products in a semi-quantitative way, we (i) determined the exponential phase of amplification by performing 25–30–35–40 cycles and (ii) amplified the gene for β-actin (15–20–25 cycles) as internal control for cDNA quantity and quality (Gros et al., 1999). cDNA template was denatured for 5 min at 94°C and amplified as the Table 1. As internal controls for the RT, samples without RNA or without reverse transcriptase were prepared in parallel, and these yield no amplification products (data not shown). As negative controls for the PCR, samples without reverse-transcribed cDNA or without Taq enzyme were used (data not shown). PCR amplifications were performed on Perkin-Elmer 2400 GeneAmp PCR System. PCR products were visualized on 1.5% agarose gels stained by ethidium bromide and

TABLE 1. Sequence and Other Information of the Primers Used for RT-PCR

Gene	Primer	Sequence (5′–3′)	Fragment size (bp)	Annealing temperature (°C)	Cycles
HIF-1α	Forward	TGG ACT CTG ATC ATC TGA CC	433	56	30
	Reverse	CTC AAG TTG CTG GTC ATC AG			
β-actin	Forward	GTG GGG CCC CCC AGG CAC CA	548	55	25
	Reverse	CTC CTT AAT GTC ACG CAC GAT TTC			

UV transillumination. The bands were analyzed using MetaView image analyzing system (version 4.50, Universal Imaging Corp., Downington, PA), and the intensity of HIF-1a bands was corrected by the comparison of corresponding β-actin levels. The cDNA was cloned into pGEM®-T Easy vector (Promega, Madison, WI). The sequencing was performed commercially (Sangon Corp., Shanghai, China) by using an Applied Biosystems Automated sequencer, ABI PRISM 377-96 (Perkin-Elmer) (data not shown).

Protein Extraction and Western Blotting

The ERK1/2 activity assay was performed by Western blotting of the whole cell extracts using antibodies, which specifically recognized phosphyorylated ERK1/2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and ERK1/2 (Santa Cruz) as the control. Briefly, the normal human cytotrophoblast cells were washed with phosphate-buffered saline before lysis in buffer containing 20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1% TritonX-100, 50 mM NaF, 2.5 mM Na_3VO_4, 1 μM aprotinin, and 1 mM PMSF. The cytoplasmic extracts were collected after centrifugation at 12,000*g* for 10 min. The analysis of HIF-1a expression used the total proteins that were extracted by Trizol reagent according to the manufacturer's instructions. The proteins extracted by Trizol were lysised in 1% SDS, and then detected the total amount of protein present in solutions by UV spectrophotometer (Beckman DU530, Fullerton State, CA). All protein samples were adjusted to the same concentration.

Proteins obtained from cell extraction were boiled in SDS/β-mercaptoethanol sample buffer and then loaded onto each lane of the 12% PAGE gels. The proteins were separated by electrophoresis and were then transferred onto microporous polyvinylidene fluoride (PVDF) membranes. Blots were blocked in 3% BSA at 37°C for 30 min and incubated overnight at 4°C with phosphyorylated ERK1/ERK2-directed antibodies in a dilution of 1:400 and HIF-1a-primary antibodies (Santa Cruz) in a dilution of 1:200, in succession. Then the corresponding secondary antibodies conjugated with AP were added at 37°C for 30 min. Color development was performed by NBT/BCIP. The bands were analyzed using MetaView image analyzing system. The intensities of HIF-1a bands were corrected by comparison of corresponding actin levels.

Analysis of VEGF Protein Production by ELISA

VEGF protein production was analyzed by ELISA using the Quantikine VEGF ELISA kit (R&D Systems, Oxford, UK) according to the manufacturer's instructions.

Statistical Analysis

Results are presented as the average ± SEM of at least three separate experiments. Statistical differences were evaluated by analysis with Student's *t*-test. Results were considered as statistically significant at $P < 0.05$.

