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Expression levels of serum mir-145-5p and Gas6 in women with early-onset preeclampsia and their clinical significance

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ABSTRACT

early preeclampsia is a pregnancy-specific clinical disease, its pathogenesis is not clear, and clinical treatment is limited. Therefore, it is particularly important to study the pathogenesis of early preeclampsia, so as to provide evidence for the diagnosis and effective treatment of early preeclampsia. This research was carried out to investigate the expression levels and clinical significance of mir-145-5p and stagflation specific gene 6 (Gas6) in serum of women with early preeclampsia. For this purpose, 142 patients with preeclampsia were divided into the early-onset group (n=78) and the late-onset group (n=64) according to the onset time. Meanwhile, 70 normal pregnant women were selected as the control group. The levels of serum Gas6, mir-145-5p and inflammatory factors were detected. Logistic regression analysis showed that the expression levels of serum gas-6 and serum mir-145-5p were valuable in the diagnosis of early preeclampsia. ROC curve was established to evaluate the diagnostic efficacy of serum Gas6 level in early-onset preeclampsia. Its sensitivity and specificity were 91.23% and 64.2%, respectively, and the cutting value was 255.71pg/mL. The serum Gas6 level was negatively correlated with systolic blood pressure (r=-0.349, P<0.05) and positively correlated with platelet count (r=0.391, P<0.05). Compared with the control group, serum levels of mir-145-5p and TNF-a in women with early-onset preeclampsia were negatively correlated (r=-0.460~0.622, P<0.05). Conclusion: the serum level of Gas6 was reduced in patients with early-onset preeclampsia, and TNF-a inhibited the invasion of SVneo cells by upregulating mir-145-5p.

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Introduction

Preeclampsia is a pregnancy-specific syndrome with hypertension as the basis and multi-system involvement. The morbidity and mortality are very high, and the incidence in China is 2% to 6%. Worldwide, PE causes more than 50,000 maternal deaths each year. In recent years, the incidence of preeclampsia is on the rise in China, which is one of the main causes of maternal and infant mortality. Preeclampsia occurs during pregnancy or early postpartum and is defined as newly diagnosed hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg) and large amounts of proteinuria (>300mg at 24h). The etiology and pathogenesis of preeclampsia have not yet been fully elucidated. Currently, it is believed that preeclampsia is a multi-factor, multi-mechanism and multi-pathway disease, which cannot be explained by monism. This is the heterogeneity of the etiology of preeclampsia (1-4).

Previous studies have shown that preeclampsia is a maternal systemic inflammatory response triggered by oxidative stress, and it is a state that favors the Th1/Th17 dominance of immune cells. An overactive immune response in early pregnancy can lead to placental abnormalities and release of immune-inflammatory substances into the maternal circulation, leading to systemic inflammation and endothelial dysfunction in the maternal body (1). Other maternal factors, such as infection or obesity, may also contribute to oxidative stress response and endothelial dysfunction, leading to preeclampsia (2). Due to the extremely complex structure and functional regulation of the immune system, previous studies on the relationship between preeclampsia and immune inflammation have focused on the detection of inflammatory markers (3). Cytokines are a kind of soluble protein with various biological activities, which are widely involved in embryo implantation, trophoblast cell growth, differentiation and delivery (4). During preeclampsia, circulating pro-inflammatory

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cytokines increase and activate endothelial cells, causing an excessive systemic inflammatory response. This is consistent with elevated levels of inflammatory factors reported in clinical studies of patients with preeclampsia, including tumor necrosis factor (TNF-a), interleukin-8 (ll-8), macrophage suppressor protein (MIP), and ifn-leukocyte, et al. (5). Preeclampsia is associated with chronic immune activation, which leads to an increase in the secretion of inflammatory cytokines by helper T cells and a decrease in regulatory and anti-inflammatory cytokines, further promoting the inflammatory state of preeclampsia (6). This imbalance between pro-inflammatory and anti-inflammatory regulatory cytokines is associated with placental ischemia in preeclampsia during pregnancy. This imbalance worsens as pregnancy progresses, further complicating pregnancy (7).

