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MMP-14 aggravates onset of severe preeclampsia by mediating soluble endoglin release

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Abstract. – OBJECTIVE: Gestational hypertension is a pregnancy complication that serious damages the maternal and child health. Early onset severe preeclampsia accounts for about 0.9% of the gestational hypertension disease. Conservative treatment is proposed in recent years to early onset severe preeclampsia through delay delivery. Therefore, it is particularly important to explore the pathogenesis of severe preeclampsia. Soluble endoglin (sEng) has been identified as a central factor to induce endothelium dysfunction of preeclampsia, while its specific mechanism is unclear.

MATERIALS AND METHODS: Matrix metallopeptidase 14 (MMP-14) and endoglin expressions and tissue localization in the placenta of preeclampsia and premature were detected by Western blot and immunohistochemistry. Endoglin level, mean arterial blood pressure (MABP), and urinary protein/creatinine ratio were analyzed for correlation to investigate their relationship and the influence of endoglin on eclampsia severity. MMP specific or broad spectrum inhibitor combining MMP-14 siRNA were used in JAR cell line BeWo to explore the regulatory role of MMP-14 on endoglin.

RESULTS: MMP-14, endoglin, and sEng expression levels significantly increased in the placenta of severe preeclampsia patients. MMP-14 and endoglin exhibited expression co-localization. Endoglin expression was positively correlated with the severity of eclampsia. MMP-14 directly mediated the release of sEng.

CONCLUSIONS: MMP-14 aggravated the onset of severe preeclampsia by mediating sEng release. MMP-14 was proposed as the effective target for the treatment of severe preeclampsia. Blocking the interaction between MMP-14 and endothelial protein may be an important treatment method.

Key Words:
MMP-14, Soluble endoglin, Severe eclampsia.

Introduction

Gestational hypertension (GH) disease is a pregnancy complication that serious damages the maternal and child health. Early onset severe preeclampsia accounts for about 0.9% of the GH disease. The pathological changes of pregnant women tend to occur before the clinical symptoms. Therefore, complications are often found when the patients are diagnosed. Since it can cause multiple organ damages in the early stage, timely termination of pregnancy is the most appropriate treatment for the mother, whereas it may increase the iatrogenic preterm birth and neonatal mortality[1,2]. In recent years, the conservative treatment is proposed to early onset severe preeclampsia through delay delivery. Therefore, it is particularly important to explore the pathogenesis of severe preeclampsia[2,3].

Early research showed that anti-angiogenesis factor soluble FMS like tyrosine kinase 1 (sFIt-1) and soluble endoglin (sEng) had been identified as the center factors inducing preeclampsia endothelial dysfunction. Their expressions increase in preeclampsia women and are related to the severity of the disease[4-6]. However, recent studies

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identified matrix metalloproteinase 14 (MMP-14) as the cleavage protease of endothelial glycoprotein in colorectal cancer and thymus carcinoma[7]. It was also found in COS-7 cell line that MMP-14 cut the binding endothelial glycoprotein adjacent to the trans-membrane structure domain to produce sEng[8]. We suspected it may be a potential therapeutic target to prevent sEng production and improve clinical features in patients with severe preeclampsia.

In this study, in order to determine whether this was also the mechanism of soluble Endoglin release in preeclampsia, we investigate the expression of MMP-14 in the placenta and its effect on the release of the soluble endothelial glycoprotein. First, we tested the expression level and tissue localization of MMP-14 and endoglin in the placenta of preeclampsia patients and matched preterm infants through WB and IHC, respectively. Endoglin level, mean arterial blood pressure (MABP), and urinary protein/creatinine ratio were analyzed for correlation to investigate their relationship and the influence of endoglin on eclampsia severity. MMP specific or broad spectrum inhibitor combining MMP-14 siRNA were used in JAR cell line BeWo to explore the regulatory role of MMP-14 on endoglin.

