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Original Article

EG-VEGF Induces Invasion of a Human Trophoblast Cell Line via PROKR2

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Extravillous trophoblast (EVT) invasion is important for embryo implantation, placental development, and successful remodeling of the uterine spiral artery. Endocrine gland derived-vascular endothelial growth factor (EG-VEGF) and matrix metalloproteinases (MMPs) are implicated in EVT invasion; however, the high concentrations found in pregnancy pathologies have not been investigated in non-tumor trophoblasts. The roles of EG-VEGF, prokineticin receptors (PROKR1/2), MMP-2, and MMP-9 in EVT invasion during spiral artery remodeling were evaluated using human EVT from HTR-8/SVneo cell lines. The expression of MMP-2, MMP-9, and mitogen-activated protein kinase (MAPK), and Akt pathways in HTR-8/SVneo cells treated with recombinant EG-VEGF alongside anti-PROKR1 and/or anti-PROKR2 antibodies was evaluated using quantitative reverse transcription-PCR and western blotting. Wound-healing and cell invasion assays were performed to assess the migration and invasion of these treated cells. Interestingly, 20 nM EG-VEGF activated ERK1/2 signaling and upregulated MMP-2 and MMP-9. This effect was suppressed by anti-PROKR2 antibody via ERK1/2 downregulation. Anti-PROKR2 antibody inhibited the migration and invasion of EG-VEGF-stimulated HTR-8/SVneo cells. Elevated concentrations of EG-VEGF enhance EVT invasion in a human trophoblast cell line by upregulating MMP-2 and MMP-9 via PROKR2. These new insights into the regulation of epithelial cell invasion may help in developing therapeutic interventions for placental-related diseases during pregnancy.

Key words: endocrine gland-derived vascular endothelial growth factor, prokineticin, extravillous trophoblast, matrix metalloproteinase, obstetric diseases

Trophoblast invasion is an important and complex process in human pregnancy. During early pregnancy, the uterine spiral artery is remodeled from a small striated vessel to a large flaccid conduit that is unresponsive to vasoconstrictors [1]. Extravillous trophoblasts (EVTs) invade the uterus and spiral arteries before the fetal-maternal circulation is established to ensure sufficient nutrient and oxygen transfer to the fetus. Inadequate trophoblast invasion underlies many harmful obstetric diseases. For example, poor trophoblast infiltration is associated with miscarriage, preeclampsia, and intrauterine fetal growth restriction [2,3].

The endocrine-gland-derived vascular endothelial growth factor (EG-VEGF), also named prokineticin 1 (PROK1), is mainly expressed in the steroidogenic glands, ovary, uterus, and placenta. The biological activity of EG-VEGF is mediated by two G-protein-coupled receptors: prokineticin receptor (PROKR) 1 and PROKR2 [4-6]. In humans, EG-VEGF is upregulated in the endometrium during pregnancy. It peaks at 8-10 weeks of gestation and gradually declines by the end of the first trimester. Placental PROKR1 shows the

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same expression pattern as EG-VEGF, with its expression peaking between the 8th and 10th weeks of gestation, whereas PROKR2 shows stable expression throughout the first trimester of pregnancy [6]. EG-VEGF expression in tissues in early pregnancy is mainly localized to the syncytiotrophoblast (ST) and cytotrophoblast (CT) layers [7, 8]. PROKR1 is mainly expressed in the CT and ST, and PROKR2 is mainly expressed in the EVT [9]. EG-VEGF has been shown to regulate placental growth, and it acts as an important endocrine factor in placental development [10]. EG-VEGF has also been shown to be strongly associated with recurrent miscarriage, gestational trophoblastic disease [11], and placental pathologies associated with fetal growth restriction [12] and/or preeclampsia [13].

EG-VEGF is an upstream inducer of several matrix metalloproteinases (MMPs) and is suggested to be the master regulator of MMP production. MMPs play major roles in trophoblast invasion into the uterine wall. Trophoblast invasion is influenced by cytokines, growth factors, and MMPs [14-16]. EG-VEGF has been shown to regulate the expression of MMP-2 and MMP-9 [17-19]. MMP-2 and MMP-9 play critical roles in the regulation of trophoblast invasion. MMP-2 and MMP-9 are considered to be important enzymes that dissociate the basement membrane, extracellular matrix, and vascular smooth muscle cell layer and mediate EVT invasion [20, 21]. MMP-2 expression and MMP-9 expression are at their highest levels in the placental bed during early pregnancy, mainly localizing to EVT cells at 6-8 weeks of gestation, and they appear to regulate the trophoblast infiltration [15]. Downregulation of MMP-2 and/or MMP-9 reduces the trophoblast invasion and causes abnormal uteroplacental artery remodeling, which has been shown to be associated with preeclampsia [14, 15, 22].

