Enhanced beta-catenin expression and inflammation are associated with human ectopic tubal pregnancy

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Submitted on January 13, 2013; resubmitted on May 1, 2013; accepted on May 7, 2013

STUDY QUESTION: Is there a molecular link between Wnt signaling in fallopian tube inflammation and ectopic tubal implantation?

SUMMARY ANSWER: Enhanced beta-catenin expression, reduced E-cadherin expression and glycogen accumulation in the tubal epithelia and hyperplasia in tubal arteries were found in ectopic tubal pregnancy, consistent with the effects induced by Wnt signaling and inflammation.

WHAT IS KNOWN ALREADY: Chronic inflammation caused by infection can alter gene expression in the fallopian tube cells possibly leading to the development of ectopic pregnancy. Knockout mouse models have shown a relationship between Wnt/beta-catenin signaling and predisposition to tubal ectopic pregnancy.

STUDY DESIGN, SIZE, DURATION: Women with ectopic tubal pregnancy (n = 18) were included in the case group, while women with chronic salpingitis (n = 13) and non-pregnant women undergoing sterilization procedures or salpingectomy for benign uterine disease (n = 10) were set as the controls. This study was performed between January 2012 and November 2012.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The ampullary segments of fallopian tubes were collected from patients. Tissues of tubal pregnancy were separated into implantation sites and non-implantation sites. Beta-catenin and E-cadherin expression were determined using immunohistological and immunofluorescence staining. Glycogen production was measured with periodic acid Schiff by staining. The diameter and wall thickness of tubal arteries were evaluated by histological analysis method.

MAIN RESULTS AND THE ROLE OF CHANCE: Immunohistological staining revealed that beta-catenin protein expression was 100% positive in the ectopic pregnant and inflamed tubal tissues, and the staining intensity was significantly higher than in non-pregnant tubal tissues. In contrast, E-cadherin expression was reduced in ectopic pregnant fallopian tubes, possibly as a consequence of increased Wnt signaling. Moreover, glycogen accumulated in the tubal cells, and hyperplasia was observed in the tubal arteries with ectopic pregnancy, which is consistent with the effects induced by Wnt signaling and inflammation. All these changes could create the permissive environment that promotes embryos to ectopically implant into the fallopian tube.

LIMITATIONS, REASONS FOR CAUTION: This finding requires a further confirmation about what activates Wnt signaling in ectopic tubal pregnancies. Also, it is generally recognized that Chlamydia infection is associated with ectopic pregnancy, and disturbs tubal epithelia via the Wnt signaling. However, the infection type in the samples used was salpingitis.

WIDER IMPLICATIONS OF THE FINDINGS: A better understanding of the underlying mechanisms leading to ectopic pregnancies may contribute to our knowledge of the pathogenesis of tubal disorders and infertility and to the prevention of tubal ectopic pregnancy.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by NSFC grant (31071054); ‘973 Project’ (2010CB529702) and NSFC grant (30971493); Guangdong Natural Science Foundation (S2011101001593); Collaborated grant for HK-Macao-TW of Ministry of Science and technology (2012DFH30060) to X.Y. The authors have no competing interests to declare.

Key words: beta-catenin / Wnt-signaling pathway / fallopian tube / ectopic pregnancy / inflammation
Introduction

Ectopic pregnancy (EP) is a potential life-threatening condition that occurs when an embryo implants outside the intrauterine cavity. Most ectopic pregnancies (>98%) occur in the fallopian tubes and are commonly known as tubal pregnancy (Shao et al., 2012). Salpingitis is a pelvic inflammatory disease and is amongst several predisposing factors that can lead to the development of tubal pregnancy. It can cause the fallopian tube to block and retain the embryo and this facilitates ectopic tubal implantation (Shaw et al., 2010). However, in the mouse, embryo retention in the oviduct itself is insufficient to result in tubal pregnancy, even if the trapped embryo is a morphologically normal blastocyst that can implant after re-transfer into a pseudo-pregnant recipient uterus (Shaw et al., 2010). These observations suggest that cellular and molecular alterations in the oviduct are necessary to create the right conditions for ectopic implantation. However, the etiology of tubal pregnancy is still not fully understood.

