The Extracellular Regulated Kinase 1/2 is Involved in the Inhibition of Matrix Metalloproteinase-9 by Transforming Growth Factor-β1 in Trophoblast Cells

Sheng-Ping Chen*, Hong-Xia Li

(*Beijing Shijitan Hospital, Department of Obstetrics and Gynecology, Beijing 100038, China*)

Abstract Normal trophoblast invasion is stringently controlled by transforming growth factor-β (TGF-β). In this study, we investigated TGF-β1-mediated regulation of matrix metalloproteinase (MMP)-2, and -9 in human normal trophoblast cells (first trimester cytotrophoblast, CTB). The results showed that TGF-β1 inhibited *MMP-9* mRNA abundance and the level of pro-enzyme secretion in CTB cells, but did not affect MMP-2 expression. Interleukin-1β (IL-1β) and TGF-β1 both showed inhibitory but mutually antagonistic effects on *MMP-9* mRNA and pro-enzyme expression. Furthermore, inhibition of the extracellular signal-regulated kinase 1/2 (ERK1/2) blocked TGF-β1-reduced *MMP-9* mRNA and pro-MMP-9 expression. These observations suggest that normal trophoblast invasion is controlled by TGF-β1 through inhibiting MMP-9 activity via ERK1/2.

Key words TGF-β; MMP; trophoblast; ERK

Trophoblastic invasion into the uterus and its vasculature is essential to achieve implantation and placentation, and for adequate fetal-maternal exchange of nutrients. However, this process differs from the infiltration of cancer cells, in which the invasive potential of trophoblasts (which is spatially limited to the endometrium and the proximal myometrium, and temporally terminated by about mid-gestation) is stringently controlled *in situ* by local molecules such as transforming growth factor-β (TGF-β). A subpopulation of cytotrophoblast (CTB) called extravillous trophoblast (EVT) infiltrates into the stroma, and its proliferation, migration and invasiveness are negatively regulated primarily by TGF-β[1].

TGF-β superfamily members transmit their signals through type II receptor binding to and subsequently phosphorylation of type I receptor[2]. Upon receptor phosphorylation, receptor-regulated Smads (R-Smads) form oligomeric complexes with Smad4 (common-partner Smad, Co-Smad), and then translocate into the nucleus where they regulate the transcription of target genes among R-Smads, Smad2 and Smad3 mediate TGF-βs/activins signals[2]. However, mitogen-activated protein kinase (MAPK) pathways, such as the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway, can also be directly activated by the TGF-β receptor[3].

It has been widely recognized that the invasion process of trophoblast cells is mediated at least in part by the action of matrix metalloproteinases (MMPs), a family of zinc-dependent neutral endopeptidases that are capable of cleaving all extracellular matrix (ECM) components[4]. Among the MMPs, MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) have been shown to be essential for the invasive ability of trophoblast cells[5,6], and regulation of their expression may play important roles in trophoblast invasion.

In vitro studies have pointed to TGF-β as an important candidate contributing to the regulation of gelatinases in trophoblast invasion. To date, evidence exists to show TGF-β-regulated MMP-2 and MMP-9 expression in trophoblast cells[7,8]. However, the underlying molecular mechanisms of these regulations re-

Received: November 24, 2010 Accepted: February 9, 2011

*Corresponding author. Tel: 86-10-63926563, E-mail: bingmchen@sina.com

main opaque. Investigations into the roles of MAPK in the egulation of MMPs by TGF-β have given insights into the signaling pathway regulating MMP expression[9~11]. However, there has been no report on the role of MAPK in the regulation of gelatinases by TGF-β in trophoblast cells.

In this study, we have examined the effects of TGF-β1 on the expression of MMP-2, and MMP-9 in primary first trimester CTB cells. The roles of IL-1β and ERK1/2 MAPK in the regulation of MMP by TGF-β1 were also investigated to advance our knowledge regarding to the molecular mechanisms of these regulations. To our knowledge, we have firstly proved that ERK1/2 is involved in the inhibition of MMP-9 activity by TGF-β1 in normal trophoblast cells.

1 Materials and Methods

1.1 Materials

Dulbecco's modified Eagle's medium (DMEM) and Ham F-12 medium were provided by Gibco BRL (Gaithersburg, MD). Recombinant human TGF-β1 and human IL-1β were purchased from PEPRO-TECH EC Ltd. (London, England). DNase I and PD98059 were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Trizol reagent and Superscript II reverse transcriptase were purchased from Invitrogen (Gaithersburg, MD). RNase-free DNase I was purchased from Roche (Indianapolis, IN). The antibodies against phosphorylated ERK1/2 and total ERK1/2 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Alkaline phosphatase (AP)-conjugated secondary antibodies were purchased from Zymed (San Francisco, CA). BioTrace™ nitrocellulose membranes were purchased from Pall Corp. (East Hills, NY). Broad range protein molecular weight marker, nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) were from Promega (Madison, WI).