However, no previous studies have revealed an association between EG-VEGF and MMPs via PROKR1/2 during EVT invasion.

Therefore, the aim of this study was to determine the relation between MMPs and cell invasion by EG-VEGF through PROKR1 and PROKR2 in HTR-8/SVneo, a human EVT cell line.

Materials and Methods

Preparation of cell culture and antibodies. The human EVT cell line HTR-8/SVneo (ATCC® CRL-3271™) was cultured at 37°C with 5% CO_2 using RPMI-1640 medium (ATCC® 30-2001™) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Anti-human prokineticin R1/PROKR1 and prokineticin R2/PROKR2 antibodies (Novus Biochemicals, Littleton, CO, USA) were used at 5 μg/mL to inhibit the PROKR-mediated signal transduction.

Quantitative reverse transcription-polymerase chain reaction and western blotting. HTR-8/SVneo cells were incubated for 24 h and treated with or without human recombinant EG-VEGF (20 nM; PeproTech), anti-PROKR1 antibody (5 μg/mL), or anti-PROKR2 antibody (5 μg/mL). Total RNA was extracted from the cells using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The cDNA was reverse-transcribed from the total RNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. MMP-2 (Hs01548727_m1), MMP-9 (Hs00957562_m1), and β-actin (Hs01060665_g1) mRNAs were assessed. β-Actin was used as an internal control. Polymerase chain reaction (PCR) was performed using the STEP ONE PCR system (Applied Biosystems) with initial denaturation at 95°C for 15 s, followed by 50 cycles of annealing at 60°C, and a final extension at 60°C for 1 min. The experiments were repeated at least three times. The results were expressed using the $2^{-\Delta\Delta Ct}$ method. Real-time PCR results were obtained using the comparative CT method.

The levels of MMPs, mitogen-activated protein kinases (MAPKs; including extracellular signal-regulated kinase (ERK) 1/2, c-jun N-terminal kinase (JNK), and p38), and Akt proteins were assessed using western blotting analysis. Cells were extracted using a lysis buffer (RIPA Lysis and Extraction Buffer; Thermo Fisher Scientific). Each protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Invitrolon PVDF, 0.45 μm; Invitrogen). After being blocked in 5% skim milk in Tris-buffered saline containing 0.1% Tween–20 for 1 h, the membranes were incubated overnight at 4°C with the following primary antibodies: anti-MMP-2 (1 : 1000) and anti-MMP-9 (1 : 1000) (Proteintech); anti-phospho (p)-ERK1/2 (1 : 2000), anti-ERK1/2 (1 : 1000), anti-p-Akt (1 : 2000), anti-Akt (1 : 1000), anti-p-p38 (1 : 1000),

anti-p38 (1 : 1000), anti-p-JNK (1 : 1000), and anti-JNK (1 : 1000) (Cell Signaling Technology); and anti-GAPDH (1 : 5000, Invitrogen). Each membrane was washed and then incubated with HRP-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at 24°C. Signals were then detected using the Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare). The experiments were repeated at least three times. Each band was quantitatively analyzed by densitometry using a GT-9500 Image Scanner (Epson, Suwa, Japan) and BioImage BQ 2.0 software (Ann Arbor, MI, USA).

The wound-healing assay (cell migration assay) and cell invasion assays. Cell migration was assessed using the CytoSelect™ 24-well Wound-healing Assay (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions. Cells were seeded into the inserts of a CytoSelect 24-well plate and cultured overnight. The inserts were carefully removed, leaving a precise wound field of 0.9 mm in diameter. The medium was aspirated, and cells treated with or without human recombinant EG-VEGF (20 nM; PeproTech), anti-PROKR1 antibody (5 μg/mL), or anti-PROKR2 antibody (5 μg/mL) were incubated for 24 h in the RPMI medium supplemented with 10% FBS. Wound closure progress was monitored using a light microscope. Cell invasion was assessed using a CytoSelect™ 24-well cell invasion assay kit (8-μm pore size; Cell Biolabs) according to the manufacturer's instructions. Cells were seeded into the upper chamber in the serum-free medium treated with or without human recombinant EG-VEGF (20 nM; PeproTech), anti-PROKR1 antibody (5 μg/mL), or anti-PROKR2 antibody (5 μg/mL). Media containing 10% FBS were added to the lower wells of the invasion plates, and the plates were incubated for 24 h. Finally, migrated cells were counted using a light microscope.

