Local Complement Regulation in Preeclampsia

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Research Article

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Abstract

Background: The objective of this study was to investigate whether local regulatory proteins are insufficient in patients with preeclampsia and whether VEGF (vascular endothelial growth factor) can regulate the expression of complement regulatory proteins on trophoblast membranes.

Methods: A case-control study was performed. The study group enrolled 20 patients with severe preeclampsia, matched with 16 patients comprising the control group. Western blotting was used to assess CD46 and CD55 protein in the placentas of the patients. Human trophoblast cells (HTR-8/SVneo) were treated with VEGF at different concentrations, and the expression of CD46 and CD55 was assessed by immunofluorescence and western blot.

Results: The CD46 protein level was significantly higher both in early-onset and late-onset severe preeclampsia group than the L-control group (P=0.028 and P=0.041, respectively). With the increase of VEGF concentration, there was no significant difference in expression of CD46 and CD55 on the HTR-8/SVneo cells.

Conclusions: No deficiency of local complement regulatory proteins was found in the patients with preeclampsia. A maternal-fetal interface feedback mechanism of CD46 may exist in severe preeclampsia. VEGF may have no effect on the expression of CD46 and CD55 on trophoblast membranes.

Background

Preeclampsia is a pregnancy-associated disorder characterized by a new onset of hypertension and proteinuria, sometimes progressing to a multiorgan syndrome, with varying clinical features[1]. Numerous hypotheses about the pathogenesis of preeclampsia have been proposed, such as vascular endothelial dysfunction, systemic inflammatory response, and immune regulatory abnormalities[2]. In recent years, researchers have found that aberrant regulation of the complement system may play an important role in the pathogenesis of preeclampsia.

Many studies have found excessive complement activation in the peripheral circulation and placentas of patients with preeclampsia[3–11]. In normal pregnancy, the complement system is regulated by complement regulatory proteins, including circulating proteins [C4b binding protein (C4BP), complement factor H (CFH), and complement factor I (CFI)] and membrane-bound proteins that are widely expressed on trophoblast membranes [decay accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), and CD59][12]. CD46 binds to both C3b and C4b, acting as a cofactor for their inactivation by C3b/C4b inactivator enzyme factor I. CD55 inhibits complement activation by binding to C3b and promoting its degradation[13]. In mice, the activity of complement receptor 1-related gene protein y (Crry) is similar to that of CD46 and CD55[14]. A deficiency of Crry in mice leads to embryonic lethality caused by complement activation[15]. This finding has emphasized the importance of complement regulation in maternal control of the tissue damage caused by complement activation. Similarly, we speculate that

CD46 and CD55 are important in complement regulation in human preeclampsia. Level of free vascular endothelial growth factor (VEGF) in the peripheral circulation was significantly lower in patients with preeclampsia than normal pregnancy[16]. Several scholars have found that VEGF can upregulate the expression of complement regulatory proteins in glomerular endothelial cell[17]. Therefore, we conducted this study to investigate whether local regulatory proteins are insufficient in preeclampsia patients and whether VEGF can regulate the expression of complement regulatory proteins on trophoblast membranes.

Methods

Patient enrollment

This was a case-control study that was approved by the ethical committee of Peking University First Hospital [No. 2019(08)]. Informed consent was obtained for placental tissue sampling. We recruited 20 patients with severe preeclampsia, including 10 patients with early-onset severe preeclampsia (EOSPE) and 10 patients with late-onset severe preeclampsia (LOSPE), who were matched with 6 patients comprising the early-onset control group (E-control) and 10 patients comprising the late-onset control group (L-control). Nine of these patients delivered in 2018 and were enrolled in the Tissue Bank Gynecology & Obstetric Department, Peking University First Hospital. The other patients delivered in 2019. All of these patients were delivered by cesarean section.

Inclusion criteria

The diagnosis of severe preeclampsia was made in accordance with both the 2015 Chinese Medical Association and the 2019 American College of Obstetricians and Gynecologists (ACOG) guidelines[18, 19] as follows: systolic blood pressure of 160 mmHg or higher or diastolic blood pressure of 110 mmHg or higher on two occasions; thrombocytopenia; impaired liver function; pulmonary edema; renal insufficiency; and cerebral or visual disturbances. Proteinuria (≥ 2.0 g/24 h) was also included as a diagnostic criterion, according to the 2015 Chinese Medical Association guidelines. Patients with preeclampsia were diagnosed as having severe preeclampsia if any of the above features were found. Preeclampsia that developed before 34 weeks of gestation was defined as early-onset preeclampsia, whereas preeclampsia that developed at or after 34 gestational weeks was defined as late-onset preeclampsia.

