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Contents of Wnt/β−catenin signaling pathway−related molecules in the preeclampsia placenta tissue and their correlation with the trophocyte apoptosis

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ABSTRACT

Objective: To study the contents of Wnt/β-catenin signaling pathway-related molecules in the preeclampsia placenta tissue and their correlation with the trophocyte apoptosis. **Methods:** A total of 56 patients who were diagnosed with preeclampsia in Ganzhou People's Hospital between March 2015 and March 2017 were selected as the preeclampsia group (PE group) of the research, and 70 healthy puerperae who gave birth in the hospital during the same period was selected as the control group of the research. After childbirth, the placenta was collected to test the contents of Wnt/β-catenin signaling pathway-related molecules, oxidative stress molecules, apoptosis molecules and cell invasion molecules. **Results:** Wnt1, Wnt3a, β-catenin, XIAP, MMP1, MMP2 and Vimentin contents in placenta tissue of PE group were significantly lower than those of control group while MDA, ox-LDL, 8-iso-PG, AOPP, Caspase-3, Caspase-9, TIMP1, TIMP2 and E-cadherin contents were significantly higher than those of control group; Wnt1, Wnt3a and β-catenin contents in placental tissues of PE group were positively correlated with the XIAP, MMP1, MMP2 and Vimentin contents, and negatively correlated with MDA, ox-LDL, 8-iso-PG, AOPP, Caspase-3, Caspase-9, TIMP1, TIMP2 and E-cadherin contents. **Conclusion:** The Wnt/β-catenin signaling pathway function is significantly inhibitedin preeclampsia placenta tissue and associated with the trophocyte apoptosis induced by oxidative stress.

1. Introduction

Preeclampsia (PE) is a common complication during pregnancy, which is mainly clincially characterized by hypertension and proteinuria, and will increase maternal and perinatal mortality. Excessive placenta trophocyte apoptosis is an important pathological feature of preeclampsia, oxidative stress reaction activation and increased oxygen free radical formation have starting effects on apoptosis, and excessive apoptosis will affect cell invasion and lead to shallow placenta implantation[1,2]. At present, the regulation mechanism of apoptosis in preeclampsia placenta is not clear. Wnt/β-catenin is an important signaling pathway in the cells, the activation of Wnt1, Wnt3a and other upstream molecules can cause intracellular β-catenin to accumulate and transfer into the nucleus, which regulates cell apoptosis, invasion, and oxidative stress reaction[3]. In vitro study shows that activation of Wnt/beta-catenin signaling pathway has protective effect on the hypoxia-reoxygenation injury of trophocytes[4]. In the following studies, we analyzed the correlation between Wnt/beta-catenin signaling pathway-related molecule levels in preeclampsia placenta and trophocyte apoptosis.

2. Case information and research methods

2.1 General case information

A total of 56 patients who were diagnosed with preeclampsia in Ganzhou People's Hospital between March 2015 and March 2017 were selected as the preeclampsia group (PE group) of the research, all patients were in accordance with the diagnostic criteria for preeclampsia, and patients combined with pre-pregnant

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hypertension were eliminated. 70 healthy puerperae who gave birth in the hospital during the same period were selected as the control group of the research, and all puerperae were without preeclampsia, gestational diabetes, intrahepatic cholestasis and other pregnancy complications. PE group were 23-34 years old, 32 cases were primiparae and 24 cases were multiparae; control group were 22-35 years old, 38 cases were primiparae and 32 cases were multiparae. There was no significant difference in general information between the two groups (P>0.05).

2.2 Research methods

2.2.1 Placenta sample collection

Within 30 min after the delivery of placenta, proper amount of placental villus tissue was collected near the central part, washed with saline to clean the residual blood after large vessels and calcification zone were removed, frozen in the liquid nitrogen for 20-30 min, then taken out and stored in -70 ℃ cryogenic refrigerator.