Statistical analysis. All data are expressed as the mean ± standard error of mean. The data analysis was performed using the Software Package for the Social Sciences (SPSS; Armonk, NY, USA). The Mann–Whitney *U*-test was used to compare variables, and a *p*-value of < 0.05 was considered statistically significant.

Results

Activation of ERK1/2 in the HTR-8/SVneo EVT cell line by EG-VEGF via PROKR2. First, we determined the protein levels of MAPKs (ERKI/2, JNK, and p38) and Akt proteins in the HTR-8/SVneo EVT cells. Western blot analysis showed that JNK, p38, and Akt proteins were not detected while ERKI/2 was present in the EVT cells (Fig. 1A). The level of phosphorylated ERKI/2 (p-ERK) appeared to be slightly increased when the cells were treated with recombinant EG-VEGF protein. As described previously, there are 2 receptors for EG-VEGF, PROKRI and PROKR2 [4-6]. To determine which receptor was used by EG-VEGF in the EVT cells,

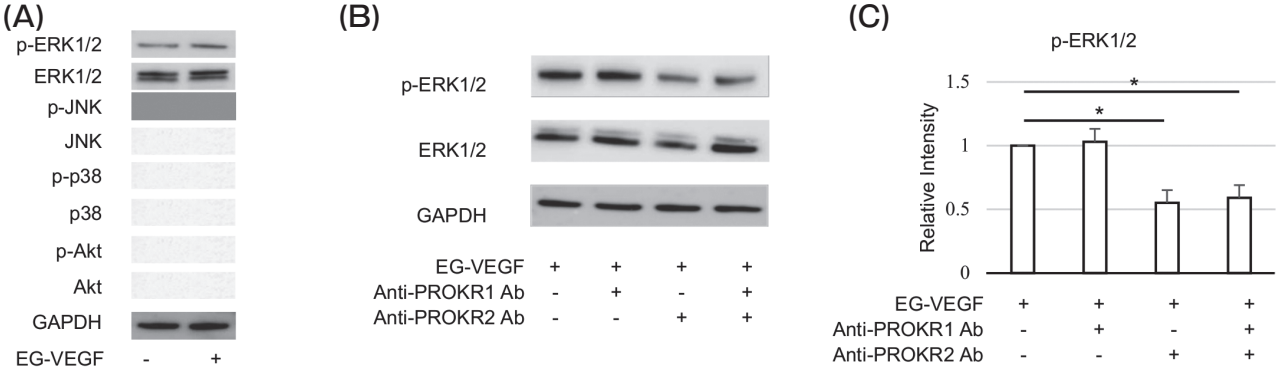

Fig. 1 (**A**) HTR-8/SVneo cells were incubated for 24 h and then treated with or without human recombinant endocrine-gland-derived vascular endothelial growth factor (EG-VEGF) (20 nM). The expression of phosphor (p)-ERK1/2 and ERK1/2 was analyzed via western blotting. GAPDH was used as an internal control. Each bar represents the mean ± SEM obtained from three independent experiments. (**B-C**) HTR-8/SVneo cells were incubated for 24 h and then treated with EG-VEGF (20 nM) with either an anti-prokineticin receptor (PROKR)1 antibody (5 μg/mL), an anti-PROKR2 antibody (5 μg/mL), or both. The expressions of ERK1/2 and p-ERK1/2 were analyzed via western blotting. GAPDH was used as an internal control. Each bar represents the mean ± SEM obtained from three independent experiments. $^{*}P < 0.05$ compared with the corresponding control as determined by the Mann–Whitney *U*-test.

we examined the alteration of ERKI/2 levels in the EG-VEGF-treated cells using blocking antibodies against PROKRI or PROKR2. We found that in the presence of anti-PROKR2, the level of activated ERKI/2 was decreased (Fig. 1B, C). In contrast, no decrease in p-ERKI/2 levels was observed in the presence of anti-PROKR1.

