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CXCR2 is decreased in preeclamptic placentas and promotes human trophoblast invasion through the Akt signaling pathway

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ABSTRACT

Introduction: CXCR2, the receptor of the CXC chemokines, plays a critical role in cell migration and invasion in many types of cancer. It is unclear what impact CXCR2 may have on Preeclampsia (PE), a pregnancy-specific disease, which is related to insufficient trophoblast invasion. The aim of this study was to investigate the expression pattern of CXCR2 in the placentas of healthy and PE pregnancies, and to investigate the molecular mechanism of CXCR2 involvement in the development of PE.
Methods: CXCR2 expression levels in newly delivered placentas from 38 pregnant women with PE and 21 healthy pregnant women were detected using quantitative real-time PCR, immunohistochemistry and Western blot assays. The effect of CXCR2 on trophoblast invasion and the underlying mechanisms were examined in two trophoblast cell lines (HTR-8/SVneo and TEV-1 cells).
Results: CXCR2 mRNA and protein expression levels were significantly decreased in preeclamptic placentas than normal control. The invasive abilities of the two trophoblast cell lines were significantly inhibited when CXCR2 was silenced, but that CXCR2 overexpression promoted trophoblast cells invasion. In addition, silencing CXCR2 reduced the expression of matrix metalloproteinase 2 and 9 (MMP2 and MMP9) and phosphorylated Akt (p-Akt). Furthermore, an Akt inhibitor suppressed the expression of MMP-2 and MMP-9.
Discussion: Our results suggest that the decreased CXCR2 may contribute to the development of preeclampsia through impairing trophoblast invasion by down-regulating MMP-2 and MMP-9 via the Akt signaling pathway.

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1. Introduction

Preeclampsia (PE) is a human disorder characterized by hypertension and significant proteinuria that develops at or after 20 weeks of pregnancy. The worldwide incidence of PE is 3%–8% [1], and PE is among the leading causes of maternal morbidity and mortality [2]. Although several varying hypotheses and theories exist regarding the etiology of PE, such as aberrant immune responses [3], oxidative stress [4], abnormal placental implantation and function [5], and genetic factors [6], the exact etiology and pathophysiology remain uncertain.

PE occurs only in the presence of the placenta and usually resolves when the placenta is delivered [7]. Human placental development is characterized by trophoblast invasion of the decidua and remodeling of the uterine maternal spiral arteries [8,9]. Previous investigations have indicated that PE is associated with insufficient trophoblast invasion and inadequate uterine spiral artery development [2,9]. PE is primarily a disorder of placental dysfunction that leads to systemic endothelial dysfunction, and the endothelial dysfunction is in turn responsible for the symptoms of PE [10]. The most commonly accepted theory describing the etiology of PE is the Two-stage Model theory. This theory proposes that poorly perfused placentas produce factor(s) that lead to the clinical manifestations of PE [11]. PE results from abnormal placental development due to insufficient trophoblast invasion, where invasive extravillous

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cytotrophoblasts fail to remodel the maternal spiral arteries in the placenta, leading to placental ischemia (stage 1). The ischemic placenta is thought to secrete soluble factors such as antiangiogenic and angiogenic factors during the third trimester that subsequently induces systemic endothelial dysfunction and the maternal syndrome of preeclampsia (stage 2) [7,12]. Therefore, the disturbance of trophoblast invasion plays a vital role in the PE process.

Chemokines exert different functions via binding to their receptors in different cell types. The chemokine superfamily is categorized into four primary groups based on the positions of conserved cysteine amino acids in the N-terminal region (CXC, CC, CX3C and C) [13]. CXCR2 is the receptor of the Glu–Leu–Arg + (ELR+) CXC chemokines CXCL1-3 and CXCL5-8; CXCL6 and CXCL8 are also ligands of CXCR1 [13]. Recent studies have shown that CXCR2 plays a critical role in tumor migration and invasion in many types of cancer, such as lung cancer, rectal cancer, ovarian cancer, prostate cancer, esophageal cancer and gastric cancer [14–19], and CXCR2 inhibitors have been reported to decrease tumor growth. In addition, immunohistochemical analyses have shown that CXCR2 is expressed in villous and decidual cells of the maternal-fetal interface [20]. However, as only one of the ELR + CXC chemokines receptors, the role of CXCR2 in the regulation of the invasive activity of trophoblast remains unknown. Because of the known role of CXCR2 in inducing invasion in tumor tissues, we hypothesized that CXCR2 would follow a pattern of expression consistent with trophoblast invasion during early pregnancy.

Given the similarities between trophoblasts and tumor cells with respect to their proliferative and invasive properties and the Two-stage Model of PE, the hypothesis that alterations in CXCR2 expression may be involved in PE is reasonable. Therefore, the aim of the present study was to investigate the expression pattern of CXCR2 in the placentas of normal and PE pregnancies and to investigate the molecular mechanism of CXCR2 involvement in the development of PE.