RESULTS

Stimulation of VEGF Secretion by TGF-β1

To determine whether TGF-β1 treatment affected VEGF secretion, the serum-starved normal human cytotrophoblast cells were exposed to TGF-β1.The quantity of VEGF protein was examined by ELISA. Treatment with exogenous TGF-β1 in different concentration (0, 0.1, 1, 5, 10 ng/ml) for 24 hr, VEGF protein secretion in normal human cytotrophoblast cells was stimulated in a dose-dependent fashion (Fig. 1a). TGF-β1 at 1 ng/ml was able to increase the VEGF protein level in normal human cytotrophoblast cells and at 10 ng/ml caused the maximal stimulation. In addition, TGF-β1 also increased VEGF protein level in a time-dependent manner (Fig. 1b). Although the base-line level of VEGF protein was low, the level increased in response to TGF-β1 (10 ng/ml) within 12 hr ($P < 0.01$) and reached a higher level by 24 hr ($P < 0.01$).

Induction of HIF-1a Protein but not HIF-1a mRNA Expression by TGF-β1

In order to study whether the expression level of HIF-1a mRNA and protein was affected by exogenous TGF-β1, the serum-starved normal human cytotrophoblast cells were treated with TGF-β1 on several different concentrations (0, 0.1, 1, 5, 10 ng/ml) for 24 hr. The semiquantitative PCR results showed that TGF-β1 could not induce HIF-1a mRNA expression (Fig. 2a). However, exposure serum-starved normal human cytotrophoblast cells to TGF-β1 for 24 hr resulted in a concentration-dependent induction of HIF-1a protein expression with a maximal effect observed in the presence of 10 ng/ml TGF-β1 ($P < 0.01$) (Fig. 2b).

Activation of ERK1/2 by TGF-β1

Previous reports have suggested that some of the biological actions of TGF-β1 are mediated by activation of MAPK signaling pathway (Atfi et al., 1997; Chin et al., 2001; Rodriguez-Barbero et al., 2002). To investigate the potential intermediate role of ERK1/2 on TGF-β1-induced accumulation of VEGF, we analyzed the

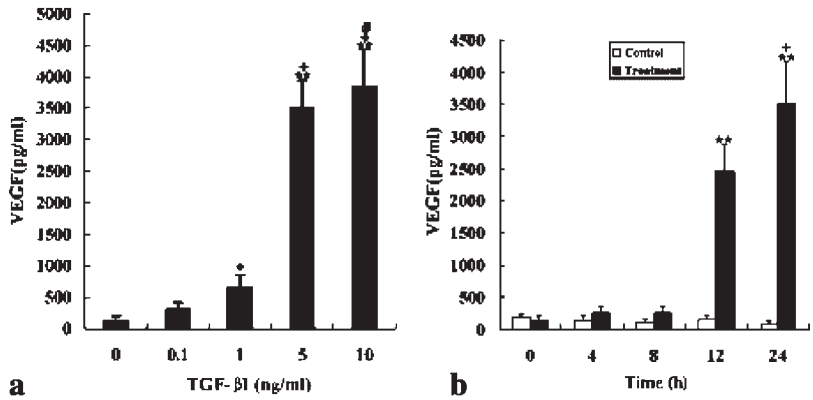

Fig. 1. VEGF protein secretion induced by TGF-β1 in normal human cytotrophoblast cells. **a**: Dose-dependence of VEGF stimulated by TGF-β1. The serum-starved normal human cytotrophoblast cells were treated with TGF-β1 (0, 0.1, 5, 10 ng/ml) for 24 hr. VEGF protein secretion in the culture media was determined by ELISA. Data are expressed as the mean of triplicate ± SEM. $^{*}P < 0.05$ versus basal; $^{**}P < 0.01$ versus basal; $+P < 0.01$ versus TGF-β1 (1 ng/ml); $\#P < 0.01$ versus TGF-β1 (5 ng/ml). **b**: Time-course of TGF-β1-induced VEGF secretion. Serum-starved normal human cytotrophoblast cells were treated with TGF-β1 (10 ng/ml), and the culture media was collected at the indicated times. The secretion of VEGF protein in culture media was quantified using ELISA. Each value represents the mean ± SEM of three experiments, each in triplicate. $^{**}P < 0.01$ versus basal; $+P < 0.01$ versus TGF-β1 (12 hr).