Blastocyst implantation normally involves several key steps: apposition, attachment and invasion (Salilew-Wondim et al., 2012). Chronic inflammation of the fallopian tube, caused by infection, can alter molecular signals emanating from tubal tissues, which compete with the uterus as a site for embryo implantation. During inflammation, a number of mediators, such as leukemia inhibitory factor (LIF) interleukin, tumor necrosis factor and IL-1β, accumulate in the tubal tissues and these have been implicated in EP (Balasubramaniam et al., 2012). In other words, in tubal pregnancy, a blastocyst receiving inductive signals from the tubal epithelium respond by adhering and implanting into the fallopian tube. EP has been serologically associated with Chlamydia trachomatis in several studies (Shibahara et al., 2003). Using C. trachomatis infection models of fallopian tube, much has been learned about pathogenic mechanisms and inflammatory response following infection. These studies demonstrated that IL-1β primes and triggers signal transduction and induces tissue destruction through MPA-kinases and Wnt signaling, which may play an important role in EP. Thus, the patients suffering with inflammatory disorders may be more susceptible to this condition (Hyvönen et al., 2007; Kessler et al., 2012).

The ability of the fallopian tube to transport the early embryo into the uterus is a prequisite for successful pregnancy. Hence, structural and functional abnormalities of the fallopian tube will interfere with the transport process that can and lead to tubal pregnancy (Nagaraja et al., 2008; Shao et al., 2012). Several rodent models that exhibit fallopian tube dysfunction have provided clues on how tubal pregnancy may develop. It was found that tubal malformation is associated with altered gene expression in tubal epithelial cells. Indeed, Dicer is a ribonuclease III enzyme required for micro-RNA processing, and experiments on transgenic mice have revealed that it regulates members of the Wnt-signaling pathway (Hong et al., 2008; Nagaraja et al., 2008). Loss of Dicer in mouse oviducts can result in embryos being trapped and lead to an increase in homeobox (Hox A10) and Wnt7a expression (Hong et al., 2008; Nagaraja et al., 2008). Expression of Hox A10 is necessary for endometrial receptivity in eutopic implantation and also in ectopic implantation. This implies that ectopic implantation may be mediated by a Hox A10-dependent mechanism (Salih and Taylor, 2004). In this context, altered tubal Wnt signaling may also play a role in tubal implantation.

Wnt signaling has been extensively studied in intrauterine pregnancy. In mice, Wnt signaling is crucially involved in blastocyst development, uterine implantation and decidualization (Sonderegger et al., 2010). Wnt7a-deficient mice do not express Hox A10 and Hox A11 in endometrial stroma (Sonderegger et al., 2010). Similarly, Hox A10 and LIF mutants do not express Wnt4. It has been reported that LIF and Hox are critical for both eutopic and ectopic pregnancies (Daikoku et al., 2004), and Wnt and LIF might interact via the Wnt-signaling pathway. Wnt signaling involves a highly organized network of ligands, receptors and downstream effectors. The key component of Wnt signaling is β-catenin (Sonderegger et al., 2010).

To date, β-catenin expression in the fallopian tube with EP has not yet been reported. Therefore, we examined β-catenin expression and its binding protein, E-cadherin, in human fallopian tubes obtained from ectopic pregnant and non-pregnant patients. Since EP is closely associated with inflammation, we also investigated fallopian tubes with salpingitis. GSK3β, a key component of Wnt signaling that functions in glucose–glycogen metabolism, was also evaluated by examining glyco- gen accumulation in the tubal tissues. Moreover, β-catenin is capable of associating with transcription factor LEF-1 to direct vascular smooth muscle cell proliferation (Couffinhal et al., 2006); hence, we histologically examined the tubal arteries. We hypothesized that the aberrant β-catenin expression in the inflamed fallopian tube maybe one of the predisposing factors leading to EP in human.