1.2 Preparation and characterization of first trimester human cytotrophoblast cells (CTB)

Trophoblastic villi were obtained from legal abortions of 6~8 weeks gestation under the agreement of patients. The project was approved by the ethics committee of the State Key Laboratory of Reproductive Biology, Institute of Zoology, the Chinese Academy of Sciences. The time of pregnancy was defined according to the first day of the last menstrual cycle. Tissues were washed with PBS until no discernible blood was visualized, and the soft villous material was cut away from the connective tissue under a dissecting microscope and minced into small fragments. After incubation in 0.25% trypsin for 45 min at 4 °C and 10 min at 37 °C, the trypsinization was terminated by the addition of a double volume of FD medium (Ham F-12: DMEM=1:1) containing 10% fetal bovine serum (FBS), followed by incubation with 15 U/ml DNase I for 15 min at 37 °C. The cells were fully suspended by rigorous pipetting and then filtered through a nylon sieve. CTB were separated from blood cells and syncytiotrophoblasts on a discontinuous BSA gradient (1%, 2%, 3%, 4%, and 5% in serum-free FD medium) and plated at 0.5×10^5~1×10^5 cells/ml in fibronectin-precoated 24-well plates. The cultures were maintained in FD medium supplemented with 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin in the presence of 10% FBS at 37 °C in a water-saturated atmosphere of 95% air and 5% CO_2. The cells showed typical monolayer epithelial morphology after adhering and were identified as being trophoblast by positive immunohistochemical staining for cytokeratin and GnRH and negative staining for vimentin.

1.3 Treatment of cells

CTB cells were plated at optimal concentrations (1×10^6 cells in 12-well plates) 24 h before reaching early confluence. The cells were then changed to serum-free medium for 12 h before treatment with fresh media containing TGF-β1 and/or IL-1β at the indicated final concentrations for the indicated periods. PD98059, a specific ERK1/2 MAPK pathway inhibitor, was added to the medium at a final concentration of 10 μmol/L or 40 μmol/L 1 h before TGF-β1 and/or IL-1β treatment. All experiments were repeated at least three times.

1.4 Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated using Trizol re-

agent according to the instructions of the manufacturer and was cleared of genomic DNA contamination by RNase-free DNase I treatment. Reverse transcription was carried out according to the method provided with Superscript II reverse transcriptase using 1 μg of total RNA. Specific primers were designed by the Primer Premier version 5.0 program (PREMIER Biosoft International, Palo Alto, CA), as shown in Table 1. Semi-quantitative RT-PCR was performed as previously described. Briefly, 1.5 μl or 3 μl of the resultant cDNAs (according to the expression level of different target genes) were amplified by PCR in a 25 μl reaction mixture containing 0.2 mmol/L of each dNTP, 1.5 mmol/L magnesium chloride, 10 pmol of sense and antisense primers, and 1 U Taq DNA polymerase. To compare the intensities of RT-PCR products in a semi-quantitative fashion, we determined the exponential phase of amplification by performing increasing cycles for *MMP-2*, *MMP-9*, and *GAPDH* genes. As a negative control, each sample was run through PCR in the absence of cDNA, or with RT product without reverse transcriptase. PCR products were resolved in 1.5% agarose gels containing 0.5 μg/ml ethidium bromide (EB) and quantified. The relative concentrations of target genes were expressed as the ratio of densitometric readings to *GAPDH*.

1.5 Zymographic analysis

Secreted gelatinases were analyzed in the conditioned medium by zymography as previously described[12]. 5 μl of 4×loading buffer (0.25 mol/L Tris-HCl, pH6.8, 40% glycerol, 8% SDS, 0.4% bromophenol blue) and 15 μl supernatants were incubated at 37 °C for 30 min prior to electrophoresis in 10% SDS-polyacrylamide gels containing 1 mg/ml gelatin. After electrophoresis, gels were washed 2×30 min at room temperature in Triton X-100 buffer (2.5% Triton X-100, 50 mmol/L Tris-HCl, pH7.5). Gels were then incubated for 20 h at 37 °C in Ca^{2+} substrate buffer (50 mmol/L Tris-HCl, 0.2 mol/L NaCl, 10 mmol/L $CaCl_2$, 1 μmol/L $ZnCl_2$, 1% Triton X-100, pH7.5), followed by staining with 0.2% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid for 60 min and destaining with 10% acetic acid. The molecular weights of the gelatinases were estimated by comparision with protein standards. Cell samples used for analysis of gelatinases expression changes were the same as those used for RT-PCR.

1.6 Western blot assay of activation and blockage of ERK1/2

Cells were lysed in lysis buffer (4 mmol/L EGTA, 3 mmol/L EDTA, pH8.0, 125 mmol/L NaF, 0.5 mmol/L Na_3VO_4, 2.5 μg/ml aprotinin, 25 μg/ml trypsin inhibitor, 25 μmol/L PMSF, 0.5% NP40, 12.5 mmol/L HEPES, 1 mmol/L DTT), boiled in 2×loading buffer for 10 min, fractioned by 15% SDS-PAGE, and transferred to a nitrocellulose membrane. Western blot was performed with antibodies against phosphorylated ERK1/2 and total ERK1/2, diluted at 1:1 000. Specific binding of antibodies was detected with AP-conjugated secondary antibodies and signals were visualized by a NBT/BCIP detection system. The levels of phosphorylated ERK1/2, as quantitated by densitometric scanning, were corrected corresponding to the levels of total ERK1/2 in the same samples.

