Expression and Influence of Galectin-3 on Missed Abortion

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Objective To explore the influence of galectin-3 on missed abortion.

Methods Forty cases of normal intrauterine early pregnancy were randomly divided into 2 groups: surgical abortion group (group A, n=20) and medical abortion group (group B, n=20). The third group was missed abortion group (group C, n=20) with the gestational age less than 13 weeks. Serum was isolated from the blood samples, collected and used for ELISA quantification of galectin-3. Villus and decidua tissues were collected from the abortus for immunohistochemical examination and real-time fluorescence relative quantitative PCR.

Results The level of galectin-3 in the serum was the lowest in missed abortion group (P<0.05). Immunohistochemistry showed that galectin-3 expression in villus of missed abortion group was significantly lower than that of surgical abortion group (P<0.01). Real-time fluorescence relative quantitative PCR showed that galectin-3 mRNA relative expression in villus of missed abortion group ($2^{\Delta\Delta Ct}=0.04 \pm 0.01$) was significantly lower than that of surgical abortion group ($2^{\Delta\Delta Ct}=1.00 \pm 0.00$). Galectin-3 mRNA relative expression in decidua of medical abortion group ($2^{\Delta\Delta Ct}=0.08 \pm 0.02$) was significantly lower than that of surgical abortion group ($2^{\Delta\Delta Ct}=1.00 \pm 0.00$) (P<0.01).

Conclusion Galectin-3 is related to the development of villus and decidua during early pregnancy. The decreased expression of galectin-3 may promote the occurrence of missed abortion.

Key words: galectin-3; early embryonic development; villus; decidua; missed abortion

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Galectin-3 belongs to a family of galectins. Its unique chimeric structure enables it to interact with a plethora of ligands and modulate diverse functions such as cell growth, adhesion, migration, invasion, angiogenesis, immune function, apoptosis and endocytosis, emphasizing its significance in the process of tumor progression[1]. There is increasing evidence that galectin-3 has important functions in several aspects of cancer biology, heart failure and infection, etc. But the research of its effects on embryonic development is still just initial. Therefore, this study intended to study the clinical significance of galectin-3 and the galectin-3 expression levels in serum and tissues of pregnant women.

Materials & Methods

Subjects and sample collection
Sixty early pregnant women were collected in family planning ward of our hospital from March to May in 2009. Among 60 women, 40 cases of normal intrauterine early pregnancy were randomly divided into 2 groups: surgical abortion group (group A) (n=20) and medical abortion group (group B) (n=20). The third group was missed abortion group (group C) (n=20). The inclusion criteria of three groups were voluntarily participating in the study, non willingness of natural pregnancy, menopause time<3 months, nearly half a year not taking hormone drugs, no tocolytic treatment history and having the request of termination. The women in group A and group C were given vacuum aspiration. The women in group B were given mifepristone and misoprostol. The study was approved by the institutional review board of International Peace Maternity and Child Health Hospital. Sample collection was performed with informed consent. Serum was isolated from the blood samples, collected and used for ELISA quantification of galectin-3. Villus and decidua tissues were collected from the subjects for immunohistochemical examination and real-time fluorescence relative quantitative PCR.

ELISA
The serum samples were diluted 1 : 4 in sample diluent, and the galectin-3 concentration was measured with human galectin-3 ELISA kit (EIAabScience, Wuhan, China) according to the manufacturer’s instructions. Three replicates were done for each sample. A standard curve ranging from 0 ng/ml to 4 ng/ml of galectin-3 was generated for the ELISA. The concentration of galectin-3 sample was determined based on the standard curve generated, by measuring the optical density of each well at 450 nm, and multiplied by the dilution factor.

Immunohistochemistry
Immunohistochemistry was performed with rabbit anti galectin-3 polyclonal antibody, unconjugated (bs-0721R, BIOS) at 1 : 500 followed by conjugation to the secondary
antibody and DAB staining. Tissue slides were hydrated by conventional dewaxing. The paraffin-embedded tissue sections were then incubated in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. The slides were incubated with normal goat serum for 20 min at room temperature. The slides were then incubated overnight at 4 °C with rabbit anti-human galectin-3 primary antibody (Beijing Boisynthesis Biotechnology Co., Ltd, Shanghai, China). Specimen in group A with PBS instead of the primary antibody was used as the negative control. The tissues were equilibrated to 37 °C for 45 min, and washed with PBS. The sample was then incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibody for 4 min at room temperature and allowed for color development. The sections were then hematoxylin-stained, dehydrated, transparentizing in xylene, and observed under an optical microscope. Expression of galectin-3 was evaluated by histochemistry score (H score).

