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Expression of β-catenin in human trophoblast and its role in placenta accreta and placenta previa

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Abstract

Objectives: To investigate the expression of β-catenin in chorionic villi, and to explore its roles in placenta accreta and placenta previa.

Methods: We compared β-catenin expression in the control group, placenta accreta group (lesion area and normal zones), and placenta previa group (placental central and placental edge zones) by immunohistochemistry, Western blotting, and RT-PCR techniques.

Results: Compared with the normal group, the placenta accreta group had a longer length of stay, greater bleeding volume, and lower newborn birth weight. Further, the expression of β-catenin was lower in both placenta previa and placenta accreta groups than in the control group, as measured by immunohistochemistry. Compared with the control group, expression of β-catenin was significantly lower in the placenta previa and placenta accreta groups by Western blotting and RT-PCR. Importantly, the level of placental β-catenin was significantly different when compared between the lesion and normal zones of placenta.

Conclusion: The expression of β-catenin in placenta accreta might play an important role in the regulation of placental cell invasion; low expression of β-catenin in placenta accreta might be responsible for excessive trophoblastic invasion.

Keywords

β-catenin, placenta accreta, placenta previa, pathogenesis, trophoblasts, placenta, birth weight, trophoblastic invasion

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Introduction

Placenta accreta (PA) is a serious complication that endangers the life of both mother and child. PA can lead to postpartum hemorrhage, coagulatory dysfunction, and puerperal infection. In serious cases of PA, the uterus must be removed, and the life of the pregnant woman can be threatened. By analyzing the pathological characteristics and pathogenesis of placenta implantation, prediction, diagnosis, and intervention can be performed in early stages of PA, thereby reducing the incidence of complications associated with placenta implantation and significantly reducing maternal morbidity, mortality, and perinatal adverse outcomes.

The human endometrium specifically expresses β-catenin, which is regulated by estrogen and progesterone; moreover, β-catenin participates in endometrial development, differentiation, and embryo implantation.[1] Several factors are produced by the placenta and play important roles in the activation and inhibition of excessive placental proliferation by apoptosis,[2] such as insulin-like protein 4,[2] matrix metalloproteinase,[3] and microRNA-29a/b/c.[4] β-catenin is an important factor involved in the differentiation, invasion, and angiogenesis of trophoblastic cells. The human endometrium specifically expresses β-catenin, regulated by estrogen and progesterone; notably, β-catenin participates in the physiological processes of endometrial development and differentiation, as well as embryo implantation.[1] Placental trophoblastic cells and tumor cells exhibit several similarities with respect to proliferation and invasion.

In this present study, we aimed to investigate the expression of β-catenin in chorionic villi, and to explore its roles in PA and placenta previa (PP). We compared β-catenin expression in the control group, PA group (lesion area and normal zones), and PP group (placental central and placental edge zones) by immunohistochemistry, Western blotting, and RT-PCR techniques.

Methods

Data sources

Pregnant women were selected who underwent regular obstetric care and gave birth by cesarean section in the Fujian Provincial Maternity and Children's Hospital between January 2015 and December 2015. The selected pregnant women were divided into three groups: PA, PP, and control (CON). Ethical approval was obtained from the Ethics Committee of the Fujian Provincial Maternity and Children's Hospital (Approval no. 2015008), and all participants provided written consent before enrolling in the study.

Specimen collection and processing

For all subjects, after delivery of the placenta, placental layers were immediately collected under sterile conditions. For groups PP and CON, portions of the central and edge zones of placenta were collected; for group PA, parts of implanted lesion central area and non-implanted lesion corresponding areas were collected. Two pieces of tissue were collected from each part, approximately $1.5\,\text{cm} \times 1.5\,\text{cm} \times 1.0\,\text{cm}$, avoiding hemorrhage, infarction, and calcification areas, with repeated sterile saline rinses and sterile gauze to absorb excess saline. One piece of tissue was fixed in 10% neutral formalin solution and used for immunohistochemical determination; the other piece of tissue was placed in an aseptic cryopreservation tube and stored in liquid nitrogen.

Experimental methods

Immunohistochemistry. The placenta tissue was cut into 3-μm-thick paraffin slices,

heated, dewaxed, and then hydrated with gradient alcohol. A β-catenin antibody (ab16051; Abcam, Cambridge, UK) was used for detection via the following protocol: a 3% peroxidase blocking agent was used to remove endogenous peroxidase activity, following by incubation with the primary antibody (described above), then with the secondary antibody (Biotin SP-AP, DGSP-A31/32; Dingguo Changsheng, Beijing, China); a fresh diaminobenzidine solution was used for staining, followed by hematoxylin staining, dehydration in a gradient alcohol series, mounting, and ultimate microscopic examination.