2. Materials and methods

2.1. Subjects and sample collection

This study was approved by the ethics committee of The First Affiliated Hospital of Sun Yat-sen University, and informed consent was obtained from every subject. Placental tissue samples were obtained from the PE cases (n = 38) and from healthy pregnancy controls (n = 21). Three small tissue pieces of approximately $1 \times 1 \times 1$ cm^3 were excised from newly delivered placentas at the time of cesarean section at a distance halfway between the base of the umbilical cord and the edge of the placental disc and then rinsed several times with ice-cold phosphate-buffered saline (PBS). Two of the tissue samples were transferred to liquid nitrogen, and the other tissue sample was transferred to 4% formaldehyde.

PE is characterized as hypertension and proteinuria that often occurs in women who are normotensive before 20 weeks of gestation. It was defined according to American College of Obstetricians and Gynecologists: Hypertension is diagnosed when 2 blood pressure readings ≥140/90 mmHg are noted 6 h apart. Gestational proteinuria is defined as urinary protein excretion of at least 300 mg per 24 h or 1 + protein or greater on a dipstick on 2 occasions at least 4 h apart but no more than 1 week apart. In the absence of proteinuria, PE is diagnosed as hypertension in association with thrombocytopenia (platelet count less than 10000/ml), impaired liver function (blood levels of liver transaminases elevated to twice the normal concentration), the new development of renal insufficiency (elevated serum creatinine greater than 1.1 mg/dL or a doubling of serum creatinine in the absence of other renal diseases), pulmonary edema, or new-onset cerebral or visual disturbances.

2.2. Reagents and antibodies

Monoclonal antibodies directed against CXCR2 (Cat. No. ab24963), Akt (Cat. No. ab109870), phosphorylated Akt (Cat. No.a b38449), ERK (Cat. No. ab196883), phosphorylated ERK (Cat. No. ab65142), Matrix metalloproteinases 2 (Cat. No. ab37150) and 9 (Cat. No. ab38898) (MMP-2 and MMP-9) and β-actin (Cat. No. ab8227) were purchased from Abcam (MA, USA), anti-CK7 antibody was obtained from Boster Biological Technology, Ltd. (Cat. No. BM1618, Wuhan, China). The small interfering RNA (siRNA) for silencing CXCR2 and a non-targeting siRNA control were purchased from RiboBio (Cat. No. Q3579-1-A, Guangzhou, China). The siRNA sequences were as follows: siRNA1, 5′-CGC UAC UUG GUC AAA UUCA dTdT -3′ (sense strand), 5′- dTdT GCG AUG AAC CAG UUU AAGU-3′ (antisense strand); and siRNA2, 5′-CAU GGA CUC CUC AAG AUUC dTdT -3′ (sense strand), 5′- dTdT GUA CCU GAG GAG UUC UAAG-3′ (antisense strand). The CXCR2 expression plasmid and empty vector were purchased from GeneChem (Cat. No. NM001557, Shanghai, China). Lipofectamine2000 reagent was obtained from Invitrogen (Carlsbad, CA, USA). The Akt inhibitor MK-2206 2HCl was obtained from Selleck (Shanghai, China).

2.3. Cell lines and culture

Two human trophoblast cell lines were used in this study. The HTR-8/SVneo cell line was obtained from Jennio Biotech Co., Ltd. (Guangzhou, China), the TEV-1 cell line was purchased from Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China). The HTR-8/SVneo cells and TEV-1cells were propagated in RPMI 1640 and Dulbecco's modified Eagle's medium (DMEM)/F12 (Gibco, Gaithersburg, MD, USA) respectively supplemented with 10% (v/v) fetal bovine serum (FBS, Sijiqing, Hangzhou, China). The cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2.

2.4. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol® (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. cDNA was synthesized from 5 mg of total RNA using a reverse transcription kit (TaKaRa, Dalian, China) and then stored at −20 °C until use. A two-step quantitative real-time PCR was performed to examine the levels of CXCR2 mRNA using an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and a SYBR Premix Ex Taq Kit (TaKaRa, Japan). The PCR primers were as follows: CXCR2, 5′-TTG CAA CCC AGG TCA GAA GT-3′ (forward), 5′-AGC TGT GAC CTG CTG TTA TTG-3′ (reverse); and GAPDH, 5′- AGA AGG CTG G GG CTC ATT TG-3′ (forward), 5′-A GG GGC CAT CCA CAG TCT TC-3′ (reverse). Relative quantification of PCR products was based on the differences in Ct values between the target and the housekeeping gene GAPDH using the △△Ct method. All experiments were performed in triplicate.

2.5. Western blot (WB) assay

Tissue and cells were lysed in 1 × SDS lysis buffer. Equal amounts of protein were resolved by sodium dodecyl sulfate (SDS)-5/10% polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene difluoride membranes. The membranes were blocked in 5% non-fat dry milk/TPBS (TBST) for 2 h at room temperature. Then, the membranes were incubated with primary monoclonal antibody (1:1000) and β-actin monoclonal antibody

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