Materials and Methods

Main Reagents and Instruments

Total protein extraction kit was purchased from Keygen (Nanjing, China). Western Blot lysis and BCA protein quantification kit were provided by Beyotime Biotech. (Shanghai, China). Rabbit anti-human MMP-14 polyclonal antibody (Catalogue No. 14552-1-AP) and rabbit anti-human endoglin polyclonal antibody (Catalogue No. 10862-1-AP) were obtained from Proteintech (Rosemont, IL, USA). Horseradish peroxidase conjugated goat anti-rabbit IgG (H + L) (Catalogue No. ZB2301) was got from ZSGB Bio. (Beijing, China).

Main Instruments

Benchtop was purchased from Boxun (Shanghai, China). UVP Multispectral Imaging System was got from UVP Co. (Sacramento, CA, USA). PS-9 semi-dry transfer electrophoresis instrument was purchased from Jim-x Scientific (Dalian, China). Carbon dioxide incubator and Thermo-354 micro-plate reader were derived from Thermo Fisher Scientific (Waltham, MA, USA). Automatic biochemical analyzer ES-480 was purchased from Nanjing Yilanbei Biotechnology Co., Ltd. (Nanjing, China).

Sample Collection

A total of 80 cases of placenta were collected from patients with severe preeclampsia or matched premature. The tissue around the fresh placenta umbilical cord root was extracted at 1 cm × 1 cm × 1 cm and stored at -80°C. All the subjects had signed the informed consent.

Prenatal Blood Pressure and Urinary Protein/Creatinine Ratio Detection

The prenatal blood pressure was obtained from the medical history. The urinary protein/creatinine ratio was tested by the automatic biochemical analyzer.

Cell Culture

BeWo cells were purchased from Wuhan university typical bio-preservation center (Wuhan, China). The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) medium containing 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. The cells were passaged when the fusion reached 70%.

Cell Transfection and Administration

MMP-14 small interfere RNA (siRNA) was designed and synthesized by Genepharma (Shanghai, China). The cells were seeded in 24-well plate and cultured when the fusion reached 30%-50%. A total of 1.25 μl siRNA (20 μM) were solved in 100 μl Opti-MEM medium as solution A, while 1 μl Lipofectamine 2000 or Lipofectamine™ RNAiMAX was solved in the Opti-MEM medium as solution B. After 5 min, solution A was mixed with solution B and set for 20 min. The cells were incubated with the mixture for 4 h and changed to DMEM medium containing 10% FBS.

MMP-13 inhibitor, MMP-2/9 inhibitor, or pan-MMP inhibitor (GM6001) were used to pre-treat BeWo cells for 6 h to investigate the influence of MMP-14 to endoglin.

Western Blot

The cells were washed with phosphate-buffered solution (PBS) for three times and added with 10 μl: phenylmethanesulfonyl fluoride (PMSF) at 100 mM. Next, the cells were lysed on ice for 5-10 min and centrifuged at 4°C and 12000 × g for 5 min.

The total protein was quantified by BCA method and boiled for 5 min. The sample was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF, Millipore, Boston, MA, USA) membrane at 300 mA for 1 h. Next, the membrane was incubated with primary antibody (1:1000) at 4°C overnight. After washed by PBS Tween-20 (PBST) for three times, the membrane was incubated with secondary antibody (1: 1000) at 37°C for 2 h. At last, the membrane was developed by chemiluminescence.

Immunohistochemistry (IHC)

IHC was adopted to investigate the cell localization. The sample was embedded by OCT, sectioned, and fixed by acetone. Then the sample was incubated with 3% hydrogen peroxide (H_2O_2) for 20 min to eliminate endogenous peroxidase activity. Next, the sample was treated with citrate buffer at 95°C for 10 min to repair the antigen. After blocked by goat serum for 20 min, the sample was incubated with primary antibody (1:100) at 4°C overnight. After labeled with secondary antibody, the sample was stained by hematoxylin and developed by diaminobezidine (DAB).

Soluble sENG Detection

sENG was measured by enzyme-linked immunosorbent assay (ELISA) according to the manual.

Statistical Analysis

The data were analyzed with statistical package SPSS Version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The data were presented as mean ± standard deviation. The Student's *t*-test was used to compare the differences between two groups. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data between groups. The pairwise comparison in the group was tested by SNK. $p < 0.05$ was depicted as statistical significance.

