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Thrombin stimulates gene expression and secretion of IL-11 via protease-activated receptor-1 and regulates extravillous trophoblast cell migration

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ABSTRACT

Extravillous trophoblast (EVT) migration and invasion is the crucial step for normal placental development. IL-11 is a cytokine regulating cell migration and invasion in cells and is a critical factor for successful implantation of an embryo. Higher expression of thrombin receptor PAR-1 was reported in early pregnancy. The precise role of thrombin in trophoblast functions is not well understood. In this study, we asked whether thrombin can induce IL-11 secretion in trophoblasts if yes, which physiological cell functions are possibly affected? In this study, HTR-8/SVneo cells, which were originally derived from first-trimester villous explants of early pregnancy were used as the extravillous trophoblast (EVT) model. BeWo cells were used as the cytotrophoblast model. For gene silencing, qPCR and ELISA, each experiment was performed in triplicates for minimum three times.

Here, we found that thrombin stimulates *IL-11* gene expression and protein secretion in HTR-8/SVneo cells but not in BeWo cells. PAR-1 was the only receptor which was highly expressed in HTR-8/SVneo cells. Thrombin-mediated expression and secretion of IL-11 were mainly activated via PAR-1 receptor. Rac1, but not Rho-kinase activation is required for thrombin-induced IL-11 secretion. We also found that thrombin stimulation significantly enhanced cell migration that was inhibited after silencing the *IL-11* gene. In conclusion, this study demonstrates the role of thrombin in regulating human EVT migration via IL-11 secretion. We propose that thrombin might regulate EVT migration through the decidua and spiral artery remodeling. Failure of thrombin-dependent EVT migration results in pregnancy disorder, such as preeclampsia.

1. Introduction

Inadequate placentation is a pathological condition that causes recurring miscarriage, intrauterine growth restriction and other pregnancy-related disorders, such as pre-eclampsia. Uterine endometrial microenvironment and trophoblast-derived factors regulate cytotrophoblast proliferation, differentiation, and extravillous trophoblast (EVT) migration and invasion during normal placental development (Knofler, 2010; Cha et al., 2012). EVT cell migration is an important step during an invasion of cells in the uterine wall and is regulated by various growth factors, such as hepatocyte growth factor and IL-8 (Cartwright et al., 2002; Jovanovic et al., 2010).

Thrombin is a multifunctional serine protease that plays a crucial role in hemostasis and elicits multiple effects, such as cytokine release, proinflammation, chemotaxis, mitogenesis, apoptosis and angiogenesis on a variety of cell types including endothelial cells and EVT (Coughlin, 2000; O'brien et al., 2003; Tsopanoglou and Maragoudakis, 2009; Leonard et al., 2013). Thrombin stimulates cells via cleavage of G-protein coupled protease-activated receptors (PARs); PAR-1, PAR-3, and PAR-4 at a specific site present in the N-terminus (Hollenberg and Compton, 2002). During early human placental development, the higher expression of PAR-1 and PAR-3 was shown in cytotrophoblasts between the 7th and 10th weeks of gestation, then gets decreased precipitously from week 11 and thereafter (Even-Ram et al., 2003; O'brien et al., 2003). The over-expression of PAR-1 receptor was reported in the placenta of preeclampsia females, suggesting PAR activation is highly regulated during proper pregnancy maintenance (Erez et al., 2008). Thrombin promotes cell migration in various cells types, such as human

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colon adenocarcinoma, renal carcinoma, C6 glioma cells, vascular smooth muscle cells and monocytes (Chiang et al., 1996; Kalmes et al., 2000; Kim et al., 2011; Gadepalli et al., 2013). Thrombin enhances the secretion of MCP-1, MMP-3 and MMP-9 in primary cultures of first trimester human decidual cells and C6 glioma cells (Mackenzie et al., 2004; Matta et al., 2007; Kim et al., 2011).

Interleukin-11 (IL-11) belongs to the IL-6 cytokine superfamily with pleiotropic biological activities in diverse cell types and transduces its signal through the IL-11 receptor α (IL11Rα) and a co-receptor gp130-mediated signaling pathway (Muller-Newen, 2003). IL-11 regulates migration of human EVT and tumor cells, such as breast cancer cells (Arihiro et al., 2000; Paiva et al., 2007). *IL-11Rα* is constantly expressed in the endometrium throughout the menstrual cycle and in the decidua of early pregnancy (Chen et al., 2002). Thrombin and IL-1β enhanced the secretion of IL11 in cultured term decidual cells (Cakmak et al., 2005). *IL-11Rα* gene knockout female mice are infertile due to defective decidualization (Bilinski et al., 1998; Robb et al., 1998).

In the present study, we investigated for the first time, whether thrombin can induce IL-11 secretion in EVT cell line HTR-8/SVneo or other cell lines, such as BeWo; if yes, which physiological cell functions are possibly affected? Indeed, we found that thrombin stimulates IL-11 secretion in HTR-8/SVneo cells but not in BeWo cells. Moreover, thrombin enhanced the EVT cell migration dependent on IL-11 secretion.

