Dual effect of transforming growth factor β1 on cell adhesion and invasion in human placenta trophoblast cells

Mei-rong Zhao[1,2,3], Wei Qiu[1,3], Yu-xia Li[1], Zhi-bin Zhang[2], Dong Li[1,3] and Yan-ling Wang[1]

[1]State Key Laboratory of Reproductive Biology, [2]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 25 Bei Si Huan Xi Road, Beijing 100080, People's Republic of China and [3]Graduate School of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Correspondence should be addressed to Y-L Wang; Email: wangyl@ioz.ac.cn

M-R Zhao and W Qiu contributed equally to this work.

Abstract

Transforming growth factor β (TGFβ) has been shown to be a multifunctional cytokine required for embryonic development and regulation of trophoblast cell behaviors. In the present study, a non-transformed cell-line representative of normal human trophoblast (NPC) was used to examine the effect of TGFβ1 on trophoblast cell adhesion and invasion. *In vitro* assay showed that TGFβ1 could significantly promote intercellular adhesion, while inhibiting cell invasion across the collagen I-coated filter. Reverse transcription (RT)-PCR and gelatin zymography demonstrated that TGFβ1 evidently repressed the mRNA expression and proenzyme production of matrix metalloproteinase (MMP)-9, but exerted no effect on mRNA expression and secretion of MMP-2. On the other hand, both the mRNA and protein expression of epithelial-cadherin and β-catenin were obviously upregulated by TGFβ1 in dose-dependent fashion, as revealed by RT-PCR and western-blot analysis. What is more, one of the critical TGFβ signaling molecules – Smad2 was notably phosphorylated in TGFβ1-treated NPC cells. The data indicates that cell invasion and adhesion are coordinated processes in human trophoblasts and that there exists paracrine regulation on adhesion molecules and invasion-associated enzymes in human placenta.

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Introduction

Successful implantation and pregnancy require the well development of a complex maternal–fetal crosstalk, during which trophoblast cell invasion to the uterus is one of the most essential events. Unlike tumor cell metastasis, trophoblast cell invasion is a highly controlled process and needs the coordination with various paracrine factors derived from maternal deciduas. Among them, transforming growth factor β (TGFβ) has been shown to be a multifunctional cytokine required for embryonic development and plays crucial roles in regulating trophoblast cell proliferation, differentiation, adhesion, and invasion/migration (Graham *et al.* 1992, Lysiak *et al.* 1995).

There has been some evidence regarding the effect of TGFβ on trophoblast cell behaviors. Choriocarcinomal cells, including JEG-3, JAR, and Bewo cell lines, were found to be TGFβ resistant. This may be partly due to the loss of Smad3, which results in the functional disruption of the TGFβ signaling pathway (Xu *et al.* 2002). By using primary cultured human cytotrophoblast (CTB) or extravillous trophoblast (EVT) cells derived from the first trimester placenta, as well as some of the transformed trophoblast cell lines, including HRT-8/SVneo, SGHP-4, and ED-27, it was revealed that TGFβ could inhibit trophoblast cell proliferation, steroid hormone production, and invasion/migration (Graham *et al.* 1995, 1997, Luo *et al.* 2002, Ma *et al.* 2002, Tse *et al.* 2002). However, the divergent mechanisms have been reported, especially in association with cell invasion regulation by TGFβ. For instance, Karmakar and Das (2002) found that TGFβ1 upregulated the expression of tissue inhibitor of metalloproteinase (TIMP)-1 and -2 and plasminogen activator inhibitors (PAI)-1 and -2, while Smith *et al.* (2001) demonstrated an inhibitory effect of TGFβ1 on TIMP-1 production in ED27 cells and Ma *et al.* (2002) reported that TGFβ1 treatment suppressed PAI-2 levels, while enhanced PAI-1 expression in HTR-8/SVneo cells. The discrepancies may result from different characteristics of various cell models used by the researchers. Therefore, a stable normal

trophoblast cell model is indispensable for elucidating the molecular mechanisms of TGFβ effects.