2.2.2 Molecule content detection

Placenta samples were taken, added in PBS and homogenized, the homogenate was centrifuged in the centrifuge for 10 min at a speed of 12 000 r/min to get the supernatant fluid, enzyme-linked immunosorbent assay kit was used to determine Wnt1, Wnt3a, β-catenin, XIAP, Caspase-3, Caspase-9, MMP1, MMP2, TIMP1, TIMP2, E-cadherin and Vimentin contents, and the radioimmunoprecipitation kit was used to determine the contents of MDA, ox-LDL, 8-iso-PG and AOPP.

2.3 Statistical methods

SPSS 21.0 software was used to input data, differences in molecule contents between two groups were analyzed by t test, the correlation between two data was by Pearson test and P<0.05 was the standard of statistical significance in differences in test results.

3. Results

3.1 Wnt/β-catenin signaling pathway-related molecule contents in placenta

Analysis of Wnt/β-catenin signaling pathway-related molecules Wnt1 (ng/mL), Wnt3a (ng/mL) and β-catenin (pg/mL) contents in placenta tissue between PE group and control group was as follows: Wnt1, Wnt3a and β-catenin contents in placenta tissue of PE group were significantly lower than those of control group. Differences in Wnt1, Wnt3a and β-catenin contents in placenta tissue were statistically significant between the two groups (P<0.05).

Table 1.

Comparison of Wnt/β-catenin signaling pathway-related molecules in placenta.

Groups	n	Wnt1	Wnt3a	β-catenin
PE group	56	1.95±0.24	1.02±0.15	98.55±10.82
Control group	70	3.36±0.49	2.78±0.35	264.52±37.29
T		12.859	16.029	15.486
P		<0.05	<0.05	<0.05

3.2 Oxidative stress molecule contents in placenta

Analysis of oxidative stress molecules MDA (nmol/mL), ox-LDL (ng/mL), 8-iso-PG (ng/mL) and AOPP (nmol/mL) contents in placenta tissue between PE group and control group was as follows: MDA, ox-LDL, 8-iso-PG and AOPP contents in placenta tissue of PE group were significantly higher than those of control group. Differences in MDA, ox-LDL, 8-iso-PG and AOPP contents in placenta tissue were statistically significant between the two groups (P<0.05). Pearson correlation analysis showed that MDA, ox-LDL, 8-iso-PG and AOPP contents in PE placenta were negatively correlated with Wnt1, Wnt3a and β-catenin contents.

Table 2.

Comparison of oxidative stress molecules in placenta.

Groups	n	MDA	Ox-LDL	8-iso-PG	AOPP
PE group	56	5.36±0.73	6.52±0.79	1.98±0.22	11.25±1.36
Control group	70	2.21±0.32	2.76±0.35	1.03±0.14	4.95±0.67
T		12.894	13.427	9.285	15.429
P		<0.05	<0.05	<0.05	<0.05

3.3 Trophocyte apoptosis molecule contents in placenta

Analysis of trophocyte apoptosis molecules XIAP (ng/mL), Caspase-3 (pg/mL) and Caspase-9 (pg/mL) contents in placenta tissue between PE group and control group was as follows: XIAP content in placenta tissue of PE group was significantly lower than that of control group while Caspase-3 and Caspase-9 contents were significantly higher than those of control group. Differences in XIAP, Caspase-3 and Caspase-9 contents in placenta tissue were statistically significant between the two groups (P<0.05). Pearson correlation analysis showed that XIAP content in PE placenta was positively correlated with Wnt1, Wnt3a and β-catenin contents while Caspase-3 and Caspase-9 contents were negatively correlated with Wnt1, Wnt3a and β-catenin contents.

Table 3.

Comparison of trophocyte apoptosis molecules in placenta.