2. Materials and methods

2.1. Materials

Primers were synthesized by Eurofins, Germany and Eurofins India. Human thrombin, Rho-kinase inhibitor Y27632 and monoclonal anti-PAR-1 antibody (Cat. No. SAB5300042) were from Sigma-Aldrich (St. Louis, MI, USA). Anti-PAR-3 antibody (Cat. No. ab66068) was from Abcam (Cambridge, UK). NSC23766, Go6976, control peptide (RLLFT-NH_2) and PAR-1 selective agonist peptide (TFLLR-NH_2) were from Tocris Bioscience (Bristol, UK). *IL-11* ON-TARGETplusSMART pool siRNA was from Dharmacon RNAi Technologies (Thermo Fisher Scientific, USA). Human IL-11 ELISA DuoSet was from R&D Systems GmbH (Wiesbaden-Nordenstadt, Germany).

2.2. Cell culture

HTR-8/SVneo cell line was a kind gift from the laboratory of Charles Graham (Graham et al., 1993). BeWo and HTR-8/SVneo cells were grown, as described previously (Goyal et al., 2013). For each experiment, cells were trypsinized and then plated in 48-wells plates (50,000 cells/well). 24 h prior to cell stimulation, cells were grown in factors-reduced growth medium (cell basal medium containing 5% charcoal-stripped FCS (dFCS). Cells were then stimulated with thrombin buffer (PBS + 0.1% BSA) or treated with inhibitors for 30 min followed by thrombin-stimulation (0.5 U/ml) in serum-starved medium (basal medium with 0.5% dFCS).

2.3. ELISA

Cell conditioned media were collected from 48-wells plates at an indicated time and then cells in each well were counted using CellTiter-Blue assay. For quantification of IL-11 secretion, human IL-11 DuoSet ELISA kit was used according to the manufacturer's instructions. Optical density of developed color was measured and data were corrected, as described previously (Brünnert et al., 2015). Data were normalized against cell count. Controls were treated as 100% in each experiment and relative secretion was calculated as % of control.

2.4. Real-time PCR

Total RNA was isolated using peqGOLDTriFast™ (Peqlab, Erlangen, Germany) or RNAiso plus reagent (DSS Takara Bio India) following the manufacturers' instructions. First-strand cDNAs were synthesized with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany) according to manufacturer's protocol.

Gene-specific primers for real-time PCR were designed and real-time PCR was performed on an ABI Prism 7300 or Light Cycler® 96 System (Applied Biosystems & Roche), as described previously (Goyal et al., 2013). Data were normalized against the reference gene, β-actin. The following primer sequences were used:

IL-11_F: TCTCTCCTGGCGGACACG
IL-11_R: AATCCAGGTTGTGGTCCCC
β-actin_F: CCTGGCACCCAGCACAAT
β-actin_R:GCCGATCCACACGGAGTACT
PAR-1_F: CCAACCGCAGCAAGAAGT
PAR-1_R: GGTCCGAAGCAAATGATGAAG
PAR-3_F: TGCTGCCATTTTTCATACTGAAG
PAR-3_R: CGCAAGTGTTGTGAACATCATG
PAR-4_F: TGCGTGGATCCCTTCATCTACT
PAR-4_R: CCTGCCCGCACCTTGTC

2.5. IL-11 gene silencing

A pool of four specific *IL-11* specific siRNAs was used to silence *IL-11* gene, as described previously (Goyal et al., 2013). A pool of non-target siRNAs was used as negative control.

2.6. Wound healing scratch assay

For wound healing assay, 200,000 HTR-8/SVneo cells were seeded in a well of a 24-wells plate using RPMI-1640 medium containing 5% dFCS. Assay was performed with control cells or after *IL-11* gene silencing, as described above. After 24 h of seeding, medium was exchanged to factors-reduced medium and after another 24 h, a scratch was created in the cell layer to generate a wound. Medium was changed to serum-starved medium and cells were stimulated with PBS + 0.1% BSA (control) or 0.5 U/ml thrombin. Cell migration for closing the wound was visualized using a Zeiss Axio Observer Z.1 microscope. Images were taken using a 10-fold magnification.

2.7. Western blot

Cell pellets were dissolved in RIPA buffer (20 mM HEPES, pH 7.9), 350 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 1% NP-40, 0.5 mM DTT, 50 mM NaF, 1 mM Na_3VO_4, 0.2 mM PMSF, and 1 μg/mL aprotinin) and samples were subjected to SDS-PAGE and then transferred to nitrocellulose membrane at 200 mA for 60 min at 4 °C using the Mini Trans-Blot electrophoresis unit (Bio-Rad GmbH, Munich, Germany). Membranes were blocked with 5% (w/v) nonfat milk and then incubated with respective primary and secondary antibodies. The dilutions of the primary antibodies were: PAR-1 mouse mAb (1:500), PAR-3 rabbit antibody (1:500). The membranes were developed with chemiluminescent substrate and exposed to CX-BL + films (Typon Röntgen-Film GmbH, Frankenthal, Germany).