It has been well accepted that cell invasion is always associated with enhanced cell motility and repressed cell-to-cell adhesion mediated by a variety of cell adhesion molecules. Epithelial-cadherin (E-cadherin), a member of the cadherin family that mediates calcium-dependent cell-to-cell adhesion (Takeichi *et al.* 1988), is mainly expressed in most epithelial cells, and is primarily responsible for initiating cell adhesion, promoting cell polarity, forming specialized cell–cell junctions in epithelial cells (Shirayoshi *et al.* 1983, Boller *et al.* 1985, McNeill *et al.* 1990). E-cadherin can interact through its cytoplasmic domain with catenins proteins, i.e. α- and β-catenin, to establish firm cell–cell adhesion. In the human placenta, both E-cadherin and β-catenin were found in trophoblasts, and they exhibited specific temporal and spatial change on the feto–maternal interface during pregnancy, indicating their involvement in the regulation of trophoblast cell adhesion, migration, and differentiation (Zhou *et al.* 1997*a*,*b*, Li *et al.* 2003). Data from Karmakar and Das (2004) showed that TGFβ1 upregulated intercellular adhesion along with an increased E-cadherin expression, and reduced cell invasiveness in both JEG-3 and trophoblast cells isolated from early and term placentae. However, their results are inconsistent with some other reports, where choriocarcinoma cells were demonstrated to be independent of TGFβ stimulation (Xu *et al.* 2001*a*,*b*). Therefore, this work, aimed to elucidate whether TGFβ1 can coordinately regulate cell invasion and adhesion in a non-transformed cell-line representative of normal human trophoblast (NPC).

NPC is a non-transformed cytotrophoblast cell line derived from human normal placental tissues in early pregnancy, and it has been proven to possess similar endocrine functions and growth regulations to those of the primary CTB in the first trimester (Li *et al.* 1996, 1997). Our previous data demonstrated the existence of Smad-2, -3, -4, and -7, as well as the inducible increase of Smad-2 and -4 expression challenged by TGFβ1 in NPC cells (Wu *et al.* 2001), indicating that the cell line may be a good model to study the role of TGFβ1 in human trophoblast cells.

In the present study, we use NPC cells to examine the effect of TGFβ1 on trophoblast cell adhesion and invasion. The production of E-cadherin and β-catenin, as well as matrix metalloproteinase (MMP)-2 and MMP-9 were detected to elucidate the mechanisms of TGFβ1 function in human trophoblasts.

Materials and Methods

Culture and treatment of normal human placenta cytotrophoblast (NPC) cell line

The NPC cells were cultured as previously described (Li *et al.* 1996, 1997). In brief, the cells were maintained in serum-free FD medium (F-12: Dulbecco's modified Eagle's medium (DMEM), 1:1; Gibco BRL) with supplement of 10 ng/ml epidermal growth factor (EGF; Sigma Chemical Co.), 10 μg/ml insulin (Sigma), 0.1% BSA (Sigma), and 2 mM glutamine (Sigma), and kept at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The culture media were refreshed every 1–2 days. Subculture at a ratio of 1:3 was performed with routine trypsinization every 5 days.

After the NPC cells were seeded in 60 mm culture dishes (Corning, NY, USA) for 24 h, EGF was withdrawn from the medium and TGFβ1 (Sigma) was added at 1–50 ng/ml for 24 h, and at least three dishes of the cells at each dosage were collected for further detection.

RNA isolation and semi-quantitative reverse transcription (RT)-PCR

Total RNA from cells was isolated using TRIzol reagent (Gibco BRL) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed in a 20 μl reaction mixture with random hexamer primers (Promega) by Moloney murine leukemia virus RT as specified by the manufacturer (Fermentas, Vilnius, Lithuania). One microliter aliquot of the RT products was amplified with specific primers (Runbio Biotechnology, Beijing, China) designed according to specific cDNA sequences in NCBI database. The primer sequences and the reaction conditions are summarized in Table 1. The 25 μl PCR system contained 2 μl RT products, 200 μmol/l dNTPs, 2 mmol/l $MgCl_2$,

Table 1 The primer sequences and reaction conditions for reverse transcription (RT)-PCR.

Gene name	Sequence of primers	Annealing temperature (°C)	Length of PCR product (bp)
MMP-9	Forward CCC TTC TAC GGC CAC TAC TGT G Backward GCA CTG CAG GAT GTC ATA G	58	612
MMP-2	Forward CAC CTA CAC CAA GAA CTT CC Backward AAC ACA GCC TTC TCC TCC TG	57	378
E-cadherin	Forward GCC AAG CAG CAG TAC ATT CTA CAC GG Backward GCT GTT CTT CAC GTGCTC AAA ATC C	55	346
β-catenin	Forward CGT GGA CAA TGG CTA CTC AA Backward GCT GTT CTT CAC GTGCTC AAA ATC C	58	396
GAPDH	Forward ACC ACA GTC CAT GCC ATC AC Backward TCC ACC ACC CTG TTG CTG TA	55	452

1 IU Taq polymerase, and 10 pmol of each primer. The template amount and cycle number were determined by a preliminary experiment to ensure that the amplification was carried out within the exponential phase of PCR. The PCR products were subjected to electrophoresis on a 1.5% agarose gel and analyzed using the Gel-Pro Analyzer (software version 4.0; United Bio., NJ USA). The relative densities of the detected genes were determined by normalization with the value of the house-keeping gene (glyceraldehyde-3-phosphate dehydrogenase, GAPDH).