Results

MMP-14 and Endoglin Expressions in Severe Preeclampsia

As shown in Figure 1A, MMP-14 level significantly increased in the placenta from severe preeclampsia compared with premature ($p < 0.05$). As shown in Figure 1B, endoglin expression obviously elevated in the placenta from severe preeclampsia compared with premature ($p < 0.05$).

MMP-14 and Endoglin Cellular Location in Severe Preeclampsia

MMP-14 and endoglin showed claybank as positive in IHC staining (Figure 2). MMP-14 was mainly stained in the membrane of syncytiotrophoblast and vascular endothelial cells. Its expression intensity on the villi side was obviously higher than that of the basement membrane, and the positive coloring was uniform. Moreover, its

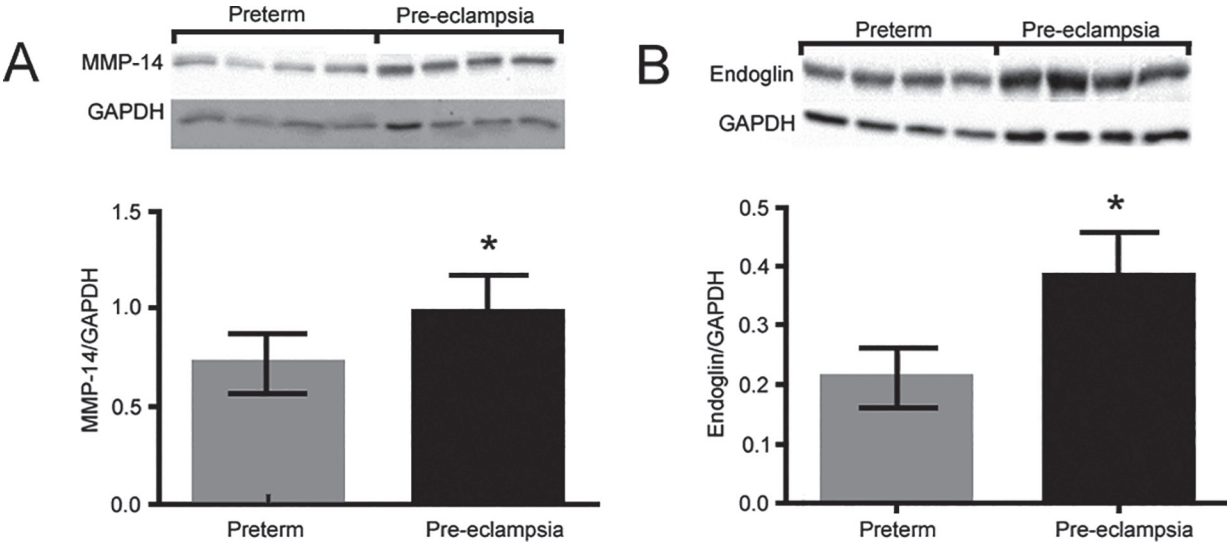

Figure 1. MMP-14 and endoglin expressions in severe preeclampsia. *$p < 0.05$, compared with premature.