2.8. Statistical analysis

Minimum three independent experiments with different passages of cells were performed. For real-time PCR and ELISA, each experiment was performed in triplicates for minimum three times. Statistical analysis was carried out with one-way or two-way analysis of variance (ANOVA), using GraphPad PRISM version 7 software (GraphPad, La Jolla, CA, USA). Results were calculated as mean ± SD, and data were shown as mean + SD.

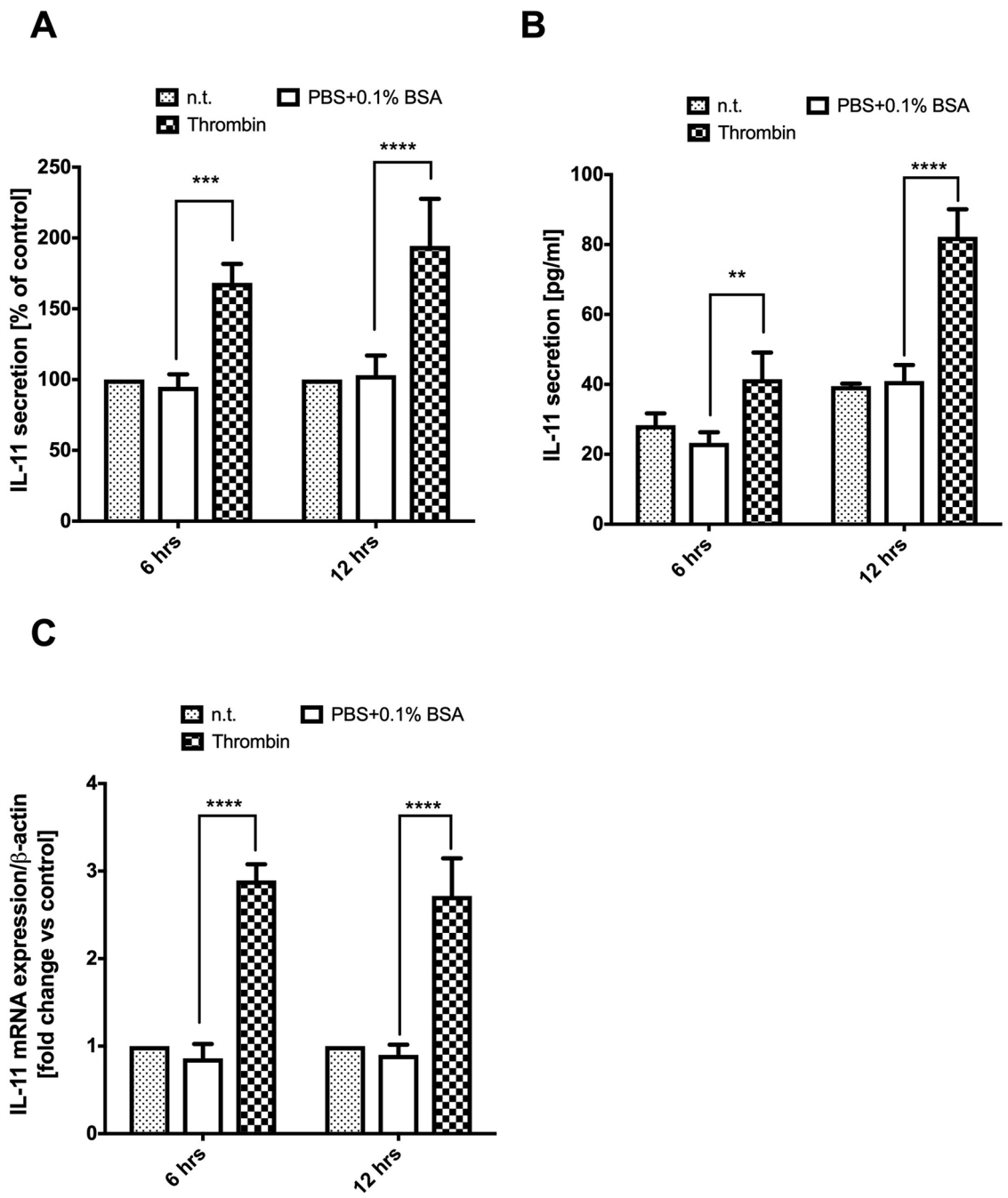

Fig. 1. Thrombin induces IL-11 secretion in HTR 8/SVneo cells. HTR-8/SVneo cells (50,000 cells/well) were plated in 48-well as described Materials and Methods. Next day, cells were grown in RPMI-1640 medium containing 0.5% dFCS and activated using 0.1% BSA in PBS (control) or 0.5 U/ml thrombin for 6 h or 12 h, respectively. IL-11 secretion was measured by ELISA. Bar diagram A) shows relative IL-11 secretion and B) shows absolute IL-11 secretion levels. Thrombin significantly increased IL-11 release after 6 h and 12 h. C) Total RNA was isolated from HTR-8/SVneo cells and the expression of IL-11 mRNA was measured by real-time PCR. Bar diagram shows the fold changes of IL-11 transcripts at 6 h and 12 h after activation with thrombin. The activation significantly increased IL-11 mRNA expression after 6 h (˜2.9-folds) and 12 h (˜2.8-folds). ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3. Results

3.1. Thrombin induces IL-11 secretion in HTR-8/SVneo cells but not in BeWo cells

To investigate the effects of thrombin on the secretion of IL-11 in various trophoblast cell lines; BeWo, and HTR-8/SVneo cells, we measured IL-11 secretion in the cell culture supernatants at 6 h and 12 h by using ELISA. We found that IL11 secretion was significantly increased ($p < 0.001$; ˜1.7 folds) within 6 h of treatment and then further slightly enhanced ($p < 0.0001$; 1.9-folds) within 12 h of thrombin stimulation of HTR-8/SVneo cells (Fig. 1A). In control state, IL-11 secretion was ˜23 pg/ml/75000 cells that significantly increased to ˜42 pg/ml/75000 cells and ˜82 pg/ml/75000 cells within 6 h and 12 h of thrombin treatment, respectively (Fig. 1B). In BeWo cells, IL-11 secretion was below the detection limit of ELISA (data not shown). These data suggest that thrombin specifically enhances the IL-11 secretion in HTR 8/SVneo cells.

Further, we asked whether thrombin-dependent IL-11 secretion was due to an effect of increased mRNA expression of *IL-11* gene? We