Western-blot analysis

After the cells were treated with TGFβ for 24 h, they were lysed with lysis buffer (20 mM Tris–HCl buffer pH 8.0, 1 mM DTT, 0.2% NP40, 100 μM PMSF, 5 mg/ml aprotinin, chymostatin, leupeptin, pristine, and trypsin inhibitor) on ice for 20 min. The lysates were centrifuged at 13 000 r.p.m. and the supernatant was collected. After measuring the protein concentration according to the method of Bradford (1976), 25 μg protein was subjected to 10% SDS-PAGE and then transferred onto a PVDF membrane. The membrane was blocked with 5% defatted milk in PBS containing 0.1% Tween-20, incubated with rabbit antibodies against human E-cadherin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), β-catenin (1:1000; Santa Cruz Bio-technology), Smad2 (1:1000; Neomarker, Montreal, Que., Canada), phosphorylated Smad2 (pho-Smad2, 1:1000; Neomarker), and β-actin (Santa Cruz Bio-technology) respectively. The membrane was further incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG, 1:2000; Santa Cruz Biotechnology). Final visualization was achieved by ECL Western Blotting Analysis System (Pierce, Rockford, IL, USA), and the signals were exposed to X-ray films (Fuji, Japan) and analyzed by the Gel-Pro Analyzer (software version 4.0; United Bio.). The relative densities of β-cadherin, β-catenin, Smad2, and pho-Smad2 were determined by normalization with density value of β-actin.

Gelatin zymography

The presence of MMP-2 and -9 in the media was demonstrated by gelatin zymography. The harvested culture media were standardized according to the protein contents of cell lysates. Ten to twenty microliter media were subjected to 10% SDS-PAGE containing 1 mg/ml gelatin. After electrophoresis, the gel was washed at room temperature for 1 h in 2.5% Triton X-100, 50 mM Tris–HCl (pH 7.5), and then incubated at 37 °C overnight in a buffer containing 150 mM NaCl, 5 mM CaCl, and 50 mM Tris–HCl (pH 7.6). The gel was subsequently stained with 0.1% (w/v) Coomassie Brilliant Blue R-250, and destained in 10% (v/v) methanol and 5% (v/v) glacial acetic acid. The results were analyzed using the Gel-Pro Analyzer (software version 4.0; United Bio.).

Cell adhesion analysis

Cell adhesion analysis was performed as previously reported (Yang *et al.* 1996). In brief, NPC cells were cultured in 96-well plates until 100% confluence as bottom cells. Some other NPC cells were treated with or without 10 ng/ml TGFβ1 for 24 h, and seeded at 1×10^5 cells/well onto the attached bottom cells in the 96-well plates. Two hours later, the non-adherent cells were discarded by washing with PBS buffer, and the remaining cells were fixed with 4% paraformaldehyde (PFA) and subjected to Giemsa staining. The cell amounts were measured by reading the absorbance at 655 nm and the value of adherent cells was calculated by deducting that of the attached bottom cells.

Transwell invasion assay

Transwell invasion assay was conducted in 24-well fitted inserts with membranes (8 μm pore size; Costar, Cambridge, MA, USA) according to the method reported previously with slight modification (Mira *et al.* 1999). Briefly, NPC cells were plated at 2×10^4 cells in transwell insert pre-coated with type I collagen (Col I; 80 μg/ml; Cell Matrix Type I-A, Institute of Biochemistry, Osaka, Japan), and incubated with FD medium supple-mented with or without 10 ng/ml TGFβ1. Lower chambers were loaded with the same medium. Twenty-four hours later (the time point was determined by a preliminary experiment when about 70% cells invade across the insert), the cells on the upper surface of membrane were completely removed, and the migrated cells were fixed with 4% PFA and stained with hematoxylin. Membranes were then cut from inserts and mounted onto glass slides. Cell invasion indices were determined by counting the number of stained cells in ten randomly selected non-overlapping fields of the membranes under light microscope.

Statistical analysis

RT-PCR, transwell invasion assay, cell adhesion analysis, and western-blot analysis were all repeated at least three times, each with at least three dishes of cells per time point or per treatment dosage. Data of RT-PCR were measured by comparing the densitometry value of MMP-2, -9, E-cadherin, and β-catenin with that of GAPDH; and the results of western blotting were measured by comparing the densitometry value of E-cadherin, β-catenin, Smad2, and pho-Smad2 with that of actin in the same experimental set. The data

were reported as the average±s.D. according to the results from three independent experiments. Comparison of the values between groups was performed by ANOVA and $P<0.05$ was considered significant.