Growth suppression-specific protein 6(Gas6) is a secreted protein encoded by a growth suppression-specific gene, which is widely found in a variety of tissues, such as the small intestine, lung, kidney, placenta and endothelial cells, and can also be detected in human serum or plasma. As a newly discovered growth factor, Gas6 plays an important role in the regulation of cell proliferation, division, growth and apoptosis (8). Gas6 also plays an important role in vascular homeostasis, apoptosis, inflammation, metabolic disorders, atherosclerosis and other pathophysiological processes (9). Gas6 can aggravate the local oxidative stress and inflammatory response of tissues by regulating angiogenesis and platelet aggregation (10). Studies have shown that the Gas6 gene expression is abnormal in preeclampsia placenta and decidua tissues (11). Since Gas6 is involved in the interaction between endothelial cells, coagulation and platelets, it can be speculated that Gas6 may be involved in the pathological process of preeclampsia (12). Related studies have shown that serum Gas6 levels in preeclampsia patients are significantly increased compared with healthy pregnant women with age matching (13). However, Toshitaka Moriet al. showed that the plasma Gas6 level of preeclampsia patients was significantly lower than that of healthy pregnant women (14). Although the etiology and pathogenesis of preeclampsia are unclear, in related cases, the elimination of clinical manifestations following placectomies clearly indicates that abnormal placental development is involved in the pathogenesis of preeclampsia (15).

Screening TNF-a can inhibit the migration of SVneo cells, so what molecular mechanism does TNF-a inhibit the migration of SVneo cells? In order to solve this problem, some transcription factors related to cell migration and invasion and regulated by TNF-a were selected through literature reviews, such as sarcoid homologous boxprotein 1(MSX1), signal transduction and transcriptional activator 3(STAT3), and Cyr61 (Cyr61) rich in cysteine as possible downstream molecules of TNF-a (16). The molecules involved in the development of preeclampsia were screened experimentally. In this study, the scratch test was used as a means to detect the migration ability of extramedullary trophoblast cells, and the effects of TGF- and TNF-a on the migration ability of SVneo cells were detected, and TNF-a was screened to have an inhibitory effect on SVneo migration (17). This study focused on the influence of TNF-a on the migration and invasion of SVneo in human extra-chorionic trophoblast cells, and the mechanisms involved (18). We found that TNF-a treatment can inhibit the migration and invasion of SVneo, which is related to the regulation of the expression of Cyr61 at the post-transcriptional level. Since post-transcriptional regulation is mostly related to miRNAs, we further detected TNF-a's expression of miRNAs that may be involved in the regulation of Cyr61 in SVneo cells by referring to a large number of literatures and bioinformatics software analysis. We found that the expression of mir-145-5p in TNF-a-treated SVneo in the detected miRNAs was up-regulated. Functional experiments confirmed the important role of mir-145-5p in TNF-a-mediated inhibition of SVneo invasion. Our results suggest that TNF - a/miR - 145-5 p/Cyr61 shaft to adjust the human chorionic trophoblast cell invasion and migration ability, may play a role in the process of the pathogenesis of preeclampsia, miR - 145-5 p and Cyr61 may as indicators of early diagnosis of preeclampsia prediction and treatment targets, we will study in the treatment of preeclampsia in the process of use of TNF-a treatment.

Materials and methods

General information

142 patients with preeclampsia were selected and divided into an early-onset group (n=78) and a late-onset group (n=64) according to the onset time. Meanwhile, 70 normal pregnant women were selected as the control group. The average age of the patients was 22~30 years old, 26~38 weeks of gestation, and the number of pregnancies was 1~3. The inclusion criteria were: all patients met the relevant diagnostic criteria of pre-eclampsia; They were all conceived naturally and did not receive assisted reproduction. Exclusion criteria: a history of abortion or fetal malformation; Patients with hypertension or diabetes during pregnancy; Acute or chronic infection, blood system, immune system, connective tissue disease. There were no statistically significant differences in age, gestational age and pregnancy times among all the patients (P>, 0.05). The experiment was approved by the hospital ethics committee and all patients gave informed consent.