Western blotting. From the frozen tissue sections, protein was extracted by using protein lysis buffer. Then, Coomassie blue was used to determine protein content. In total, 80 μg of protein was separated by 10% polyacrylamide gel electrophoresis and electrotransferred to a polyvinylidene-difluoride membrane. The membranes were blocked with 5% skim milk, then incubated with the primary antibody (β-catenin, 1:1000; Beyotime, Shanghai, China) overnight at 4°C with gentle shaking. After the membranes had been washed in Tris-buffered saline (TBS), they were incubated with the secondary antibody (rabbit anti-mouse, VT687, 1:4000; Beyotime) for 2 hours at room temperature with gentle shaking. The membranes were then washed in TBS, developed and fixed in a darkroom, and gray values were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The relative protein content was identified by the ratio of the integral light density of β-catenin to that of β-actin.

Real-time PCR. From the frozen tissue sections, total RNA was extracted by using Tranzol UP (TransGen, Beijing, China), and β-catenin cDNA was obtained by reverse transcriptase, in accordance with the manufacturer's instructions. Primer sequences and PCR product lengths were as follows: β-catenin, amplified fragment length 169 bp (gene), upstream: 5′-AGC CACAAGATTACAAGAAAC-3′, downstream: 5′-CCAGAGTGAAAAGAACG ATAG-3′; β-actin primer, amplified fragment length 318 bp (internal control), upstream: 5′-ATCATGTTTGAGACCTT CAACA-3′, downstream: 5′-CATCTCT TGCTCGAAGTCCA-3′. DNA was amplified by using the following procedures, with the Trans DNA maker II (TransGen): an initial denaturizing step at 94°C (2 minutes); followed by 35 cycles of a thermal step protocol comprising 94°C (30 s), 60°C (30 s) and 72°C (30 s); the final step utilized 72°C for 10 minutes.

Statistical data

All data were statistically analyzed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp. Armonk, NY, USA). Measurement data were expressed in terms of $\bar{x} \pm s$. Differences among multiple groups were compared by one-way ANOVA. A t-test or Kruskal-Wallis test was used to analyze differences between two groups, and Spearman correlation analysis was used for bivariate correlation analysis with $\alpha=0.05$; $P < 0.05$ was considered to indicate statistical significance.

Result

General clinical comparison among three groups of pregnant women

A total of 60 pregnant women were enrolled in this study. Ten women were diagnosed with PA (group PA) by clinical and pathological examination. Twenty women were examined by ultrasound and diagnosed with PP (group PP). Thirty women underwent cesarean section due to uterine scarring, fetal abnormalities, or social factors (group CON).

There were no significant differences in the numbers of abortions or deliveries among the three groups of pregnant women. The age, number of pregnancies, and number of uterine operations in groups PA and PP were all higher than those in group CON ($P < 0.05$ for all), and the gestational age of delivery in groups PA and PP was significantly earlier than that in group CON ($P < 0.05$ for both); however, there were no significant differences in any of these comparisons between groups PA and PP. Hospital costs for pregnant women and the length of stay in the Intensive Care Unit (ICU) in groups PA and PP were both significantly higher than in group CON ($P < 0.05$ for both). Compared with group CON, group PA had longer length of stay, greater bleeding volume, and lower newborn birth weight; these differences were statistically significant ($P < 0.05$ for all). However, in comparisons of these data between groups PA and PP, as well as between groups PP and CON, none were statistically significant. Placental weight did not significantly differ among groups (Table 1, Table 2).

Comparison of placental β-catenin expression among the three groups

Localization and expression of β-catenin in placenta. Immunohistochemistry analysis showed that β-catenin was expressed in placental tissues of all three groups of pregnant women. Expression of β-catenin in placental tissue of groups PA and PP was lower than that of group CON, with lighter staining. Positive staining showed brown-yellow granules in the tissue (Figure 1).

β-catenin expression in placental tissues of the three groups of pregnant women

Comparison of overall *β*-catenin expression in placental tissue among the three groups of pregnant women. Protein expression and gene expression of β-catenin in placental tissues of the three groups of pregnant women were measured by Western blotting and real-time PCR, respectively. The results indicated that β-catenin was

Table 1. Comparison of clinical characteristics among the three groups of pregnant women.

