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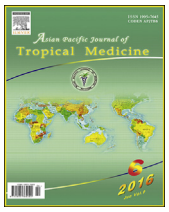

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Effect of hypoxia on expression of placental trophoblast cells SATB1 and β-catenin and its correlation with the pathogenesis of preeclampsia

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ABSTRACT

Objective: To study the effect of hypoxia on the expression of placental trophoblast cells SATB1 and β-catenin and its correlation with the pathogenesis of preeclampsia.
Methods: Trophoblastic cell lines HRT8/SVneo were cultured, SATB1 and β-catenin expression and cell biological behavior were determined after hypoxia reoxygenation treatment; cell biological behavior and the expression of related genes were determined after the transfection of SATB1 and β-catenin siRNA; preeclampsia placenta and normal placenta tissues were collected and the expression of SATB1 and β-catenin were determined.
Results: OD value, cell migration rate, mRNA contents of SATB1 and β-catenin of H/R group were significantly lower than those of Nor group, cell apoptosis rate was higher than that of Nor group and the number of invasive cells was less than that of Nor group; OD value and bcl-2 mRNA content of SATB1-siRNA group were lower than those of NC group; cell apoptosis rate as well as Bax, Caspase-3, Caspase-6 and Caspase-9 mRNA contents were higher than those of NC group; cell migration rate as well as CTSB, CTSD, MMP2 and MMP9 mRNA contents of β-catenin-siRNA group were lower than those of NC group; the number of invasive cells was less than that of NC group; the expression levels of SATB1 and β-catenin in preeclampsia placenta tissue were significantly lower than those in normal placenta tissue.
Conclusions: Hypoxia can inhibit the expression of SATB1 and β-catenin in the pathogenesis of preeclampsia, which can affect the proliferation, apoptosis, migration and invasion of cells.

1. Introduction

Preeclampsia is the idiopathic disease during pregnancy, and is still one of the leading causes of maternal and perinatal death in our country. At present, the etiology and pathogenesis of preeclampsia have not been elucidated. Studies have confirmed that the placenta hypoxia is an important characteristic of preeclampsia women. Local hypoxia, excessive cell apoptosis, inadequate invasion and other pathological characteristics influence each other [1–3]. However, it is not yet clear which molecules are involved in the mutual influence between placenta hypoxia and cell apoptosis, invasion as well as other biological behavior. Special AT-rich sequence binding protein 1 (SATB1) is a high-level organizer of chromatin structure, β-catenin is the key protein of Wnt/β-catenin pathway, and two molecules have a regulatory effect on the expression of multiple genes, which affects the cell biological function [4,5]. In this research, the effect of hypoxia on the expression of placental trophoblast cells SATB1 and β-catenin and its correlation with the pathogenesis of preeclampsia were analyzed.

2. Materials and methods

2.1. Placenta origin and information

Placenta samples were from 35 cases of preeclampsia women and 40 cases of normal pregnant women who labored in

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Obstetrics Department of our hospital from May 2012 to August 2015. Preeclampsia women met the diagnostic criteria for the disease, (31 ± 4) years old, (37.4 ± 4.1) gestational weeks, BMI (25.1 ± 2.9) kg/m^2, systolic pressure (158.3 ± 16.4) mmHg, diastolic pressure (98.6 ± 10.2) mmHg, and urine protein (0.782 ± 0.104) mg; normal pregnant women were excluded of pregnant diseases, (30 ± 4) years old, (37.1 ± 3.8) gestational weeks, BMI (24.7 ± 2.5) kg/m^2, systolic pressure (126.6 ± 13.9) mmHg, diastolic pressure (83.4 ± 9.4) mmHg, and urine protein (0.084 ± 0.010) mg. The age, gestational weeks, BMI and other information have no difference between preeclampsia women and normal pregnant women.

2.2. Experimental materials

Trophoblastic cell lines HRT8/SVneo were purchased from the cell bank of Chinese Academy of Sciences, high-glucose DMEM, fetal bovine serum and trypsin for cell culture were purchased from Gibco Company, SATB1, β-catenin, negative control (NC) siRNA and transfection reagents were synthesized by Shanghai GenePharma Company, cell viability MTS kits were purchased from Promega Company, cell apoptosis kits were purchased from BD Company, and RNA extraction and PCR detection kits were purchased from Beijing Tiangen Company.