Results

Effect of TGFβ1 on cell–cell adhesion in NPC cells

With the cell adhesion assay, it was shown that the treatment of TGFβ1 could significantly promote intercellular adhesion of NPC cells, with the adherent cell amount being approximately 2.9-fold more than that of the untreated cells (Fig. 1A).

Effect of TGFβ1 on cell invasion in NPC cells

To understand the role of TGFβ1 on NPC cell migration/invasion, Col I-coated transwell filter invasion assay was performed. In the previous work of Li and Zhuang (1997), it was shown that TGFβ1 alone did not influence NPC cell growth. Accordingly, we did not account for the effect of TGFβ1 on cell proliferation in the invasion assay here. Figure 1B displayed graphically the results of invasion assay. Challenging with 10 ng/ml TGFβ1 could repress NPC cell invasion through the Col I membrane by approximately 76% when compared with the control (Fig. 1B and C).

Influence of MMP-2 and MMP-9 production in NPC cells by TGFβ1

Both RT-PCR and gelatin zymography were performed to determine the change of MMP-2 and -9 production in NPC cells treated with TGFβ1. It was revealed that both MMP-9 mRNA expression and latent MMP-9 secretion were decreased by TGFβ1 in a dose-dependent manner. The maximal inhibition was observed at 50 ng/ml TGFβ1, when the mRNA expression and latent MMP-9 production decreased to 24.7 and 7.3% of those of control respectively. However, no effects of TGFβ1 on active MMP-9 secretion or MMP-2 mRNA and protein productions were detected in NPC cells (Figs 2 and 3).

Stimulation of E-cadherin and β-catenin expression by TGFβ1 in NPC cells

E-cadherin and β-catenin have been well accepted to be involved in mediating intercellular adhesion. In NPC cells, both the mRNA and protein expression of these molecules have been shown to be obviously upregulated by TGFβ1 in dose-dependent fashions, as revealed by RT-PCR and western-blot analysis with values of GAPDH and β-actin for normalization respectively. TGFβ1 at 1–50 ng/ml were effective to stimulate the expression of E-cadherin and β-catenin. For mRNA expressions, 50 ng/ml TGFβ1 led to maximal stimulations, with the levels of E-cadherin and β-catenin being 2- and 3.5-fold of control respectively. For protein expressions, the optimal effect of TGFβ1 was observed at 10 ng/ml, and the relative density of E-cadherin and β-catenin was 5.8- and 3.1-fold of the control respectively (Figs 4 and 5).