Group	Case	Age (years)	Gestations (N)	Childbirths (N)	Length of gestation (weeks)	Abortions (N)	Uterine operations (N)
PA	10	31.7 ± 2.1*	3.7 ± 0.6*	2.1 ± 0.3	35.3 ± 1.28*	1.5 ± 0.5	0.7 ± 0.7*
PP	20	30.3 ± 1.3*	2.5 ± 0.3*	0.7 ± 0.1	36.2 ± 0.55*	0.9 ± 0.3	0.5 ± 0.8*
CON	30	30.2 ± 0.7	2.0 ± 0.2	0.6 ± 0.1	39.2 ± 0.2	0.4 ± 0.1	0.4 ± 0.6

Note: Compared with Group CON, *P<0.05; PA, placenta accreta; PP, placenta previa; CON, control.

Table 2. Comparison of treatments among the three groups of pregnant women.

Group	N (cases)	Hospitalization expenses (thousand yuan)	Hospitalization (days)	Intraoperative hemorrhage (mL)	Stay in ICU (n)	Placental weight (g)	Birth weight of newborns (g)
PA	10	22.1 ± 5.1*	8.0 ± 1.4*	1280.0 ± 416.4*	10*	551.0 ± 46.7	2441.0 ± 288.9*
PP	20	12.3 ± 0.6*	6.5 ± 0.7	543.0 ± 40.2	18*	577.8 ± 23.8	2651.1 ± 144.2
CON	30	9.4 ± 0.2	5.4 ± 0.3	383.3 ± 23.9	1	673.6 ± 26.9	3414.6 ± 96.6

Note: Compared with Group CON, *P<0.05; PA, placenta accreta; PP, placenta previa; CON, control.

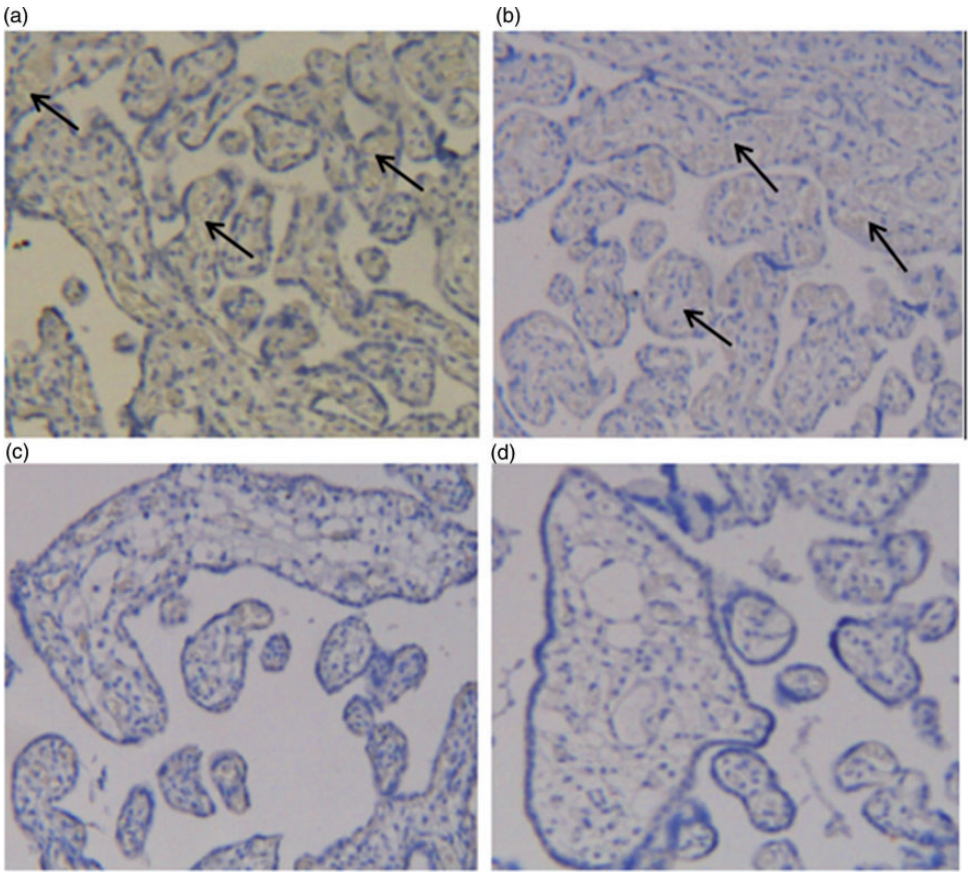

Figure 1. Expression and distribution of β-catenin in placental tissue.
Note: a) and b) are negative control (100×); c) and d) show expression of β-catenin in placenta tissues in groups PA and PP, respectively (100×). Positivity is indicated by brown cytoplasmic staining. PA, placenta accreta; PP, placenta previa; CON, control.

expressed in placental tissues of all three groups. Differences in the expression of β-catenin between groups PA and CON, and between groups PP and CON, were statistically significant ($P < 0.05$); the difference in expression of β-catenin between groups PA and PP was not statistically significant (Table 3).