Experimental methods

8mL of fasting elbow venous blood was collected from each group in the morning. It was allowed to stand for 20 to 30 min at room temperature and then centrifuged for 25 min at 3500r/1min. The supernatant was retained and stored in the refrigerator at -80 ℃ for examination. Serum Gas6 and levels of inflammatory factors were detected by enzyme-linked immunosorbent assay. The Gas6 detection kit was purchased from Haihuzhen biotechnology co., LTD. All operations were performed according to the kit instructions.

Scratch experiment

(i) Cell suspension was prepared, and SVneo cells in the logarithmic phase were taken as the research object. Add 5mlPBS to gently wash the cells and suck PBS. 1ml trypsin-EDTA digestive juice (0.25%) was added, gently shaken for 10s, and about half of the trypsin was absorbed, with the remaining half in the cell culture dish. Digestion was carried out in incubators at 37 °C. after the 30s, a complete medium containing 10% serum was added to neutralization of trypsinase activity. The cell suspension was gently blown evenly and cells were counted. The cell concentration was adjusted to 4x105 /ml and seeded into 24-well plates with 500 livl cell suspension for each well and 3 additional Wells for each cell group.

(ii) The cells were incubated at 5% CO2, 37 ℃ for 24 hours, and then the cells were fully spread. Use the horizontal line drawn vertically by the 10 living l spearhead, and draw a straight line across the center of the circle. Wash it twice with PBS. After removing the cells, observe and take photos under the microscope.

(iii) Serum-free medium was added along the pore wall to continue the cell culture. In this experiment, mitomycin C 10 living g/ml was added to the medium to inhibit cell proliferation. At different time points, the migration of the cells in the scratch was recorded under the 100-fold field of view of an inverted microscope.

Statistical treatment

SPSS 23.0 software was used to process and analyze the data. Normally distributed measurement data were represented as x±s, t-test was used for comparison between groups, and one-way analysis of variance was used for comparison among multiple sample means. Spearman correlation analysis was used to evaluate the correlation between serum Gas6 level and other indicators. The receiver operating characteristic curve (ROC curve) was established based on Gas6 and the diagnosis of preeclampsia, and the sensitivity and specificity of Gas6 for the diagnosis of preeclampsia were analyzed. P<0.05 was considered statistically significant.

Results and discussion

Morphological observation of SVneo cells

After digestion and passage of SVneo cells, they were observed under an inverted light microscope. After 4h, almost all the cells sank to the bottom of the petri dish or culture bottle, but they were still round and did not have a strong adherence to the wall. 6~8h, the cells have been adherent to the wall, about 30% of the cells have slowly spread to the surrounding. After 24h, the cells were completely adherent to the wall. SVneo cells are polygonal, irregular in shape, with fine spines protruding, and the boundaries between cells are obvious (see Figure 1). The newly divided cells are in a bright circle, with each passage passing from one to two, and the cells are passed every other day.

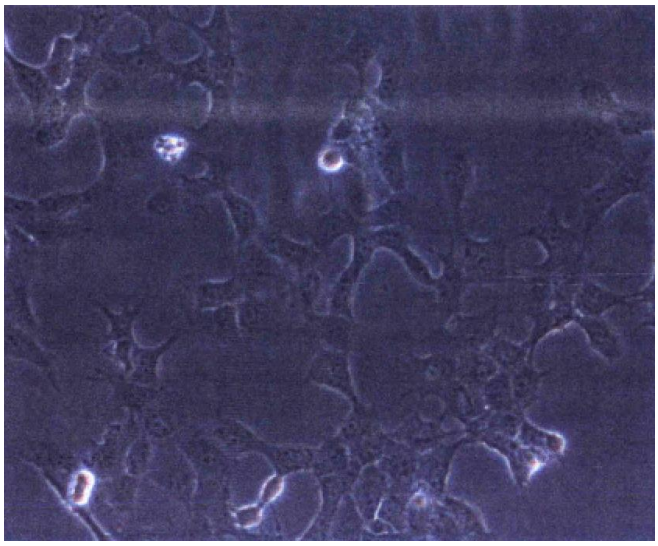

Figure 1. Morphology of SVneo cells (100X)

Effect of TNF-a on SVneo cell migration

The effect of TNF-a on the migration ability of SVneo cells was observed by cell scratch experiment. SVneo cells were cultured with TNF-a at different final concentrations (0, 1, 10, and 100 ng/ml) for 24h, and scratch healing was observed. The results showed that 1 ng/ml TNF-a had no significant effect on the mobility of SVneo compared with the 0 ng/ml TNF-a-treated SVneo cell group in the control group. However, compared with the control group with 0ng/ml TNF-a, the HTR8/SVneo cell healing rate was significantly reduced in the groups with final concentrations of 10ng/ml and 100 ng/ml TNF-a treatment (see Figure 2).

