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MXRA5 is decreased in preeclampsia and affects trophoblast cell invasion through the MAPK pathway

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ABSTRACT

Preeclampsia causes gestational failure in a significant number of women annually. Insufficient trophoblast cell invasion plays an essential role in preeclampsia pathogenesis. Matrix-remodeling associated 5 (MXRA5) is a proteoglycan involved in adhesion and matrix remodeling. This study sought to explore the role of MXRA5 in trophoblast cell invasion. Preeclamptic villi were obtained for the delineation of MXRA5 expression. Specific MXRA5 siRNA and pcDNA3.1/MXRA5 were used to manipulate MXRA5 expression in HTR-8/SVneo. Cell viability was determined by MTT and apoptosis by flow cytometry. Cell invasion was evaluated using Matrigel invasion assay. MXRA5 expression was lower in preeclamptic villi and cytotrophoblasts. Silencing MXRA5 expression in HTR-8/SVneo decreased cell viability and invasion, which were augmented by MXRA5 overexpression. Furthermore, MXRA5 modulated N-cadherin, E-cadherin, MMP-2, and MMP-9 expression through p38 MAPK and ERK1/2 signaling transduction. In addition, the expression of MXRA5 was influenced by exogenous TNF-α but not by IFN-γ. Overexpression of MXRA5 attenuated HTR-8/SVneo apoptosis induced by TNF-α. MXRA5 is down-regulated in preeclamptic cytotrophoblasts and can regulate trophoblast cell invasion via the MAPK pathway.

1. Introduction

Preeclampsia (PE) is an adverse pregnancy condition, which is a potentially fatal threat for pregnant women and their fetuses (Bartsch et al., 2016; Mol et al., 2016). The traits of this complex syndrome include the occurrence of hypertension (≥140/90 mm Hg) and significant proteinuria (≥300 mg in 24-h urine) in a healthy woman on or after the 20th week of gestation (Pauli and Repke, 2015). This disease may affect around 2–8% of pregnancies and cause about 50,000 deaths worldwide every year (Duley, 2009). Accumulated evidence has predicted many factors responsible for the occurrence of PE, i.e., PE history, multifetal gestations, obesity, diabetes mellitus, systemic lupus erythematosus, older age in pregnancy, and smoking (Eiland et al., 2012). Researchers have also reviewed the pathophysiological causes participating in PE; factors such as unsuccessful placentation, abnormal trophoblast development, defective vascular remodeling, and aberrantly maternal immune responses have been regarded as the main causes (Wang et al., 2009; Berzan et al., 2014; Steegers et al., 2010).

Following the implantation of blastocysts, cytotrophoblasts continually proliferate and differentiate into extravillous cytotrophoblasts and villous cytotrophoblasts (Gupta et al., 2016). During villous cytotrophoblast differentiation, these cells can further generate invasive endovascular cytotrophoblasts (eCTBs) and interstitial cytotrophoblasts (iCTBs) (Gupta et al., 2016). These cytotrophoblasts play critical roles in maternal decidua invasion and uterine blood vessel invasion, helping to establish blood vessels within the lining of the maternal endothelium (Huppertz et al., 2014; Fitzgerald et al., 2010). Studies have established that insufficient trophoblast invasion, abnormal spiral arterial remodeling, and inadequate placental supply are the sine qua non of PE (Lyall et al., 2013). In particular, trophoblast invasion is greatly

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susceptible to extracellular stimulation. Growth factors such as EGF, vEGF, PDGF, placental growth factor (PLGF), CSF-1, IGF-I, and IGF-II positively influence trophoblast proliferation and invasion (Knofler, 2010; Bischof et al., 2000; Lala and Hamilton, 1996). Likewise, inflammatory factors, including IL-1β, IL-6, IL-8, IL-11, IL-15, IP-10, and RANTES, can enhance trophoblast proliferation and invasion (Sharma et al., 2016). In contrast, factors such as IL-10, IL-12, TNF-α, and interferon-γ can impede trophoblast invasion and cause apoptosis (Otun et al., 2011). With the stimulation of these growth factors and inflammatory factors, the activation of p38 MAPK, ERK1/2, PI3K/Akt, JAK2/STAT3, and canonical Wnt signaling contribute to trophoblast cell invasion, while signaling transduction mediated by TGF-β/smad2 plays a negative role in trophoblast cell invasion (Gupta et al., 2016; Massimiani et al., 2015).

Matrix-remodeling associated 5 (MXRA5) is a member of the MXRA protein family, participating in cell adhesion and extracellular matrix remodeling (Rodningen et al., 2008). This protein is expressed in primates but not in rat or mouse. The function of this protein remains elusive. Previous research has characterized the MXRA5 function resembling the vascular endothelial growth factor (VEGF) receptor and noted its higher expression in patients with osteoarthritis (Walker and Volkmuth, 2003). Studies on tumor research have found that MXRA5 plays an important part in tumorigenesis. For instance, somatic mutations of MXRA5 are found in patients with non-small cell lung cancer (NSCLC), highlighting the effect of matrix remodeling in the etiology of NSCLC (Xiong et al., 2012); MXRA5 is also aberrantly expressed in colorectal cancer (CRC) tissues, and serves as a critical biomarker for the diagnosis and prevention of CRC (Wang et al., 2013). Likewise, in the process of trophoblast invasion, matrix remodeling occurs progressively, and facilitates extravillous trophoblast migration and invasion (Busch et al., 2009; Renaud et al., 2014). Importantly, the expression of MXRA5 was shown to be downregulated in in the placentas of preeclampsia patients using microarray technology (Yan et al., 2013). However, the effect of MXRA5 on trophoblast invasion is still unknown.