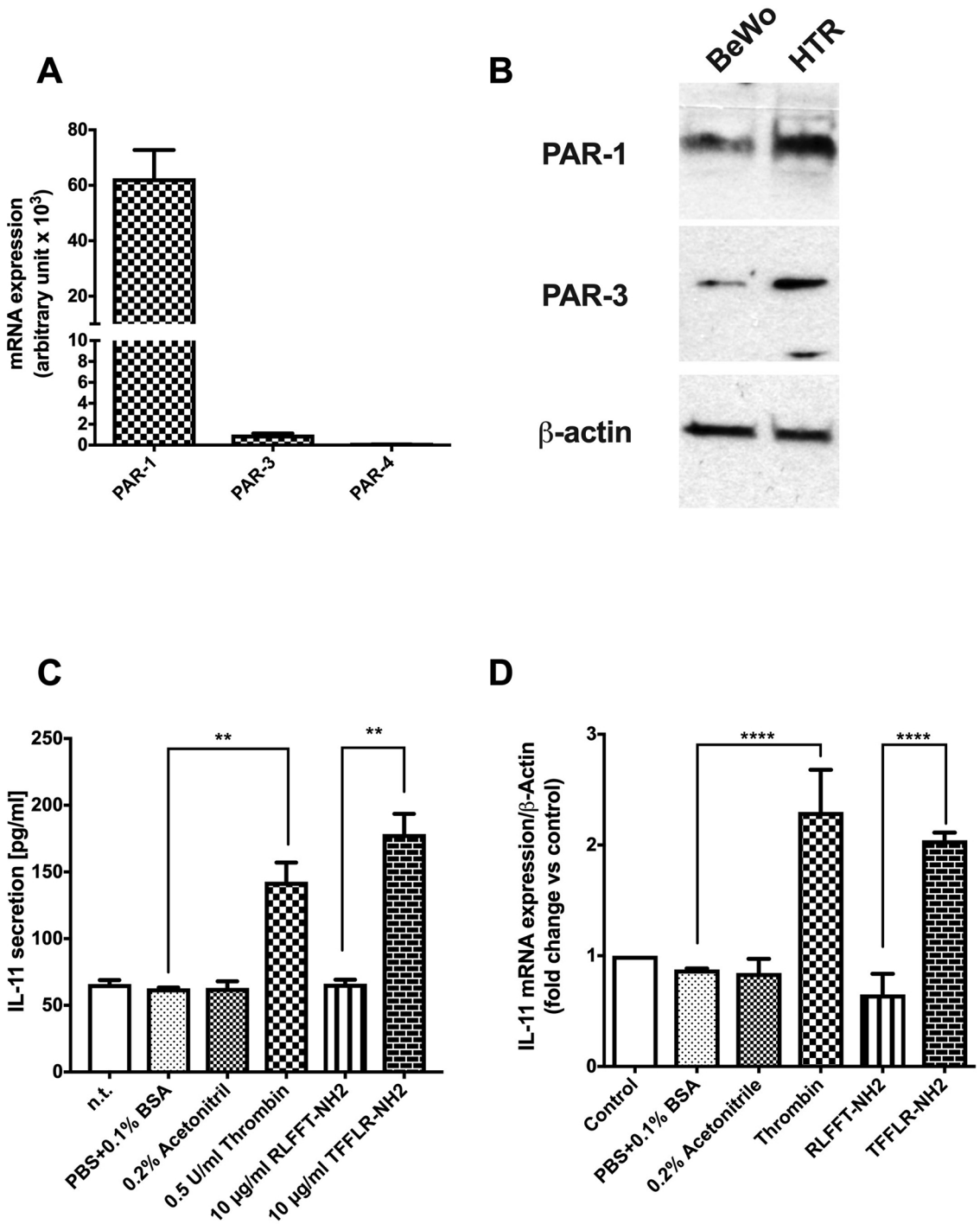

Fig. 2. Thrombin acts via PAR-1 receptor. A) RNA was isolated from HTR-8/SVneo cells and then expression of thrombin receptor transcripts was measured by real-time PCR. A) Bar diagram shows the relative expression of known thrombin receptors (PAR-1, -3, -4). The expression is normalized against β-actin. B) HTR 8/SVneo cells (HTR) and BeWo cells were lysed in RIPA buffer and 30 µg of the cell lysates were subjected to immunoblotting using specific PAR-1, PAR-3 antibodies. Specific bands of PAR-1 and PAR-3 were detected in both cell types C) and D) HTR-8/SVneo cells were grown in RPMI-1640 medium containing 0.5% dFCS and activated with 0.1% BSA in PBS, 0.2% acetonitrile, 0.5 U/ml thrombin, control peptide RLFFT-NH_2 (10 µg/ml) or the PAR-1 agonist TFFLR-NH_2 (10 µg/ml) for 6 h. C) The PAR-1 agonist TFFLR-NH_2 induced IL-11 protein secretion whereas its control peptide did not alter the IL-11 secretion. D) Treatment of cells with PAR-1 agonist TFFLR-NH_2 induced IL-11 transcript expression whereas its control peptide RLFFT-NH_2 did not affect the same. ** $p < 0.01$, **** $p < 0.0001$.

measured the expression of mRNA in control and thrombin-induced HTR 8/SVneo cells by real-time PCR. Indeed, the expression of *IL-11*mRNA was significantly upregulated ($p < 0.0001$; 2.9-folds) by thrombin treatment within 6 h while the expression was slightly decreased within 12 h (Fig. 1C). These data suggest that thrombin-stimulation rapidly enhances the *IL-11* gene expression leading to increased protein secretion in HTR-8/SVneo cells.

3.2. PAR-1 receptor mediates thrombin-induced IL-11 secretion in HTR8/SVneo cells

To examine the expression of known thrombin receptor subtypes (PAR-1, -3, -4) in HTR-8/SVneo cells, we measured the abundance of receptor mRNAs by using real-time PCR. Among all the thrombin receptor subtypes, PAR-1 was the only highly expressed receptor in HTR-