Exclusion criteria

Patients complicated with diseases before pregnancy, such as systemic lupus erythematosus (SLE), anti-phospholipid syndrome (APS), chronic hypertension, or chronic kidney disease (CKD), were excluded. Multiple pregnancy was also excluded.

Control group recruitment

Control groups were selected to match EOSPE and LOSPE cases based on weeks of delivery. Patients with the above diseases were excluded.

The indications for delivery before 34 weeks in the E-control group were as follows: 1 case of fetal hemolysis, 3 cases of placental implantation, 1 case of premature rupture of the fetal membrane and 1 case of inevitable preterm labor. Pathological examinations showed no cases of chorioamnionitis in the E-control group.

The indications for delivery in the L-control group were as follows: 3 cases of fetal distress, 2 cases of threatened uterine rupture and 5 cases of scarred uterus. Five patients in the L-control group delivered before term, and no cases of chorioamnionitis were noted.

Placenta collection

Placental tissue blocks (2 cm^3) from the central parts of the maternal side were collected immediately after the delivery of placentas, washed in ice-cold phosphate-buffered saline (PBS) 3 times to clear the blood, snap-frozen, transferred in liquid nitrogen and stored at -80°C for protein analysis.

Western blot

Western blot analysis was performed as described in a previous study[20]. The primary antibodies included CD46 and CD55 (Thermo Fisher Scientific, Rockford, IL, USA). The secondary antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Quantitative analysis of the gray value of the bands in western blots was performed using ImageJ analysis software (National Institutes of Health, USA). Data were corrected for background, expressed as the gray value and then normalized with β-actin expression.

Immunofluorescence

Immunofluorescence analysis was performed as described in a previous study[9]. Cells were washed with PBS, fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.25% Triton X- 100 for 10 minutes, and then incubated with primary antibodies to CD46 and CD55. Secondary antibodies were subsequently incubated. Nuclei were stained with DAPI. Slides were observed and imaged with a laser scanning confocal microscope.

Cell culture

Human extravillous trophoblastic cell line HTR-8/Svneo (purchased from ATCC, USA) was cultured in RPMI 1640 culture medium containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin and incubated at 37°C with 5% CO_2. Cells were starved in serum-free medium for 6 hours, then treated VEGF at different concentrations (0, 1, 10, 20, 50 ng/ml) for 24 hours. Subsequently, expression of CD46 and CD55 was tested by immunofluorescence and western blot.

Statistical analysis

The statistical software program SPSS 20.0 (IBM, Armonk, NY, USA) was used for statistical analysis. We used the Shapiro-Wilk test to analyze the normality of continuous variables. Normally distributed data are presented as the means ± standard deviation. Skewed data are described as medians (Q1-Q3). An independent t-test (normally distributed data) or a Mann-Whitney U test (data with a skewed distribution) was used to determine the difference between two groups. A *P* value of less than 0.05 was considered to be significant.

Results

Clinical Data

The clinical characteristics of each group are shown in Table 1. No difference was observed in gestational age at delivery, BMI, maternal age, gravidity and parity between the preeclampsia group and the corresponding control group. As expected, blood pressure and proteinuria were significantly higher in the preeclampsia group than those in the control group. The newborn birthweight in the EOSPE group was significantly lower than that in the E-control group. The serum creatinine, blood alanine aminotransferase (ALT) and aspartate transaminase (AST) levels in the preeclampsia group were much higher than those in the control group, whereas the blood platelet count was lower in the LOSPE group than that in the L-control group.

Table 1

Clinical characteristics of the EOSPE, E-control, LOSPE, and L-control groups.