1.7 Statistical analysis

Signal intensities were scanned and determined by MetaView image analyzing system version 4.50 (Universal Imaging Corp., Downington, PA) and all bands were evaluated using the same pre-set standards. Values are expressed as means±SEM of at least three experiments. Statistical differences between groups were analyzed by one-way ANOVA using the SPSS 10.0 software program (SPSS Inc., Chicago, IL) and differences were considered significant at $P<0.05$.

2 Results

2.1 Semi-quantitative RT-PCR and zymographic analysis of the dose- and time-dependency of TGF-β1 regulation of *MMP-2* and *-9* mRNA abundance and secretion in CTB cells

We first examined the effect of increasing doses of TGF-β1 treatment of CTB cells for 24 h, and alternatively, 10 ng/ml TGF-β1 for different time periods, on *MMP-2* and *-9* mRNA abundance and secretion

using semi-quantitative RT-PCR and zymography. Dose- (Fig.1A, $P<0.05$ versus basal at doses over 1 ng/ml) and time-dependent (Fig.1B, $P<0.05$ versus corresponding basal at 12 h, 24 h, and 48 h) decreases in *MMP-9* mRNA abundance were observed after TGF-β1 treatment. The level of pro-MMP-9 secretion was inhibited significantly (Fig.1A, $P<0.05$ versus basal at doses of 5 ng/ml, 10 ng/ml, and 20 ng/ml) in dose-dependent manner, while expression of the pro-MMP-9 heterodimer was interestingly increased (Fig.1A, $P<0.05$ versus basal at doses of 5 ng/ml, 10 ng/ml, and 20 ng/ml). Similarly, when cells were exposed to 10 ng/ml TGF-β1 for 6 h, 12 h, 24 h, and 48 h, the latent form of *MMP-9* was also reduced significantly (Fig.1B, $P<0.05$ versus corresponding basal at 24 h and 48 h), while the level of pro-MMP-9 heterodimer secretion was again enhanced (Fig.1B, $P<0.05$ versus corresponding basal at 6 h, 12 h, 24 h, and 48 h). On the contrary, neither *MMP-2* mRNA abundance nor the secretion of latent/active enzyme was affected significantly by increasing doses of TGF-β1 (Fig.1A, $P>0.05$). There was a basal increase in the secretion of latent/active *MMP-2* when cells were cultured for 6 h, 12 h, 24 h, and 48 h, and exposure to 10 ng/ml TGF-β1 for increasing time intervals did not affect *MMP-2* message or pro-/active enzyme levels (Fig.1B, $P>0.05$).