Materials and Methods

Patients and tissue collection

The ampullary segments of fallopian tubes were collected from patients of the First Affiliated Hospital of Jinan University, Guangzhou, China. The study was approved by the local Research Ethics Committee. All enrolled patients had regular menstrual cycles (21–35 days), did not have previous history of EP nor had taken any hormonal preparations 3 months prior to surgery. In addition, their menstrual cycle coincided with endometrial histology according to the staging scheme developed by Noyes et al. (1975). Demographic and obstetrical features of the patients were recorded. Tubal pregnancy was diagnosed by pelvis ultrasound for the presence of an adnexal mass and no intrauterine implantation. Patients who had used an intrauterine device or received mephitrexate treatment before an operation were excluded from the study. Only ectopic pregnancies where embryos have implanted into the ampullary region were included (n = 18). In each sample from patients with ectopic tubal pregnancy, we investigated both the tubal implantation site (area within 5 mm of the gestational mass) and the non-implantation site (10 mm beyond the gestational mass; Lam et al., 2004).

As controls, fallopian tubes were obtained during sterilization procedures or salpingectomy for benign uterine disease (n = 10) in normal non-pregnant women and in women confirmed to have salpingitis by a pathologist after surgery (n = 13). The tissues were fixed, embedded in wax, sections and stained with Hematoxylin and Eosin (H&E) dye. Serial sections (5 μm thick) were also prepared for immunohistological and immunofluorescent staining. Some representative sections were stained with periodic acid Schiff (PAS) for assessing glycogen accumulation.
Immunohistological and immunofluorescent staining

Tissue sections in citrate buffer (pH 6.0) were heated in a microwave for antigen retrieval before exposure to the primary antibody. For immunohistochemistry, the tissue sections were treated with mouse monoclonal β-catenin antibody (1:100 dilution, Maxin, Fuzhou, China) or mouse monoclonal E-cadherin antibody (1:100 dilution, BD, USA) at 4°C overnight. Biotinylated goat anti-mouse serum IgG was used as the secondary antibody. To visualize the staining, a streptavidin–peroxidase system (KIT-9901, Elivision plus, Maxin, Fuzhou, China) or diaminobenzidine tetrahydrochloride substrate (DAB kit, Maxin) were used. For immunofluorescent staining, the slides were incubated with mouse monoclonal E-cadherin antibody (1:100 dilution, BD, USA), followed by a specific secondary antibody mixture coupled with Alexa Fluor 555 anti-mouse IgG (1:1000, Invitrogen, CA, USA), and then counterstained with DAPI (4′,6-diamidino-2-phenylindole, 1:1000, Invitrogen) for 1 h at room temperature. In co-localization staining, rabbit monoclonal β-catenin antibody was also used as a primary antibody, and Alexa Fluor 488 anti-mouse IgG and Alexa Fluor 555 anti-rabbit IgG were used as the secondary antibodies. For the negative controls, the primary antibody was replaced with isotype control antibody.

The intensity of β-catenin or E-cadherin labeling was established using semi-quantitative immunohistochemical reactive scores (IRS; Zappulli et al., 2012). The staining intensity was multiplied by the percentage of positive cells to achieve a score between 0 and 12. The percentage of positive cells was determined via observation of five randomly selected high-power fields (×400) on each histological section. The percentage of positively stained cells was divided into five groups: 0 = no labeling, 1 ≤ 10% of cells, 2 = 10–50% of cells, 3 = 51–80% of cells and 4 ≥ 80% of cells in which labeling could be detected. Staining intensity ranged from 0 = negative, 1 = weak, 2 = moderate and 3 = intense labeling. A final IRS score of ≥3 was regarded as a positive reaction and ≥9 was regarded as strongly positive. All slides were visually screened by two observations. Statistical analysis was performed using one-way analysis of variance analysis. Only P-values below 0.05 was considered to be significant.

Histological evaluation of tubal arteries

Histological sections were stained with H&E and examined and photographed using a digitized light microscope (Olympus IX51, Japan). The muscularis and serosal layers of arteries in ectopic pregnant and non-pregnant tubal tissues were measured and recorded. These arteries were easily identified because they were derived from the branches of ovary and uterus (Matson et al., 2000). Five samples were randomly selected from each tissue group and six fields per sample were photographed at ×100 magnification (Page et al., 2002). The photographs were then evaluated by an observer who had no knowledge of the origin of the tissue origin.