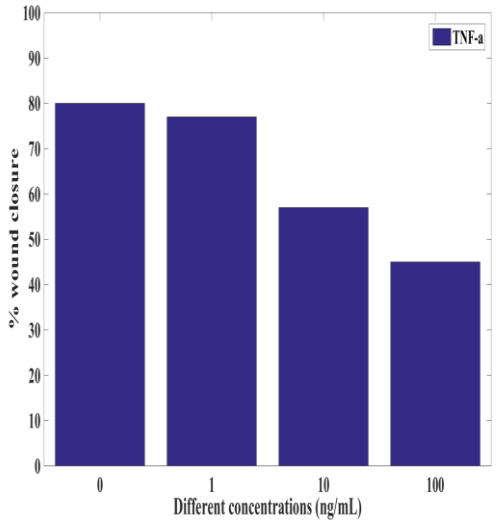

Figure 2. Effects of different concentrations of TNF-a on SVneo cell migration

Comparison of serum Gas6 and mir-145-5p expression levels between preeclampsia patients and normal pregnant women

The expression levels of serum Gas6 and mir-145-5p were compared as shown in Table 1 and Figure 3. As can be seen from the figure, the serum Gas6 level of pre-eclampsia patients was lower than that of the control group, while the expression of mir-145-5p was higher than that of the control group, with statistically significant differences (t=10.728, 9.8483, P<0.05).

Table 1. Comparison of serum Gas6 and mir-145-5p expression levels between the patient group and the normal pregnant women group

Group	n	Gas6(ρ/μg·L^{-1})	miR-145-5p (ρ/μg·L^{-1})
Patient group	142	13.52±2.16	1.19±0.17
Normal pregnant group	70	9.15±2.14	1.84±0.25

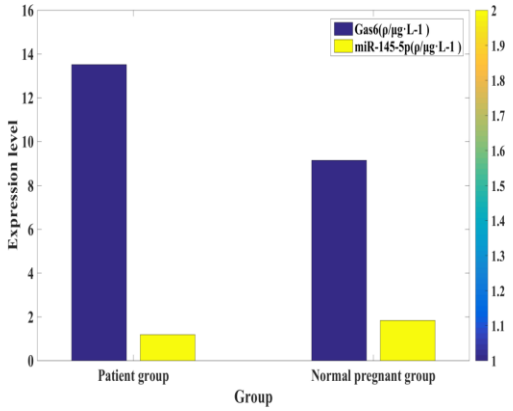

Figure 3. Comparison of serum Gas6 and mir-145-5p expression levels between the patient group and the normal pregnant women group

Comparison of the expression levels of serum Gas6 and mir-145-5p in preeclampsia patients at different onset times

The expression levels of serum Gas6 and mir-145-5p in preeclampsia patients at different onset times were compared as shown in Table 2 and Figure 4. It is not difficult to find that the serum Gas6 level of patients in the early hair style group is higher than that in the late hair style group, while the expression level of mir-145-5p is lower than that in the late hair style group, with significant differences (t=7.873, 4.481, P<0.05).