Groups	n	XIAP	Caspase-3	Caspase-9
PE group	56	2.32±0.35	215.65±33.41	175.52±20.35
Control group	70	6.61±0.79	93.46±10.39	75.62±9.35
T		18.389	12.672	13.029
P		<0.05	<0.05	<0.05

3.4. Trophocyte invasion molecule contents in placenta

Analysis of trophocyte invasion molecules MMP1 (ng/mL), MMP2 (ng/mL), TIMP1 (ng/mL), TIMP2 (ng/mL), E-cadherin (pg/mL) and Vimentin (pg/mL) contents in placenta tissue between PE group and control group was as follows: MMP1, MMP2 and Vimentin contents in placenta tissue of PE group were significantly lower than those of control group while TIMP1, TIMP2 and E-cadherin contents were significantly higher than those of control group. Differences in MMP1, MMP2, TIMP1, TIMP2, E-cadherin and Vimentin contents

Table 4.

Comparison of trophocyte invasion molecules in placenta.

Groups	*n*	MMP1	MMP2	TIMP1	TIMP2	E-cadherin	Vimentin
PE group	56	0.78±0.09	1.55±0.18	0.92±0.11	0.73±0.08	125.5±14.9	106.7±13.8
Control group	70	2.74±0.35	5.82±0.79	0.41±0.07	0.35±0.06	69.34±9.32	252.8±31.9
T		24.598	21.385	12.309	10.895	9.285	13.673
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

in placenta tissue were statistically significant between the two groups ($P<0.05$). Pearson correlation analysis showed that MMP1, MMP2 and Vimentin contents in PE placenta were positively correlated with Wnt1, Wnt3a and β-catenin contents while TIMP1, TIMP2 and E-cadherin contents were negatively correlated with Wnt1, Wnt3a and β-catenin contents.

4. Discussion

The excessive apoptosis and insufficient invasion of placental trophocytes are the important characteristics of preeclampsia, oxidative stress reaction activation is the important pathological factor affecting trophocyte apoptosis and invasion, but the specific regulatory mechanism is not yet clear. Wnt/β-catenin is the important signaling pathway in cells regulating apoptosis, invasion and oxidative stress, Wnt1 and Wnt3a are the classic molecules that start the signaling pathway, they can inhibit the degradation of downstream molecule β-catenin and make it accumulate in the cells, and the continuously accumulated β-catenin can further transfer into the nucleus and regulate the expression of multiple genes[5,6]. Existing cellular experimental study has confirmed that activation of Wnt/β-catenin signaling pathway can reduce placental trophocyte injury induced by hypoxia reoxygenation[4], but there is no clear report about the changes of Wnt/β-catenin signaling pathway function in preeclampsia progression. In the study, in order to define the correlation between Wnt/β-catenin signaling pathway function change and the onset of preeclampsia, the Wnt/β-catenin signaling pathway molecule levels in preeclampsia placenta were analyzed, and the results showed that Wnt1, Wnt3a and β-catenin contents in placenta tissue of PE group were significantly lower than those of control group. This means that Wnt/β-catenin signaling pathway inhibition is closely related to the onset of preeclampsia, and the signaling pathway may affect the cell apoptosis, invasion and oxidative stress to participate in the occurrence and development of preeclampsia.

Oxidative stress is an important biological link affected by Wnt/β-catenin signaling pathway, and the excessive activation of oxidative stress is an important pathological feature in the onset of preeclampsia, which is characterized by the increased formation of oxygen free radicals as well as the peroxidation damage of trophocytes and endothelial cells[7,8]. Lipid is the composition in cellular structure that is most vulnerable to oxygen free radical attack, and the oxidation reaction between oxygen free radicals and lipids in cell membrane structure and organelle membrane structure will cause the cellular structure and function injury, and also generate MDA, ox-LDL, 8-iso-PG and other lipid peroxidation products[9,10]. The proteins in the cells are also attacked by oxygen free radicals and then generate the protein oxidation product AOPP[11]. In the study, analysis of the oxidative stress molecule contents in preeclampsia placenta showed that MDA, ox-LDL, 8-iso-PG and AOPP contents in placenta tissue of PE group were significantly higher than those of control group. This indicates that the activation of oxidative stress response and the excessive generation of oxygen free radicals are related to the occurrence of preeclampsia. Further analysis of the correlation between the Wnt/β-catenin signaling pathway function and oxidative stress response showed that MDA, ox-LDL, 8-iso-PG and AOPP contents in PE placenta were negatively correlated with Wnt1, Wnt3a and β-catenin contents. This indicates that the inhibition of Wnt/β-catenin signaling pathway in preeclampsia placenta will result in the increased generation of oxygen free radicals and cause oxidative stress damage in trophocytes.