There have been no reports concerning the size of tubal arteries during EP. We wanted to establish whether the size of arteries and thickness of their walls changed after embryo implantation. To quantify the arteries, the distance between the external edges of each vessel wall and was measured perpendicular to the long-axis of the wall (Fiel et al., 2010). The ‘Set Scale’ function in ImageJ was used to convert pixel numbers to standard units. Data were presented as mean ± SEM. The measurements were carried out on six different areas for every specimen, and the mean values of all specimens were elucidated. Statistical comparisons were performed using an unpaired Student t-test by a SPSS (version 17.0) software. P-values < 0.05 were considered to be statistically significant.

Results

Histology of normal, ectopic pregnant and inflamed fallopian tubes

The demographic information and obstetrical profile of patients are presented in Table I. The mean age and obstetrical parameters amongst the groups were established to be similar. The fallopian tubes obtained from all patients were examined by routine histology. In non-pregnant women, the fallopian tubes were determined to be composed of an internal mucosal layer, an intermediate muscular layer and an external serosa layer (Fig. 1A). The mucosa was made up numerous longitudinal folds that project into the lumen of the ampulla. The lamina propria of each fold was covered by a simple columnar epithelium, some of which were ciliated (Fig. 1B). In chronically inflamed specimens, the epithelial cells were flattened and sparsely ciliated, some being so devoid of cilia that they were difficult to identify. The lamina propria of these specimens were engorged with blood vessels (Fig. 1C), and the smooth muscle (SM) fibers were reduced, atrophic and replaced by fibrous tissues. Inflammation, such as extravasations of blood cells, could easily be seen in the lamina propria (Fig. 1D). In the EP group (outside the tubal implantation site), the mucosal folds were dramatically increased in size and complexity. The muscularis was thickened and composed of a thick inner circular layer and an outer longitudinal layer, and the serosal layer was also thickened (Fig. 1E and F). In the tubal implantation site of the EP group, the stromal fibroblasts appeared to be larger and polygonal-shaped (containing large quantity glyogen and lipids), much like decidual cells (Fig. 1G and H).

β-Catenin expression in normal, ectopic pregnant and inflamed fallopian tubes

In non-pregnant fallopian tube, β-catenin was expressed at relatively low level in the tall columnar epithelial layer (Fig. 2A). β-Catenin expression increased significantly when the fallopian tube was inflamed (Fig. 2B) and in ectopic pregnancies (Fig. 2C and D). In these specimens, the cytoplasm of epithelial cells and cells in the lamina propria were strongly β-catenin immunoreactive, which could be more obviously observed in the high-magnification images as shown in Fig. 2A and D. Furthermore, it appears that the tubal epithelial cells at the implantation site were stained more intensively for β-catenin than those outside of the implantation site (Fig. 2D). We quantified the intensity of β-catenin staining between the different groups (Fig. 2E). The IRS score for the non-pregnant group (6.3 ± 0.6) was significantly lower than (P < 0.05) than the EP and inflamed groups. The IRS scores for non-implantation site in the EP group and in the inflamed group were similar (8.8 ± 0.5 and 9.1 ± 0.5, respectively). In contrast, the IRS score for the implantation site (10.9 ± 0.6) was significantly higher than the non-implantation site and also the inflamed group (P < 0.05; shown in Fig. 2E).

E-cadherin expression in tubal epithelium

β-Catenin can form a complex with E-cadherin at the cell membrane to enhance cell adhesion (Zappulli et al., 2012). Hence, we postulate that increased β-catenin expression may alter E-cadherin expression in the fallopian tube. Using immunohistochemistry, we investigated E-cadherin expression in the various tissue sections of fallopian tubes (Fig. 3). In non-pregnant fallopian tube, E-cadherin was strongly expressed in the cell membrane of epithelial cells (Fig. 3A–C). In contrast, it was weakly expressed in the epithelial cell membrane of ectopic pregnancy patients (Fig. 3D–F).
expressed in the epithelial cells of ectopic pregnant fallopian tubes at both non-implantation (Fig. 3D–F) and implantation (Fig. 3G–I) sites. The reduction of E-cadherin expression at the cell membrane might reflect activation of Wnt signaling during inflammation and EP in the fallopian tubes.

