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Differential expression of GPR30 in preeclampsia placenta tissue and normal placenta tissue and its clinical significance

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ABSTRACT

Objective: To study the differential expression of GPR30 in preeclampsia placenta tissue and normal placenta tissue and its clinical significance. **Methods:** Preeclampsia placenta tissue and normal placenta tissue were collected and GPR30 expression levels were detected; human umbilical vein endothelial cells were cultured and processed with GRP30 inhibitor and GRP30 agonist combined with hypoxia-reoxygenation respectively, and cell apoptosis as well as pro-angiogenesis molecule and apoptosis molecule contents were detected. **Results:** mRNA content and protein content of GRP30 in preeclampsia placenta tissue were significantly lower than those in normal placenta tissue; apoptosis rate of G15 group was significantly higher than that of control group, VEGF and bFGF contents in supernatant were significantly lower than those of control group, and mRNA contents of *Bax*, *Caspase−3* and *Caspase−9* in cells were significantly higher than those of control group; apoptosis rate of H/R group was significantly higher than that of control group, VEGF and bFGF contents in supernatant were significantly lower than those of control group, and mRNA contents of *Bax*, *Caspase−3* and *Caspase−9* in cells were significantly higher than those of control group; apoptosis rate of G1 group was significantly lower than that of H/R group, VEGF and bFGF contents in supernatant were significantly higher than those of H/R group, and mRNA contents of *Bax*, *Caspase−3* and *Caspase−9* in cells were significantly lower than those of H/R group. **Conclusions:** Low expression of GPR30 in placenta tissue is closely associated with the occurrence of preeclampsia, enhancing GPR function can reduce endothelial cell apoptosis and increase the contents of pro-angiogenesis factors, and it has endothelial protection effect.

1. Introduction

Preeclampsia is an idiopathic disease during pregnancy that causes serious harm to both maternal and fetal health and safety. With the end of pregnancy and the delivery of placenta and fetus, symptoms of both hypertension and proteinuria should disappear, and the main means of clinical treatment of the disease is symptomatic treatment, maintenance of pregnancy and maximum protection of maternal and fetal safety. However, for patients with more serious symptoms and unable to maintain pregnancy, conventional symptomatic treatment is invalid and pregnancy has to be terminated[1]. At present, the pathogenesis of preeclampsia is still to be elucidated and the disease is also short of specific treatment methods. According to existing study, endothelial dysfunction is an important link in the pathogenesis of preeclampsia and how to reduce endothelial dysfunction and protect endothelial function has become an important topic in the treatment of preeclampsia[2]. G protein coupled receptor 30 (GPR30) is a G protein coupled receptor newly discovered in recent years, and it has regulating effect on cardiovascular system and endothelial function in tumor tissue[3,4]. In the following research, the differential expression of GPR30 in preeclampsia placenta tissue and normal placenta tissue and its clinical significance were analyzed.

2. Materials and methods

2.1. Enrolled subjects

Subjects enrolled in the research included 30 cases of pregnant women with preeclampsia and 30 cases of healthy pregnant women, and pregnant women with preeclampsia had hypertension and proteinuria and met basic diagnostic criteria; healthy pregnant women were excluded of pregnancy complications through prenatal examination. Pregnant women with preeclampsia were (28.49±3.32)

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years old and BMI was (24.33±2.85) kg/m^2; healthy pregnant women were 2(8.81±2.99) years old and BMI was (24.19±2.49) kg/m^2. Comparison of general information between two groups showed no differences.

2.2. Research materials

Human umbilical vein endothelial cells (HUVEC) were purchased from the ATCC cell bank, GPR30 agonist G1 and GPR30 inhibitor G15 were purchased from Sigma Company, RNA extraction and detection kits were from Beijing Tiangen Company, and antibodies for Western-blot were purchased from Abcam Company.

2.3. Research methods

2.3.1. Placenta tissue collecting and detecting methods

After delivery, appropriate amount of placenta tissue was cut, cleaned with plenty of normal saline and then divided into two, one was used for RNA extraction and PCR amplification and the other was used for protein extraction and Western-blot detection. PCR amplification curve and Western-blot protein bands were obtained, and then mRNA contents and protein contents were calculated.