Upregulation of MMP-2 and MMP-9 expression in the HTR-8/SVneo EVT cell line by EG-VEGF via PROKR2. We next examined the MMP-2 and MMP-9 mRNA levels in the EVT cells treated with recombinant EG-VEGF using quantitative reverse transcription-PCR (RT-qPCR). The RT-qPCR results showed that the relative expression levels of MMP-2 and MMP-9 were significantly upregulated after EG-VEGF treatment (Fig. 2A). The expression levels of MMP-2 and MMP-9 mRNA were significantly suppressed when the cells were co-treated with the anti-PROKR2 antibody (Fig. 2B). Furthermore, western blot analysis showed that the expression of MMP-2 and MMP-9 proteins was significantly suppressed when the cells were co-treated with the anti-PROKR2 antibody (Fig. 2C).

Migration and invasion of the HTR-8/SVneo cell line were enhanced by EG-VEGF signaling. To determine whether the migration of HTR-8/SVneo cells was enhanced by EG-VEGF signaling, we performed a wound-healing assay (Fig. 3A) and calculated the percentages of cells capable of migration (Fig. 3B). Compared with recombinant EG-VEGF alone (50.6%), the percentages of cells capable of migration significantly decreased to 32. 0% and 35.0% in the assays in which anti-PROKR2 antibody and both anti-PROKR1 and anti-PROKR2 antibodies, respectively, were used (Fig. 3B).

The invasion of HTR-8/SVneo cells was further examined by a cell invasion assay (Fig. 3C). The percentages of cells capable of invasion decreased to 92.4%, 51.4%, and 56.0% in the presence of the anti-PROKR1, anti-PROKR2, and both antibodies, respectively. The percentages of the cells capable of invasion showed especially large and significant decreases in the presence of the anti-PROKR2 antibody and both antibodies (Fig. 3D).

These results suggest that EG-VEGF upregulated the expression of MMP-2 and MMP-9 and facilitated the invasion and migration of EVT via PROKR2 (Fig. 4).

Discussion

In this study, we used immortalized extravillous CTs isolated from human placenta (HTR-8/SVneo) and anti-PROKR antibodies to elucidate the role of the EG-VEGF signaling pathway in EVT invasion. EG-VEGF induced upregulation of ERK1/2 and MMP-2. MMP-9, EVT migration, and invasion were inhibited by the anti-PROKR2 antibody. The anti-PROKR1 antibody did not inhibit the expression of MMPs or cell invasion, suggesting that PROKR1 signaling is not dominant in EVT. These results suggest that an elevated EG-VEGF level induces the expression and activation of MMP-2 and MMP-9 and promotes EVT infiltration via PROKR2 signaling.

The role of EG-VEGF in human pregnancy is strongly associated with trophoblast invasion and migration. EG-VEGF signaling is mediated by two G-protein-coupled receptors, PROKR1 and PROKR2, and results in the phosphorylation of MAPK and Akt serine/threonine kinase. PROKR1 is abundantly expressed in CTs, placental microvascular endothelial cells (HPECs), and Hofbauer cells (Ho), whereas PROKR2 is mainly expressed in STs, HPECs, Ho, and EVT. EG-VEGF regulates trophoblast infiltration in human gestational tissues. The mechanism by which this regulation occurs is thought to involve the activation of oxygen tension, human chorionic gonadotropin, inflammatory cytokines, and metalloproteinases [13].

Wang *et al.* showed that EG-VEGF activates ERK1/2 signaling, and then upregulates MMP-2 and MMP-9 in human EVT HTR-8/SVneo cells [18]. Zhang *et al.* showed that elevated miR-141 and miR-200a levels inhibit the expression of EG-VEGF and the downstream ERK1/2, MMP-2, and MMP 9 signaling, as well as cilia formation, thereby leading to defective trophoblast invasion [19]. Traboulsi *et al.* showed that choriocarcinoma development and metastasis were significantly suppressed in pregnant mice after treatment with PROKR1 or PROKR2 antagonists. They also found that PROKR2 antagonists had a stronger inhibitory effect than did PROKR1 antagonists [11]. However, Hoffmann *et al.* demonstrated that EG-VEGF inhibits trophoblast migration and invasion, both in HTR and explant models [9]. Because in this study they used a concentration of EG-VEGF that mimics the physiological levels found during the first trimester, one can speculate