phosphorylation status of ERK1/2 by using antibodies that specifically recognize the phosphorylated form of ERK1/2. As shown in Figure 3, exogenous TGF-β1 (10 ng/ml) increased the phosphorylation of ERK1/2 at 10 min of stimulation and reached a maximum at 40 min. The amount of the total ERK1/2 remained unchanged during the experiment, as indicated by Western blotting of the lysates using antibodies that detect total (phosphorylation state-independent) ERK1/2 protein.

Inhibition of TGF-β1-Stimulated VEGF Secretion and HIF-1a Protein Expression by ERK1/2 Inhibition

To further characterize the role which ERK1/2 signaling plays in TGF-β1-induced-VEGF secretion, serum-starved normal cytotrophoblast cells with various concentration PD98059 (0, 1, 10, 50, 100 μM) and incubated in the presence (+) or absence (−) of exogenous TGF-β1 (10 ng/ml) for 24 hr. The data indicated

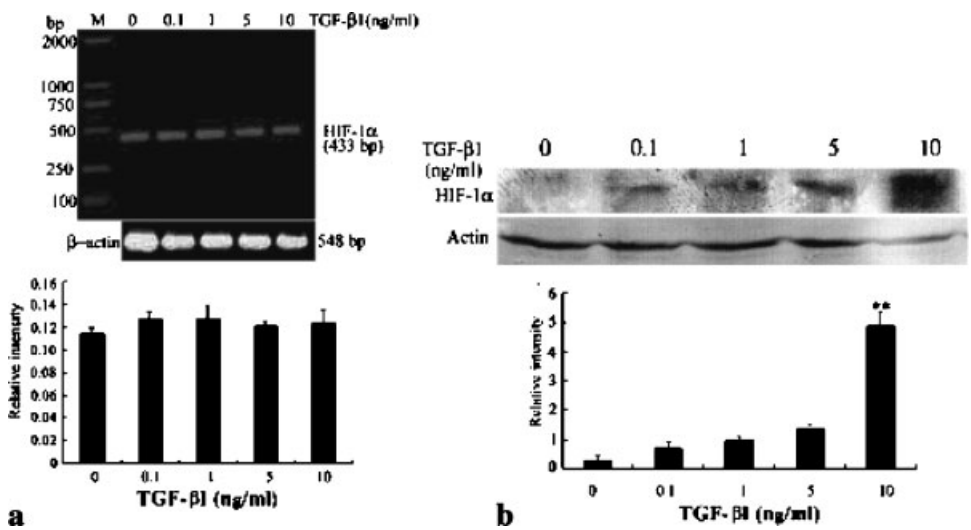

Fig. 2. The effect of TGF-β1 on HIF-1a mRNA expression and HIF-1a protein expression in normal human cytotrophoblast cells. **a**: Total RNA was extracted from the normal human cytotrophoblast cells, which were treated by different concentrations of TGF-β1 (0, 0.1, 5, 10 ng/ml) for 24 hr. Representative semi-quantitative RT-PCR experiments were performed as described in the "Materials and Methods." The optic densities of the DNA bands were analyzed by optical scanning densitometry. Bar graphs shown the intensity of the bands, which was corrected by comparison of β-actin levels. M: Marker. **b**: Serum-starved normal human cytotrophoblast cells were exposed to different concentrations of TGF-β1 (0, 0.1, 5, 10 ng/ml) for 24 hr. Then the whole cell lysates were subject to Western blotting assay to detect the expression of HIF-1a protein. Bar graphs show the levels of HIF-1a quantity corrected for Actin. Bars represent the mean ± SEM of values from three independent experiments. $^{**}P < 0.01$ versus basal.