2.3.2. Cell culturing and processing methods

Human umbilical vein endothelial cells (HUVEC) were recovered, cultured with high glucose DMEM medium, amplified, then seeded in cell plate and processed with different drugs. Processing methods of GPR30 agonist were as follows: cells were processed with 0 μmol/L and 10.0 μmol/L of G1 respectively, placed in hypoxic environment (5%CO_2 and 95%N_2) and cultured for 4 h, and then transferred to normoxic environment (21%O_2 and 5%CO_2) and cultured for 18h; processing methods of GPR30 inhibitor were as follows: cells were processed with 0 μmol/L and 10.0 μmol/L of G15 respectively and continuously cultured for 24 h.

2.3.3. Detecting methods of related indexes in cells

After drug processing, cells and cell supernatant were collected. Cell specimens were taken, Annexin-V-FITC and PI kits were used for staining, and then flow cytometer was used to analyze apoptosis rate; RNA extraction kits were used to extract RNA, and after PCR amplification, mRNA contents were calculated. Supernatant specimens were taken, and ELISA kits were used to detect VEGF and bFGF contents.

2.3.4. Statistical methods

SPSS22.0 software was used to input data, comparison of measurement data between two groups was by *t* test and comparison of measurement data among groups was by *chi*-square test. Differences were considered to be statistically significant at a level of $P< 0.05$.

3. Results

3.1. GPR30 expression levels in placenta tissue

Analysis of mRNA contents of *GRP30* in preeclampsia placenta tissue and normal placenta tissue was as follows: mRNA content of *GRP30* in preeclampsia placenta tissue was significantly lower than that in normal placenta tissue; analysis of protein contents of GRP30 in preeclampsia placenta tissue and normal placenta tissue was as follows: protein content of GRP30 in preeclampsia placenta tissue was significantly lower than that in normal placenta tissue, shown in Figure 1.

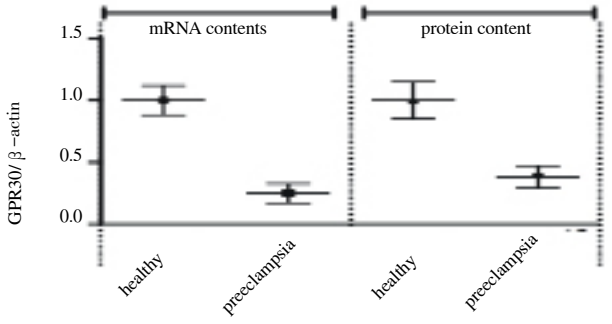

Figure 1. GPR30 expression levels in preeclampsia placenta tissue and normal placenta tissue.

3.2. Effect of GRP30 agonist and inhibitor on HUVEC apoptosis rate

Analysis of the effect of GRP30 inhibitor on HUVEC apoptosis rate was as follows: after GRP30 inhibitor G15 processing, HUVEC apoptosis rate was significantly higher than that of control group, shown in Figure 2A. Analysis of the effect of GRP30 agonist on HUVEC apoptosis rate was as follows: HUVEC apoptosis rate of H/R group was significantly higher than that of control group, and after GRP30 agonist G1 processing, HUVEC apoptosis rate was significantly lower than that of H/R group, shown in Figure 2B.

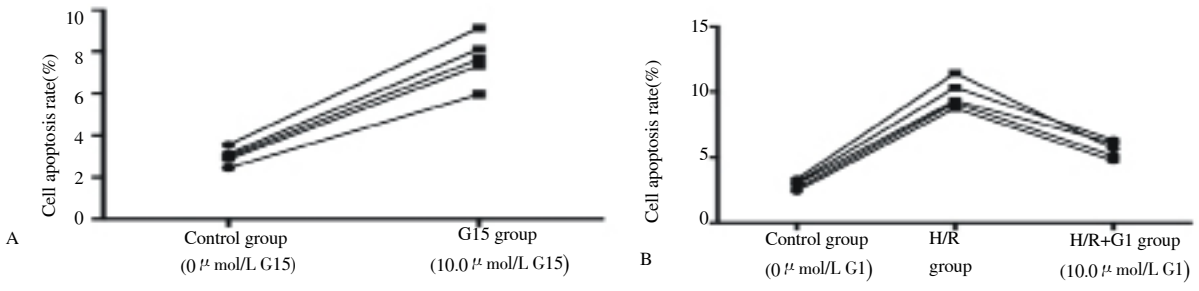

Figure 2. Effect of GRP30 agonist and inhibitor on HUVEC apoptosis rate.