Activation of Smad2 by TGFβ1 treatment in NPC cells

Data from western blotting revealed that treatment of 1–50 ng/ml TGFβ1 showed no obvious influence on Smad2 expression, but evidently increased the level of pho-Smad2. The maximal stimulation of pho-Smad2, which was 7.8-fold more than that of control, was observed at 10 ng/ml TGFβ1 (Fig. 6).
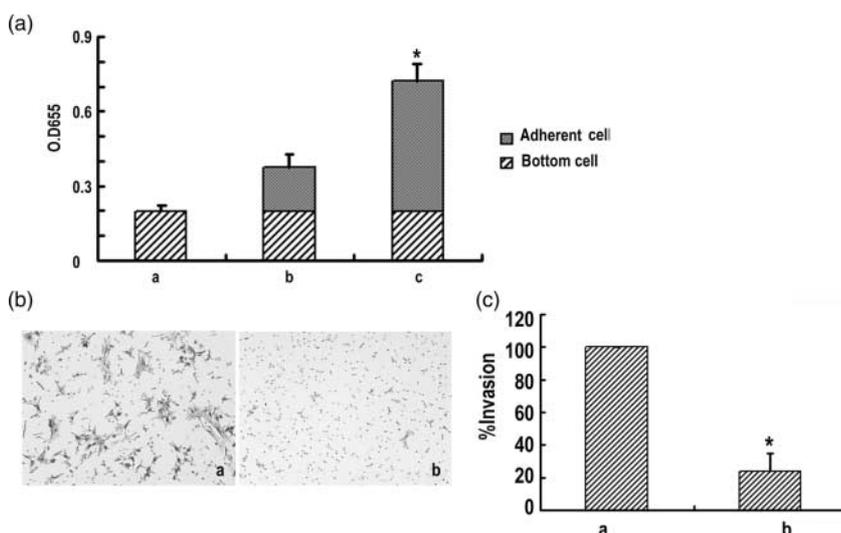
Figure 1 Effect of transforming growth factor (TGF)β1 on intercellular adhesion and cell invasion in normal human trophoblast (NPC) cells. (A) Cell-to-cell adhesion assay in NPC cells. Statistical analysis by ANOVA was performed according to three independent experiments, and the value was presented as mean±s.D. (a) Negative control with only bottom cells; (b) adhesion between untreated cells and bottom cells; (c) adhesion between TGFβ1-treated cells to bottom cells. *Compared with (b), $P<0.05$. (B) and (C) Transwell insert invasion assay in NPC cells. The cells were treated with 0 ng/ml TGFβ1 (a) or 10 ng/ml TGFβ1 (b) for 24 h. Statistical analysis by ANOVA was performed according to three independent experiments, and the value was presented as mean±s.D. *Compared with (a), $P<0.05$.
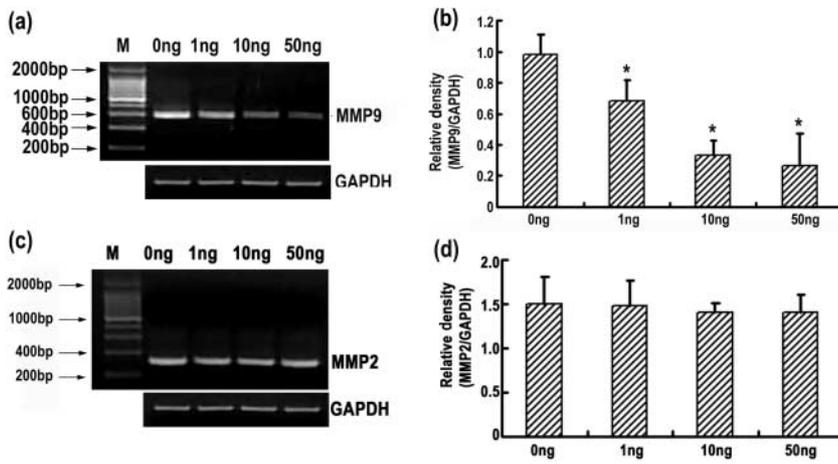
Figure 2 Semi-quantitative reverse transcription (RT)-PCR to manifest the dose-dependent regulation on matrix metalloproteinase (MMP)-9 and -2 mRNA expression by TGFβ1 in NPC cells. (a) and (c) Products of a typical RT-PCR were subjected to 1.5% agarose gel electrophoresis. 0, 1, 10, and 50 ng represent RT-PCR products using mRNA derived from the NPC cells treated by 0, 1, 10, and 50 ng/ml TGFβ1 separately. (b) and (d) Statistical analysis by ANOVA for the semi-quantitative RT-PCR according to three independent experiments. The values of MMP-9 and -2 were normalized with that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) respectively, and the relative amount was presented as mean ± s.D. *Compared with 0 ng, $P < 0.05$.

Discussion

It has been well accepted that TGFβ1 can inhibit trophoblast cell invasion. However, discrepancies exist regarding the involved molecular mechanisms, mainly due to the different properties of cell models used by various investigators. In the present study, we used NPC cell line that was previously established in this laboratory as the *in vitro* cell model. NPC has been characterized as a non-transformed cytotrophoblast cell line derived from human normal placenta villi, and proven to maintain most of the endocrine functions, growth regulations of normal CTB at the first trimester (Li *et al.* 1996, 1997). Some of our unpublished data revealed little endogenous production of TGFβ1 in NPC cells, while the response of NPC cells to exogenous TGFβ1 challenging was demonstrated (Wu *et al.* 2001). The production of MMPs and their invasive abilities *in vitro* shown in this study further indicate the EVT cell properties of NPC cells. Therefore, we propose that the NPC cell line may be a good model to investigate TGFβ1 function in human trophoblasts.

MMPs have been considered critical for trophoblast cell migration/invasion. The effect of TGFβ1 on MMP-9 and -2 production in NPC cells reported here is inconsistent with previous reports by Meisser *et al.* (1999) and Lash *et al.* (2005). By using the primary