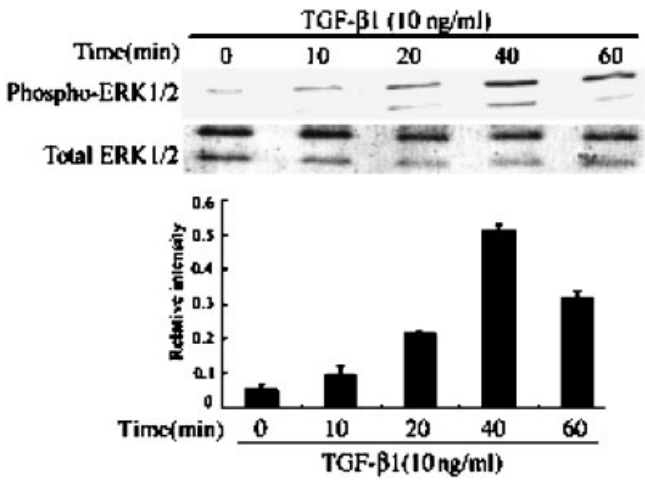

Fig. 3. ERK1/2 pathway activation by TGF-β1. Normal human cytotrophoblast cells were incubated with exogenous TGF-β1 (10 ng/ml) for the times indicated. The figure shows a representative of three experiments in the same conditions. Phosphorylation of ERK1/2 MAPK from cellular extracts was determined using Western blotting with phospho-p44/42 MAPK antibody (phospho-ERK1/2). Western blotting of total ERK1/2 protein (total ERK1/2) in cellular extracts is also shown. Bar graphs show the levels of activated ERK1/2 quantity corrected for total ERK1/2. Bars represent the mean ± SEM of values from three independent experiments.

the concentration of PD98059 that effectively inhibited TGF-β1-stimulated-VEGF secretion is 50 μM ($P < 0.01$) (Fig. 4). In addition, the same treatment was applied to 12 normal human cytotrophoblast cell with PD98059 and TGF-β1, and the data showed that TGF-β1-induced-HIF-1a expression was inhibited by PD98059 (Fig. 5a). Moreover, 50 μM PD98059 efficiently prevented the TGF-β1-induced-activation of the ERK1/2 in the normal human cytotrophoblast cells (Fig. 5b).

DISCUSSION

Previous studies have demonstrated that VEGF, an endothelial mitogen and potent vasopermeability factor,

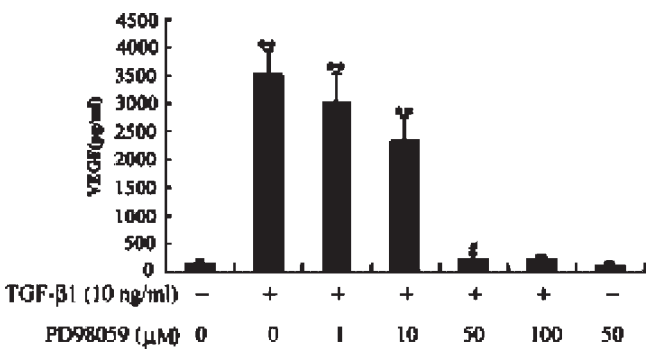

Fig. 4. The effect of PD98059 on TGF-β1-induced VEGF secretion. Serum-starved normal human cytotrophoblast cells were pretreated for 1 hr with PD98059 (0, 1, 10, 50, 100 μM), and then incubated in the presence (+) or absence (−) of exogenous TGF-β1 (10 ng/ml) for 24 hr. The secretion of VEGF protein in the culture media was quantified using ELISA. Each value represents the mean ± SEM of three experiments, each in triplicate. $^{**}P < 0.01$versus basal; $\#P < 0.01$ versus PD98059 (10 μM).