To further investigate the location of β-catenin and E-cadherin, we performed co-localization staining, in which β-catenin and E-cadherin clearly co-localized on cell membrane (Supplementary data, Fig. S2).

We additionally performed DAB staining (Supplementary data, Fig. S1), which demonstrated that the IRS score for the non-pregnant group (6.3 ± 0.8) was significantly higher than that of the pregnant groups (P < 0.05), but no difference was found between ectopic implantation sites (3.2 ± 0.5) and non-implantation sites (2.9 ± 0.3) (P > 0.05; Supplementary data, Fig. S1).

Association between glycogen storage and β-catenin expression in tubal epithelium

Glycogen metabolism and β-catenin are interlinked through activation of the Wnt signal pathway. Moreover, GSK3β is not only a modulator of β-catenin localization in the cytoplasm but also a key regulatory enzyme of glycogen storage (Maranghi et al., 2010). To establish whether there is an association between β-catenin and glycogen metabolism in fallopian tubes, the glycogen content in fallopian tubes was evaluated via PAS staining (Fig. 4). There were few PAS+ cells found in the epithelium and connective tissue of non-pregnant fallopian tubes, suggesting that there was only a small quantity of glycogen stored in the cells (Fig. 4A and B). In the EP group, there was a dramatically increase in PAS+ cells in the tubal epithelium and muscularis (Fig. 4C and D). The intensity of PAS staining for inflamed fallopian tubes was in between the staining intensity for the non-pregnant and EP groups (Fig. 4E and F).

We compared the glycogen and β-catenin expression patterns between the different specimens. In ectopic pregnant fallopian tube, there was heavy accumulation of glycogen and strong β-catenin expression in the epithelium (Figs 2C and D and 4C and D) compared with non-pregnant fallopian tubes (Figs 2A and 4A and 4B). However, in the inflamed fallopian tube, there was no direct association as where β-catenin expression was strong (Fig. 2B), but there was only moderate glycogen accumulation (Fig. 4E and F).

Table I

<table>
<thead>
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<th>Non-pregnancy (n = 10)</th>
<th>Pregnancy (n = 18)</th>
<th>Inflammation (n = 13)</th>
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<tr>
<td>Age year</td>
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<td>31.3 ± 4.0</td>
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<td>Parity (number)</td>
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<td>1.6 ± 0.7</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>Gestation days</td>
<td>40.6 ± 8.8</td>
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The values stand for the mean ± SD.

Figure 1

Histology of fallopian tubes. Transverse section of non-pregnant (A and B) and inflamed (C and D) fallopian tubes, as well as ectopic pregnant tubal tissues outside (E and F) and within the embryo implantation site (G and H). In the implantation site (framed area in G), the stromal fibroblasts appeared large and polygonal-shaped like decidual cells. These cells also contained large quantities of glycogen and lipids. Scale bar = 500 μm in A, C, E, G and 50 μm in B, D, F, H.
Figure 2  β-catenin expressions in fallopian tubes. Immunohistological staining was performed to elucidate β-catenin expression in the ampullary region of the human fallopian tube. β-catenin expression in non-pregnant group (A), salpingitis (inflammation) group (B), ectopic pregnancy group outside the embryo implantation site (C) and ectopic pregnancy group at the implantation site (D). (E) The intensity of the immunohistological staining was given an IRS score, as mean ± SEM. The results show β-catenin expression was stronger in the ectopic pregnancy and inflammation groups than in the non-pregnant groups. Scale bar = 50 μm in A–D. IRS, immunohistochemical reactive score.