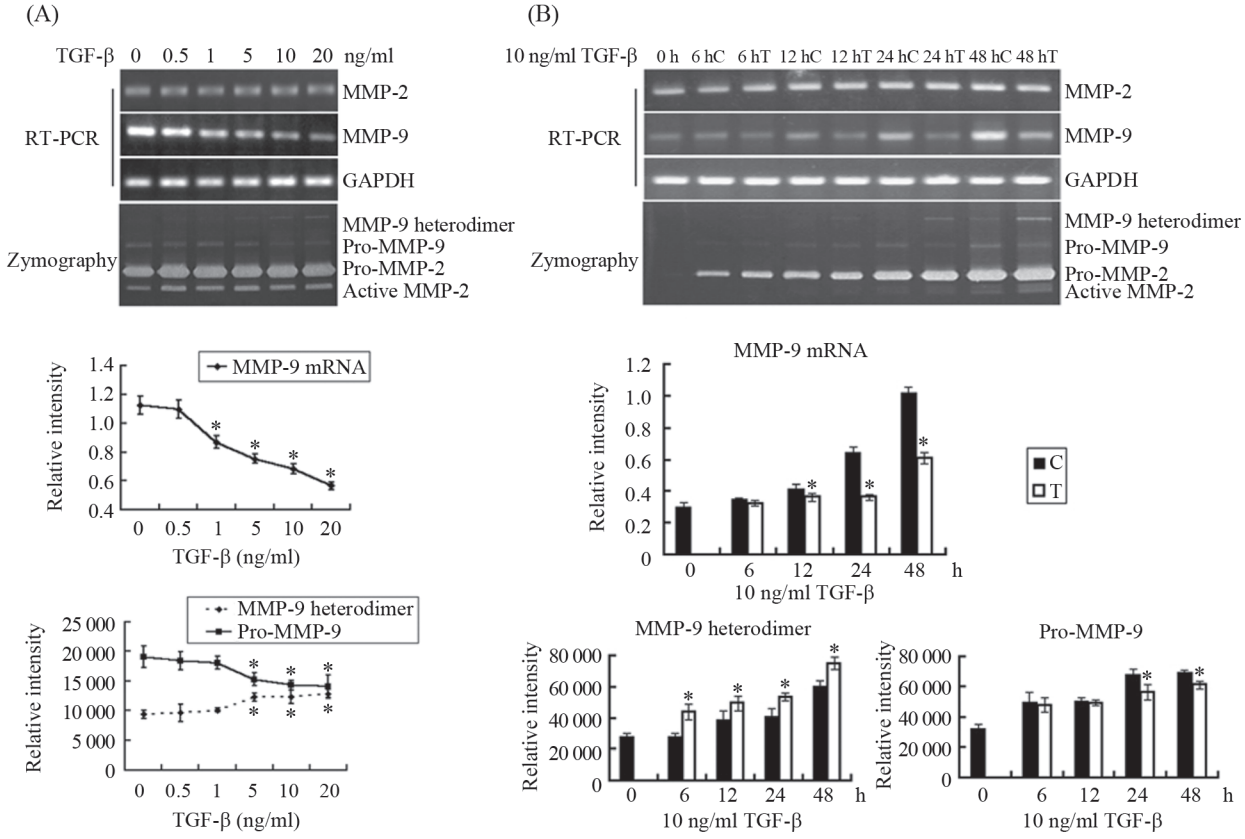

Fig.1 Semi-quantitative RT-PCR and zymographic analysis of the dose- and time-dependent regulation of *MMP-2* and *-9* mRNA abundance and secretion in response to TGF-β1 in CTB cells

A: serum-starved cells were treated with increasing concentrations of TGF-β1 (0 ng/ml, 0.5 ng/ml, 1 ng/ml, 5 ng/ml, 10 ng/ml, and 20 ng/ml) for 24 h; B: cells were treated with vehicle or 10 ng/ml TGF-β1 for 6 h, 12 h, 24 h, and 48 h; C, vehicle control; T, 10 ng/ml TGF-β1. At the end of the treatment, total cellular RNA was extracted and subjected to semi-quantitative RT-PCR analysis, and the conditioned media were collected and analyzed for gelatinases activity by zymography. The results are representative of at least three independent experiments. The relative levels for *MMP-2* or *MMP-9* mRNA were determined as the ratio of *MMP-2*/*-9*: *GAPDH* measured by densitometry. Secretion of MMP-2 or MMP-9 detected by zymography was quantified by densitometry. Each value represents the means±SEM of at least three independent experiments. Differences are considered to be significant at $P<0.05$ (*) versus corresponding basal.

2.2 The role of IL-1β on the regulatory effect of TGF-β1 on MMP-9 expression in CTB cells

Because some lines of evidence shows that IL-1 plays intermediary or antagonistic roles in TGF-β1-regulated MMP expression, the effects of IL-1β on the regulation of *MMP-9* expression were examined. Similar to the effects of TGF-β1, IL-1β could also reduce *MMP-9* mRNA abundance and latent enzyme secretion (P<0.05 versus basal at 10 ng/ml), as shown by RT-PCR and zymographic analysis (Fig.2). However, when treating the cells with both IL-1β and TGF-β1, IL-1β opposed the inhibitory effect of TGF-β1 on *MMP-9* mRNA and latent enzyme expression (P<0.05 versus 10 ng/ml TGF-β1 treatment at 1 ng/ml, 5 ng/ml, and 10 ng/ml). Conversely, IL-1β upregulated pro-MMP-9 heterodimer expression (P<0.05 versus basal at 10 ng/ml), but showed no effects when combined with TGF-β1 (P>0.05 versus 10 ng/ml TGF-β1 treatment).