8/SVneo cells whereas PAR-3 mRNA expression was 50–folds less than that of PAR-1 (Fig. 2A). PAR-4 gene was undetectable in HTR-8/SVneo cells (Fig. 2A). To analyze the protein expression of PAR-1 and PAR-3 receptor subtypes, we performed a Western blot analysis. Specific bands of PAR-1 and PAR-3 were detected in both cell types (HTR-8/SVneo and BeWo cells) confirming their expression in these cells (Fig. 2B). The expression of both PAR-1 and PAR-3 in HTR-8/SVneo cells was much higher than BeWo cells (Fig. 2B).

To explore the role of PAR-1 in thrombin-dependent IL-11secreation, we treated the cells with a highly selective PAR-1 agonist, TFLLRN-NH_2. A scrambled peptide (RLFFT-NH_2) was used as a control. Indeed, TFLLRN-NH_2-induced secretion of IL-11 was similar to that induced by thrombin (Fig. 2C). Furthermore, we investigated whether PAR-1 activation enhances the *IL-11* gene expression. Indeed, activation of PAR-1 by TFLLRN-NH_2 could enhance the *IL-11* mRNA expression (Fig. 2D). These data suggest that PAR-1 is the major receptor in HTR8/SVneo cells that is activated by thrombin leading to IL-11 gene expression and secretion.

3.3. Activation of Rac-1, but not Rho-kinase regulates IL-11 secretion

In many cell types, thrombin activates RhoGTPases, such as Rho-dependent Rho-kinase and Rac1 (Coughlin, 2000; Pandey et al., 2009). We asked whether thrombin-dependent activation of Rho/Rho-kinase pathway could be involved in regulating IL-11 secretion. To inhibit Rho/Rho-kinase pathway, we used a highly specific Rho-kinase inhibitor; Y27632. Cells were treated with Y27632 for 30 min and then activated with thrombin for 6 h. We found that the effect of Y27632 on thrombin-induced IL-11 secretion was not significant (Fig. 3A). Interestingly, Rho-kinase inhibition could partially inhibit the thrombin-induced *IL-11* gene expression to ˜68% ($p < 0.05$; Fig. 3B).