Figure 3  E-cadherin expressions in fallopian tubes. Immunofluorescent staining of non-pregnant fallopian tubes show E-cadherin was strongly expressed in the cell membrane of epithelial cells (A–C). In contrast, it was weakly expressed in the epithelial cells of ectopic pregnant fallopian tubes at both the non-implantation (D–F) and implantation (G–I) sites. Scale bar = 50 μm in A–I.

Figure 4  Glycogen accumulations in fallopian tubes. PAS staining of was performed on non-pregnant (A and B), ectopic pregnant (C and D) and inflamed (E and F) fallopian tissues to establish extent of glycogen accumulation in the epithelium and muscularis. In non-pregnant fallopian tubes, only a few PAS-stained cells were found in the epithelium and connective tissue (A and B). However, there was a dramatic increase in PAS-stained cells in the tubal epithelium and muscularis of the ectopic pregnant group (C and D). The intensity of PAS staining for inflamed fallopian tubes was in between the staining intensity for non-pregnant and ectopic pregnant groups (E and F). Scale bar = 500 μm in A, C, E and 100 μm in B, D, F.
In the muscularis and serosa, the thickness of tubal artery walls in the ectopic pregnant group was found to be increased by \(~\sim 3 - 4\) fold compared with the non-pregnant group (muscularis: 0.03 ± 0.00 versus 0.01 ± 0.00 mm, \(P = 0.04\); serosa: 0.07 ± 0.02 versus 0.02 ± 0.00 mm, \(P = 0.04\)). Measurement of the EP tubal artery diameter revealed that there was approximately an 8-fold increase in the muscularis (0.39 ± 0.07 versus 0.05 ± 0.00 mm, \(P = 0.009\)) and 3-fold increased at serosa (0.57 ± 0.07 versus 0.20 ± 0.02 mm \(P = 0.001\)) compared with the non-pregnant fallopian tubes. It has previously been reported that the arteries in the uterus of ectopic pregnant patients was 1 – 4 mm in diameter (Anwar et al., 1999). Our results showed that the ectopic pregnant fallopian tube was mostly composed of small caliber arteries and arterioles (\(\leq 1\) mm in diameter).

**Discussion**

**β-Catenin and implantation**

Previous studies have demonstrated that Wnt signaling is involved intrauterine implantation, where blastocyst attachment induces β-catenin signaling in the uterine epithelium and SM layers. However, some researchers have reported that β-catenin was dispensable for Wnt signaling and that it involved a non-canonical Wnt pathway during implantation (Daikoku et al., 2011). β-Catenin normally acts as a central regulator of the canonical Wnt-signaling pathway and tissues that expresses high level of β-catenin might indicate that this pathway plays an important role in the biology of that tissue (Zappulli et al., 2012). To date, β-catenin expression and function have not been reported in the fallopian tube with EP. Hence, we performed immunohistochemistry on the fallopian tube and found that β-catenin was expressed in the tubal epithelium (which included secretory and ciliated cells). It was also seen in the cilia of ciliated epithelial cells. All of these epithelial cells expressed an increased level of β-catenin when the tissue samples were isolated from patient with tubal pregnancy. β-Catenin immunoreactivity was especially strong at the ectopic implantation site which indicates that β-catenin expression was partly influenced by the implanted embryo. Alternatively, it might also suggest that dysregulated β-catenin expression might promote tubal implantation. Although nuclear localization of β-catenin is considered to be the hallmark of Wnt activation (Matsuzaki et al., 2010), it is only barely detectable in the nucleus in many tissues. Similar recruitment of β-catenin has also been reported for the plasma membrane following Wnt induction (Wang et al., 2009). Thus, given
our observation that β-catenin is recruited in ectopic epithelia, we reasoned that the Wnt/β-catenin signaling pathway might be activated in the tissue of fallopian tube with EP.