	EOSPE	E-control	LOSPE	L-control	P-value
Age (years)*	33.6±5.0	33.0±3.8	34.5±5.3	33.2±3.1	>0.05
BMI (kg/m^2) *	30.1±3.6	27.1±2.8	29.4±4.1	26.6±2.9	>0.05
Gravidity#	3.00(1.00-4.25)	4.00(2.00-5.00)	1.50(1.00-3.00)	3.00(1.00-4.00)	>0.05
Parity#	1.00(0-1.00)	1.00(0.75-1.25)	1.00(0-1.00)	1.00(0-1.00)	>0.05
Highest SBP (mmHg)#	173(164-181)	125(120-135)	173(167-179)	116(107-120)	<0.05[a,b]
Highest DBP (mmHg)#	105(102-111)	72(68-80)	97(93-105)	71(70-80)	<0.05[a,b]
Proteinuria (g/24 h) #	6.57(2.38-10.79)	0	0.83(0.31-2.72)	0	<0.05[a,b]
ALT (IU/L) #	18.0(14.3-129.0)	10.5(7.0-14.3)	14.0(10.3-27.8)	9.5(7.0-13.3)	<0.05[a,b]
AST (IU/L) #	22.5(14.8-118.5)	13.0(11.8-16.8)	20.5(16.8-28.8)	14.0(12.0-19.3)	<0.05[b]
Platelet count (10^9/L) *	165.4±50.5	201.1±33.8	160.0±47.5	201.3±36.2	<0.05[a,b]
Serum creatinine (μmol/L) *	67.0±14.4	45.3±9.4	60.8±10.0	51.6±7.6	>0.05
Gestational age at delivery (weeks)*	30.8±1.8	31.8±1.5	37.0±1.2	37.2±1.1	<0.05[a]
Fetal birth weight (g)*	1280±288	1793±385	2760±436	2908±255	

*Data are the mean ± SD.

#Data are the median (Q1~Q3).

aP: EOSPE versus E-control

bP: LOSPE versus L-control.

EOSPE, early-onset severe preeclampsia; E-control, controls for early-onset severe preeclampsia; LOSPE, late-onset severe preeclampsia; L-control, controls for late-onset severe preeclampsia.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate transaminase.

Expression of CD46 and CD55 in placentas

Western blot images of CD46 and CD55 in placentas of the EOSPE, LOSPE, E-control, and L-control groups are shown in Figure 1.

We quantified CD46 and CD55 protein expression by calculating the CD46/β-actin and CD55/β-actin gray value ratios. The data are shown in Figure 2 and Figure 3.

There was no significant difference in the CD46/β-actin ratio between the severe preeclampsia group and the control group [0.68 (0.47-0.92) vs. 0.39 (0.29-0.71), *P*=0.052]. When the groups were subdivided into the EOSPE, LOSPE, E-control and L-control groups, we discovered that the CD46/β-actin ratio was significantly higher in the LOSPE group than in the L-control group [0.52 (0.45-1.00) vs. 0.33 (0.12-0.69), *P*=0.041]. The CD46 protein level was significantly higher in the EOSPE group than that in the L-control group [0.74 (0.42-0.93) vs. 0.33 (0.12-0.69), P=0.028]. There was no significant discrepancy between the EOSPE and LOSPE groups [0.74 (0.42-0.93) vs. 0.52 (0.45-1.00), *P*=0.821] or between the EOSPE and E-control groups [0.74(0.42-0.93) vs. 0.57(0.40-1.06), *P*=0.745].

The CD55/β-actin gray value ratio was comparable between the severe preeclampsia group and the control group [0.92 (0.50-1.21) vs. 1.38 (0.67-1.57), *P*=0.08]. When the groups were subdivided into the EOSPE, LOSPE, E-control and L-control groups, no significant differences were found in the CD55/β-actin gray value ratio between the EOSPE and E-control groups [0.78 (0.46-1.09) vs. 1.53 (0.54-1.70), *P*=0.159] or between the LOSPE group and L-control group [1.03 (0.49-1.29) vs. 1.16 (0.70-1.61), *P*=0.406]. No significant discrepancy existed between the EOSPE and LOSPE groups [0.78 (0.46-1.09) vs. 1.03 (0.49-1.29), *P*=0.450].

VEGF had no effect on the expression of CD46 and CD55 on trophoblast membranes.

CD46 and CD55 expressed widely on the surface of HTR-8/Svneo cells. With the increase of VEGF concentration, there was no significant difference discovered in the expression of CD46 and CD55 on the HTR-8/SVneo cell surface. Immunofluorescence and western blot data were showed in Figure 4, Figure 5 and Figure 6.

Discussion

In this study, we found a significant upregulation of CD46 protein expression in the EOSPE and LOSPE group. No difference in CD46 protein expression was observed between the EOSPE and E-control groups or between the EOSPE and LOSPE groups. Additionally, no significant difference in CD55 protein expression was discovered between the preeclampsia group and the control group.