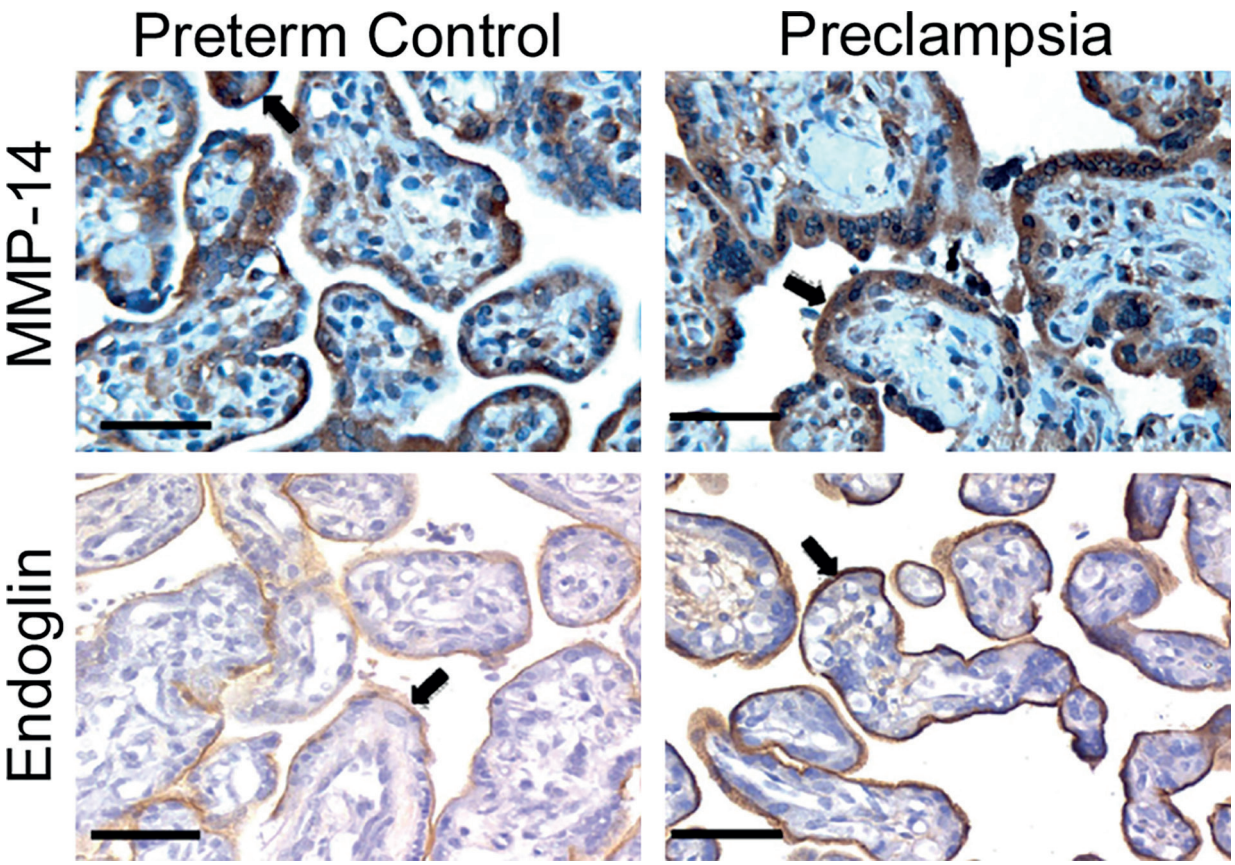

Figure 2. MMP-14 and endoglin cellular location in severe preeclampsia (× 20).

expression position and distribution were in accordance with Endoglin expression and location. Combined with a previous study[7], we speculated that MMP-14 and endoglin may have a regulatory relationship between each other.

The influence of Endoglin Release to Severe Eclampsia Degree

MABP and urinary protein/creatinine ratio are important indicators to assess the severity of severe eclampsia[9-11]. We examined the expression levels of Endoglin in 40 cases of severe preeclampsia and premature, and analyzed the correlation between blood pressure and urinary protein/creatinine ratio (Table I). The results showed that the levels of Endoglin, MABP, and urinary protein/creatinine in patients with severe preeclampsia were significantly higher than those in premature ($p < 0.05$). There was a positive correlation between the expression of Endoglin and MABP ($\gamma = 0.698$, $p < 0.05$). There was also a positive correlation between Endoglin expression and urinary protein/creatinine ratio ($\gamma = 0.698572$, $p < 0.05$).

The impact of MMP-14 Down-Regulation on Endoglin Release

To demonstrate the relationship between MMP-14 and endoglin, we used MMP-13 inhibitor, MMP-2/9 inhibitor, and MMP-14 specific in-

Table I. Endoglin, MAPB, and urinary protein/creatinine ratio in severe preeclampsia and premature.

