Early Human Development 90 (2014) 657–664

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Dysregulated expression of matrix metalloproteinases and their inhibitors may participate in the pathogenesis of pre-eclampsia and fetal growth restriction

JiaYu Zhu, Mei Zhong, ZhanJun Pang *, YanHong Yu **

Department of Obstetrics and Gynecology, Nanfang Hospital of Southern Medical University, Guangzhou 510515, China

ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 5 July 2014
Accepted 9 August 2014

Keywords:
Placenta
Trophoblast invasion
Matrix metalloproteinase
Tissue inhibitor of metalloproteinase
Pre-eclampsia
Fetal growth restriction
Trophoblast-related diseases

ABSTRACT

Background: Trophoblast invasion into the maternal endometrium serves an important function in human pregnancy. Dysregulation of the finely controlled process of trophoblast invasion can result in a wide spectrum of pregnancy abnormalities.
Aims: We aimed to elucidate the relationship between the expression of matrix metalloproteinases and pregnancy complication.
Study design: The study group consisted of placental bed biopsy tissues obtained from normal vaginal deliveries (N = 15), normal cesarean deliveries (N = 15), pre-eclampsia (N = 24) and fetal growth restriction (FGR) (N = 10). We evaluated the expressions of MMP-2, -8, -9, -11, -19, -15 (MT2-MMP), -16 (MT3-MMP), and -24 (MT5-MMP), as well as TIMP-1 and -3, by applying Western blot and immunohistochemistry methods.
Subjects: Human placental tissues were used for this study.
Outcome measures: The expressions of MMP-2, -8, -9, -11, -19, -15 (MT2-MMP), -16 (MT3-MMP), and -24 (MT5-MMP), as well as TIMP-1 and -3 in human placenta tissues.
Results: Compared with those in normal pregnancies, the expression of MMP-2, -8, -9 and -11 was downregulated in villous tissues of pre-eclampsia and FGR cases ($p < 0.05$). TIMP-1 and -3 were increased in pre-eclampsia and FGR ($p < 0.05$). No significant difference was found between normal vaginal deliveries and cesarean deliveries.
Conclusions: We speculate that the change in invasion-associated proteinase expression will affect placental development and may thus contribute to the development of complicated pregnancies.

1. Introduction

Invasion of trophoblast cells into the endometrial stroma and inner third of the myometrium is an essential process for the development of definitive maternal–fetal circulation and for pregnancy success in humans [1]. The invasive growth of trophoblast is similar to that of malignant tumors in many respects. However, unlike tumor invasion, trophoblast invasion is precisely regulated, confined spatially to the uterus and temporally to early pregnancy. Dysregulation of the finely controlled process of trophoblast invasion can result in a wide spectrum of pregnancy abnormalities [2–4]. Shallow invasion and failure in the trophoblast invasion process are believed to be associated with the development of gestational diseases, as severe forms of intrauterine growth restriction (FGR) and pre-eclampsia, which can induce the adverse long-term outcome called Fetal Origins of Adult Disease [5–7].

Invasive processes are generally characterized by a fine balance between the production/activation of proteolytic pro-enzymes in particular matrix metalloproteinases (MMPs) and their inhibitors, i.e., tissue inhibitors of metalloproteinases (TIMPs) [4,8,9]. MMPs belong to a family of structurally related zinc-dependent endopeptidases capable of degrading and remodeling specific components of the extracellular matrix (ECM). Over 20 human MMPs have been described, including collagenases (MMP-8 and -19) that cleave native fibrillar collagen types I, II, III, and IV; stromelysin (MMP-11), which has a broad substrate specificity; gelatinases (MMP-2 and -9) that degrade denatured collagens and basement membrane components; and membrane-associated MMPs. The membrane-type matrix metalloproteinases (MMP-15/MT2-MMP, -16/MT3-MMP, and -24/MT5-MMP) are unique in that they have a hydrophobic amino acid stretch at the C-terminal end that can pass into the cell membrane and act as a trans-membrane domain localizing the enzymes at the cell surface [9–14]. TIMPs, which comprise a family of five endogenously expressed extracellular proteins, exert their effect either directly by binding to MMPs or indirectly by activating nuclear transcription factors that control the expression of select MMP genes. TIMPs include TIMP-1, which binds in a stoichiometric manner to form a complex with activated interstitial collagenase, namely, stromelysin, and inhibits the active form of all MMPs. TIMP-3 has been shown to have inhibitory activity against stromelysin-1 and collagenase-1 [15–19].

