Original Article
Expression of co-stimulatory molecule B7-H3 in patients with recurrence spontaneous abortion

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Abstract: To study the expression of B7-H3 in the chorion and decidua of patients with voluntarily abortion (within three months after normal pregnancy) and with recurrence spontaneous abortion (within three months after miscarriage) and to investigate its biological significance in gestational immunity for providing experimental evidence in the early diagnosis of spontaneous abortion. 90 normal pregnant patients including 45 cases in voluntarily abortion group and 45 in recurrence spontaneous abortion group were randomly collected from department of obstetrics and gynecology, the Second Affiliated Hospital of Soochow University. Surgical specimens of chorionic and decidual tissues from those patients were performed for immunohistochemical test to analyze the expression of B7-H3. There was no significant difference in the immunohistochemistry scores for the expression of B7-H3 in chorionic tissues between voluntarily abortion group and recurrence spontaneous abortion group (P>0.05). However, there were significant differences in the expression of B7-H3 in decidual tissues of both groups, and its expression in voluntarily abortion group was significant higher than that in recurrence spontaneous abortion group (P<0.05). And B7-H3 was expressed in part of the chorion but the whole decidua. In the decidua, the expression of B7-H3 in voluntarily abortion group is significantly higher than that in recurrence spontaneous abortion group, which suggests that B7-H3 gets involved in gestational immunity and may be associated with the occurrence of recurrence spontaneous abortion.

Keywords: B7-H3, recurrence spontaneous abortion, pregnancy, co-stimulatory molecule

Introduction
Recurrence spontaneous abortion (RSA), also known as miscarriage, refers to the pregnancy loss of an embryo or fetus (the weigh <1000 g) before it is able to survive for 28 weeks of gestation. It is a refractory disease of obstetrics and gynecology and may directly affect the reproductive health of patients [1, 2]. RSA may occur for many complicated reasons, such as chromosomal abnormalities, endocrine abnormalities, and some risk factors in immunity, infection and anatomy [3, 4]. About 50% of RSA occurs with unknown etiology, which is called unexplained recurrence spontaneous abortion (URSA) [5]. In recent years, immunological factors have aroused our concern, and it is believed that such patients may suffer from alloimmune disorders [6]. From the viewpoint of reproductive immunology, pregnancy is viewed as a kind of unusually successful semi-allograft, while recurrence spontaneous abortion is described as maternal transplant rejection.

The success of pregnancy profits from maternal immune tolerance against the embryonic semi-alloantigen. And yet, immune tolerance of normal pregnancy involves mutual influence and restraint of many factors, so any mistake is likely to lead to a pregnant failure [7, 8]. Domestic and foreign researches have shown that B7H3 is related to recurrent spontaneous abortion. As co-stimulatory molecules, members of B7 family play a key role in the immune-mediated immune response and tolerance. This study investigated the effect of B7-H3, the newest member of B7 family, on the pathogenesis of recurrence spontaneous abortion.

Subjects and methods
Patients and specimens

90 patients with normal pregnancy including 45 cases in voluntarily abortion group and 45 in recurrence spontaneous abortion group were
randomly collected from department of obstetrics and gynecology, the Second Affiliated Hospital of Soochow University from August 2011 to July 2013. 45 cases in voluntarily abortion group included 45 chorionic specimens and 45 decidual specimens, while 45 in recurrence spontaneous abortion group included 45 chorionic specimens and 45 decidual specimens. The average age of each groups were 23.25 ± 2.90 and 25.87 ± 2.34 years respectively, and there was no significant difference in age between the two groups (P>0.05).

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the Second Affiliated Hospital of Soochow University. Written informed consent was obtained from all participants.

**Immunohistochemical assays**

All tissue specimens were fixed in 10% formalin, routinely dehydrated in ethanol, cleared in xylene and embedded in paraffin. Above tissues were cut at 3 μm thickness. After deparaffinized and rehydrated in a graded series of alcohol, the paraffin sections were heat-repaired, and the endogenous peroxidase in them was closed with 3% H₂O₂ solution. Along with primary antibody (B7-H3 monoclonal antibody, Soochow University Institute of Biotechnology) was added, the mixture was placed in refrigerator at 4°C overnight. Next day, after rabbit/rat universal secondary antibody was added, the mixture was incubated in a thermostat. Then the sections were washed for three times to remove excess antibodies, and diaminobenzidine (DAB) was used as the chromogen; after thoroughly rinsed with distilled water, they were counterstained with hematoxylin and differentiated with 1% hydrochloric acid-alcohol. Next above sections was dehydrated with graded series of alcohol and mounted with neutral resin after they were dried in a fume hood.

**Assessment of immunohistochemical results**

The specimens of 45 chorionic tissues and 45 decidual tissues from voluntarily abortion group and recurrence spontaneous abortion group were separately stained. Expression of B7-H3 in chorion and decidua are shown in Figure 1. The expression of B7-H3 in the chorion and decidua. A: The chorion; B: The decidua. +, ++, +++: expression intensity; ×50, ×200: magnification.