Group	Endoglin (pg/mL)	MABP (mmHg)	Urinary protein/creatinine ratio (mg/mmol)
Preterm control	152.6 ± 4.4	82.7 ± 7.2	14.87 ± 3.76
Preeclampsia	167.8 ± 3.8*	129.3 ± 9.3*	43.25 ± 4.22*

*$p < 0.05$, compared with premature.

hibitor GM6001 or MMP-14 siRNA to investigate the expression of sEng, respectively. As shown in Figure 3, no significant sEng change was found after MMP-13 inhibitor or MMP-2/9 inhibitor treatment, while it markedly declined after MMP-14 inhibitor GM6001 or MMP-14 siRNA intervention ($p < 0.05$). It indicated that MMP-14 down-regulation can inhibit sEng release.

Discussion

Gestational hypertension (GH) disease is a pregnancy complication that serious damages the maternal and child health. Early onset severe preeclampsia accounts for about 0.9% of the GH disease. Timely termination of pregnancy is the most appropriate treatment for mother, whereas it may increase the iatrogenic preterm birth and neonatal mortality[1,2]. Conservative treatment is proposed in recent years to early onset severe preeclampsia through delay delivery. Therefore, it is particularly important to explore the pathogenesis of severe preeclampsia[3].

The previous study demonstrated that increased endoglin release is associated with the onset of eclampsia, while the regulatory mechanism is unknown. Venkatesha et al[13,14] adopted microarray and found that endoglin mRNA expression was significantly increased in the placental tissue of the preeclampsia patients. In this study, we also confirmed that endoglin elevated in placental tissue and mainly expressed in the syncytiotrophoblast of severe preeclampsia patients through WB