In recent years, many studies have revealed that circulating C4d, C3a, C5a, and sC5b-9 levels were higher in patients with preeclampsia than in healthy pregnant women[3–7, 21]. Meanwhile, complement-activated fragments, including C4d, C5a and MAC, were expressed at much higher levels in placentas of patients with preeclampsia than in normal pregnant women[8–11]. Considering these findings, we estimated that

the upregulation of CD46 protein expression in severe preeclampsia was a maternal-fetal interface feedback mechanism to attempt to limit local complement activation.

Our recent study found that excessive activation of the complement system through the alternative and classical pathways was observed as early as the first trimester in patients with preeclampsia later in pregnancy[22]. Then, the concentrations of complement components became similar to those in normal pregnancy thereafter, even in late pregnancy. Complement activation was not core factor in the pathogenesis of preeclampsia. So, upregulation of CD46 protein expression could not prevent the progression of preeclampsia.

In this study, we did not discover significant differences in CD46 and CD55 expression between the EOSPE group and the E-control group. In fact, it was difficult to enroll a standardized E-control group without any potential factor of complement activation. In this study, we enrolled patients with preterm delivery, premature rupture of membranes, placental implantation and fetal hemolysis in the E-control group. No placenta infection occurred in this group. Complement activation was previously reported to be involved preterm labor[23, 24], and CD55 expressed on white blood cells was elevated in patients with preterm labor[25, 26]. Overexpression of complement regulatory protein may have also occurred in placentas of patients with preterm delivery and premature rupture of membranes in the E-control group. Therefore, the expression of complement regulatory proteins in the EOSPE group requires further study.

To date, several studies have examined the expression of complement regulatory protein in placentas of preeclampsia patients. Lokki et al.[10] found that membrane-bound regulatory proteins (CD46, CD55, and CD59) were widely expressed on the syncytiotrophoblast layer by immunofluorescence staining and histochemistry. There was no difference observed in the expression pattern of any regulatory protein between the preeclampsia and control groups or between the early-onset and late-onset preeclampsia groups. Another study by Buurma et al.[11] discovered that the mRNA expression of CD55 and CD59, but not CD46, was much higher in early-onset preeclampsia patients than in normal pregnant women with term delivery. However, this study did not enroll late-onset preeclampsia and recruited the early-onset preeclampsia group without strict standards, including 8 cases of chronic hypertension and 2 cases of inherited thrombophilia. Perhaps due to the complex regulatory pathways, our results were inconsistent.

In addition to mRNA and protein expression, some researchers have focused on the mutations of CD46 that predispose patients to preeclampsia. Salmon et al.[27] confirmed the relationship between hypomorphic variants of CD46 and non-autoimmune preeclampsia. However, Lokki et al.[28] did not identify an association between genetic polymorphisms of CD46 and preeclampsia. Further study should focus on the role of functional CD46 isoforms in the pathogenesis of severe preeclampsia.

In cellular experiments, we found no significant difference in expression of CD46 and CD55 on the HTR-8/SVneo cell surface after VEGF treatment at different concentrations. Mason et al. found that human umbilical vein endothelial cells and dermal endothelial cells expressed more CD55 after VEGF treatment, but not CD46 and CD59[29]. However, another study has found that VEGF upregulated the expression of

factor H, CD46 and CD55 in glomerular[17]. Extravillous trophoblasts invade and replace endothelial cells of spiral artery to remodel blood vessels[30]. Perhaps limited concentration gradient may result in negative results. Furthermore, we speculated that complement regulatory proteins' response to VEGF in heterogeneous endothelial cells may vary between different vascular beds.

Our study had the following limitations: (i) As previously mentioned, E-control group enrollment was not strict, and perhaps CD46 and CD55 were upregulated in some patients. Additional time will be needed to enroll a standardized group of patients. (ii) This study had a relatively small sample size, and further studies with larger sample sizes need to be implemented. (iii) In cellular experiments, HTR-8/SVneo cells had a tendency to express more CD46 with the increase of VEGF concentration, but without statistical difference. We should set higher concentration of VEGF and examine circulating regulatory proteins in further experiment.