Table 2. Comparison of serum Gas6 and mir-145-5p expression levels in preeclampsia patients at different onset times

Group	n	Gas6(ρ/μg·L-1)	miR-145-5p (ρ/μg·L-1)
Early group	78	6.16±1.03	2.16±0.83
Late group	64	8.78±1.27	1.77±0.66

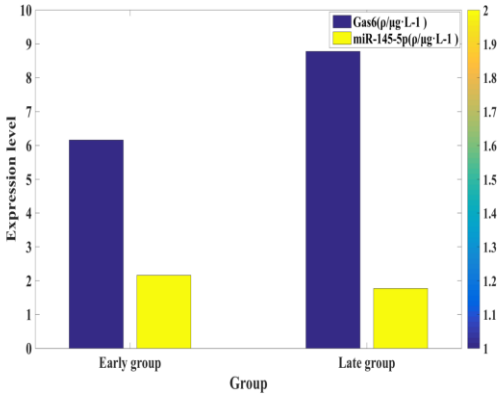

Figure 4. Comparison of the expression levels of serum Gas6 and mir-145-5p in preeclampsia patients at different onset times

Comparison of clinical data between preeclampsia patients and normal pregnant women

The comparison results of clinical data between the two groups are shown in Table 3. The gestational weeks and platelet counts at the time of delivery of preeclampsia patients were significantly lower than those of normal pregnant women, and the semi-quantitative values of systolic blood pressure, diastolic blood pressure, blood creatinine and urine protein were significantly higher than those of the control group, with statistically significant differences (P<0.05). The correlation analysis results of Gas6 with other relevant clinical indicators are shown in Table 4. The results showed that the serum Gas6 level was negatively correlated with systolic blood pressure (P<0.05) and positively correlated with platelet count (P<0.05).

Table 3. Comparison of clinical data between the two groups

Group	n	Gestational age	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Platelet count ($\times10^9$/L)	Urine protein	Creatinine (u mol/L)	ALT(U/L)
Patient group	142	34.2±2.7	160.3±17.3	102.5±11.6	201±69.2	+-+++	98.4±31.6	21.5±7.2
Normal pregnant group	70	37.3±2.9	115.3±17.8	76.4±15.2	195±59.3	-+	55.3±27.1	13.7±4.6

Table 4. Correlation analysis between Gas6 and other relevant clinical indicators

Item	r	p
Gestational age	0.026	0.842
Systolic blood pressure (mm Hg)	-0.347	0.019
Diastolic blood pressure (mm Hg)	-0.052	0.72
Platelet count ($\times10^9$/L)	0.395	0.005
Urine protein	-0.077	0.603
Creatinine (u mol/L)	-0.121	0.362
ALT(U/L)	0.017	0.915

The level of Gas6 in preeclampsia patients was significantly lower than that in the normal pregnant women group, and the difference was statistically significant (t=6.372, P<0.05). The ROC curve was established with Gas6 as the diagnostic factor of early preeclampsia (see Figure 5). The area under the ROC curve was 0.862(P<0.001), and the 95% CI was 0.784-0.931. In the statistical data table, the maximum value of the sum of sensitivity 71.9% and specificity 83.0% was selected as the cutting point, and the cutting value was 257.36pg /mL, suggesting that the serum level of Gas6 detected by this kit could be used for diagnostic reference of preeclampsia with early onset.

Given that preeclampsia is a pregnancy-specific disease, its pathogenesis is still unclear and clinical treatment methods are limited, in-depth understanding of the molecular mechanism of preeclampsia is the key to prevention and treatment of preeclampsia. However, the current research sites that down-regulate the expression of Cyr61 through mir-145-5p believe that the limitation of migration and invasion of placental trophoblast cells is a key link in the pathogenesis of preeclampsia (19). Therefore, it is one of the focuses of preeclampsia research to study the molecular mechanism of trophoblast cell migration and limited invasion ability. A full understanding of the molecular changes in this process is expected to provide theoretical basis and experimental support for the prevention and treatment of preeclampsia (20).