Comparison of β-catenin expression in different zones of placental tissue among the three groups of pregnant women. In group PA, the expression of β-catenin in the PA area and non-accreta area zones was statistically significantly different ($P < 0.05$). As shown in Table 4, in groups PP and CON, the expression of β-catenin in the placental central and placental edge zones was statistically significantly different ($P < 0.05$). There were no statistically significant differences in the expression of β-catenin between any two areas within each group.

Discussion

In the present study, when PA occurred, extravillous trophoblast cells exhibited

strong invasion and migration, accompanied by reversible changes in a large number of cell adhesion molecules. Because β-catenin is important in cell adhesion, its decline constituted an early indicator of enhanced cell invasion.

The role of β-catenin in normal gestation trophoblastic cells

Senoi et al. showed that β-catenin is involved in the differentiation and invasion of normal gestational trophoblastic cells.[1] Tian et al. showed that β-catenin binds to E-cadherin to form catenin-cadherin complexes, which play an important role in the maintenance of epithelial cell polarity and adhesion.[2] Zhang et al.[3] have demonstrated that E-cadherin expression levels are significantly reduced during placental implantation. As a signaling molecule, β-catenin plays an important role in the classical Wnt pathway, and is an important link in the signal transduction of Wnt.[4] The Wnt pathway is activated to promote cell growth and proliferation, as well as inhibition of apoptosis.[5] Therefore, these prior studies suggest that changes in the expression levels of β-catenin and E-cadherin are associated with PP and PA.

Table 3. Comparison of the expression level of β-catenin ($\bar{x} \pm s$) among the three groups.

Group	Protein β-catenin	β-catenin mRNA
PA	0.382 ± 0.0178*	1.295 ± 0.3084*
PP	0.595 ± 0.0685*	1.526 ± 0.1942*
CON	1.204 ± 0.0349	2.886 ± 0.0835

Note: Compared with Group CON, *P<0.05; PA, placenta accreta; PP, placenta previa; CON, control.

β-catenin and trophoblast invasiveness

After embedding of the blastocyst, trophoblastic cells proliferate and differentiate into syncytiotrophoblasts and cytotrophoblasts. Cytotrophoblasts have capabilities of differentiation, proliferation, and invasion. There are two main methods of differentiation and development, comprising the extravillous and villous trophoblast pathways. In the extra villous pathway, cytotrophoblasts lose intercellular tight junctions, become separated from anchoring villi, and invade the maternal decidua, thus obtaining the ability to adhere to and invade maternal epithelial cells.[6] The invasive migration behavior of extravillous trophoblast cells is a fundamental element of placental formation, development, and maintenance

Table 4. Comparison of the expression level of β-catenin among the three groups ($\bar{x} \pm s$).

Group	Protein β-catenin	β-catenin mRNA
Group PA		
Accreta area	0.357 ± 0.0351	1.116 ± 0.092
Non-accreta area	0.406 ± 0.0432	1.474 ± 0.134
Group PP		
Placental central zone	0.581 ± 0.0423*	1.494 ± 0.5394*
Placental edge zone	0.609 ± 0.0374*	1.558 ± 0.7572*
Group CON		
Placental central zone	1.195 ± 0.0372	2.737 ± 0.2726
Placental edge zone	1.213 ± 0.0461	3.035 ± 0.0831

Note: Compared with Group CON, *P<0.05; PA, placenta accreta; PP, placenta previa; CON, control.

during normal pregnancy; the loss of intercellular closely, which is similar to the process of epithelial-mesenchymal transition (EMT).[7] During EMT, epithelial cells undergo multiple biochemical changes to achieve the mesenchymal phenotype; prior to EMT, epithelial cells are polarized and intercellular connections are tightly connected by adhesion complexes, which are located in the basement membrane.[8]