<table>
<thead>
<tr>
<th>Table 1. Scoring criteria of immunohistochemical results</th>
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<tr>
<td>Expression area percentage [X (%)]</td>
</tr>
<tr>
<td>X&lt;10%</td>
</tr>
<tr>
<td>10%&lt;X≤25%</td>
</tr>
<tr>
<td>25%&lt;X≤50%</td>
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<tr>
<td>50%&lt;X≤75%</td>
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<td>X&gt;75%</td>
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<tr>
<th>Table 2. Average score of B7-H3 expression in voluntarily abortion group and recurrence spontaneous abortion group (points)</th>
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<tr>
<td>Groups</td>
</tr>
<tr>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>Voluntarily abortion group</td>
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<tr>
<td>Recurrence spontaneous abortion group</td>
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Note: *P<0.05.
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group and 45 chorionic tissues and 45 deciduous tissues from recurrence spontaneous abortion group were collected for immunohistochemical assays. Specific scoring criteria was shown in Table 1 with some relevant literature as reference [9]. Two indices of expression area percentage (X) and expression intensity (Y) were scored and their points were added together as the criteria. The total points <3 was denoted as +; the total points between 4 and 6 was denoted as ++; the total points >6 was denoted as +++.

Statistical analysis

All statistical analyses were performed using GraphPad Prism5 software. Comparison between the two groups was analyzed using non-paired t-test, whereas multiple data was analyzed by the chi-square test. The data were shown as the mean ± SD from at least three independent experiments. Values of P less than 0.05 were considered statistically significant.

Results

Comparison of the expression of B7-H3 in chorionic and decidual tissues

The expression pictures of B7-H3 in chorionic and decidual tissues, of which the total score was denoted as +, ++, +++ respectively were selected for analysis. As shown in Figure 1, B7-H3 was expressed in both tissues to different degrees. However, B7-H3 was expressed in part of the chorion but the whole decidua (Figure 1).

Comparison of the expression of B7-H3 between voluntarily abortion group and recurrence spontaneous abortion group

The score results of B7-H3 expression voluntarily abortion group and recurrence spontaneous abortion group was shown in Table 2 and Figures 2, 3. In chorionic tissues, the average score of B7-H3 expression of voluntarily abortion group was 5.93 ± 1.163, while its average score of recurrence spontaneous abortion group was 5.68 ± 1.552. That is to say, the expression of B7-H3 existed in chorionic tissues of both groups, but there was no significant difference between the two groups (P>0.05). In decidual tissues, the average score of B7-H3 expression of voluntarily abortion group was 5.83 ± 1.239, while its average score of recurrence spontaneous abortion group group was 3.63 ± 1.138. This indicated that the expression of B7-H3 in voluntarily abortion group was significant higher than that in recurrence spontaneous abortion group and there were significant differences between both groups (P<0.05; Table 2).

Discussion

Pregnancy is equivalent to allogeneic transplantation, and a fetus and its appendages need to avoid maternal rejection until their maturity in the uterus as long as the mother forms immune tolerance. During normal preg-
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...nancy, the maternal immune function changes, and in the mother-fetal immune microenvironment, there are a variety of cytokines involved in this immune regulation [10, 11]. B7-H3, as a co-stimulatory signal, plays an important role in maternal immune rejection against embryonic antigens caused by abortion. B7-H3, also called CD276, is a member of immune co-stimulatory molecule B7 superfamily [12, 13]. The result of sequence analysis shows that B7-H3 is a kind of type I transmembrane protein with 316 amino acids, which contains one N-terminal signal peptide, one IgV sample area, one IgC sample area, one transmembrane protein region and a 45-amino acid cytoplasmic tail. What's more, the abnormal expression of B7-H3 was correlated with many diseases, such as infectious diseases, autoimmune diseases and tumor, and it contains two expression forms of membrane B7-H3 (mB7-H3) and soluble B7-H3 (sB7-H3) [14-16]. In order to investigate the correlation between B7-H3 and recurrence spontaneous abortion, surgical specimens of chorionic and decidual tissues from normal pregnant patients with induced abortion and spontaneous abortion were performed for immunohistochemical test to analyze the expression of B7-H3.

The experimental results showed that the expression of B7-H3 existed in chorionic tissues of voluntarily abortion group and spontaneous abortion group, but there was no significant difference between the two groups. However, in decidual tissues, the expression of B7-H3 in voluntarily abortion group was significant higher than that in recurrence spontaneous group and there were significant differences between both groups. In addition, Chorionic and decidual villi are important to normal pregnancy, and mB7-H3 expression exists in both of them. The chorion is formed by extraembryonic mesoderm and the two layers of trophoblast that surround the embryo and other membranes. And after the implantation of the embryo into the uterine wall, the embryo undergoes rapid proliferation and forms numerous processes. Thus the placenta develops from the chorion frondosum and the decidua basalis with pcytotrohoblast as the inner layer and syncytiotrophoblast as the outer layer [17-19]. Because normal pregnant patients with voluntarily abortion and recurrence spontaneous abortion underwent the early gestational process, and the chorion does matter in this process, B7-H3 was expressed in part of the chorion during early pregnancy. With the development of early gestation, the decidua begins to emerge, and there were significant differences in the expression of B7-H3 in decidual tissues of patients with voluntarily abortion and recurrence spontaneous abortion. This suggested that B7-H3 could be associated with the occurrence of recurrence spontaneous abortion to some degree. B7-H3 is a kind of co-stimulatory molecules, and its expression level will increase during gestational process, which has a positive effect on normal pregnancy because of its negative regulation with reducing the maternal immune rejection of the fetus to ensure normal pregnancy.

In conclusion, it is believed that B7-H3 is a kind of co-stimulatory molecules which was...
expression of the chorion and decidua. During gestational process, the level of mB7-H3 in decidua will increase while there is no obvious change in its level in chorion, which indicates that B7-H3 may be associated with the occurrence of recurrence spontaneous abortion to some degree. Finally, B7-H3 will provide a warning for occurrence of miscarriage in clinical work and it also has positive significance for reducing or avoiding miscarriage.

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Disclosure of conflict of interest

None.

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