Conclusions

In conclusion, no deficiency of local complement regulatory proteins was found in the patients with preeclampsia. A maternal-fetal interface feedback mechanism of CD46 may exist in severe preeclampsia. VEGF may have no effect on the expression of CD46 and CD55 on trophoblast membranes.

Abbreviations

C4BP C4b binding protein

CFH complement factor H

CFI complement factor I

DAF decay accelerating factor

MCP membrane cofactor protein

Crry complement receptor 1-related gene protein y

VEGF vascular endothelial growth factor

EOSPE early-onset severe preeclampsia

LOSPE late-onset severe preeclampsia

E-control early-onset control group

L-control late-onset control group

ACOG American College of Obstetricians and Gynecologists

SLE systemic lupus erythematosus(), chronic hypertension

APS anti-phospholipid syndrome

CKD chronic kidney disease,

PBS phosphate-buffered saline

ALT alanine aminotransferase

AST aspartate transaminase

Declarations

Ethical approval and consent to participate

This study was approved by the Ethics Committee of Peking University First Hospital prior to the commencement of the study. The study was conducted according to the principles of the Declaration of Helsinki and its amendments.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent form is available for review by the Editor of BMC Pregnancy and Childbirth, if needed.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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None.

Authors' contributions

MW and ZL drafted and revised the manuscript, and YH, QC and HY provided critical revisions of the report for important intellectual content. All authors participated in the care of the patient and approved the final version of the manuscript for submission.

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Figures

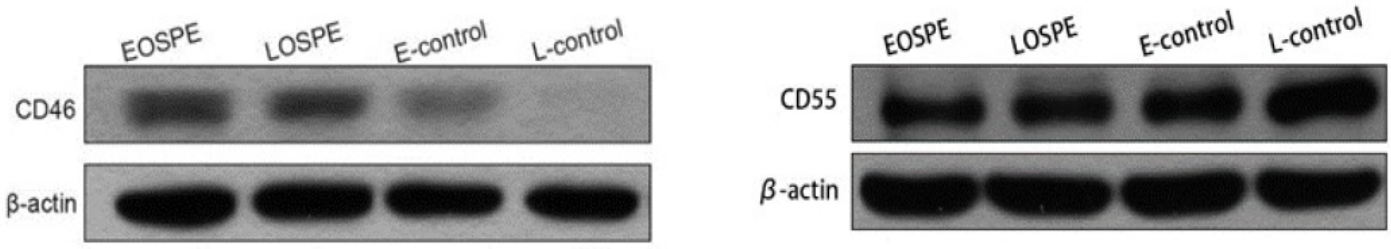

Figure 1

Western blot images of CD46 and CD55 in placentas of the EOSPE, LOSPE, E-control, and L-control groups.