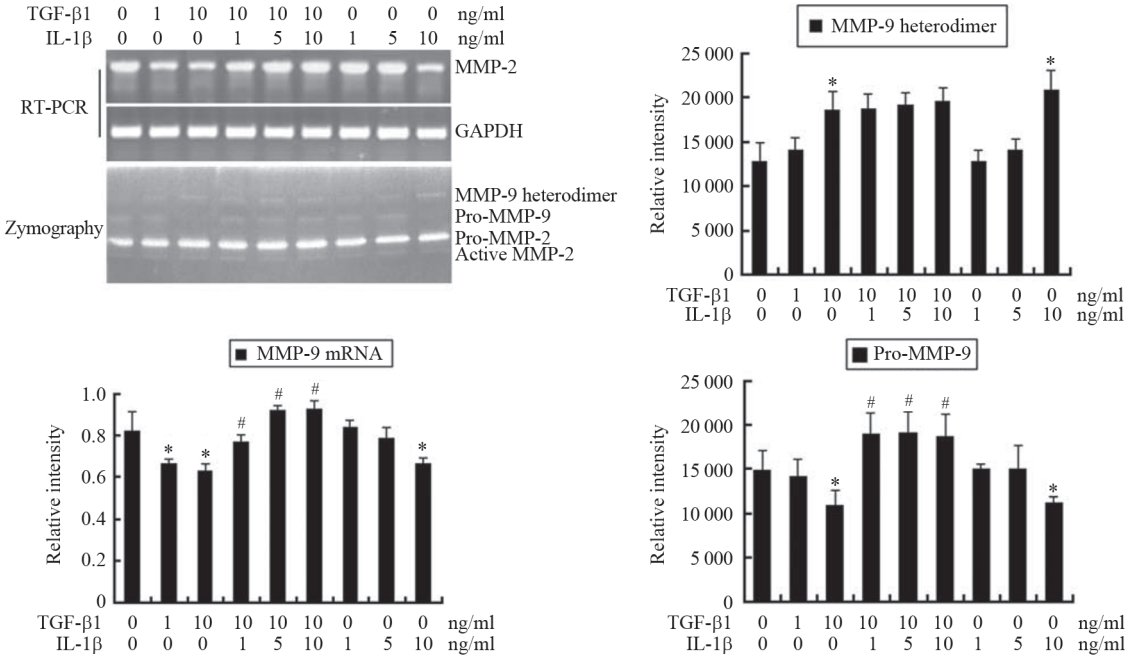

Fig.2 The role of IL-1β in TGF-β1-mediated MMP-2 and -9 regulation in CTB cells

Cells were treated with TGF-β1 alone (1 ng/ml or 10 ng/ml), IL-1β alone (1 ng/ml, 5 ng/ml, 10 ng/ml), or 10 ng/ml TGF-β1 together with increasing concentrations of IL-1β (1 ng/ml, 5 ng/ml, 10 ng/ml) for 24 h. Total cellular RNA was extracted and subjected to semi-quantitative RT-PCR analysis of *MMP-9*. The conditioned media were collected and subjected to zymographic analysis. The results are representative of at least three different experiments. The relative levels for *MMP-9* mRNA were determined as the ratio of *MMP-9*: *GAPDH* measured by densitometry. Secretion of MMP-9 detected by zymography was quantified by densitometry. Each bar represents the means±SEM of at least three independent experiments. Differences are considered to be significant at P<0.05 (*) versus basal and at P<0.05 ($^{\#}$) versus 10 ng/ml TGF-β1 treatment.

2.3 TGF-β1 and IL-1β promoted ERK1/2 phosphorylation in CTB cells

Because it is becoming increasingly clear that TGF-β receptors can activate the ERK1/2 MAPK signaling cascades[13], we first confirmed abundant mRNA expression of type I and II TGF-β receptors in CTB cells (data not shown) using the primers reported previously[14]. We then examined whether TGF-β1 could activate the ERK1/2 MAPK pathway by performing Western blot with phosphorylated ERK1/2 and total ERK1/2 antibodies. As shown in Fig.3, TGF-β1 promoted ERK1/2 phosphorylation, with maximal activity at 1 h and 6 h (P<0.05 versus activity at 0.25 h). IL-1β could also activate ERK1/2, with maximal activity occurred at 1 h or 6 h (P<0.05 versus activity at 0.25 h). Co-treatment of TGF-β1 and IL-1β had similar effect on ERK1/2 phosphorylation (P<0.05 versus activity at 0.25 h), as compared to TGF-β1 or IL-1β treatment alone.

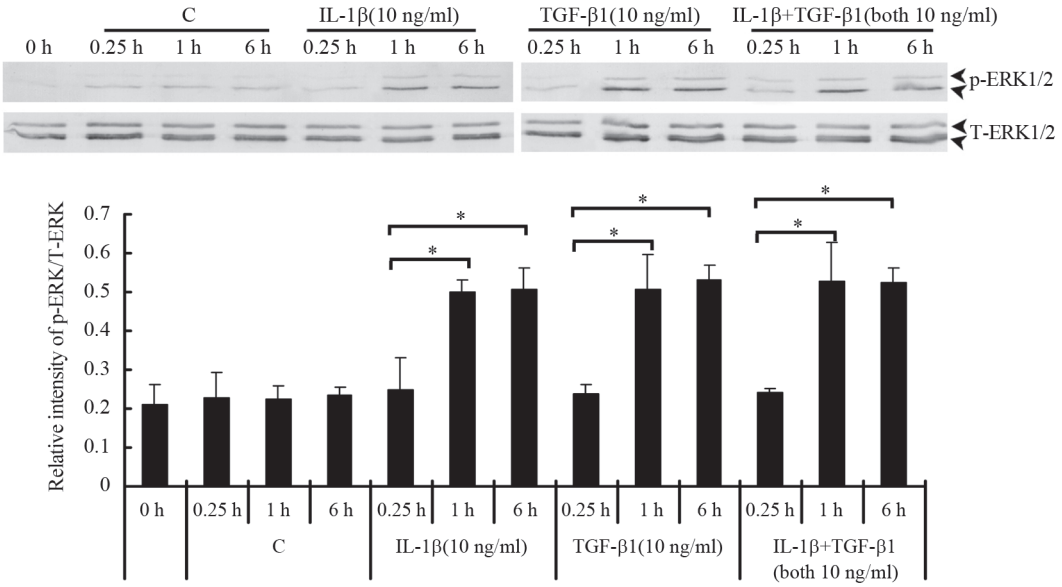

Fig.3 TGF-β1 promotes ERK1/2 phosphorylation in CTB cells

Cells were treated with vehicle (C), 10 ng/ml TGF-β1, 10 ng/ml IL-1β, or 10 ng/ml TGF-β1 together with 10 ng/ml IL-1β for 0 h, 0.25 h, 1 h, or 6 h. Cell lysates were subjected to western blotting to detect phosphorylated ERK1/2 (p-ERK1/2) levels and total ERK1/2 (T-ERK1/2) levels. The results are representative of three independent experiments. Ratio of p-ERK1/2 and T-ERK1/2 was quantified by densitometry. Each bar represents the means±SEM. Differences are considered to be significant at $P<0.05$ (*) versus corresponding control at 0.25 h.