β-catenin is a marker of the epithelial phenotype and is widely expressed on the surface of epithelial cells; it plays an important role in the maintenance of cell stability and intercellular connections, as well as regulation of polarity. Prior studies have shown reversible changes in adhesion molecules[9]: when epithelial cells are transformed into mesenchymal cells, β-catenin expression is significantly reduced on the cell surface; moreover, interstitial cell connections are loosened, and cellular capabilities for migration and invasion are significantly increased.[10]

β-catenin and PA

On the basis of physiological characteristics, EMT is divided into three types: type 3 EMT is associated with the transformed phenotype of malignant tumor, and epithelial cells are formed by the migration of mesenchymal cells with type 3 EMT, promotion of tumor metastasis, formation of tumor stem cells, and ability of malignant tumor cells to migrate and invade.[11] One study[12] found that the number of epithelial cells, including noninvasive multinucleated giant cells in the myometrium, is reduced, while the number of invasive trophoblast cells is significantly increased along with increased volume. The expression of intervillous trophoblast was increased, concurrent with increased invasiveness, suggesting that patients with PA have abnormalities in the regulation of EMT or mesenchymal-to-epithelial transition (MET).

Li et al.[13] showed that β-catenin in the maternal surface of the placenta is involved in the regulation of gestational trophoblastic invasion: syncytiotrophoblast β-catenin expression level is higher; however, when the regulation mechanism is abnormal, trophoblast cells become separated from villous tissues and invade the decidual stroma, resulting in a reduction in β-catenin. In gestational trophoblastic diseases, as well as during malignant transformations, the expression levels of β-catenin mRNA were reduced. In the present study, our results showed that in groups PP and PA, β-catenin expression levels at the maternal surface were significantly lower than corresponding levels in group CON; in group PA, the β-catenin expression level was lower in the PA area zone than in the non-accreta area zone, which indicated that the degree of placental trophoblast invasion was negatively correlated with reduction in the level of β-catenin. When the level of β-catenin decreased, trophoblastic cells would exhibit strong capabilities for migration and invasion. Therefore, in PA patients, an irreversible downregulation of the expression of β-catenin in placental trophoblast cells occurred, which caused persistent low expression in late pregnancy; thus, EMT could not be converted into MET, as the invasion ability of trophoblast cells continued to strengthen, leading to excessive placental invasion and PA. Changes in trophoblast cell surface β-catenin expression or distribution played an important role in the occurrence and development of gestational trophoblastic cells during normal placenta formation; abnormal expression of β-catenin was associated with a series of gestational trophoblastic diseases. When the expression of β-catenin was reduced on the trophoblast surface, trophoblastic cell invasiveness increased; conversely, when the expression

of β-catenin increased, invasiveness was reduced.

The regulation of the expression of uterine decidua and β-catenin

The expression of β-catenin is regulated primarily in the following ways: cell adhesion, cell invasion, angiogenesis, and estrogen stimulation. The decidua defect results in a damaged or absent Nitabuch's layer between the trophoblast and the decidua. Similarly, decidual vascular growth defects result in disorders of placental blood supply and vascular remodeling, thus meeting the needs of the maternal-fetal interface during pregnancy; excessive blood supply results in increased trophoblast invasiveness. In Japan, an immunohistochemical study of 19 postoperative PA patients showed that the E-cadherin expression level in the PA area zone was significantly lower than that in the non-accreta area zone; TGF-β and Snail expression were significantly increased in decidual tissue corresponding to the PA area zone.[14] The present study showed that placental β-catenin protein and mRNA expression levels in the central and edge areas of two parts of placental tissue in group PP were respectively lower than corresponding expression levels in group CON, suggesting that in the decidua deficient area, the expression of β-catenin was downregulated, resulting in reduced placental blood supply. To ensure adequate nutrition support and oxygen supply, trophoblast cells proliferate and/or undergo excessive invasion of the uterine myometrium. β-catenin is also involved in the regulation of various angiogenic factors; a large number of studies have confirmed that[15] normal vascular β-catenin exhibits stable expression, and is associated with high expression of Wnt proteins, which suggests that the Wnt/β-catenin signaling pathway may be involved in the regulation of angiogenesis.

Abnormal and reversible changes in the placental expression of β-catenin were involved in the regulation of trophoblastic cell invasiveness. Decidual microenvironment defects led to irreversible downregulation of β-catenin expression on the surface of trophoblast cells, enhanced trophocyte invasiveness, and abnormal vascular remodeling in the maternal-fetal interface, which was involved in PP and PA.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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