**Real-time fluorescence relative quantitative PCR (qRT-PCR)**

The qRT-PCR was used to determine the galection-3 in villus and deciduas. A little of organization was taken and ground into a powder in liquid nitrogen. Trizol reagent method was used to extract total RNA of cells. The cDNA was obtained in accordance with the RT-PCR kit (Toyoba company) specifications for reverse transcription step operation. 18s ribosome was used as an endogenous control to normalize for differences in the amount of total RNA in each sample. The primer sequences and the sizes of the amplified fragments were as follows, *galectin*-3 (93 bp): 5'-CTTCCACTTTAACCACGCTTCAA-3' (sense), 5'-TGTCTTTCTCCCTTCCCCAGTTATT-3' (anti-sense); 18s *ribosome* (252 bp): 5'-CAGCCACCCGAGATTGAGCA-3' (sense), 5'-TAGTAGCGACGGGCGGTGTG-3' (anti-sense).

Primers were synthesized by Shanghai Sangon Biological Engineering Technology Service Co., Ltd. The qRT-PCR reaction system was as follows: the total volume 20 μl, upstream primer 1.2 μl, downstream primer 1.2 μl, template DNA 2 μl, SYBR green I 10 μl, plus solution 2 μl and double steaming water. Fluorescence quantitative PCR instrument is the Eppendorf realplex 4. Reaction conditions were as follows: 95 °C modified 2 min, 95 °C degeneration 15 s, 58 °C annealing 30 s, 72 °C stretching 42 s, a total of 40 circulations, and maked the melt curve analysis. The target gene was evaluated by comparison threshold method. The relative expression quantity of target gene is $2^{-\Delta\Delta Ct}$, $\Delta\Delta Ct = Ct_{test \ group} - Ct_{control \ group}$.

**Statistical analysis**

The difference between serum galectin-3 levels in three groups was determined by t-test. On the other hand, the difference of galectin-3 protein expression in villus and deciduas was compared by Wilcoxon rank-sum test. All the data were processed by SAS8.2 statistical software. The result of qRT-PCR was expressed as mean ± standard error ($\bar{x} \pm s$) and others were expressed by mean ± standard deviation ($\bar{x} \pm s$). $P<0.05$ was considered significant.
Results

The age of the groups A, B and C was 28.5 ± 5.4 years, 30.6 ± 5.5 years and 29.0 ± 4.8 years, respectively.

Galectin-3 serum levels in three groups

The levels of galectin-3 in the serum of group A, group B and group C were 1.62 ± 0.91 (0.89–2.15) ng/ml, 1.75 ± 0.80 (0.92–2.24) ng/ml and 0.72 ± 0.58 (0.23–1.36) ng/ml, respectively. The mean serum galectin-3 level of group C was significantly lower than that of group A and group B (P<0.05).

Immunohistochemical analysis of galectin-3

The expression of galectin-3 in villus

Galectin-3 protein was mainly expressed in the cell membrane, cell cytoplasm and nuclear membrane of villous trophoblast (Figure 1). Sixty cases of villous cells were positive expression. The mean scores for groups A, B and C were 120.90, 112.62 and 50.84. Expression of galectins-3 protein was significantly the lowest in group C (P<0.01).

The expression of galectin-3 in decidua

Galectin-3 protein was mainly expressed in the cell membrane, cell cytoplasm and nuclear membrane of decidua cells (Figure 2). Sixty cases of decidua cells were positive expression. The mean scores for groups A, B and C were 82.15, 75.46 and 68.85, respectively. The differences among 3 groups were not statistically significant (P>0.05).

The results of qRT-PCR

Each melting curve of galectin-3 and 18s ribosome had a single peak. The melting temperature was at about 80 °C and 91 °C, respectively. Amplification curves prompt that all amplification cycle number was less than 30 circulations. qRT-PCR showed that galectin-3 mRNA relative expressions in villus of group C (2^(ΔΔCt)=0.04±0.01) was significantly lower than that of group A (2^(ΔΔCt)=1.00±0.00) (P<0.01) (Figure 3). Galectin-3 mRNA relative expressions in deciduas of group B (2^(ΔΔCt)=0.08±0.02) was significantly lower than that of group A (2^(ΔΔCt)=1.00±0.00) (P<0.01) (Figure 3).

Discussion

The level of galectin-3 in the serum and the villus is was lower in the women of missed abortion

This study examined galectin-3 expression in the serum of three groups of pregnant female and found that the level of galectin-3 in the serum was the lowest in the group of missed abortion. This study also found that galectin-3 was markedly decreased in villus in the group of missed abortion through immunohistochemical analysis. The reason may be that
galectin-3 promotes embryo cultivation and development mainly through enhancing adhesion and erosion of trophoblast cells.