2.4 Effect of ERK1/2 blockage on the regulatory effects of TGF-β1 on MMP-9 expression in CTB cells

To further examine whether the ERK1/2 pathway is involved in the regulatory effects of TGF-β1 or IL-1β on MMP-9 in CTB cells, we examined changes in gene/protein expression levels resulting from the addition of a specific ERK1/2 pathway inhibitor, PD98059, to the medium. As shown in Fig.4, treating the cells with PD98059 blocked TGF-β1-reduced *MMP-9* mRNA and pro-enzyme expression ($P<0.05$ versus TGF-β1 treatment at 10 μmol/L and 40 μmol/L). Furthermore, the addition of PD98059 blocked IL-1β-reduced *MMP-9* mRNA and pro-MMP-9 expression ($P<0.05$ versus IL-1β treatment at 10 μmol/L and 40 μmol/L), and blocked IL-1β+TGF-β1-enhanced *MMP-9* mRNA and pro-MMP-9 expression ($P<0.05$ versus IL-1β+TGF-β1 treatment at 10 μmol/L and 40 μmol/L). There was no blockage on TGF-β1-, IL-1β-, and IL-1β+TGF-β1-enhanced pro-MMP-9 heterodimer secretion ($P>0.05$ versus treatment of TGF-β1, IL-1β, or IL-1β+TGF-β1).

3 Discussion

The invasive potential of trophoblast cells is associated with the remodeling of ECM mediated by MMPs, among which MMP-2 and MMP-9 are considered to be of prime importance[5,6]. In present study, we found that TGF-β1 inhibited *MMP-9* mRNA abundance and the level of pro-enzyme secretion in normal trophoblast cells, but did not affect MMP-2 expression, in line with previous findings by Graham[15] and Meisser[16]. These data suggest that the penetration of normal trophoblast cells is stringently controlled by TGF-β1 through inhibiting MMP-9 activity. MMP-2 is not involved in this process. In addition, the discrepancy between TGF-β1's effects on the pro-MMP-9 heterodimer and pro-MMP-9 forms may be caused by TGF-β1's effect on neutrophil gelatin-associated lipocalin, which remains to be further elucidated.

Evidence exists to suggest that IL-1 and TGF-β may play converse roles in the regulation of *MMP-9* mRNA expression or its enzymatic activity[15,17] in some cell types. Alternatively, IL-1 and TGF-β may also play intermediary roles in trophoblast invasion

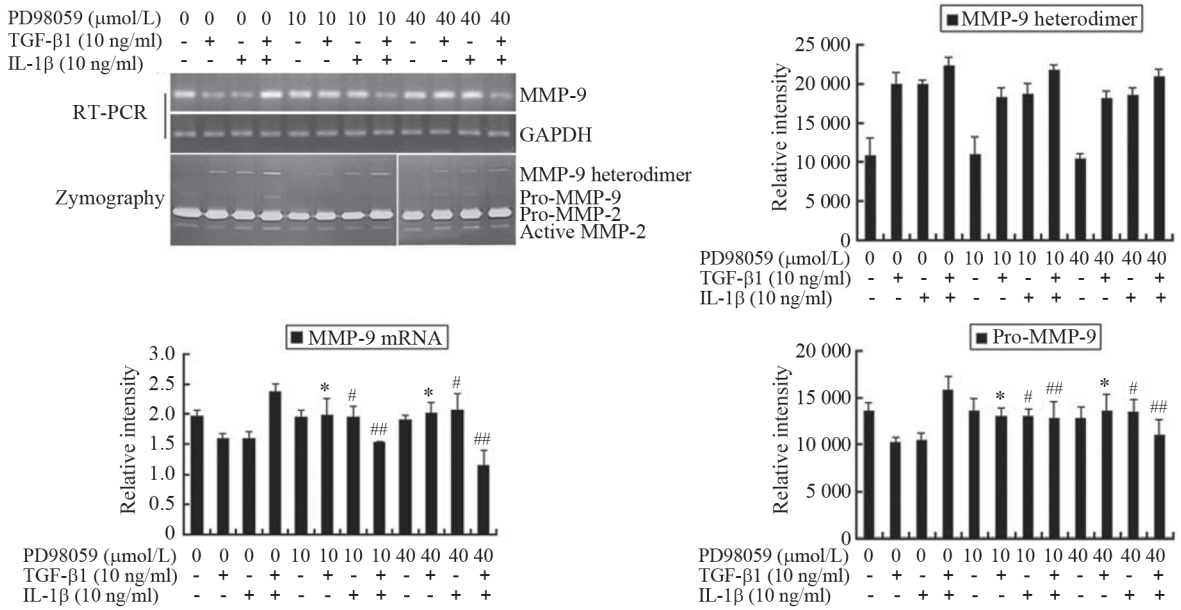

Fig.4 The effects of an ERK1/2 pathway inhibitor, PD98059, on TGF-β1-mediated MMP-2 and -9 expression in CTB cells

Cells were incubated for 24 h in the presence(+) or absence(-) of 10 ng/ml TGF-β1 or 10 ng/ml IL-1β, or in combination with PD98059 (10 μmol/L or 40 μmol/L) added 1 h prior to TGF-β or IL-1β. Total cellular RNA was extracted and subjected to semi-quantitative RT-PCR analysis for *MMP-9*. *GAPDH* was used as the internal control. The conditioned media were collected and subjected to zymographic analysis. The results are representative of at least three different experiments. The relative levels for *MMP-9* mRNA were determined as the ratio of *MMP-9*: *GAPDH* measured by densitometry. Secretion of MMP-9 detected by zymography was quantified by densitometry. Each bar represents the means±SEM of at least three independent experiments. Differences are considered to be significant at $P<0.05$ (*) versus 10 ng/ml TGF-β1 treatment, ($^{\#}$) versus 10 ng/ml IL-1β treatment and ($^{\#\#}$) versus 10 ng/ml TGF-β1+IL-1β treatment.