cultured CTB and EVT cells from placental explants respectively, they demonstrated that TGFβ1 could inhibit MMP-9 activity along with the repressed cell invasion ability, but was without effect on MMP-2 activity. In contrast, Graham *et al.* (1993) reported that TGFβ1 could increase the level of MMP-2 mRNA, but did not influence MMP-2 activity in HTR-8 cells, although the cell invasion was evidently inhibited. The effect of TGFβ1 on MMP-9 production can hardly be detected in HTR-8 cells due to little MMP-9 production in the cells. Librach *et al.* (1991) have demonstrated that the invasiveness exhibited *in vitro* by human normal trophoblast cells depends on the production of MMP-9. Our previous work in normal CTB cells also showed that cell migration was significantly promoted by vitronectin, accompanied by increase in MMP-9 concentration and activity, while no change in MMP-2 and membrane type (MT)-MMP-1 productions (Xu *et al.* 2001a,b). Furthermore, Morgan *et al.* (1998) found that human trophoblast cell lines that secreted MMP-9 had high invasive ability, whereas the BeWo cell line, which produces mainly MMP-2 and little MMP-9, was non-invasive. Therefore, it is likely that MMP-9 is more critical for human trophoblastic cell invasion. Recently, MMP-26 was suggested to function as an intracellular activator of
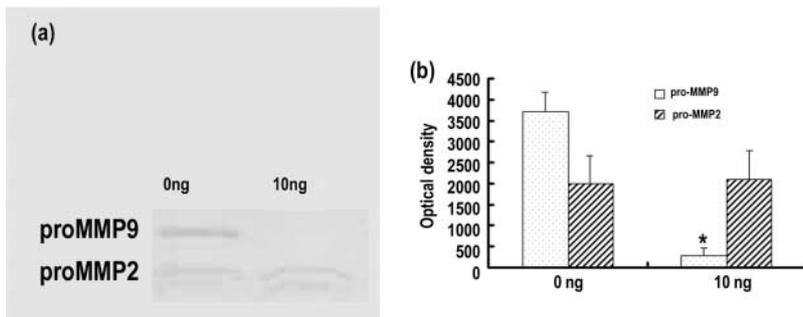
Figure 3 Gelatin zymography to measure secretion of MMP-2 and -9 in NPC cells treated with 0 ng/ml (0 ng) or 10 ng/ml (10 ng) TGFβ1. (a) A typical result of gelatin zymography. (b) Densitometric analysis of gelatin zymography. Statistical analysis was performed by ANOVA according to three independent experiments, and the values of proenzymes (proMMP-2 and -9) are presented as means ± s.D. *Compared with 0 ng, $P < 0.05$.
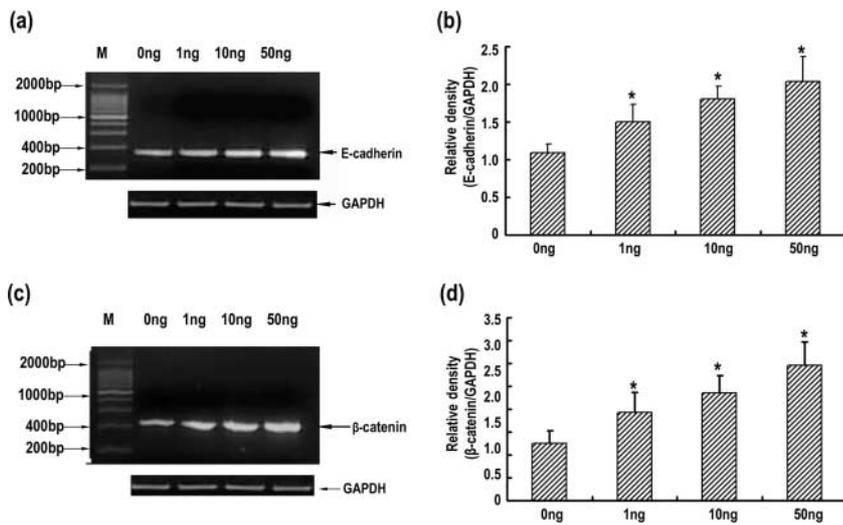
Figure 4 Semi-quantitative RT-PCR to manifest the dose-dependent regulation on epithelial-cadherin (E-cadherin) and β-catenin mRNA expression by TGFβ1 in NPC cells. (a) and (c) Products of a typical RT-PCR were subjected to 1.5% agarose gel electrophoresis. 0, 1, 10, and 50 ng represent RT-PCR products using mRNA derived from NPC cells treated by 0, 1, 10, and 50 ng/ml TGFβ1 separately. (b) and (d) Statistical analysis by ANOVA for the semi-quantitative RT-PCR according to three independent experiments. The value of E-cadherin and β-catenin were normalized with that of GAPDH, and the relative amount was presented as mean ± S.D. *Compared with 0 ng, $P < 0.05$.

MMP-9 (Zhao *et al.* 2003). Some of our unpublished data revealed that TGFβ1 could inhibit MMP-26 expression in NPC cells, and MMP-26 itself had potential to promote NPC cell invasion (M-R Zhao unpublished data). Taken together, we propose that TGFβ1 may inhibit trophoblast cell invasion mainly through downregulating MMP-9 expression and/or activity, as well as influencing interactions between MMP-9 and other MMPs like MMP-26.