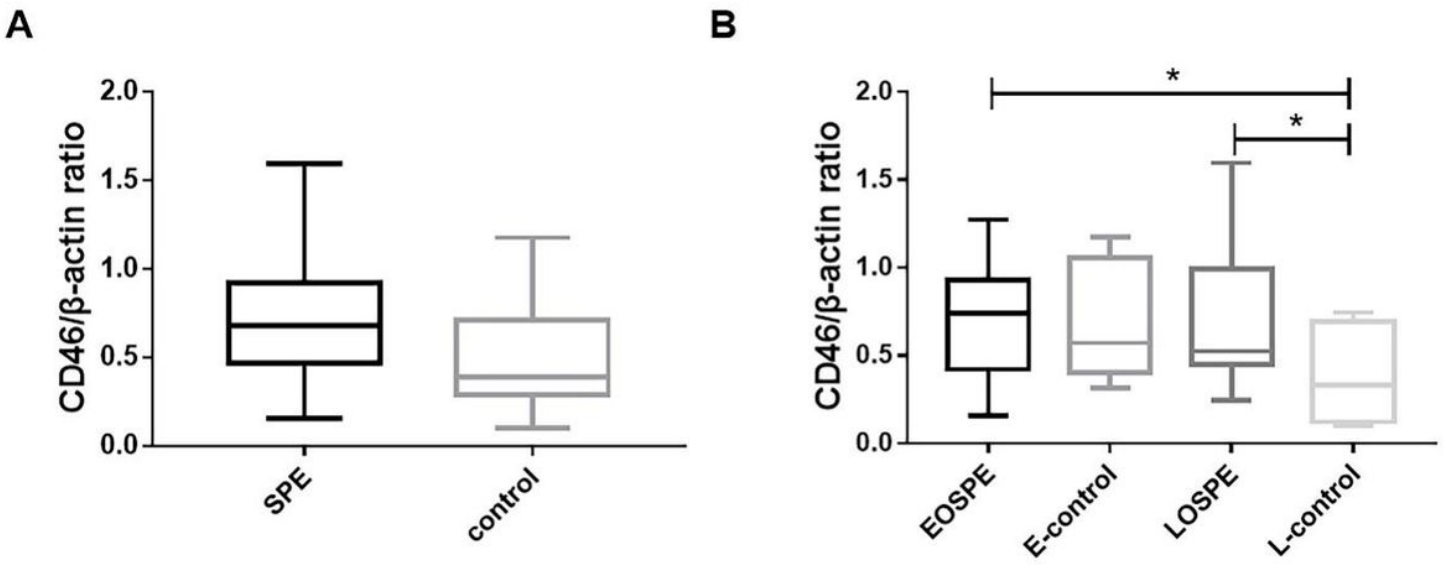

Figure 2

A. Quantification of CD46 expression in placentas of the severe preeclampsia group (n=20) and control group (n=16). There was no significant difference between the two groups. B. Quantification of CD46 expression in placentas of the EOSPE, LOSPE, E-control, and L-control groups. *P<0.05.

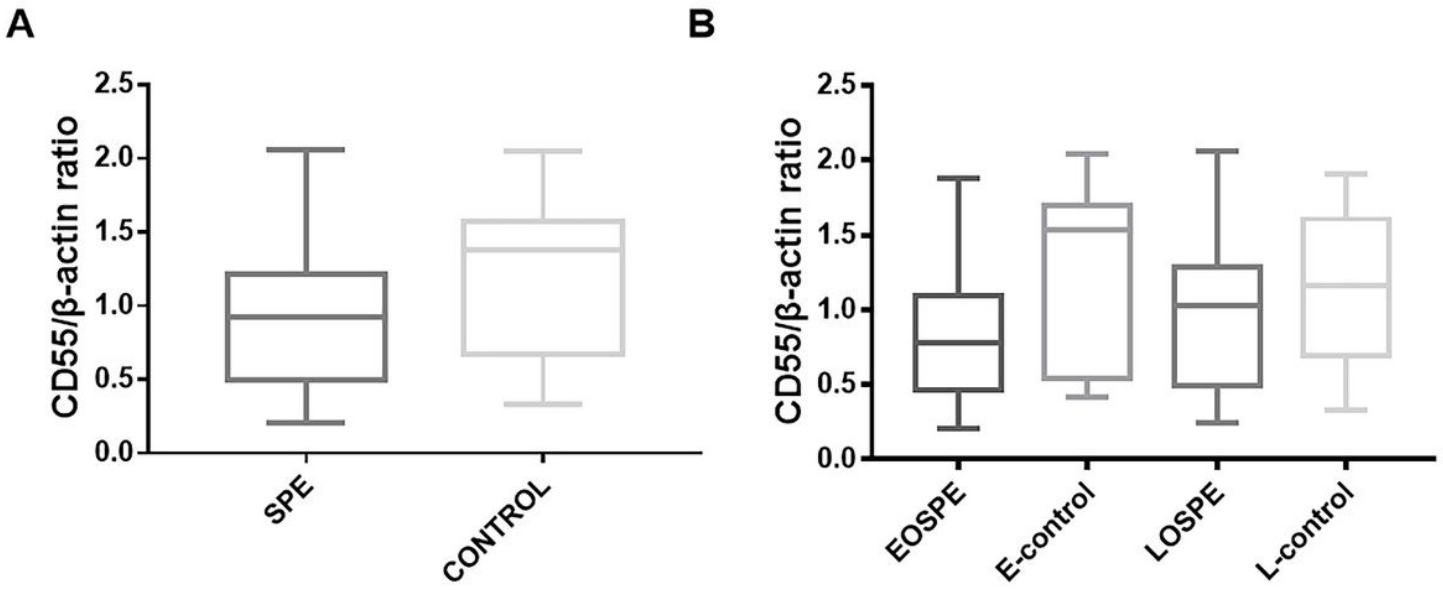

Figure 3

A. Quantification of CD55 expression in placentas of the severe preeclampsia group (n=20) and control group (n=16). There was no significant difference between two groups. B. Quantification of CD55 expression in placentas of the EOSPE, LOSPE, E-control, and L-control groups. There was no significant difference between any two groups.