This study aimed to explore the expression of MXRA5 in human trophoblast cells of PE patients and clarify its role in trophoblast cell invasion using a cell line derived from first-trimester human extravillous cytotrophoblast. Our study provides a novel insight into the pathogenesis of PE.

2. Materials and methods

2.1. Tissue samples

Forty-one pregnant women treated at our hospital were enrolled in the study (Table 1). All women included in our sample delivered via elective Cesarean section without labor. Patients were diagnosed with PE as their blood pressure was higher than 140/90 mm Hg at two different times (6 h intervals), with urinary excretion of >300 mg protein per 24 h. Patients with a history of diabetes mellitus, vaginal infections, and other systemic illnesses were excluded from the study. All placentas in this study were obtained by cesarean section. This study was reviewed and approved by the Institutional Ethical Committee (No. ZZ2016-0226). Informed consent was obtained from each donor.

2.2. Primary cytotrophoblast isolation

Primary cytotrophoblasts were isolated from normal and PE placentas in line with a previous study (Kliman et al., 1986). Briefly, minced fragments of placentas were extensively washed and then subjected to three cycles of digestion with 1.25 mg/mL of trypsin and 100 mg/mL of DNase, followed by a discontinuous concentration (5–70%) of Percoll (Sigma) gradient centrifugation. Cytotrophoblasts containing cell suspensions were recovered from 35 to 50% gradient layers and the purity of cytotrophoblasts was analyzed using anti-cytokeratin 7 antibody (Abcam, Cambridge, MA, USA).

2.3. HTR-8/SVneo culturing and treatment

HTR8/SVneo trophoblasts were cultured in Dulbecco's modified Eagle's medium (DMEM)/nutrient mixture F-12 Ham medium (DMEM/F12; Thermo Fisher) containing 100 U/mL penicillin, 100 g/mL streptomycin, and 10% fetal bovine serum (FBS; Thermo Fisher). Cells were cultured at 37 °C, 5% CO2/air atmosphere, and the medium was changed every other day. To inhibit the activity of p38 MAPK and ERK1/2 in HTR-8/SVneo trophoblasts, their specific inhibitors SB203580 (10 μmol/L) and PD98059 (10 μmol/L) were added to HTR-8/SVneo trophoblasts culture and incubated for 2 h, respectively. To study the effect of inflammatory factors on HTR-8/SVneo trophoblasts, TNF-α (0,10, 20, 40, and 80 pg/mL) and IFN-γ (0, 10, 20, 40, and 80 pg/mL) were added to HTR-8/SVneo trophoblast culture and incubated for 2 h.

2.4. Cell viability detection

Cell viability detection was conducted using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Thermo Fisher). HTR8/SVneo cells were seeded in 24-well plates (2×10^5 cells/well in 1 mL of medium) and incubated as indicated for 1–4 days. Cells were then incubated with 0.5 mg/mL MTT for 4 h, the medium was replaced with 1 mL dimethylsulfoxide (DMSO), and the absorbance was measured at 490 nm using a microplate reader.

2.5. Cell invasion assay

The invasive ability of trophoblasts was evaluated using Matrigel invasion assay. Briefly, DMEM with 10% fetal bovine serum was placed in the lower chambers. Then, HTR-8/SVneo trophoblasts (2×10^5 in 1 mL of medium) with different treatments were resuspended in serum-free medium and placed into the upper chambers. Cells were incubated at 37 °C for 48 h, then the inserts were removed, washed in ice-cold PBS, and noninvading cells together with extracellular matrix were removed from the upper surface of the filter by wiping with a cotton bud. Trophoblasts on the lower surface of the inserts were fixed in 4% paraformaldehyde, stained with crystal violet, and observed using an inverted phase-contrast microscope (Olympus, Japan).

2.6. MXRA5 siRNA and plasmid transfection

To suppress the expression of MXRA5, cells were transfected with 50 nM MXRA5 siRNA (si-MXRA5) using Lipofectamine

Table 1
Characteristics of pregnant women enrolled in the study.

	Preeclampsia group	Control group
n	22	19
Mean age (years)	30.2 ± 4.3	31.3 ± 2.5
Blood pressure (mm Hg)	172 ± 6/100 ± 5	121 ± 3/72 ± 6
Proteinuria	+++	none
Mean gestational age (weeks)	35.6 ± 1.1	38.9 ± 0.9
Fetal weight (kg)	2.31 ± 0.24	3.24 ± 0.35
Parity	1.3 ± 0.1	1.6 ± 0.5