* Corresponding author. Tel.: +86 20 6278 7290; fax: +1 718 6164 8070.
** Corresponding author. Tel.: +86 20 6164 8678; fax: +1 718 6164 8070.
E-mail addresses: zjpang@126.net (Z. Pang), yuyh_1010@126.com (Y. Yu).

http://dx.doi.org/10.1016/j.earlhumdev.2014.08.007
0378-3782/

Several molecular studies show that, unlike normal human pregnancy in which trophoblast cells proliferate and differentiate to form the placenta in a regulated manner, members of the MMP and TIMP family have been implicated in trophoblast-induced changes, as pre-eclampsia and FGR [20–22].

Although the volume of literature on MMPs and TIMPs in pregnancy has recently increased, no study has simultaneously compared the protein levels of MMPs and TIMPs in groups from pregnancy complications, normal vaginal and cesarean deliveries. Therefore, this clinical–pathological correlation study aimed to investigate the relationship between these invasion-associated proteins and the pathogenesis of these two complicated pregnancies.

2. Materials and Methods

2.1. Patients and tissue collection

Samples of human placental tissues were obtained from pregnancies hospitalized in Nanfang Hospital (Guangzhou, China) between January 2012 and June 2013, with the approval of the local Ethics Committee. All participants provided their informed consent. Common patient characteristics are described in Table 1. Patients with pre-eclampsia and FGR were diagnosed according to ACOG standard criteria. All cases in pre-eclampsia group were diagnosed as mild pre-eclampsia, which was defined as diastolic blood pressure of 90 mm Hg or higher measured on two occasions at least 6 h apart in a woman who was normotensive until at least 20 weeks of gestation and if proteinuria exists (>300 mg total protein in a 24 hour urine collection or >30 in a spot urine protein: creatinine ratio) [23]. Sonography-based definition of FGR is a weight below the 10th percentile for gestational age [24]. For normal pregnancies that delivered healthy neonates at term, fetal growth was documented by ultrasound in utero and confirmed by birth weight between the 10th and 90th percentile. The patients with pre-eclampsia or FGR were from a mixture of cesarean and vaginal delivery.

Placental tissue was immediately removed and washed briefly in sterile phosphate-buffered saline (PBS). Six 1 cm × 1 cm × 1 cm tissue blocks were taken from the central parts in the maternal face of placentas and dissected free of conception products and blood clots. Three were snap-frozen in liquid nitrogen and stored at −80 °C for protein extraction and Western blot. The rest portions were fixed in 10% buffered formalin for immunohistochemistry.

2.2. Western blot

Proteins were extracted from 30 mg of cryo-cut tissue samples, which were lysed in Ripa buffer (Beyotime, Shanghai, China) containing protease inhibitors (complete, Roche, Germany) and PMSF (Beyotime, Shanghai, China). The mixture was incubated in ice for 30 min. The tissue lysate was ultracentrifuged (13000 *g*, 20 min, 4 °C), and an aliquot of the supernatant was assayed for protein concentration. Protein concentration was determined by the Bradford method [25]. Thereafter, the samples were mixed in 5× loading buffer (Protein Loading Buffer, Fermentas, Canada), denatured at 100 °C for 5 min, chilled quickly on ice, and stored at −20 °C for further analysis.