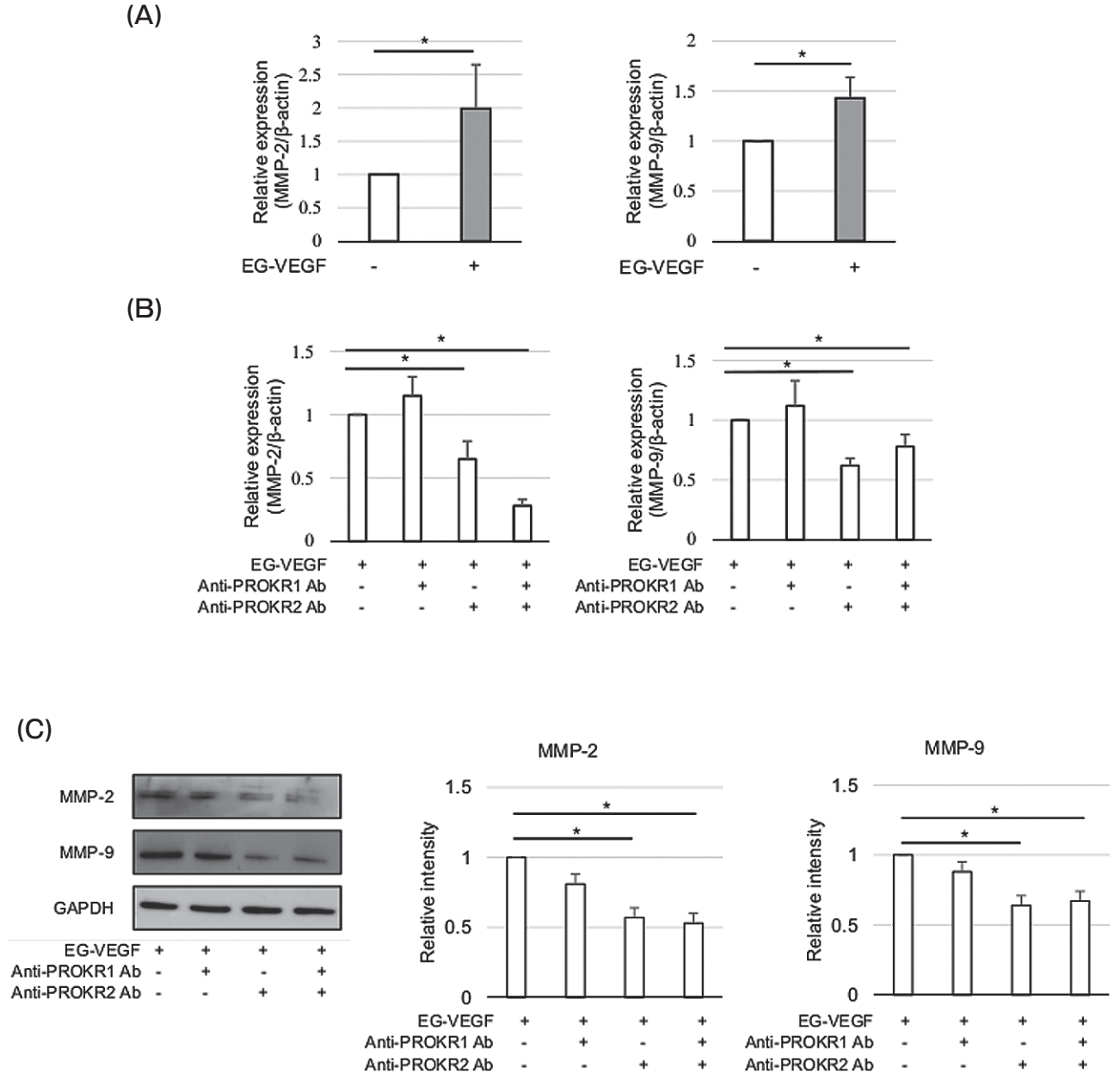

Fig. 2 (**A**) HTR-8/SVneo cells were incubated for 24 h and then treated with or without human recombinant endocrine-gland-derived vascular endothelial growth factor (EG-VEGF) (20 nM). The expressions of matrix metalloproteinase (MMP)-2 and MMP-9 were analyzed via RT-qPCR. Data are presented as the mean ± SEM. (**B**) HTR-8/SVneo cells were incubated for 24 h and then treated with EG-VEGF (20 nM) with either an anti-prokineticin receptor antibody (PROKR)1 (5 μg/mL), an anti-PROKR2 antibody (5 μg/mL), or both. The expressions of MMP-2 and MMP-9 were analyzed via RT-qPCR. Data are presented as the mean ± SEM. (**C**) HTR-8/SVneo cells were incubated for 24 h and then treated with EG-VEGF (20 nM) with either an anti-PROKR1 antibody (5 μg/mL), an anti-PROKR2 antibody (5 μg/mL), or both. The expressions of MMP-2 and MMP-9 were analyzed via western blotting. GAPDH was used as an internal control.
The data are presented as the mean ± SEM. $^{*}P<0.05$ compared with the corresponding control as determined by the Mann–Whitney *U*-test.

