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The increased level of Tspan5 in villi suggests more proliferation and invasiveness of trophoblasts in tubal pregnancy

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ABSTRACT

Objective: This study was to determinate the expression of Tspan5 in tubal ectopic implantation sites and to explore the correlation of the expressive level of Tspan5 at maternal-fetal interface and the occurrence of tubal ectopic pregnancy.

Study Design: This is a retrospective study. Trophoblastic and endometrial tissues were collected from tubal ectopic pregnancy(Total of 40), and intrauterine pregnancy(Total of 41), who had voluntary abortion, non-pregnancy women(Total of 12), who recieved an diagnostic uterine curettage before IVF-ET for male infertility. All samples were collected from women aged 23–40 years, from February 2012 to January 2014.

Results

1. In human villi

Tspan5 was primarily located in cytoplasm and on the surfaces of cytotroblasts(CTs) and extravillous trophoblast(EVCTs). The intensity of Tspan5 in tubal pregnancy was significantly higher than that in normal intrauterine pregnancy, showing significant differences (Mean of IOD:109.39 ± 61.84 Vs. 89.04 ± 36.44;t = 2.33, $P = 0.023$).

2. In human deciduas of intrauterine pregnancy or endometrium of tubal pregnancy and non-pregnancy

Tspan5 expressed in cytoplasm and membrane of glandular epithelial cells. The expressive level of this protein was increased in tubal pregnancy than that in intrauterine pregnancy and non-pregnancy(Mean of IOD:144.18 ± 106.22 Vs. 93.43 ± 67.10, $P = 0.037$; 144.18 ± 106.22 Vs. 88.56 ± 33.24, $P = 0.018$).

Conclusion: Our study indicated that the trophoblasts in tubal pregnancy showed more proliferative and invasive characteristics. Dysregulation of Tspan5 in decidual microenvironment may relate to the retention of embryo in fallopian tube.

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Introduction

Ectopic pregnancy remains a leading cause of maternal mortality in the first trimester of pregnancy, occurring at a rate of 1–2% worldwide [1]. Approximately 98% of ectopic pregnancies occur in the fallopian tube [2]. As we know in IVF-ET, when the thickness of endometrium is less than 8 mm, it is difficult for embryo to implant. When the endometrial thickness is less than 5 mm, almost no embryo can implant. The diameter of fallopian tube is only 1.5 cm and lamina propria of fallopian tube is much thinner than the endometrium. So, why and how can the embryo implant in the tube?

Transmembrane 4 superfamily(TM4SF) also called tetraspanins or tetraspans, are cell-surface proteins that span the membrane four times. They display numerous physiological properties, such as cell adhesion, motility, activation and proliferation [3]. Recent studies have showed that some familymembers of TM4SF, such as CD82, CD63, CD9 and Tspan5, expressed in maternal-fetal interface [4–6]. These findings indicate that TM4SF proteins may be involved in the process of embryo implantation.

Tspan5, also named TM4SF9, is a transmembrane protein, that belongs to the transmembrane 4 superfamily. Study had shown that Tspan5 was involved in both the developmental and the

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functional maturation of the brain [7]. Jubin examined the spatial and temporal gene expression of four related tetraspanins(Tspan3, Tspan4, Tspan5 and Tspan7) during the embryonic development of Xenopus laevis [8]. Interestingly, both of the two studies had demonstrated the expression of Tspan5 in the cells with a high migratory potential, such as the neural crest cells, migrating external granular cells and migrating Purkinje cells. It suggested the role of Tspan5 in the regulation of migration processes. Our previous study has found the increasing expression of Tspan5 in trophoblasts of early pregnancy, hydatidiform mole, invasive hydatidiform mole and chorionic carcinoma tissue [9]. In vitro experiments, we found that the proliferation and migration of choriocarcinoma cells were reduced after using RNAi technology to knockdown Tspan5 expression, suggesting that the expressive level of this protein reflects proliferation and invasiveness of trophoblasts [10]. In this study, we observated the location of Tspan5 in human tubal ectopic implantation sites, and compared the expressive levels between intrauterine and tubal pregnancy.

Materials and methods

Objects

1) Group of Tubal Pregnancy:

We collected the specimens of fallopian tube, villi and endometrium from the tubal pregnancy patients (Total of 40, mean age:27.35 ± 3.13 years), who underwent a salpingectomy of the troubled oviduct and a diagnostic uterine curettage was performed before salpingectomy.