**β-Catenin and deciduation**

At the ectopic implantation site, we discovered that β-catenin expression was strongly increased in the stromal cells of the lamina propria. This suggests that β-catenin plays a specific role in the implantation process. During normal pregnancy, the uterine stromal cells undergo extensive proliferation, differentiation and remodeling, in a process known as decidualization. This hormonally induced cellular transformation is an essential prerequisite for embryo implantation, especially stimulation from progesterone (Marx et al., 1999). Several earlier reports showed a distinct expression of progesterone receptor (PR) and estrogen receptor (ER) in human fallopian tube epithelia (Natu et al., 2009). Interestingly, PR and ER are frequently lost in the fallopian tube with EP (Shao et al., 2012). This may offer an explanation for poor decidualization, which has been observed in tubal pregnancy and associated with the absence of PR (Marx et al., 1999). Additionally, other investigators have shown that progesterone or estrogen can regulate Wnt signaling in uterine stroma by selectively regulating specific Wnt-signaling components during the process of uterine decidualization (Daikoku et al., 2004). Furthermore, β-catenin deficient mice uteri exhibit decidualization defects (Shao et al., 2012), although little is known about Wnt ligands and Wnt signaling-related genes in the context of the stroma cells during tubal pregnancy. These previous observations are in agreement with our result in that β-catenin increased expression was seen in the stroma of the pregnant fallopian tube, and was associated with decidualization process within the implantation site.

**β-Catenin and inflammation**

Infection is the biggest risk factor for development of tubal pregnancy. The incidence of EP increases in the presence of salpingitis or other inflammatory processes. Histological and molecular studies have demonstrated that inflammatory changes occur at the site of tubal pregnancy (King et al., 2009). Implantation is known to induce a local proinflammatory response that helps to carry the embryo towards the implantation site in a manner similar to leukocyte infiltration (Bondza et al., 2008). It has been reported that production of cytokines, IL-8 and IL-1, are induced in response to the inflammatory process in human fallopian tube (Balasubramaniam et al., 2012). ILF might also be one of the factors that cause inflammation and make the fallopian tube more susceptible to tubal implantation (Guney et al., 2008). However, previous studies have only compared ectopic pregnant tissues with non-pregnant tissues. Currently, there is a distinct lack of molecular data on systemic tissues isolated from patients with tubal pregnancy that have developed from tubal infections (Salih and Taylor, 2004). In this study, we have demonstrated that β-catenin expression was up-regulated in chronically inflamed fallopian tubes compared with non-pregnant controls. This was also the case when comparing EP fallopian tubes with non-pregnant tubes. This suggests that abnormal β-catenin expression may be engaged in the inflammatory process of salpingitis and likewise involved EP. Hitherto, if development of inflammation and EP are linked, it is tempting to speculate that Wnt activation and induced inflammation contribute to tubal implantation.

**β-Catenin, E-cadherin and implantation**

It is now well established that β-catenin plays multifunctional roles in cells, functioning as an intermediate in the canonical Wnt-signaling pathway or binding with E-cadherin as a cell membrane complex (Matsuzaki et al., 2010). Hence, we were not surprised to observe in our specimens a reduction in E-cadherin expression accompanied by an increase in β-catenin expression. The cadherin/catenin complex is a component of adherens junctions on epithelial cells. We expect that a reduction in the cadherin/catenin complex will probably result in cells being less adhesive. Indeed, it has been reported that during EP E-cadherin is lost from the tubal epithelium and that it allows the trophoblasts to invade, sharing a feature of invasive carcinoma cells (Al-Nasiry et al., 2009). Reduction in E-cadherin expression has been considered as a crucial step in trophoblast invasion of the tubal epithelia. Our findings support this idea since we establish that E-cadherin expression in tubal epithelium was reduced in tubal pregnancy compared with non-pregnant samples. Moreover, this reduction in E-cadherin expression was also found in the tubal tissues outside the ectopic implantation site. This loss of epithelial membrane E-cadherin probably created the right environment (decreased cell–cell interactions and cell polarity) for trophoblast invasion and implantation.