Previous studies showed that galectin-3 took part in cell proliferation\cite{2} and galectin-3

Figure 1 The expression of galectin-3 protein in villus by immunohistochemistry (400 ×)

A: surgical abortion group
B: medical abortion group
C: missed abortion group
D: negative control group

Figure 2 The expression of galectin-3 protein in decidua by immunohistochemistry (400 ×)
could be secreted into the extracellular environment to induce cell apoptosis\cite{3}. Galectin-3-induced apoptosis of Jurkat cells is regulated by both O-glycans and N-glycans on CD45\cite{4}. Bozic’s research showed that galectin-3 significantly increased in gestational trophoblastic disease, which also prompted galectin-3 was related to the infiltration and metastasis of cells\cite{5}. Noël et al.\cite{6} analyzed the expression of galectin-3 in peritoneal endometriosis, ovarian endometriosis, deeply infiltrating endometriosis and eutopic endometrium, carried out by immunohistochemistry, and found that galectin-3 overexpressed in various forms of endometriosis, suggesting that galectin-3 was related to the proliferation and adhesion of endometrial cells. The study by Markowska further told that galectin-3 was a mediator of vascular endothelial growth factor (VEGF)- and basic fibroblast growth factor (bFGF)-mediated angiogenic response, and that galectin-3 promoted angiogenesis by interacting with complex N-glycans on \(\alpha_v\beta_3\) integrin and activating integrin signaling pathways that influence VEGF and bFGF angiogenic activity\cite{7}.

**Galectin-3 involves in the immune response**

Galectin-3, \(\beta\)-galactoside-binding lectin, plays multiple roles in the regulation of immune and inflammatory responses. The embryo is just as allogeneic grafts for mother. Embryo survival needs maternal immune escape. Our research found that the expression of galectin-3 was higher in the normal pregnancy and it may suggest that galectin-3 involves in the maternal-fetal immune. Galectin-3 is involved in the differentiation and proliferation of several immune cells. It activates both lymphoid and myeloid cells, such as T cells, mast cells, monocytes and neutrophils\cite{8}. Galectin-3 is also a negative regulator of immune cell function by controlling the anergic state of T cells\cite{9}. Galectin-3 suppresses Th17 responses by regulating dendritic cell (DC) cytokine production\cite{10}. The research by Sato et al.\cite{11} proposed that galectin-3 can hinge two areas of the innate immune recognition system, damage associated molecular pattern (DAMP) and pathogen associated molecular pattern (PAMP) pathways in the early host responses against various pathogens. And Vanderstraeten et al.\cite{12} reported the presence of the immune checkpoints PD-11/PD-12 and B7-H4 in uterine tumors as well
as the presence of the immune inhibitory molecules galectin-3 in conjunction with uterine cancer.

The correlation of galectin-3 and reproductivity

Galectin-3 is a β-galactoside-binding protein which plays a role in variety of biological processes, including cell growth and differentiation, cell adhesion, and apoptosis[13]. In 1994, Vandenbrule et al.[14] found that galectin-3 existed in human placenta. A study found that the expression level of galectin-3 in villi tissues and deciduas increased with the pregnant progress during the first trimester[15]. The current study showed that expression of galectin-3 in both endometrial epithelial cells and endometrial stromal cells could be regulated by hCG in an intricate manner, and indicated that galectin-3 might be regulated by hCG in preparing the endometrium for embryonic implantation[16]. Our study found that the mean galectin-3 serum level of the the missed abortion group was significantly lower than that in the groups of normal pregnancy. It indicates that galectin-3 may be important to embryo implantation. Recent reports have shown that the number of embryos implanted decreased substantially when galectin-3 was knocked down in mouse endometrium and the galectin-3 is related to endomerium receptivity due to its expression at the time of embryo implantation[17]. Yang’s study also showed that estradiol and progesterone up-regulated galectin-3 expression, which in turn decreased the apoptotic rate of endometrial cells, and the results strongly suggested that hormonal activation of galectin-3 was involved in inhibiting endometrial cell apoptosis, playing key roles in embryo implantation[18].

In conclusion, this study suggested that galectin-3 was closely related to the development of villi and decidua in early pregnancy. Decreased expression of galectin-3 may induce missed abortion. Further investigation is necessary to explore and define if galectin-3 could be one of the indicators of early embryonic development through blood monitoring.

References


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