To investigate whether thrombin-dependent IL-11 secretion was regulated through the activation of Rac1, cells were treated with the Rac1-specific inhibitor; NSC23766 that blocks the Rac1 activation (Gao et al., 2004; Brünnert et al., 2015). NSC23766 alone significantly reduced the basal level of IL-11 secretion to ˜33% ($p < 0.001$) at 6 h after cell treatment (Fig. 3A). Furthermore, thrombin-induced IL-11 secretion was significantly inhibited in cells pretreated with NSC23766 (Fig. 3A). Notably, *IL-11* gene expression was unaffected by Rac1 inhibition at the basal level (Fig. 3B). Interestingly, the thrombin-dependent increase of IL-11 gene expression was partially inhibited by Rac1 inhibition (Fig. 3B). These data indicate that Rac1, but not Rho-kinase activation is required for thrombin-induced IL-11 secretion.

3.4. Thrombin enhances cell migration in HTR-8/SVneo cells via IL-11 secretion

As cell migration is a crucial step for cell invasion, we checked whether thrombin regulates the HTR-8/SVneo cell migration using a wound healing scratch assay. We found that thrombin stimulation significantly enhanced cell migration and area covered by cell migration was ˜ 60% ($p < 0.05$) more as compared to that in unstimulated cells (Fig. 4A, B). These data imply that thrombin is involved in the migration processes of EVTs. To explore the Rho-kinase and Rac1 pathways in cell migration, cells were treated with Rac1 and Rho-kinase inhibitors. Within 24 h of treatments, cell morphology was severely affected which was shown as reduced size and round or elongated shape (Fig. 4A).

To address the question whether thrombin-dependent IL-11 secretion regulates the cell migration, we specifically knocked down *IL-11* gene using a pool of four specific siRNAs or control siRNAs with an efficiency of 75–85% (Supplementary Fig. 1) and also performed wound healing scratch assay for 24 h. We could show that after *IL-11* gene knockdown, thrombin could not enhance cell migration (Fig. 4A right panel and 4C). The migration level was significantly lower ($p < 0.05$) than that of thrombin-stimulated control cells. These data indicate that thrombin regulates cell migration via IL-11 dependent pathway.