2.3. Experimental methods

2.3.1. Placenta tissue collecting

Within 5 min after delivery of placenta, placenta tissues were collected and cut into tissue blocks of 1 cm × 1 cm × 1 cm, and after cleaned of blood with normal saline, tissue blocks were placed in sterile and enzyme-deactivated freezing tubes, rapidly frozen in liquid nitrogen tank for half an hour and then transferred into −80 °C refrigerator.

2.3.2. Cell culturing and processing

HRT8/SVneo cell lines were cultured with high-glucose medium containing 10% fetal bovine serum, and hypoxia/reoxygenation (H/R) processing was as follows: cells were processed in 95% N_2–5% CO_2 incubator for 8 h of hypoxia processing, and then put back in 95% air–5% CO_2 incubator for 16 h of normoxia processing, repeating it once; siRNA transfection was as follows: cells cultured in normoxia condition were collected, and SATB1, β-catenin, NC siRNA was mixed with transfection reagents according to appropriate proportion and added in the medium for 24 h of continuous processing.

2.3.3. Cell behavior detecting

Cell proliferation detection was as follows: cells were inoculated in 96-hole cell plate, and MTS reagents were added for staining after H/R processing or siRNA transfection, and absorbance (OD value) at 490 nm wavelength was read from microplate reader.

Cell apoptosis detection was as follows: cells were inoculated in 24-hole cell plate, and after H/R processing or siRNA transfection, cells were digested with trypsin, collected and stained with Annexin V/PI kits, and the proportion of apoptotic cells was detected in flow cytometer.

Cell migration detection was as follows: cells were inoculated in 12-hole cell plate, a scratch was marked in the middle of cell plate, photographed and recorded before H/R processing or siRNA transfection, it was photographed again after H/R processing or siRNA transfection, and scratch area and migration rate were calculated.

Cell invasion detection was as follows: cells were inoculated in Transwell chamber, high-glucose medium containing 10% fetal bovine serum was added in the lower layer for induction, unpenetrated cells in the upper layer were removed after H/R processing or siRNA transfection, and after staining, the number of cells in 5 high power fields (×400) was observed under microscope.

2.3.4. Gene expression detecting

mRNA content detecting: Cells were inoculated in 12-hole cell plate, culture medium was discarded after H/R processing or siRNA transfection, and RNA extraction kits were used to extract total RNA in cells and reverse-transcribe it to get cDNA in cells processed with different conditions; placenta tissue samples were collected and grinded, and then RNA extraction kits were used to extract total RNA in tissues and reverse-transcribe it to get cDNA in placenta; cDNA samples were collected and PCR detection kits were used to detect the contents of SATB1, β-catenin, Bcl-2, Bax, Caspase-3, Caspase-6, Caspase-9, CTSB, CTSD, MMP2 and MMP9. Placenta tissue samples were collected, grinded and then centrifuged to get protein suspension, and Elisa kits were used to detect the contents of SATB1 and β-catenin.

2.4. Statistical methods

SPSS23.0 software was used to input and analyze data, comparison between two groups was by t test, and differences were considered to be statistically significant at the level of $P < 0.05$.

3. Results

3.1. Effect of normoxia and hypoxia/reoxygenation on placental trophoblast cell proliferation, apoptosis, migration and invasion

OD value and cell migration rate of H/R group were significantly lower than those of Nor group, cell apoptosis rate was higher than that of Nor group and the number of cells invading to under Transwell chamber membrane was less than that of Nor group (Table 1).

Table 1
Cell proliferation, apoptosis, migration and invasion of Nor group H/R group.

Groups	OD value	Apoptosis rate (%)	Migration rate (%)	Invasive number
Nor group	0.982 ± 0.103	6.14 ± 0.77	78.34 ± 9.27	29.58 ± 3.23
H/R group	0.477 ± 0.058	15.45 ± 1.78	41.38 ± 5.48	14.47 ± 1.77
T	10.374	14.172	8.978	11.038
P	<0.05	<0.05	<0.05	<0.05

3.2. mRNA expression levels of SATB1 and β-catenin in placental trophoblast cells under normoxia and hypoxia/reoxygenation

mRNA contents of SATB1 and β-catenin of H/R group were significantly lower than those of Nor group (Table 2).

3.3. Effect of siRNA inhibition of SATB1 on placental trophoblast cell proliferation and apoptosis under normoxia

OD value and bcl-2 mRNA content of SATB1-siRNA group were lower than those of NC group, and cell apoptosis rate as well as Bax, Caspase-3, Caspase-6 and Caspase-9 mRNA contents were higher than those of NC group (Table 3).