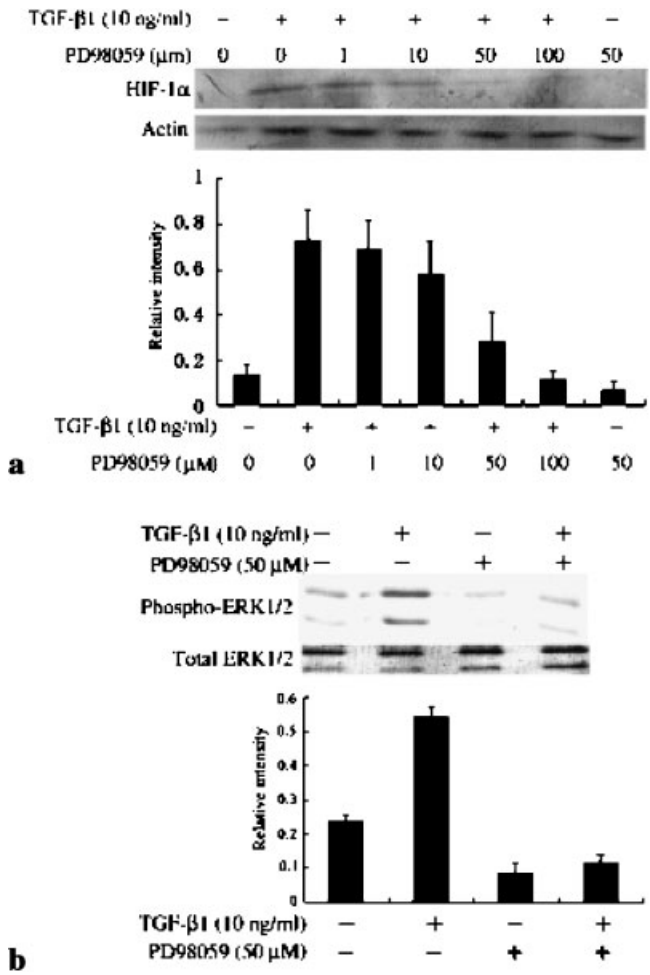

Fig. 5. Inhibition of the ERK1/2 MAPK pathway with PD98059 blocks TGF-β1-induced HIF-1a protein expression. **a**: Serum-starved normal human cytotrophoblast cells were pretreated for 1 hr with PD98059 (0, 1, 10, 50, 100 μM), and then incubated in the presence (+) or absence (−) of exogenous TGF-β1 (10 ng/ml) for 24 hr. Cells were harvested for analysis of HIF-1a protein by Western blotting. Bar graphs show the levels of HIF-1a quantity corrected for Actin. Bars represent the mean ± SEM of values from three independent experiments. **b**: Representative blot phosphorylation of ERK from cellular extracts pretreated for 1 hr with PD98059 (50 μM), and then exposed to TGF-β1 (10 ng/ml) for 24 hr, as determined using Western blotting with anti-phospho-ERK antibody (phospho ERK1/2). Western blotting of total ERK1/2 protein (total ERK1/2) in cellular extracts is also shown. Where indicated with Bar graphs show the levels of activated ERK1/2 quantity corrected for total ERK1/2. Bars represent the mean ± SEM of values from three independent experiments.

is expressed in villous syncytiotrophoblasts and extravillous cytotrophoblast throughout gestation and in decidual cells during the first trimester (Breier et al., 1992; Sharkey et al., 1993; Jackson et al., 1994; Ahmed et al., 1995; Chung et al., 2000). It has been well known that HIF-1 is one of the major transcriptional activators of VEGF gene (Semenza et al., 1997). Several cytokines have been proved to regulate HIF-1-mediated VEGF expression (Shih and Claffey, 2001; Fukuda et al., 2002; Stiehl et al., 2002). TGF-β1, a pro-angiogenic cytokine, has been found to express in a variety of tissues

including human trophoblasts and endometrium and regulates VEGF expression in human trophoblast cell (Tazuke and Giudice, 1996; Chuang et al., 2000). However, little is known about the molecular mechanism of TGF-β1-regulated VEGF secretion in normal human cytotrophoblast cells. In the current study, we addressed a possible route that TGF-β1 regulated HIF-1-mediated VEGF secretion for the first time in the normal human cytotrophoblast cells.