A: GRP30 inhibitor G15 processing; B: GRP30 agonist G1 combined with H/R processing.

3.3. Effect of GRP30 agonist on angiogenesis molecule and apoptosis molecule contents

Analysis of the effect of GRP30 agonist on angiogenesis molecule contents was as follows: VEGF and bFGF contents in cell supernatant of H/R group were lower than those of control group, and after GRP30 agonist G1 processing, VEGF and bFGF contents in cell supernatant were higher than those of H/R group; analysis of the effect of GRP30 agonist on apoptosis molecule expression was as follows: mRNA contents of *Bax, Caspase-3* and *Caspase-9* in cells of H/R group were significantly higher than those of control group, and after GRP30 agonist G1 processing, mRNA contents of *Bax, Caspase-3* and *Caspase-9* in cells were lower than those of H/R group, shown in Table 1.

3.4. Effect of GRP30 inhibitor on angiogenesis molecule and apoptosis molecule contents

Analysis of the effect of GRP30 inhibitor on angiogenesis molecule contents was as follows: after GRP30 inhibitor G15 processing, VEGF and bFGF contents in cell supernatant were lower than those of control group; analysis of the effect of GRP30 inhibitor on apoptosis molecule expression was as follows: after GRP30 inhibitor G15 processing, mRNA contents of *Bax, Caspase-3* and *Caspase-9* in cells were higher than those of control group, shown in Table 2.

Table 1

Effect of GRP30 agonist on angiogenesis molecule and apoptosis molecule contents.

	Angiogenesis molecules in supernatant		Apoptosis molecules in cells		
Groups	VEGF (pg/mL)	bFGF (pg/mL)	Bax (/ β -actin)	Caspase-3 (/ β -actin)	Caspase-9 (/ β -actin)
Control group	47.59±5.41	103.33±13.56	1.00±0.12	1.00±0.09	1.00±0.13
H/R group	20.32±2.52	42.48±5.49	2.32±0.26	1.97±0.22	2.77±0.32
G1 group	36.65±4.11	79.41±8.49	1.45±0.17	1.22±0.13	1.61±0.19
F	11.382	8.494	13.328	7.235	9.449
P	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2

Effect of GRP30 inhibitor on angiogenesis molecule and apoptosis molecule contents.

Groups	Angiogenesis molecules in supernatant		Apoptosis molecules in cells		
	VEGF (pg/mL)	bFGF (pg/mL)	Bax (/ β -actin)	Caspase-3 (/ β -actin)	Caspase-9 (/ β -actin)
Control group	52.14±5.77	106.14±12.78	1.00±0.13	1.00±0.11	1.00±0.12
G15 group	28.22±2.92	62.47±7.92	1.79±0.20	1.91±0.26	1.68±0.18
t	8.922	7.338	9.182	9.833	6.492
P	<0.05	<0.05	<0.05	<0.05	<0.05

4. Discussion

At present, the pathogenesis of preeclampsia has not been fully elucidated, and therefore there are no specific means and methods of treating the disease. Systemic arteriolar spasm and abnormal placental hemodynamics are important pathological changes in patients with preeclampsia. Abnormal placental hemodynamics and local tissue ischemia will aggravate systemic arteriolar spasm and lead to maternal high blood pressure [5]; maternal high blood pressure will compensatory-increase placental perfusion and cause ischemia-reperfusion, causing oxidative stress injury of vascular endothelial cells and abnormal completion of angiogenesis process in placenta, and ultimately causing abnormal development of placental vascular bed and occurrence of corresponding symptoms and signs of preeclampsia [6,7].