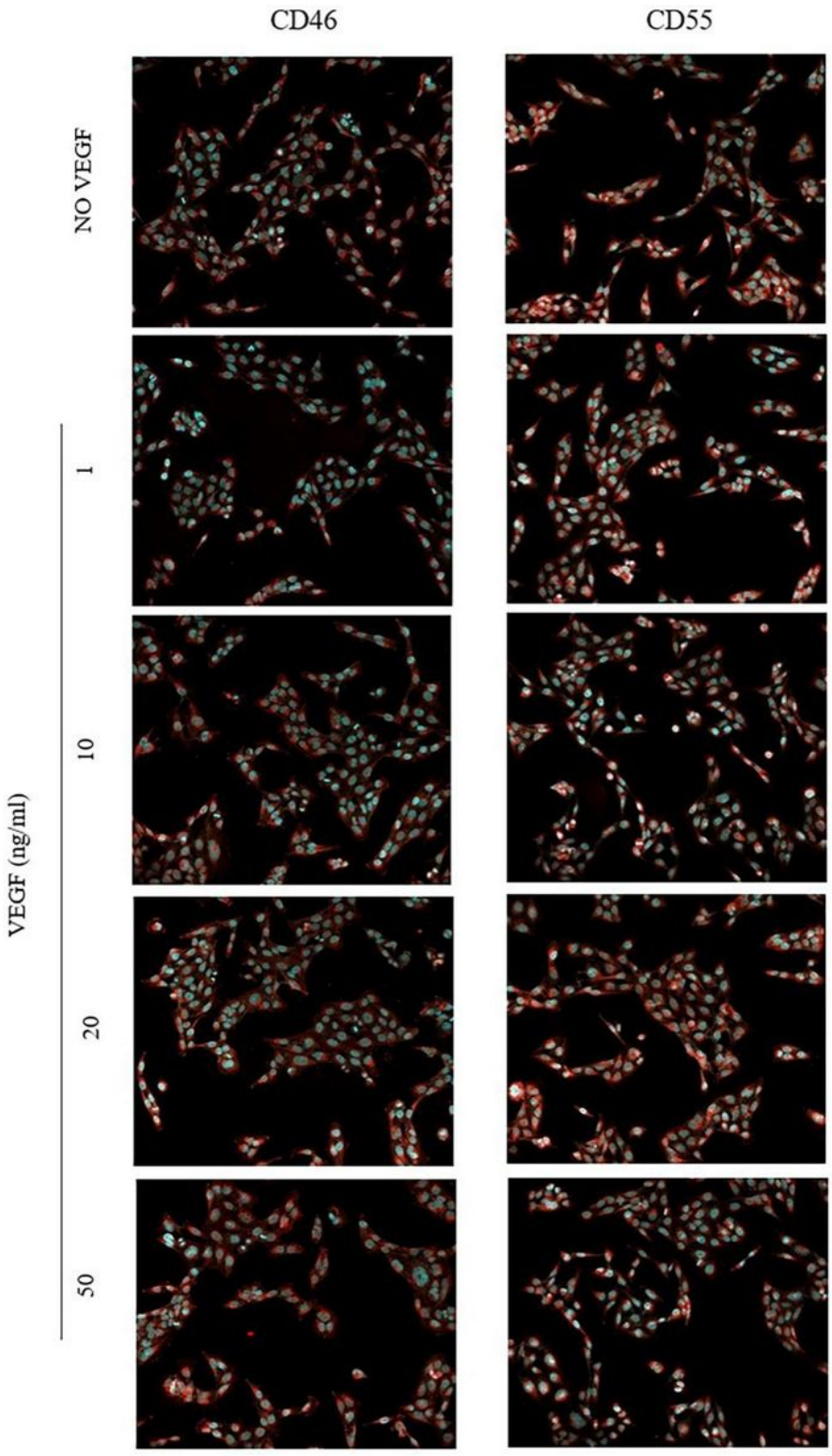

Figure 4

Immunofluorescence staining showed no significant difference in CD46 and CD55 expression on HTR-8/Svneo after VEGF treatment (CD46 red, CD55 red, DAPI blue).