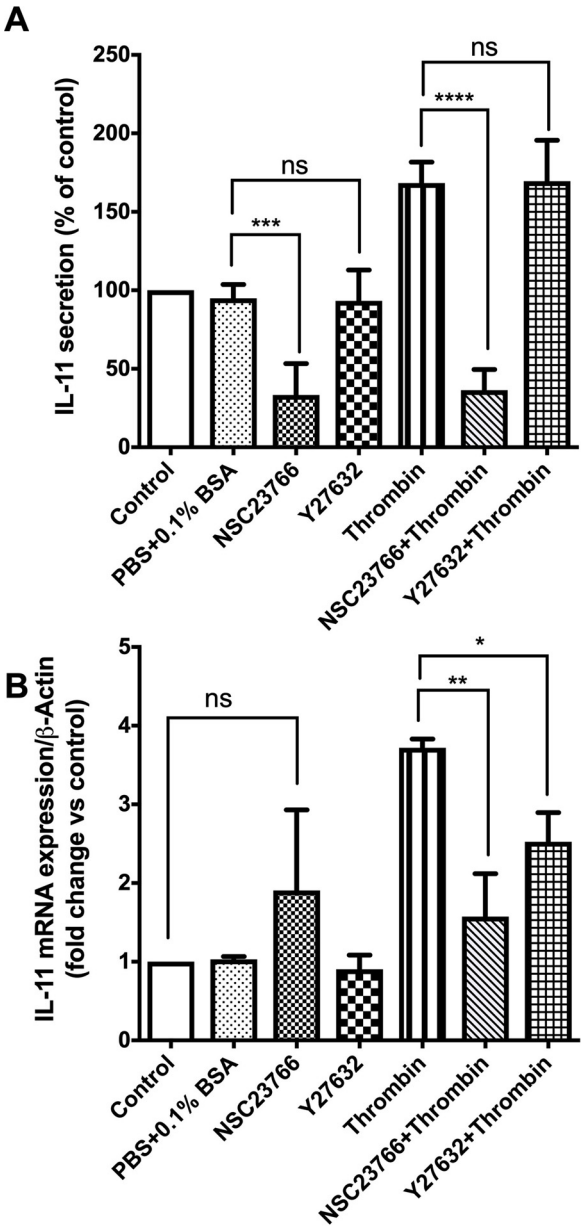

Fig. 3. Rac-1 activation, but not Rho-kinase is required for thrombin-induced IL-11 secretion. HTR-8/SVneo cells were pretreated in cell medium (RPMI1640 basal medium with 0.5% dFCS), 0.1% DMSO (control), 0.1% BSA in PBS, Rac-1 inhibitor NSC23766 (200 μM) or Rho kinase inhibitor Y27632 (20 μM) and then activated with 0.5 U/ml thrombin for 6 h. A) Stimulation of cells with NSC23766 drastically reduced thrombin-activated IL11 release. Y27632 did not alter the thrombin-dependent IL-11 secretion. B) Total RNA was isolated from HTR-8/SVneo cells and IL-11 mRNA expression was measured by real-time PCR. Bar diagram shows the fold changes of IL-11 transcripts at 6 h. NSC23766 significantly inhibited thrombin-dependent IL-11 mRNA expression. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns = not significant.

4. Discussion

Several molecules derived from the endometrium and placental cells have individually been shown to regulate the trophoblast cell functions during pregnancy. IL-11 is one of the major cytokines that has a crucial role in trophoblast, endometrial, epithelial and stromal cell functions,

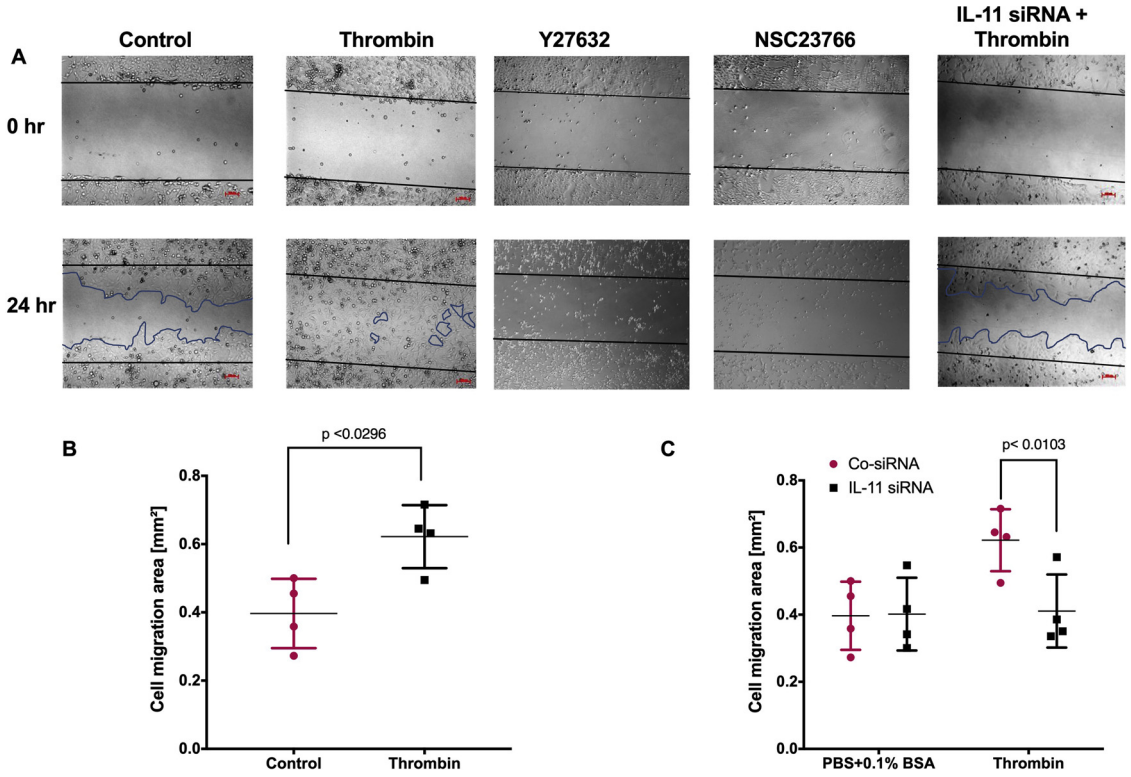

Fig. 4. Thrombin enhances cell migration in HTR-8/SVneo cells via IL-11 secretion. HTR-8/SVneo (200,000) cells were plated in a 24-well plate and grown in factors-reduced medium. Cells were then activated using 0.1% BSA (control) or 0.5 U/ml thrombin. Gene knockdown was performed using 30 pmol of control siRNAs or 30 pmol IL11 specific siRNAs pool. A) a wound scratch assay was performed for 24 h to analyze migration behavior of the cells. Thrombin significantly enhanced migration of HTR-8/SVneo cells (second panel) whereas Y27632 (third panel) and NSC23766 (fourth panel) severely affected the cell morphology. IL-11 gene silencing led to a significant decrease of thrombin-induced migration of cells (fifth panel).
Scatter plot shows the cell migration area covered after 24 h of stimulation B) with thrombin C) after IL-11 gene silencing and thrombin stimulation. Data are mean ± SD of 4 independent experiments with different passages of HTR-8/SVneo cells and representative images are shown.

and in pregnancy maintenance (Paiva et al., 2007; Marwood et al., 2009; Sonderegger et al., 2011). In the present study, we demonstrate that thrombin regulates EVT migration via activation of PAR-1 receptor and then IL-11 secretion.