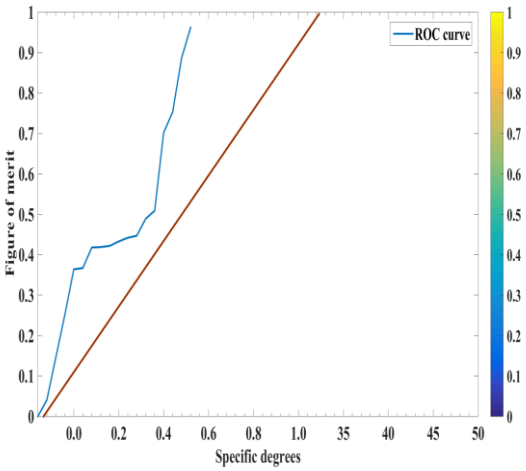

Figure 5. ROC curve of early eclampsia diagnosed by Gas6

Cell scratch test is an effective method to detect cell movement. It is mainly used to detect and evaluate the invasion and metastasis ability of adherent tumor cells. The principle is to lay an appropriate number of cells in a 6-hole, 24-hole or 48-hole plate. Generally, the cells should be fully laid, but not too much. It is best to lay a single layer just fully laid (21). Then use sterile spear was covered in cell on the board draw a line, due to the effect of mechanical force, in the process of marking, cells have been crossed out, and then use PBS gently wash the cells of the row fall into culture medium containing mitomycin C inhibition of cell proliferation, ruled out due to the proliferation of the cell free region growing (22). According to the need, the factors used to treat cells can also be added into the culture medium. This leaves a blank, cell-free area in the cell-lined board. If the cells are cultured for a period of time, as required, they can be observed at visible light under an inverted microscope to see if the cells are invading the empty space in the middle (23). The software was used to analyze the changes in the blank area of the control group and the experimental group after scratch 0h and scratch for a period of time to determine the changes in the invasion rate of cells and understand the migration ability of cells.

Mir-145-5p is a targeted negative regulator of Cyr61, and mir-145-5p has a potential role in regulating trophoblast invader in preeclampsia. Previous studies have shown that mir-145-5p regulates the aggressiveness of cancer cells. For example, mir-145-5p inhibited the invasion and metastasis of gastric cancer cells by targeting n-cadherin. In breast cancer cells, mir-145-5p also inhibited cell proliferation, invasion, and invasion (24). In addition to malignant cells, certain types of normal cells can also be regulated by mir-145-5p. Mir-145-5p reduced the invasion of vascular smooth muscle cells induced by high glucose (25). This study demonstrated that the overexpression of mir-145-5p can significantly reduce the migration and invasion of SVneo trophoblast cells. In addition, the low expression of mir-145-5p significantly weakens the TNF-a-mediated inhibition of trophoblast cell invasion and invasion, suggesting that mir-145-5p is an important mediator of TNF-a-induced anti-trophoblast cell invasion.

The severity of early-onset preeclampsia included elevated blood pressure, neurologic abnormalities, involvement of various organs and fetal involvement, etc. Specific laboratory indicators included proteinuria, serum creatinine, platelet count, ALT, etc. The differences in the above laboratory indicators between the case group and the control group were compared in this study. Only the difference in ALT was not statistically significant (P>, 0.05), and the elevated liver enzyme was a manifestation of liver injury in preeclampsia. Therefore, this result may be related to the small sample size. In addition, gestational week is an important factor affecting the prognosis of maternal and infant. The average onset of gestational week in the case group was less than that in the control group, suggesting that maternal and infant outcomes were worse than normal. Correlation analysis results showed that Gas6 was negatively

correlated with systolic blood pressure and positively correlated with platelet count. Changes in serum Gas6 level could indicate the severity of sPE. The area under the ROC curve established by the sPE for the diagnosis of Gas6 is large and has high sensitivity, which is of reference value for the diagnosis of early preeclampsia. Therefore, Gas6 is considered as a new serum marker for the diagnosis and evaluation of the severity of pre-eclampsia. About Gas6 levels in case group to reduce the phenomenon, there is literature from gene-level Gas6 gene level of the possible mechanism is analyzed, the factors related to Axl, Axl Gas6 is the main ligand, preeclampsia patients affected by systolic blood pressure and proteinuria, plasma sAxl level is higher, increase sAxl can be combined with more Gas6, resulting in a decrease of free Gas6 in the serum.

In summary,GAS6 can be used as a serological marker molecule to evaluate the severity of patients with early-onset preeclampsia due to changes in plasma levels. In the subsequent studies, the combined analysis of Gas6 and serum uric acid and cystatin C can improve the diagnostic ability of early preeclampsia.

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Conflict interest

The authors declare no conflict of interest.

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