E-cadherin and β-catenin are the main mediators of intercellular adhesion in epithelial cells. *In vivo*, the loss of E-cadherin expression is correlated with aggressiveness and metastasis in different cancer types. E-cadherin is, therefore, considered to act as an invasion suppressor. The idea has been supported by some studies in various types of carcinoma cells. Using an *in vivo* human airway epithelial xenograft model in nude mice as well as the *in vitro* cell culture system, Nawrocki-Raby *et al.* (2003) demonstrated that E-cadherin transfected invasive bronchial tumor cell-line BZR were less invasive than control vector-transfected cells. On the maternal–fetal interface during normal pregnancy, E-cadherin was extensively distributed in villous CTB and column trophoblasts. Along with the invasive pathway, the level of E-cadherin was downregulated gradually following the differentiation toward the invasive trophoblasts *in vitro* and *in vivo* (Babaawale *et al.* 1996, Zhou *et al.* 1997*a,b*). The expression pattern may also be altered in pathological conditions related to trophoblastic invasion. Trophoblast shallow invasion of the uterus and endovascular is believed to play a critical role in pathogenesis of preeclampsia. It was shown that there was an apparent downregulation of E-cadherin and β-catenin expression in interstitial trophoblasts and vascular trophoblasts colonizing maternal blood vessels (Zhou *et al.* 1997*a,b*, Li *et al.* 2003). Furthermore, our present study and some of the others (Karmakar & Das 2002) demonstrated that trophoblast invasion and adhesion were reversely influenced by paracrine factors like TGFβ1. All these *in vivo* and *in vitro* data indicated
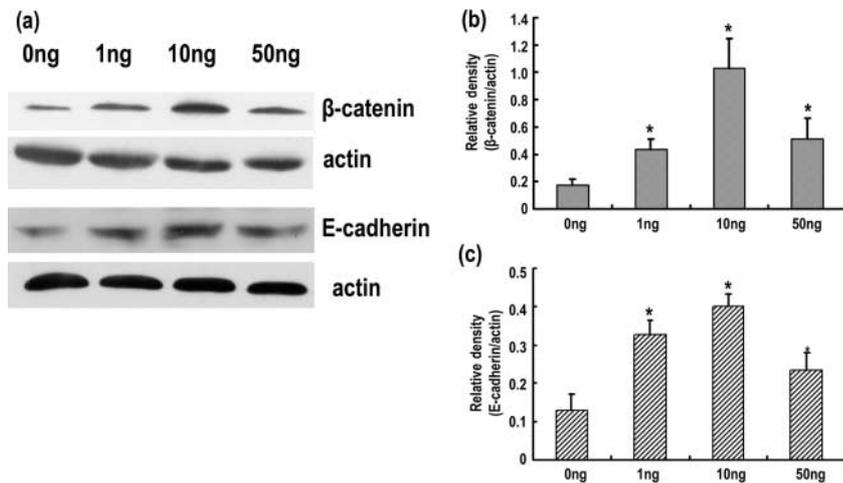
Figure 5 Western-blot analysis to show the dose-dependent regulation on E-cadherin and β-catenin protein expression by TGFβ1 in NPC cells. (a) Typical results of western blotting. 0, 1, 10, and 50 ng represent proteins derived from NPC cells treated by 0, 1, 10, and 50 ng/ml TGFβ1 separately. (b) and (c) Statistical analysis by ANOVA for western blottings according to three independent experiments. The value of E-cadherin and β-catenin was normalized by that of actin, and the relative amount was presented as mean ± S.D. *Compared with 0 ng, $P < 0.05$.
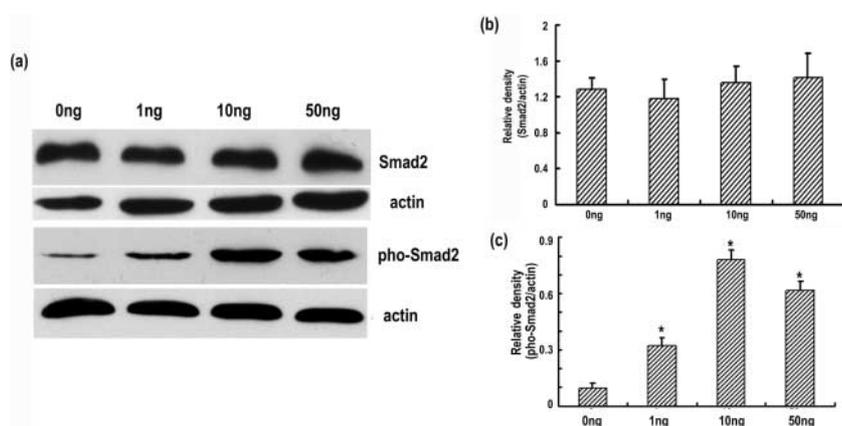
Figure 6 Western-blot analysis to show the regulation on Smad2 and phosphorylated Smad2 (pho-Smad2) by TGFβ1 in NPC cells. (a) Typical results of western blotting. 0, 1, 10, and 50 ng represent proteins derived from NPC cells treated by 0, 1, 10, and 50 ng/ml TGFβ1 separately. (b) Statistical analysis by ANOVA for western blotting of Smad2 according to three independent experiments. The value of Smad2 was normalized by that of actin, and the relative amount was presented as mean±s.d. (c) Statistical analysis by ANOVA for western blotting of phosphorylated Smad2 (pho-Smad2) according to three independent experiments. The value of pho-Smad2 was normalized by that of actin, and the relative amount was presented as mean±s.d. *Compared with 0 ng, $P<0.05$.