Based on the above understanding, human umbilical vein endothelial cells (HUVEC) were taken as research subjects of the research, and the processing of hypoxia-reoxygenation (H/R) was adopted to simulate endothelial injury process in patients with preeclampsia. After hypoxia-reoxygenation processing, HUVEC apoptosis rate was detected to reflect the degree of endothelial cell injury, and analysis results showed that apoptosis rate of H/R group was significantly higher than that of control group. It indicated that hypoxia-reoxygenation processing could induce endothelial cell injury, thus successfully simulating endothelial injury in patients with preeclampsia. After endothelial cell hypoxia-reoxygenation injury model was established, it was used to explore the pathogenesis of preeclampsia and seek the molecules that played important roles in the development process.

G protein coupled receptor 30 (GPR30) is an estrogen receptor newly discovered in recent years, study has confirmed that GPR30 is largely expressed in vascular endothelial cells, and abnormal expression of it is closely related to the occurrence and development of a variety of cardiovascular diseases and tumor diseases[8]. The receptor is membrane receptor, consists of 375 amino acids and is with typical 7-transmembrane, and combination with corresponding ligand can activate signaling pathway mediated by downstream kinases such as ERK and MAPK, and thereby cause rapid non-genomic effect[9]. Related study about essential hypertension confirms that decreased expression of GPR30 in vascular endothelial cells will cause endothelial contraction and vasospasm[10]; related studies of malignant tumors confirm that increased expression of GPR30 in vascular endothelial cells will cause increased new blood vessels[11,12]. Above studies indicate that abnormal expression of GPR30 is closely associated with endothelial cell function, and

therefore it was speculated that the receptor might be involved in the occurrence and development of preeclampsia.

In order to clarify whether GPR30 was associated with the occurrence and development of preeclampsia, GRP30 expression levels in preeclampsia placenta tissue and normal placenta tissue were detected at first, and results showed that mRNA content and protein content of GRP30 in preeclampsia placenta tissue were significantly lower than those in normal placenta tissue. It indicated that low expression of GRP30 was involved in the occurrence of preeclampsia. As has been noted, endothelial function injury is the key link of the occurrence of preeclampsia and GPR30 has regulating effect on endothelial cell function. After GPR30 inhibitor G15 was used to process conventionally cultured HUVEC, it was observed that G15 could significantly increase cell apoptosis rate; after GPR30 agonist G1 was used to process HUVEC after hypoxia-reoxygenation, it was observed that G1 could significantly decrease cell apoptosis rate. It indicated that inhibiting GPR30 function could induce HUVEC apoptosis and enhancing GPR30 function could reduce HUVEC apoptosis caused by hypoxia-reoxygenation.

GPR30 is G protein coupled receptor located on cell membrane, and it can regulate the expression of a variety of molecules through signaling pathway mediated by downstream kinases such as ERK and MAPK. In the case of endothelial cells, pro-angiogenesis molecules VEGF and bFGF that are synthesized by them have definite endothelial protection function, and can promote endothelial cell proliferation and migration and induce the formation of local new blood vessels[13,14]. In the research, analysis of pro-angiogenesis molecule contents in HUVEC showed that GPR30 inhibitor G15 could decrease VEGF and bFGF contents in supernatant while GPR30 agonist G1 could increase VEGF and bFGF contents in supernatant of hypoxia-reoxygenation HUVEC. Endothelial cell apoptosis is mainly mediated by Bax, Caspase-3, Caspase-9 and other molecules[15,16], analysis in the research showed that GPR30 inhibitor G15 could increase Bax, Caspase-3 and Caspase-9 contents in cells while GPR30 agonist G1 could decrease Bax, Caspase-3 and Caspase-9 contents in hypoxia-reoxygenation HUVEC.

To sum up, low expression of GPR30 in placenta tissue is closely associated with the occurrence of preeclampsia, enhancing GPR function can reduce endothelial cell apoptosis and increase the contents of pro-angiogenesis factors, and it has endothelial protection effect.

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