Chung et al. (2000) have reported that culture of a first trimester trophoblast cell line (HTR-8/SVneo) in the presence of TGF-β1, resulted in the increase of VEGF secretion in culture media. In this study, we obtained the similar results in the normal human cytotrophoblast cells. Our data showed that culture of the primary normal human cytotrophoblast cells in the presence of TGF-β1 resulted in the secretion of significant levels of VEGF in culture media in a time-dependent fashion. In addition, we found that the normal human cytotrophoblast cells showed a dose-dependent increase in VEGF secretion in the culture media when the presence of TGF-β1. This finding is in line with previous findings by Saadeh et al. (1999) who found that TGF-β1 stimulated a dose-dependent increase in VEGF protein production by MC3t3 E1 osteoblastic cells. Our results provide the further evidence that TGF-β1 stimulate VEGF secretion in normal human cytotrophoblast cells. In addition, TGF-β1 may decrease VEGF expression to reduce angiogenesis, and counteract the overexpression of VEGF in pre-eclampsia in correspondence with Caniggia and Winter (2002) and Madazli et al. (2003). Moreover, our observations indicated that the up-regulation of VEGF by TGF-β1 might induce angiogenesis, and compensate for the low blood flow during embryo implantation.

It has been reported that signaling by transforming growth factor family members regulate gene transcription through functional cooperatives and associations with other DNA-binding proteins, and analysis of the human HIF-1a promoter activity indicates that HIF-1a cooperates with the elements of TGF-β signaling (Sanchez-Elsner et al., 2001). We found that HIF-1a protein synthesis was increased by a dose-dependent manner, when the normal human cytotrophoblast cells were treated with TGF-β1. However, TGF-β1 cannot induce HIF-1a mRNA expression, demonstrating specific effects of TGF-β1 on HIF-1a protein expression. These findings led us to hypothesize that TGF-β1 stimulated HIF-1a expression in protein levels may be one of the reasons for the increasing of VEGF secretion in the culture media when the normal human cytotrophoblast cells were administrated with TGF-β1. It has been known that MAP kinase cascades participate in the TGF-β signaling (Mulder, 2000). In this study, ERK1/2 signaling was regarded as an important factor to be examined. Treatment of the normal cytotrophoblast cells with exogenous TGF-β1 resulted in ERK1/2 phosphorylation. Our findings demonstrated that a rapid activation of ERK1/2 in the normal human cytotrophoblast cells occurred within 10–60 min of TGF-β1 addition, and reached a maximum at 40 min. These data indicate that activation of ERK1/2 MAPK signal cascade is an early event in normal human cytotrophoblast cells in response to TGF-β1 stimulation. Then we also evaluated the effect of inhibiting the ERK1/2 pathway on this process. We found that PD98059, a specific inhibitor of ERK1/2, can decrease the expression of HIF-1a in protein level which was stimulated by the exogenous TGF-β1 in the normal human cytotrophoblast cells in a dose-dependent fashion. Meanwhile, the increased VEGF secretion in the culture media was reduced because of the presence of PD98059. It indicates that PD98059 prevents not only the ERK1/2 activity, but also the expression of HIF-1a protein and the secretion of VEGF that were induced by exogenous TGF-β1 in the normal human cytotrophoblast cells. These novel findings raise possibility that ERK1/2 pathway may participate in the induction of HIF-1-mediated-VEGF secretion by TGF-β1 in the normal human cytotrophoblast cells.

Taken together, our investigation implicate a likely route that TGF-β1, as a pro-angiogenic cytokine, stimulates HIF-1-mediated VEGF secretion that may be dependent on ERK1/2 signal pathway in normal human cytotrophoblast cells. These observations will permit further investigation of the precise mechanism of TGF-β1 regulated VEGF secretion on human placental vasculture.

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