Protein extract and molecular mass marker were denatured and exposed to 10% polyacrylamide gel (SDS-PAGE). Proteins were electrotransferred to 0.45 μm polyvinylidene fluoride membranes (Millipore, USA). To avoid unspecific binding, the membrane was blocked with 10% non-fat milk protein in TBST at room temperature (RT) for 1 h. Subsequently, the membrane was incubated with the appropriate primary antibody diluted in primary antibody dilution buffer (Beyotime, Shanghai, China) at 4 °C overnight. Polyclonal rabbit anti-MMP-2, -8, -9, -11, -15, -16, and -24, as well as TIMP-1 and -3 primary antibodies (Bioworld, USA), were used at a 1:500 concentration diluted in primary antibody dilution buffer (Beyotime, Shanghai, China). After washing with TBST, the membrane was incubated with species-specific horseradish peroxidase-conjugated secondary antibodies for 1 h at RT and then detected by enhanced chemiluminescence (ECL, Millipore, USA). The bands were quantified using a densitometer system with Gel-Pro analyzer 4.0 software (Media Cybernetics, USA).

2.3. Immunohistochemistry

Tissues were sectioned (4 μm) from formalin-fixed, paraffin-embedded tissue blocks, mounted on glass-slides, and dried for 2 h at 58 °C. Paraffin sections were deparaffinated twice in xylene and rehydrated in a graded series of ethanol solutions. After rinsing in PBS, the sections were predominantly stained with pretreatment for antigen demasking in a microwave oven in 0.01 M of citrate buffer solution (pH 6.0) at 700 W to 100 °C for 8 min. The slides were allowed to cool at RT. The sections were then incubated overnight at 4 °C with one of the respective primary polyclonal antibodies as before, except for biotinylated secondary antibody (biotinylated goat anti-rabbit IgG, Zhongshan, China), and then diluted in PBS at a 1:100 concentration for 30 min at RT. Intervening PBS washing was necessary between every two steps. Diluted DAB (DAB; BOSTER, Wuhan, China) was used as chromogen, which resulted in brown staining. Sections were rinsed with distilled water, counterstained with hematoxylin (BOSTER, Wuhan, China), and mounted with histomount (Zymed Lab Inc., San Francisco, CA). The slides were analyzed using a light microscope (Olympus, Japan). Positive control expreriments were conducted as follows: lung squamous carcinoma tissues were detected for MMP-2 and TIMP-3, breast ductal carcinoma for MMP-8 and -11, MT2-MMP, and MT3-MMP, endometrial carcinoma for MMP-9 and TIMP-1, and ovarian carcinoma for MMP-19 and MT5-MMP. Negative control experiments were conducted by staining with PBS as primary antibodies.

Semi-quantitative analysis of protein expression based on the combination of staining intensity of immunohistochemical images with

Table 1
Clinical characteristics of study subjects.

Characteristics	NC	ND	PE	FGR
Sample size	N = 15	N = 15	N = 24	N = 10
Mother				
Age (years)	27 (17,38)	28 (23,32)	30 (17,38)	29.5 (17,37)
Pre-pregnancy BMI (kg/m^2)	25.5 (15.9,31.5)	25.3 (15.9,31.5)	23.1 (20.3,25)	23 (18.5,32.3)
Postgravid BMI (kg/m^2)	29.7 (18.36.4)	28 (19.1,35.5)	27 (19,32.5)	27 (21.1,33.1)
Gestation (weeks)	38.6 ± 1.0	39.6 ± 1.0	38.3 ± 1.1	38.7 ± 2.2
Fetus				
Fetal weight (g)	3044 (2502,3986)	3053 (2516,3950)	3033 (2508,3949)	1907 (1641,2450)*
Placental weight (g)	599 (355,608)	585 (366,615)	577 (358,596)	529.5 (316,596)*

Normal cesarean deliveries (NC), normal vaginal deliveries (ND), pre-eclamptic (PE), FGR pregnancies (FGR).
Unpaired Student's test was applied for gestation which was normally distributed. Kruskal–Wallis test followed by the Bonferroni test was applied for the maternal age, pre-pregnancy BMI, postgravid BMI, fetal weight and placental weight which were mostly distributed non-normally.
BMI, body mass index.
* $p < 0.05$ versus normal vaginal deliveries group.