In this study, we screened the secretion of selected cytokines in trophoblast cell lines including BeWo and HTR-8/SVneo cells (Supplementary Fig. 2) after thrombin treatment. Indeed, thrombin enhanced the secretion of IL-11 protein and the gene expression specifically in HTR-8/SVneo cells, but not in BeWo. In primary EVT, expression of thrombin receptor subtypes (PAR1 and PAR-3) was reported in early pregnancy (Even-Ram et al., 2003; O'brien et al., 2003). In this study, we found that PAR-1 was highly expressed in HTR-8/SVneo cells, which were originally derived from first trimester villous explants of early pregnancy (Graham et al., 1993), suggesting a role of thrombin and PAR-1 in the regulation of EVT functions, independent from that in coagulation pathway. Various studies have shown that the activation of PAR-1 regulates cytokine secretion, tumor progression and cell migration in various cell types (Even-Ram et al., 1998; Sambrano and Coughlin, 1999; Martin et al., 2001). The specific peptide agonist of PAR-1 receptor could activate the gene expression and protein secretion of IL-11 similar to thrombin-dependent activation. These data suggest that thrombin-mediated IL-11 gene expression and protein secretion is mainly regulated via PAR-1 receptor. The present study elucidated, for the first time the role of thrombin-dependent IL-11 secretion in EVTs mainly via PAR-1. Activation of PAR-1 receptor via thrombin induces RhoGTPases (Rho and Rac1) that mediates the secretion in various cells including platelets (Coughlin, 2000; Pandey et al., 2009). In this study, we found that inhibition of Rac1, but not Rho/Rho-kinase reduces the secretion of IL-11 at basal level and completely inhibits the thrombin-induced secretion. Furthermore, thrombin-dependent expression of *IL-11* gene was inhibited by Rac1 inhibitor, but the basal expression was unaffected. We propose that a thrombin/PAR-1/Rac1 axis regulates the IL-11gene expression and protein secretion in EVT.

Regulation of IL11 secretion during pregnancy is crucial for implantation, placental development and pregnancy maintenance (Robb et al., 1998; Chen et al., 2002). Abnormal increase of IL11 levels was associated with preeclampsia in human decidua cells (Basar et al., 2010). The thrombin-dependent secretion of IL-11 might be an important event for EVT function and early placental development. Thrombin stimulation results in an increasing invasion level in EVT whereas a PAR-1 specific antibody could inhibit invasion (O'brien et al., 2003). These studies suggest that precise regulation of IL-11 is required for placental development and pregnancy maintenance. This is in line with our data that HTR8/SVneo cells stimulation with thrombin could also enhance cell migration that is a crucial step for cell invasion. A study showed that Rho/Rho-kinase regulates the extravillous trophoblast cell migration wherein, Rho-kinase and Rac1 inhibitors could block the cell migration in the controls (Shiokawa et al., 2002). Furthermore, IL-11 has been shown to enhance the migration and proliferation of chondrosarcoma cells (Te-Mao et al., 2012). Here, we show that thrombin increases cell migration via enhancing IL-11 secretion. After specific IL-11 knockdown, thrombin is not able to enhance cell migration over a basal level of untreated cells. We proposed that thrombin-dependent IL-11 secretion enhanced the EVT migration. Interestingly, we couldn't find any significant increase of cell migration by recombinant IL-11 (data not shown). This result suggests that IL-11 is one of the crucial factors responsible for thrombin-induced cell migration. Thrombin-induced cell migration may require additional factors besides IL-11 secretion.

Thrombin plays an essential role in tumor progression and

metastasis, and is associated with cell migration leading to invasion. Furthermore, thrombin acts as a regulator of trophoblast invasion during placental development (O'brien et al., 2003). In this study, we could show a regulatory link in between thrombin and the cytokine IL-11 in HTR-8/SVneo cells.

In conclusion, this study demonstrates the role of thrombin in regulating human EVT migration via IL-11 secretion. We suggest that thrombin might regulate EVT migration through the decidua and spiral artery remodeling and failure of thrombin-dependent EVT migration might result in spontaneous abortion during early pregnancy.

Authors' contribution

Conceived and designed the experiments: P.G and D.B. Performed the experiments: P.G., D.B. and J.E. Analyzed the data: P.G., D.B., I.S., and K.R.C. Manuscript drafting and critical discussion: P.G., D.B. I.S.J.P., J.K.Y. K.R.C. and M.Z.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2019.03.001.

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