3.4. Effect of siRNA inhibition of β-catenin on placental trophoblast cell migration and invasion under normoxia

Cell migration rate as well as CTSB, CTSD, MMP2 and MMP9 mRNA contents of β-catenin-siRNA group were lower than those of NC group, and the number of invasive cells was less than that of NC group (Table 4).

3.5. SATB1 and β-catenin expression levels in preeclampsia and normal placenta tissues

mRNA content and protein content of SATB1 as well as mRNA content and protein content of β-catenin in preeclampsia placenta tissue were significantly lower than those in normal placenta tissue (Table 5).

Table 2
Expression levels of SATB1 and β-catenin in cells of Nor group H/R group.

Groups	SATB1	β-Catenin
Nor group	1.000 ± 0.108	1.000 ± 0.114
H/R group	0.372 ± 0.048	0.315 ± 0.037
T	15.866	17.752
P	<0.05	<0.05

Table 5
SATB1 and β-catenin expression levels in preeclampsia and normal placenta tissues.

Groups	mRNA contents		Protein contents (μg/mg protein)	
	SATB1/β-actin	β-Catenin/β-actin	SATB1	β-Catenin
Preeclampsia placenta	0.35 ± 0.06	0.42 ± 0.07	16.65 ± 1.93	31.35 ± 4.46
Normal placenta	1.00 ± 0.12	1.00 ± 0.18	32.52 ± 4.73	54.57 ± 6.73
T	20.385	15.857	9.283	7.686
P	<0.05	<0.05	<0.05	<0.05

4. Discussion

Placenta is the fetal subsidiary organ during pregnancy, and its structure and function have adjusting effect on maternal adaptive response, material-fetal exchange, pregnancy maintenance and labor. Trophoblast cells are the most important functioning cells in the placenta. In the process of normal pregnancy, trophoblast cells can invade the muscular layer of uterine spiral arteries and the decidua layer of uterine, and cause spiral artery reconstruction, lumen expansion, decreased resistance and increased blood flow. But in the pathologic process of preeclampsia, uterine spiral artery reconstruction is significantly insufficient. The blood vessels are still in a state of high resistance and low capacity, and blood flow in placental tissue is insufficient and in hypoxia condition [6–8]. In recent years, a growing number of studies have confirmed that the placenta tissue ischemia and hypoxia are important pathologic characteristics of preeclampsia [9,10]. In this research, *in vitro* hypoxia/reoxygenation conditions were used to process trophoblast cells to simulate the pathological condition of hypoxia in preeclampsia placenta tissue. And analysis of the biological characteristics of cells showed that cell viability OD value of H/R group was significantly lower than that of Nor group; cell apoptosis rate was higher than that of Nor group;

Table 3
Placental trophoblast cell proliferation and apoptosis after siRNA inhibition of SATB1.

Groups	Proliferation and apoptosis		mRNA contents of related genes				
	OD value	Apoptosis rate (%)	Bcl-2/β-actin	Bax/β-actin	Caspase-3/β-actin	Caspase-6/β-actin	Caspase-9/β-actin
SATB1-siRNA	0.338 ± 0.041	11.36 ± 1.26	0.42 ± 0.06	2.13 ± 0.24	2.56 ± 0.28	1.93 ± 0.20	2.71 ± 0.29
NC-siRNA	0.762 ± 0.093	5.29 ± 0.65	1.00 ± 0.13	1.00 ± 0.11	1.00 ± 0.15	1.00 ± 0.09	1.00 ± 0.08
T	12.771	11.784	13.474	10.083	17.484	9.282	16.586
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 4
Placental trophoblast cell migration and invasion after siRNA inhibition of β-catenin.

Groups	Migration and invasion		mRNA contents of related genes			
	Migration rate (%)	Invasive number	CTSB/β-actin	CTSD/β-actin	MMP2/β-actin	MMP9/β-actin
SATB1-siRNA	49.67 ± 5.12	17.66 ± 2.13	0.37 ± 0.05	0.44 ± 0.06	0.29 ± 0.04	0.56 ± 0.08
NC-siRNA	82.21 ± 9.35	28.84 ± 3.26	1.00 ± 0.12	1.00 ± 0.18	1.00 ± 0.16	1.00 ± 0.09
T	8.968	7.382	17.587	12.575	23.185	9.384
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

cell migration rate was lower than that of Nor group; and the number of cells invading to under Transwell chamber membrane was less than that of Nor group. It indicated that there were pathological conditions of inhibited proliferation, excessive apoptosis as well as weakened migration and invasion in trophoblast cells under hypoxia conditions.