that trophoblast cell invasion and adhesion are two tightly associat processes during gestation.

The coordination between the processes of cell adhesion and invasion may result from the interactions between MMPs/TIMPs and E-cadherin/β-catenin. More and more data indicate the regulation of MMPs by E-cadherin and/or β-catenin. Llorens *et al.* (1998) and Ara *et al.* (2000) demonstrated that MMP-9 and MT1-MMP were regulated by E-cadherin in mouse skin and human tongue squamous carcinoma cells respectively. Recently, Nawrocki-Raby *et al.* (2003) revealed that the transfection of E-cadherin in bronchial BZR tumor cells induced a decrease of MMP-1, -3, -9, and MT1-MMP production *in vitro* and *in vivo*. On the other hand, MMP/TIMP proteolytic axis was involved in affecting cell–cell adhesion. In Swiss 3T3 fibroblasts, Ho *et al.* (2001) found that upregulation of TIMP-1 or treatment with synthetic MMP inhibitor (MMPi) could increase E-cadherin protein levels and localization at cell–cell contacts, as well as the calcium-dependent cell–cell aggregation in association with β-catenin. They proposed that MMPi could prevent cadherin ectodomain cleavage and thus stabilize cadherin-mediated cell–cell contacts and their association with actin cytoskeleton through β-catenin. In the present study, the increased expression of E-cadherin/β-catenin and repressed MMP-9 production occurred simultaneously after TGFβ1 challenging in NPC cells, indicating the interaction between cell adhesion molecules and invasion-associated enzymes in human trophoblasts. As a matter of fact, β-catenin is a shared target of cadherins and Wnt signaling pathway. It transmits Wnt signal to the nucleus and forms a complex with transcription factors of the Tcf/LEF family to activate specific Wnt target genes (Gumbiner 1995, Grosschedl & Birchmeier 1996, Huber & Weis 2001). Several members of MMP family, including MT1-MMP, MMP-7, and -26 have been demonstrated to be Wnt target genes (Brabletz *et al.* 1999, Takahashi *et al.* 2002, Marchenko *et al.* 2004). E-cadherin binding will prevent β-catenin nuclear localization and transactivation of transcription. Thus, β-catenin signaling through Wnt pathway may be a mechanistic link between MMP-proteolysis and intercellular adhesion, and this sharing of a critical component between two fundamental processes – cell adhesion and signaling of cell motility – may reflect a need for coordinated control between them (Mariann 2005). However, it is yet to be determined how MMP-9 and E-cadherin/β-catenin interact in trophoblast cells challenged with TGFβ1.

Smad proteins play central roles in manifestation of the biological activities of TGFβ signaling (Kohei *et al.* 2003). Our previous work revealed that TGFβ1 could induce modest increases in Smad2 and Smad4 mRNA levels without affecting Smad3 mRNA expression in NPC (Wu *et al.* 2001). The data presented here further elucidate promoted phosphorylation of Smad2, but unchanged the protein expression of Smad2 by TGFβ1 treatment in NPC cells. All these data indicated that Smad2 might be involved, at least partly, in activating the downstream signaling molecules to coordinately regulate cell invasion and adhesion in human trophoblast cells.

In summary, the present study elucidated the promoted intercellular adhesion and repressed cell invasion by TGFβ1 in the human cytotrophoblast NPC cell line. Meanwhile, the upregulated expression of E-cadherin and β-catenin as well as the downregulated MMP-9 production were demonstrated. The data indicated the existence of paracrine regulation on the coordinated processes of cell invasion and adhesion in human trophoblasts.

Acknowledgements

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