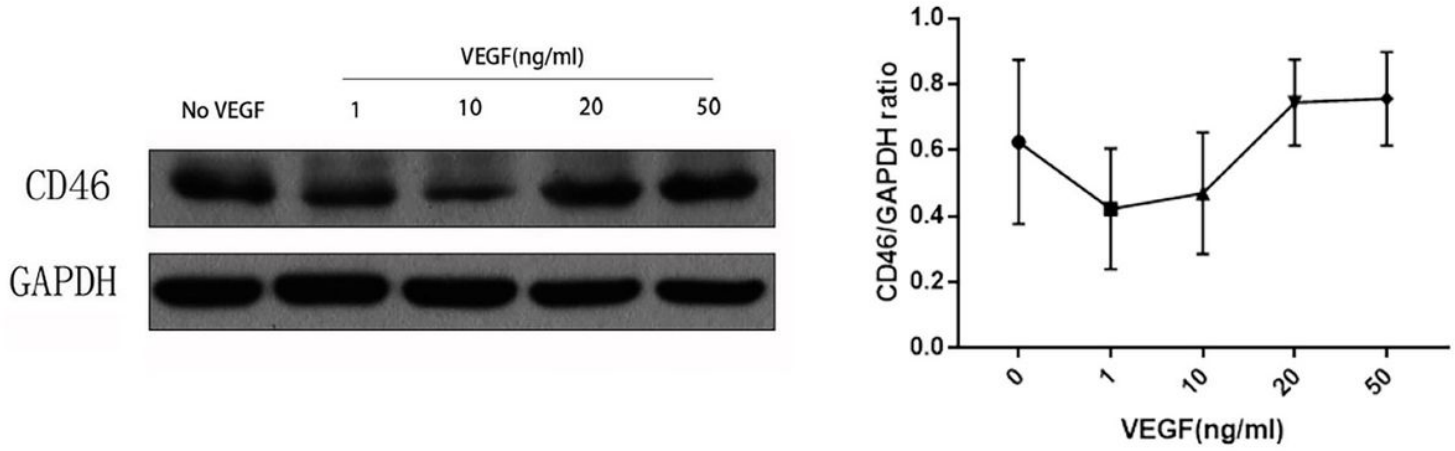

Figure 5

Expression of CD46 on HTR-8/Svneo after VEGF treatment.

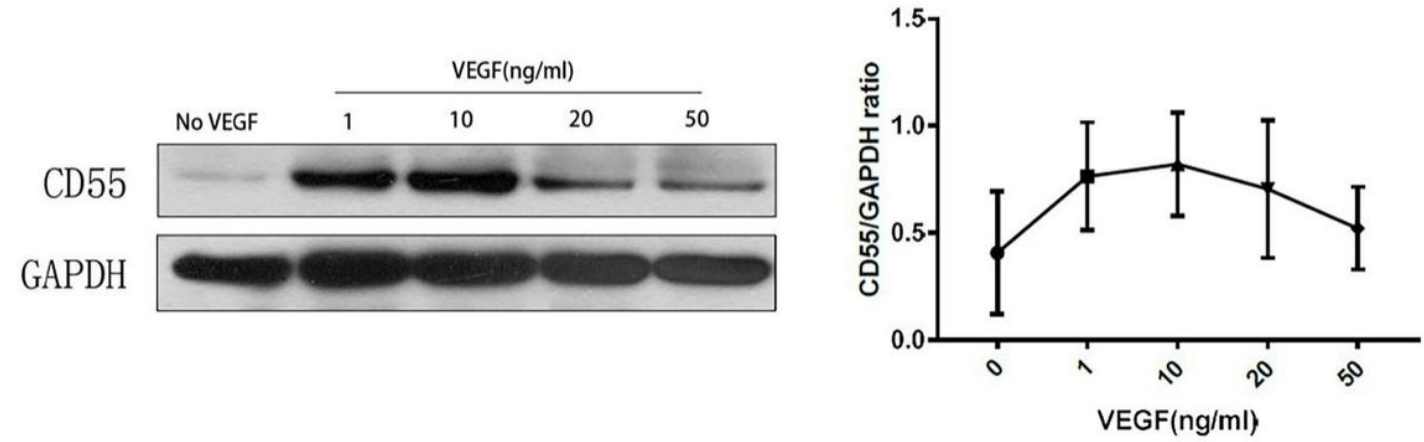

Figure 6

Expression of CD55 on HTR-8/Svneo after VEGF treatment.

Supplementary Files

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