Special AT-rich sequence binding protein 1 (SATB1) is also known as matrix attachment region binding protein 1. It can target recognize and combine with the AT sequence on DNA matrix attachment region, thus recruiting auxiliary activator protein or auxiliary repressor protein to regulate gene expression [11,12]. β-Catenin is the core molecule of the Wnt/β-catenin signaling pathway. The activation of Wnt molecules can inhibit the degradation of β-catenin and make β-catenin gathered in the cytoplasm. Translocate into the nucleus at the same time, and regulate the expression of a variety of downstream gene [13,14]. Studies have confirmed that in the process of pregnancy, SATB1 and β-catenin expression levels in placental tissue show dynamic change and have regulating effect on the function of trophoblast cells and the maintenance of pregnancy [15,16]. In the research, analysis of the effect of hypoxia/reoxygenation conditions on trophoblast cells SATB1 and β-catenin expression levels showed that mRNA contents of SATB1 and β-catenin of H/R group were significantly lower than those of Nor group. It indicated that placental trophoblast cell hypoxia would inhibit the expression of SATB1 and β-catenin, which might affect cell function to be involved in the occurrence of preeclampsia.

In order to further confirm whether the change of SATB1 and β-catenin expression levels would affect the function of trophoblast cells, siRNA transfection was adopted to suppress SATB1 and β-catenin expression in trophoblast cells. Then cell proliferation, apoptosis, migration and invasion were analyzed. Studies have confirmed that SATB1 participates in the regulation of tumor cell proliferation and apoptosis. And β-catenin is mainly associated with epithelial–mesenchymal transition and invasive growth of tumor cells. After SiRNA transfection inhibited SATB1 expression, trophoblast cell OD value significantly decreased and apoptosis rate increased significantly; and after β-catenin expression was inhibited, trophoblast cell migration rate decreased significantly and the invasive number significantly decreased. Thus it confirmed that SATB1 could regulate the proliferation and apoptosis of trophoblast cells; β-catenin could regulate the migration and invasion of trophoblast cells.

Both SATB1 and β-catenin are able to be combined with DNA sequences to regulate gene expression. Cell proliferation, apoptosis, migration and invasion processes are regulated by multiple genes [17,18]. In the occurrence and development of preeclampsia, abnormal expression of Bcl-2/Bax, Caspase-3, Caspase-6 and Caspase-9 are associated with trophoblast cell apoptosis [19]. The abnormal expression of matrix metalloproteinases MMP2 and MMP9, as well as cathepsin CTSB and CTSD are associated with the shallow implantation of trophoblast cells [20]. Bcl-2 and Bax keep constant proportion, Bcl-2 is with apoptosis inhibition effect while Bax can antagonize the antiapoptotic effect of Bcl-2. Decreased Bcl-2 content and increased Bax content can cause Caspase cascade and lead to apoptosis [21,22]. MMPs and CTS can degrade a variety of components in extracellular matrix, which are key molecules mediating trophoblast cell migration and infiltration to decidua layer and spiral artery muscle layer, while insufficient MMPs and CTSs expression can cause insufficient trophoblast cell invasion [23–26].

In the research, the analysis of the expression levels of proliferation, apoptosis, migration and invasion-related genes in cells after siRNA transfection showed that bcl-2 mRNA content of SATB1-siRNA group was lower than that of NC group, and Bax, Caspase-3, Caspase-6 and Caspase-9 mRNA contents were higher than those of NC group; CTSB, CTSD, MMP2 and MMP9 mRNA contents of β-catenin-siRNA group were significantly lower than those of NC group. It confirmed that SATB1 could regulate the expression of Bcl-2/Bax, Caspase-3, Caspase-6 and Caspase-9 in trophoblast cells, thereby affecting cell proliferation and apoptosis; β-catenin could regulate the expression of CTSB, CTSD, MMP2 and MMP9 in trophoblast cells, thereby affecting cell migration and invasion. At last, the expression levels of SATB1 and β-catenin were verified in preeclampsia placenta tissue, and results showed that the expression levels of SATB1 and β-catenin in preeclampsia placenta tissue were significantly lower than those in normal placenta tissue.

To sum up, hypoxia can inhibit the expression of SATB1 and β-catenin in the pathogenesis of preeclampsia, which can affect the proliferation, apoptosis, migration and invasion of cells.

Conflict of interest statement

We declare that we have no conflict of interest.

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