Table 1 Primer sequences used for RT-PCR

Genes	Primers (5'→3')	Location	PCR product	GenBank accession No.	Annealing temperature
MMP-2	Sense: ATT TGG CGG ACT GTG ACG	1 766 to 1 783	442 bp	NM_004530	55 °C
	Antisense: GCT TCA GGT AAT AGG CAC	2 190 to 2 207			
MMP-9	Sense: GGG ACG GCA ATG CTG ATG	855 to 872	320 bp	NM_004994	58 °C
	Antisense: CCA CTT CTT GTC GCT GTC	1 157 to 1 174			
GAPDH	Sense: AGC CAC ATC GCT CAG ACA C	41 to 59	315 bp	M33197	55 °C
	Antisense: TGG ACT CCA CGA CGT ACT C	337 to 355			

by augmenting MMP-9 secretion[18]. In our case, we could detect similar but mutually antagonistic effects on *MMP-9* mRNA and pro-enzyme expression in CTB cells. Investigations have shown that the expression of IL-1 receptor can be reduced by TGF-β treatment in chondrocytes. A more interesting report is that IL-6 stimulates TGF-β receptor partitioning resulting in the enhancement of TGF-β1 signaling[19]. Taken together, these data may advance our understanding of the biological roles of these two factors, and their mutually antagonistic or contributory mechanisms still deserve to be further elucidated.

In recent years, the contributions of the ERK1/2 pathway in the regulation of MMPs have been evaluated in various cell types[20,21]. Regarding to the role of ERK1/2 in TGF-β-regulated MMPs expression, it has been reported that the ERK1/2 pathway is potently involved in TGF-β-enhanced MMP-1 and MMP-9 production in transformed squamous epithelial cells[22]. Our results suggest that the activated ERK1/2 pathway is involved in TGF-β1- and IL-1β-mediated MMP-9 inhibition in CTB cells. These data help to explain the signaling pathway involved in TGF-β1-regulated MMP-9 expression in normal trophoblast invasion. It has been

well established that phosphorylated TGF-β receptors can directly or indirectly activate MAPK pathway through the cross-talk with Smad signaling pathway[23]. Therefore, TGF-β-stimulated ERK1/2 pathway can activate AP-1 or Smads transcriptional factors, which subsequently control the transcription and expression of MMP-9 during normal trophoblast invasion.

In summary, we have studied TGF-β1-mediated MMP-2 and -9 regulation in normal trophoblast cells. Our data suggest that the trophoblast cells are stringently controlled by TGF-β1 through the restriction of MMP-9 activity. IL-1β and TGF-β1 show similar but mutually antagonistic effects on *MMP-9* mRNA and pro-enzyme expression. The ERK1/2 pathway is involved in TGF-β1-mediated *MMP-9* mRNA and pro-MMP-9 inhibition in CTB cells.

References

1 Jones RL, Stoikos C, Findlay JK, Salamonsen LA. TGF-beta superfamily expression and actions in the endometrium and placenta. Reproduction 2006; 132(2): 217-32.

2 Moustakas A, Heldin CH. The regulation of TGFbeta signal transduction. Development 2009; 136(22): 3699-714.

3 Javelaud D, Mauviel A. Crosstalk mechanisms between the mitogen-activated protein kinase pathways and Smad signaling downstream of TGF-beta: Implications for carcinogenesis. Oncogene 2005; 24(37): 5742-50.

4 Cohen M, Bischof P. Factors regulating trophoblast invasion. Gynecol Obstet Invest 2007; 64(3): 126-30.

5 Staun-Ram E, Goldman S, Gabarin D, Shalev E. Expression and importance of matrix metalloproteinase 2 and 9 (MMP-2 and -9) in human trophoblast invasion. Reprod Biol Endocrinol 2004; 2: 59.

6 Isaka K, Usuda S, Ito H, Sagawa Y, Nakamura H, Nishi H, *et al*. Expression and activity of matrix metalloproteinase 2 and 9 in human trophoblasts. Placenta 2003; 24(1): 53-64.

7 Lash GE, Otun HA, Innes BA, Bulmer JN, Searle RF, Robson SC. Inhibition of trophoblast cell invasion by TGFB1, 2, and 3 is associated with a decrease in active proteases. Biol Reprod 2005; 73(2): 374-81.

8 Zhao MR, Qiu W, Li YX, Zhang ZB, Li D, Wang YL. Dual effect of transforming growth factor beta1 on cell adhesion and invasion in human placenta trophoblast cells. Reproduction 2006; 132(2): 333-41.

9 Ravanti L, Toriseva M, Penttinen R, Crombleholme T, Foschi M, Han J, *et al*. Expression of human collagenase-3 (MMP-13) by fetal skin fibroblasts is induced by transforming growth factor β via p38 mitogen-activated protein kinase. FASEB J 2001; 15(6): 1098-100.