The activation of oxidative stress in the placenta of preeclampsia can not only directly cause the damage to trophocytes and endothelial cells, but also induce apoptosis of trophocytes through mitochondrial pathway[12,13]. Oxygen free radical attack and damage on the mitochondrial membrane will cause the massive cytochrome C release from the mitochondria into the cytoplasm, activate caspase-9, lead to cascade amplification of apoptosis, and eventually mediate apoptosis by activating Caspase-3[14-16]. XIAP is an important anti-apoptotic molecule in cells, which can be combined with many kinds of Caspase molecules and inhibit the over-activation of apoptosis[17]. In the study, analysis of the changes in trophocyte apoptosis molecule contents in preeclampsia placenta showed that XIAP content in placenta tissue of PE group was significantly lower than that of control group while Caspase-3 and Caspase-9 contents were significantly higher than those of control group. This indicates that the excessive apoptosis of trophocytes and the weakening of anti-apoptotic capacity are associated with the occurrence of preeclampsia. Further analysis of the correlation between the Wnt/β-catenin signaling pathway function and the trophocyte apoptosis showed that Wnt1, Wnt3a and β-catenin contents in PE placenta were positively correlated with XIAP content and negatively correlated with Caspase-3 and Caspase-9 contents. This indicates that the inhibition of Wnt/β-catenin signaling pathway function in preeclampsia placenta can cause the excessive apoptosis of trophocytes and the weakening of anti-apoptotic capacity.

There will be functional damage in the placenta of preeclampsia

after the excessive apoptosis of trophocytes, and there is a close relationship between the invasive function injury and the occurrence of disease. MMPs and TIMPs are the key molecules that affect extracellular matrix degradation and adjust cell invasion, MMP1 and MMP2 can hydrolyze extracellular matrix and promote cell invasion, and the TIMP1 and TIMP2 can inhibit MMPs hydrolysis activity and inhibit cell invasion[18-20]. Epithelial mesenchymal transition is an important mechanism for cells to obtain invasion and movement performance, epithelial phenotype marker molecule E-cadherin can maintain intercellular polarity and inhibit cell invasion, and mesenchymal phenotype marker molecule Vimentin can make cells obtain strong movement performance and promote cell invasion[21,22]. In the study, analysis of the changes of trophocyte invasion molecule contents in preeclampsia placenta showed that MMP1, MMP2 and Vimentin contents in placenta tissue of PE group were significantly lower than those of control group while TIMP1, TIMP2 and E-cadherin contents were significantly higher than those of control group. This indicates that the insufficiency of trophocyte invasion is related to the occurrence of preeclampsia. Further analysis of the correlation between Wnt/β-catenin signaling pathway function and trophocyte invasion showed that Wnt1, Wnt3a and β-catenin contents in PE placenta were positively correlated with MMP1, MMP2 and Vimentin contents, and negatively correlated with TIMP1, TIMP2 and E-cadherin contents. This indicates that the inhibition of Wnt/β-catenin signaling pathway function in preeclampsia placenta can cause the deficiency of trophocyte invasion.

In this study, it is believed that the Wnt/β-catenin signaling pathway function in the placenta of preeclampsia is significantly inhibited; the inhibition of this signaling pathway can lead to hyperactivation of oxidative stress, and then result in excessive apoptosis and insufficient invasion.

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