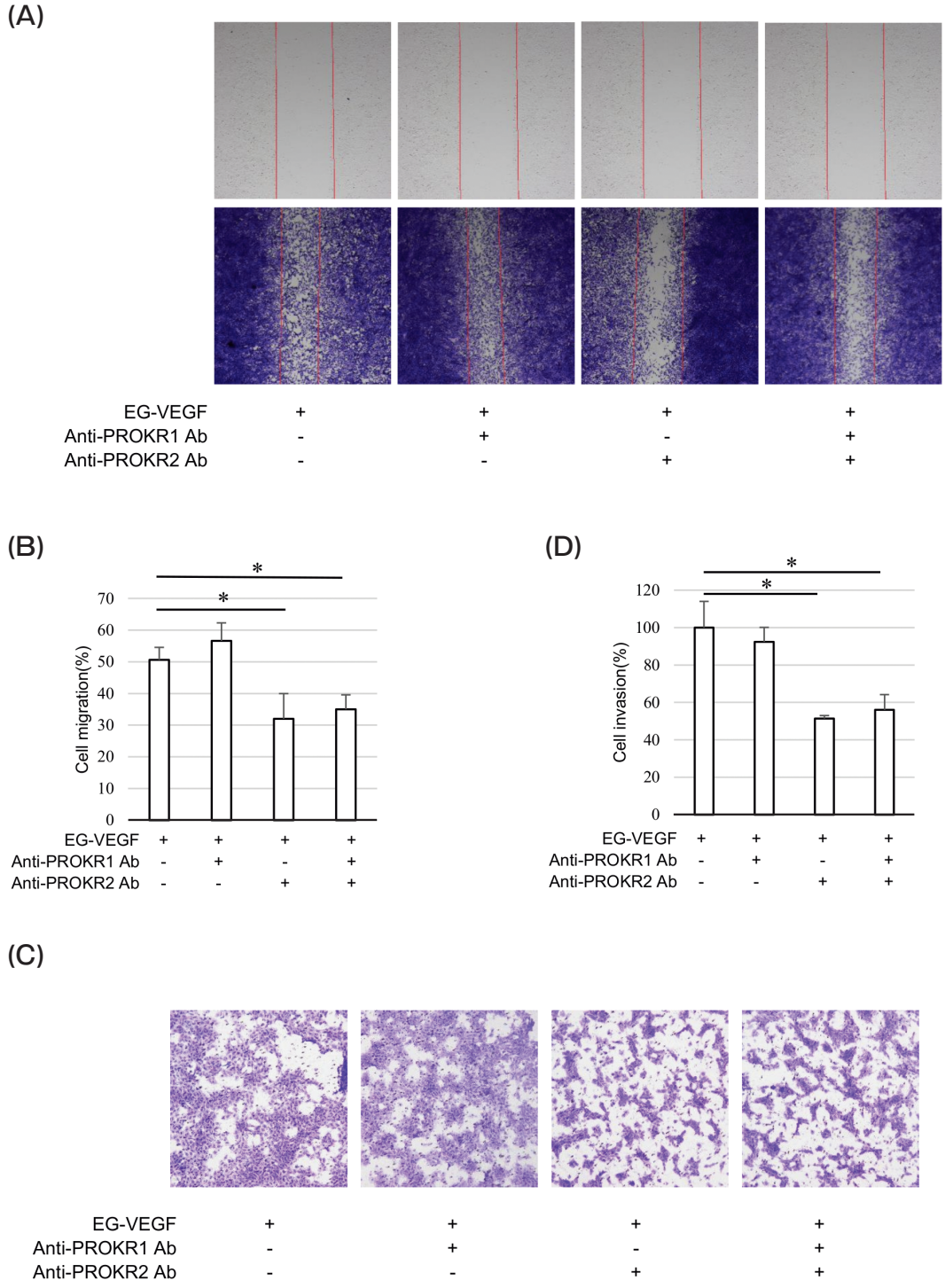

Fig. 3 (**A**-**B**) The migration of HTR-8/SVneo cells was assessed using a CytoSelect™ 24-well wound-healing assay kit. HTR-8/SVneo cells were treated with endocrine-gland-derived vascular endothelial growth factor (EG-VEGF) (20 nM) with either an anti-prokineticin receptor antibody (PROKR)1 (5 μg/mL), an anti-PROKR2 antibody (5 μg/mL), or both.
(**C**-**D**) The invasion of HTR-8/SVneo cells was assessed using a CytoSelect™ 24-well cell invasion assay kit. HTR-8/SVneo cells were treated with EG-VEGF (20 nM) with either an anti-PROKR1 antibody (5 μg/mL), an anti-PROKR2 antibody (5 μg/mL), or both. Data are presented as the mean ± SEM. $^{*}P<0.05$ compared with the corresponding control as determined by the Mann–Whitney *U*-test.

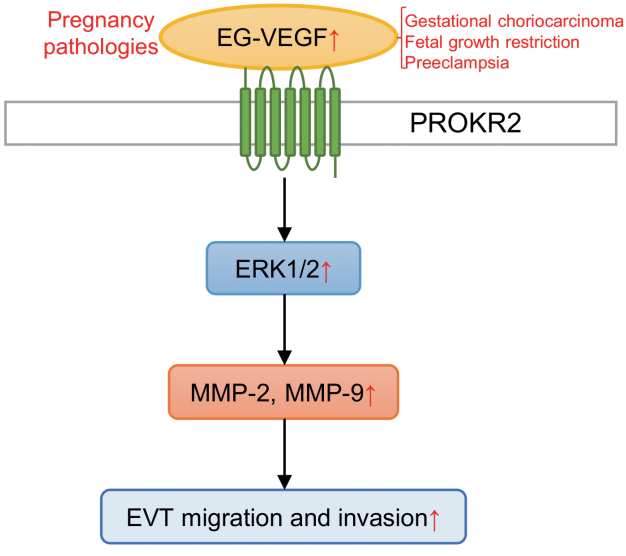

Fig. 4 Relationship between EG-VEGF signaling and EVT invasion. EG-VEGF enhances EVT invasion through the activation of MMP-2 and MMP-9 via PROKR2 in a human trophoblast cell line.

that EG-VEGF inhibits trophoblast invasion at low concentrations and exerts an opposite effect under pathological conditions when its concentrations are elevated, such as choriocarcinoma and growth restriction [11, 12]. The association between PROKR1/2 and MMPs during EVT invasion remains largely unexplored. Interestingly, we found that the expressions of ERK1/2, MMP-2, and MMP-9 were attenuated after anti-PROKR2 antibody treatment, indicating that suppressing the PROKR2 pathway inhibited the functions of EVT, possibly in HTR-8/SVneo cells.

One limitation of the present study is our heavy reliance on the HTR-8/SVneo cell line in the experiments. Although HTR-8/SVneo cells are derived from healthy human placentas, it would be ideal to use primary cells to mimic in vivo placental invasion.

In conclusion, our results demonstrated that EG-VEGF upregulates MMP-2 and MMP-9 via ERK1/2 and PROKR2 to induce EVT invasion. Thus, abnormal PROKR2 expression may impair EVT invasion during pregnancy and cause placental-related diseases.