**Glycogen accumulation and implantation**

It has been reported that implantation of the embryo requires a ‘window of receptivity’ supplied by the uterus or oviduct, representing a hospitable change in components of the epithelium and stroma. One of these key changes is an increase in glycogen content regulated by glycogen synthase and progesterone (Salameh et al., 2006). The glycogen synthase activator is controlled by GSK3β and inhibition of this enzyme is crucial for glycogen synthase activation to elevate glycogen synthesis (Tilgner et al., 2009). GSK3β expression has been reported in the endometrium and fallopian tube and inactivation of GSK3β induces glycogen production and the potential for embryo implantation (Salameh et al., 2006). In this context, our PAS and β-catenin staining results suggest that there may be a direct association between the high levels of β-catenin and glycogen synthesis during tubal pregnancy.

**Vasculogenesis and ectopic pregnancy**

During ectopic implantation, the maternal vasculature must adapt to the changes that is vitally important for a successful pregnancy. Vascular endothelial growth factor (VEGF) and its receptors have been shown to be present at higher levels in the serology and tissues of ectopic pregnancies compared with non-pregnant tissues. It is thought that increased VEGF induced by hypoxia promotes the supply of oxygen to the embryo favoring tubal implantation (Felemban et al., 2002). Although VEGF and Wnt signaling are generally studied individually, reports have demonstrated that they can interact or be dependent on each other through transforming growth factor beta signaling (Clifford et al., 2008). Hypoxia inducible factor (HIF-1), which is known to be activated by hypoxia, is downstream of the Wnt pathway, and may contribute to VEGF production (Lee et al., 2009).

In addition, a series of hormones have been implicated to regulate this vasculature adaptation during pregnancy (Burton et al., 2009), although direct evidence for vascular remodeling remains rather unclear. Progesterone has been shown to affect Wnt7a and DKK1 expression in endometrial cells (Satterfield et al., 2008; Wang et al., 2009). It has been
Inflammation in fallopian tube
↓
Wnt signaling activation
↓
E-Cad in fallopian tube
↓
Angiogenesis in fallopian tube
↓
Glycogen accumulation
↓
Appropriate environment in fallopian tube
↓
Ectopic pregnancy

Figure 6 Proposed mechanism of how inflammation may induce ectopic pregnancy in the fallopian tube.

reported that the uterine vasculature changes morphologically to increase uterine blood flow by at least 40-fold in the human (Burton et al., 2009). During pregnancy, the walls of uterine arteries undergo hypertrophy and hyperplasia that results in an increase in artery diameter and length (Burton et al., 2009). In this study, we observed in tubal pregnancy a dramatic increase in the diameter of arteries and thickness of the arterial wall. The endothelial and SM cells in these arteries were enlarged, providing evidence for vascular remodeling. Changes in tubal artery supply to the ectopic implantation site have been reported to increase blood flow by >20% (Szabo et al., 2003). However, these changes have not been characterized. Given that vascular adaptation is indispensable for both eutopic and ectopic implantation, it is reasonable to suggest that Wnt signaling is involved in the vascular remodeling process, which ultimately provides the permissive environment of embryo implantation.

Conclusion

We have schematically summarized the results of our study in Fig. 6, which depicts a model by which chronic inflammation in the fallopian tube could generate molecular mediators which up-regulate β-catenin expression. The subsequent activation of Wnt signaling could result in a loss of membrane E-cadherin as a result of decreased cadherin/β-catenin complexes. Glycogen could accumulate as a result of GSK3β inhibition. These changes, along with the vessel wall hyperplasia, may eventually lead to ectopic tubal pregnancy.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/

Acknowledgements

We would like to thank Dr Ben Du and for providing us with the Fallopian tubes, and Ms Pei-er Zheng and Ms Li Qin for technological assistance.

Authors’ roles

P. L., Z.-L.M., G.W. and Y.C. performed the experiments and collected the data; H.P. contributed the materials; W.-J.Z. and X. Y. designed the study and analyzed the data with input from P.L.; K.K.H.L. critically read the manuscript; P.L. and X.Y. wrote the manuscript.

Funding

This study was supported by NSFC grant (31071054); ‘973 Project’ (2010CB529702) and NSFC grant (30971493); Guangdong Natural Science Foundation (S201101001593); Collaborated grant for HK-Macao-TW of Ministry of Science and technology (2012DFH30060) to X.Y.

Conflict of interest

None declared

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