10 Kim ES, Kim MS, Moon A. TGF-β-induced upregulation of MMP-2 and MMP-9 depends on p38 MAPK, but not ERK signaling in MCF10A human breast epithelial cells. Int J Oncol 2004; 25(5): 1375-82.

11 Munshi HG, Wu YI, Mukhopadhyay S, Ottaviano AJ, Sassano A, Koblinski JE, *et al*. Differential regulation of membrane type 1-matrix metalloproteinase activity by ERK 1/2- and p38 MAPK-modulated tissue inhibitor of metalloproteinases 2 expression controls transforming growth factor-β1-induced pericellular collagenolysis. J Biol Chem 2004; 279(37): 39042-50.

12 Wang H, Li Q, Shao L, Zhu C. Expression of matrix metalloproteinase-2, -9, -14, and tissue inhibitors of metalloproteinase-1, -2, -3 in the endometrium and placenta of rhesus monkey (Macaca mulatta) during early pregnancy. Biol Reprod 2001; 65(1): 31-40.

13 Mulder KM. Role of Ras and Mapks in TGFβ signaling. Cytokine Growth Factor Rev 2000; 11(1-2): 23-35.

14 Xu G, Chakraborty C, Lala PK. Expression of TGF-β signaling genes in the normal, premalignant and malignant human trophoblast: loss of Smad 3 in choriocarcinoma cells. Biochem Biophys Res Commun 2001; 287(1): 47-55.

15 Graham CH, Connelly I, MacDougall JR, Kerbel RS, Stetler-Stevenson WG, Lala PK. Resistance of malignant trophoblast cells to both the anti-proliferative and antiinvasive effects of transforming growth factor-β. Exp Cell Res 1994; 214(1): 93-9.

16 Meisser A, Chardonnens D, Campana A, Bischof P. Effects of tumour necrosis factor-α, interleukin-1α, macrophage colony stimulating factor and transforming growth factor β on trophoblastic matrix metalloproteinases. Mol Hum Reprod 1999; 5(3): 252-60.

17 Huang HY, Wen Y, Irwin JC, Kruessel JS, Soong YK, Polan ML. Cytokine-mediated regulation of 92-kilodalton type IV collagenase, tissue inhibitor or metalloproteinase-1 (TIMP-1), and TIMP-3 messenger ribonucleic acid expression in human endometrial stromal cells. J Clin Endocrinol Metab 1998; 83(5): 1721-9.

18 Karmakar S, Das C. Regulation of trophoblast invasion by IL-1beta and TGF-beta1. Am J Reprod Immunol 2002; 48(4): 210-9.

19 Zhang XL, Topley N, Ito T, Phillips A. Interleukin-6 regulation of transforming growth factor (TGF)-β receptor compartmentalization and turnover enhances TGF-β1 signaling. J Biol Chem 2005; 280(13): 12239-45.

20 Endo H, Watanabe T, Sugioka Y, Niioka M, Inagaki Y, Okazaki I. Activation of two distinct MAPK pathways governs constitutive expression of matrix metalloproteinase-1 in human pancreatic cancer cell lines. Int J Oncol 2009; 35(6): 1237-45.

21 Zhang Z, Song T, Jin Y, Pan J, Zhang L, Wang L, *et al*. Epidermal growth factor receptor regulates MT1-MMP and MMP-2 synthesis in SiHa cells via both PI3-K/AKT and MAPK/ERK pathways. Int J Gynecol Cancer 2009; 19(6): 998-1003.

22 Johansson N, Ala-Aho R, Uitto V, Grenman R, Fusenig NE,

Lopez-Otin C, *et al*. Expression of collagenase-3 (MMP-13) and collagenase-1 (MMP-1) by transformed keratinocytes is dependent on the activity of p38 mitogen-activated protein kinase. J Cell Sci 2000; 113(Pt 2): 227-35.

23 Massague J. How cells read TGF-β signals. Nat Rev Mol Cell Biol 2000; 1(3): 169-78.

ERK1/2信号通路参与人滋养层细胞中转化生长因子-β1对MMP-9表达的抑制作用

陈升平* 李红霞

(北京世纪坛医院妇产科, 北京 100038)

摘要 正常滋养层细胞的侵润受转化生长因子-β (TGF-β)的调控。该文研究了人正常细胞滋养层细胞(CTB)中TGF-β1对MMP-2和-9表达的调控。结果表明, TGF-β1抑制CTB细胞中*MMP-9* mRNA的表达和酶原MMP-9的分泌, 但不影响MMP-2 mRNA和蛋白的表达。IL-1β和TGF-β1均能抑制*MMP-9* mRNA的表达和酶原MMP-9的分泌, 但二者的效应互相拮抗。抑制ERK1/2信号通路导致TGF-β1对*MMP-9* mRNA和酶原MMP-9的抑制作用受阻。以上结果表明ERK1/2信号通路参与TGF-β1对人滋养层细胞MMP-9表达的抑制作用。

关键词 转化生长因子-β; 基质金属蛋白酶; 滋养层; ERK

收稿日期: 2010-11-24 接受日期: 2011-02-09

*通讯作者。Tel: 010-63926563, E-mail: bingmchen@sina.com