Requirements:

1) Without a past history of tubal disease or fallopian tube operation, such as tubal sterilization surgery, anastomosis of fallopian tube or tubal patency test;
2) Without any intrauterine device;
3) with no history of spontaneous abortion or other abnormal pregnancy.

2) Group of Intrauterine Pregnancy:

41 age-matched normal early intrauterine pregnancy women (gestational age, 6-12weeks), who went through voluntary abortion for no medical reason, were included as a group of intrauterine pregnancy.

Requirements:

1) has a history of full-term childbirth.
2) with no history of spontaneous abortion or other abnormal pregnancy.
3) without any endocrine disorder, anatomic abnormality, autoimmune diseases (such as autoimmune thyroiditis and systemic lupus erythematosus), mycoplasma or chlamydia genital duct infections.
4) there was no foreboding abortion symptoms such as vaginal bleeding or abdominal pain.
5) the ultrasound demonstrate a normal intrauterine pregnancy.
6) All these women had regular menstruation and were estimated clinically.

3) Group of Non-Pregnancy

In the group of non-pregnancy, we selected 12 age-matched normally fertile women, who recieved an diagnostic uterine curettage before IVF-ET for male infertility.

All the cases were selected from archival material of the department of pathology of Guangdong Second Provincial General Hospital from February 2012 to January 2014. All procedures involving participants in this study were approved by Human Research Ethics Committee of Guangdong Second Provincial General Hospital, Guangzhou, China.

Methods

Immunocytochemistry

Immunocytochemistry was used to detect the expression of Tspan5. Tissues were gained after operations, washed in normal saline, then formalin-fixed and paraffin wax-embedded for further experiments. Paraffin-embedded samples were incised into tissue sections of 4 μm thickness. Then de-waxed in xylene, rehydrated and subjected to antigen retrieval by pressure cooking for 5min40 s in 10 mm sodium citrate (pH 6.0), before blocking endogenous peroxidase with 3% hydrogen peroxidase. An avidinebiotin block and protein block(BOSTER) were performed prior to overnight incubation with 1/50-diluted anti-Tspan5 (rabbit IgG, Santa Cruz Biotechnology, USA). Sections were incubated with biotinylated secondary antibody(GT) and DAB-Elite. Sections were counterstained in Mayer's Hematoxylin and mounted using resinene.

Immunostaining analysis

All stained cells were analyzed at ×400 magnifications. Image-Pro Plus software was used to determine the integrated optical density(IOD). Colours of yellow and tan were identified as positive signal. Five horizons were chosen for each slide and calculated the average.

Statistical analysis

Data were reported as mean value ± standard error of the mean (SEM). Data was analyzed by Independent-Samples *t*-test, which followed a normal distribution and homogeneity of variance. Satterthwaite *t*-test was used when data does not obey homogeneity of variance. Wilcoxon Test was used when data was not in line with a normal distribution. Statistical analysis was performed using SPSS statistics version 13.0. Differences were considered to be statistically significant if $P < 0.05$.

Results

Basic characteristics of patients

Detailed characteristics of the patients in the two groups were showned in Table 1. There was no difference between the two groups regarding age, gravidity, or gestational ages (Table 1).

The expressive level of Tspan5 in human villi of tubal pregnancy is increased

Tspan5 was detected in the cytoplasm and cell membrane of cytotroblasts and Extravillous Cytotrophoblas (EVCT) but not in the syncytiotrophoblasts (Fig1 a–d). The intensity of Tspan5 in villi of tubal pregnancy was significantly higher than that in intrauterine pregnancy, showing significant differences (Mean of IOD: 109.39 ± 61.84 Vs 89.04 ± 36.44; t = 2.33, $P = 0.023$) (Table. 2).

Table 1
Differences of the patients' basic characteristics(mean ± sem).

	n	Age (years)	Gestational age (days)	Gravidity (frequency)
Tubal Pregnancy	40	27.35 ± 3.13	54.00 ± 15.51	2.98 ± 1.29
Intrauterine Pregnancy	41	27.61 ± 4.42	58.17 ± 11.69	2.56 ± 1.45
t		−0.306#	−1.369	1.356
P		0.761	0.175	0.179

#Satterthwaite *t*-test.
Sem:standard error of the mean.