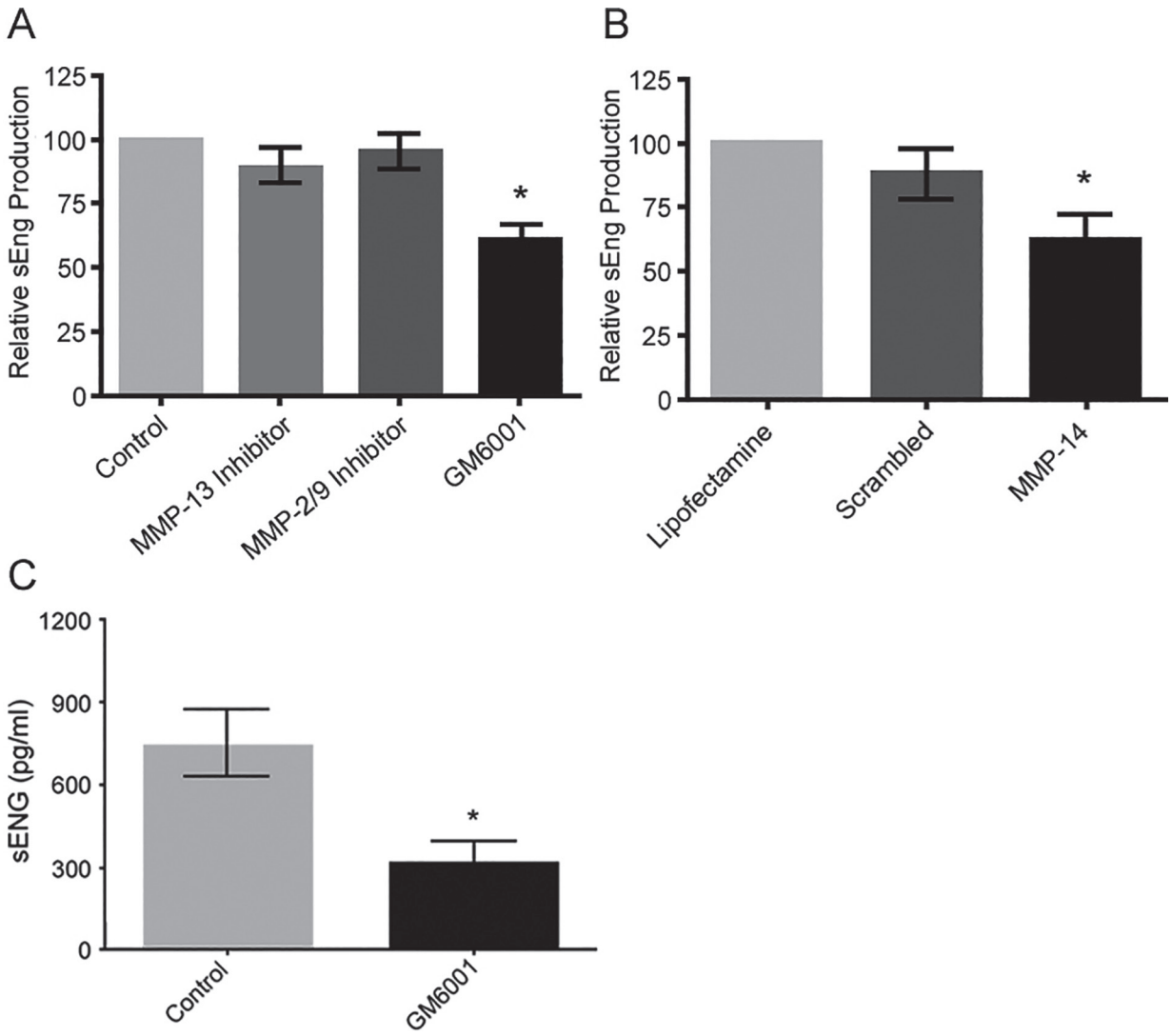

Figure 3. MMP-14 downregulation inhibited endoglin release. *$p < 0.05$, compared with control.

method and IHC staining method. Some scholars speculated that placental endoglin upregulation may be due to compensation mechanism, that is, placental trophoblast invasion is too shallow in preeclampsia, resulting in poor placental formation, ischemia. Thus, endoglin compensatory over-expresses to promote transforming growth factor (TGF-β), and facilitate trophoblast invasion and migration to compensate placental trophoblastic ischemia[15,16]. Our results suggested that MMP-14 is a cleavage protease of placental endothelin. Endoglin is co-localized with MMP-14 in syncytiotrophoblast cells and is significantly upregulated in placental tissue of patients with preeclampsia. We, therefore, proposed that MMP-14 cleavage on Endoglin may occur in the maternal-fetal cross-section of the placenta and, then, release its extracellular domain into the maternal circulation to form sEng. Although enhanced Endoglin expression may be responsible for a significant increase in clinical symptoms in women with severe preeclampsia, MMP-14 cleavage on Endoglin remains in normal placenta[17,18].

Up to now, there are some animal experiment data indicated that in the severe preeclampsia sEng overexpression played an important role in causing mother organ damage[19]. However, the direct molecule mechanism of sEng upregulation is still unclear. Cudmore et al[20] suggested that Akt signaling pathway activation can reduce the release of sEng, while Akt upstream inhibitor, such as phosphatase and tonic protein, is associated with increased release of sEng. Furthermore, heme oxygenase HO-1 depending on Akt is also involved in the inhibition of sEng release[21]. These studies revealed related signaling pathway for the release of sEng, whereas the specific way of sEng cleavage was unclear. This study proposed that MMP-14 is a cleavage protease of placental endoglin.

In this study, we also used small molecule inhibitors and siRNA to provide strong evidence of MMP-14-mediated sEng production in the placental tissue. It is worth mentioning that MMP-14 monoclonal antibody has been developed for the treatment of breast cancer in clinical trials[22,23]. MMP-14 is speculated as an effective target for the treatment of severe preeclampsia. Moreover, specific preventing the interaction between MMP-14 and endothelin can also be an important treatment. To further elucidate the role of MMP-14 in severe preeclampsia, further study is needed to explore the way of MMP-14 in cleaving sEng at animal or cellular levels.

Conclusions

MMP-14 aggravated the onset of severe preeclampsia by mediating sEng release. MMP-14 was proposed as the effective target for the treatment of severe preeclampsia. Blocking the interaction between MMP-14 and endothelial protein may be an important treatment method.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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