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References

1. Pijnenborg R, Vercruysse L and Hanssens M: The uterine spiral arteries in human pregnancy: facts and controversies. Placenta (2006) 27: 939–958.
2. Roberts JM: Pathophysiology of ischemic placental disease. Semin Perinatol (2014) 38: 139–145.
3. Tessier DR, Yockell-Lelièvre J and Gruslin A: Uterine spiral artery remodeling: the role of uterine natural killer cells and extravillous trophoblasts in normal and high-risk human pregnancies. Am J Reprod Immunol (2015) 74: 1–11.
4. Ferrara N, Frantz G, LeCouter J, Dillard-Telm L, Pham T, Draksharapu A, Giordano T and Peale F: Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries. Am J Pathol (2003) 162: 1881–1893.
5. Fraser HM, Bell J, Wilson H, Taylor PD, Morgan K, Anderson RA and Duncan WC: Localization and quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum. J Clin Endocrinol Metab (2005) 90: 427–434.
6. Hoffmann P, Feige JJ and Alfaidy N: Expression and oxygen regulation of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy. Endocrinology (2006) 147: 1675–1684.
7. Battersby S, Critchley HO, Morgan K, Millar RP and Jabbour HN: Expression and regulation of the prokineticins (endocrine gland-derived vascular endothelial growth factor and Bv8) and their receptors in the human endometrium across the menstrual cycle. J Clin Endocrinol Metab (2004) 89: 2463–2469.
8. Evans J, Catalano RD, Morgan K, Critchley HO, Millar RP and Jabbour HN: Prokineticin 1 signaling and gene regulation in early human pregnancy. Endocrinology (2008) 149: 2877–2887.
9. Hoffmann P, Saoudi Y, Benharouga M, Graham CH, Schaal JP, Mazouni C, Feige JJ and Alfaidy N: Role of EG-VEGF in human placentation: physiological and pathological implications. J Cell Mol Med (2009) 13: 2224–2235.
10. Brouillet S, Hoffmann P, Feige JJ and Alfaidy N: EG-VEGF: a key endocrine factor in placental development, Trends Endocrinol Metab (2012) 23: 501–508.
11. Traboulsi W, Sergent F, Boufettal H, Brouillet S, Slim R, Hoffmann P, Benlahfid M, Zhou QY, Balboni G, Onnis V, Bolze PA, Salomon A, Sauthier P, Mallet F, Aboussaouira T, Feige JJ, Benharouga M and Alfaidy N: Antagonism of EG-VEGF receptors as targeted therapy for choriocarcinoma progression in vitro and in vivo. Clin Cancer Res (2017) 23: 7130–7140.
12. Brouillet S, Murthi P, Hoffmann P, Salomon A, Sergent F, De Mazancourt P, Dakouane-Giudicelli M, Dieudonné MN, Rozenberg P, Vaiman D, Barbaux S, Benharouga M, Feige JJ and Alfaidy N: EG-VEGF controls placental growth and survival in normal and pathological pregnancies: case of fetal growth restriction (FGR). Cell Mol Life Sci (2013) 70: 511–525.
13. Alfaidy N, Hoffmann P, Boufettal H, Samouh N, Aboussaouira T, Benharouga M, Feige JJ and Brouillet S: The multiple roles of EG-VEGF/PROK1 in normal and pathological placental angiogenesis. BioMed Res Int (2014) 2014: 451906.
14. Luo J, Qiao F and Yin X: Hypoxia induces FGF2 production by vascular endothelial cells and alters MMP9 and TIMP1 expression in extravillous trophoblasts and their invasiveness in a cocultured model. J Reprod Dev (2011) 57: 84–91.
15. Suman P and Gupta SK: Comparative analysis of the invasion-

associated genes expression pattern in first trimester trophoblastic (HTR-8/SVneo) and JEG-3 choriocarcinoma cells. Placenta (2012) 33: 874–877.
16. Yang W, Wagener J, Wolf N, Schmidt M, Kimmig R, Winterhager E and Gellhaus A: Impact of CCN3 (NOV) glycosylation on migration/invasion properties and cell growth of the choriocarcinoma cell line Jeg3. Hum Reprod (2011) 26: 2850–2860.
17. Su MT, Tsai PY, Tsai HL, Chen YC and Kuo PL: miR-346 and miR-582-3p-regulated EG-VEGF expression and trophoblast invasion via matrix metalloproteinases 2 and 9. BioFactors (2017) 43: 210–219.
18. Wang CY, Tsai HL, Syu JS, Chen TY and Su MT: Primary cilium–regulated EG-VEGF signaling facilitates trophoblast invasion. J Cell Physiol (2017) 232: 1467–1477.
19. Zhang ZH, Li MY, Wang Z, Zuo HX, Wang JY, Xing Y, Jin C, Xu G, Piao L, Piao H, Ma J and Jin X: Convallatoxin promotes apoptosis and inhibits proliferation and angiogenesis through cross talk between JAK2/STAT3 (T705) and mTOR/STAT3 (S727) signaling pathways in colorectal cancer. Phytomedicine (2020) 68: 153172.
20. Nagase H and Woessner Jr. JF: Matrix metalloproteinases. J Biol Chem (1999) 274: 21491–21494.
21. Chen J and Khalil RA: Matrix metalloproteinases in normal pregnancy and preeclampsia. Prog Mol Biol Transl Sci (2017) 148: 87–165.
22. Myers JE, Merchant SJ, Macleod M, Mires GJ, Baker PN and Davidge ST: MMP-2 levels are elevated in the plasma of women who subsequently develop preeclampsia. Hypertens Preg (2005) 24: 103–115.