Tubal origin of ovarian endometriosis

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Endometriosis is a puzzling and debilitating disease that affects millions of women around the world. Ovary is the most common organ site involved by endometriosis. Despite various hypotheses about its cell of origin, uncertainty remains. On the basis of our clinicopathologic observations, we hypothesize that fallopian tube may contribute the histogenesis of ovarian endometriosis. To examine if the hypothesis, tubal origin of ovarian endometriosis, has scientific supporting evidence, we identified a set of novel genes, which are either highly expressed in the normal fallopian tube or in the endometrium through a gene differential array study. Among many differentially expressed genes, *FMO3* and *DMBT1* were selected as the initial biomarkers to test the hypothesis. These biomarkers were then validated in ovarian sections with foci of endometriosis by comparing their expression levels in the fallopian tube and the endometrium within the same patients with real-time PCR, western blot and immunohistochemistry analysis. *FMO3* was highly expressed in the tubal epithelia while low in the paired endometrium. In contrast, *DMBT1* was high in the endometrium but low in the fallopian tube. In 32 ovarian endometriosis cases analyzed by real-time PCR, 18 (56%) showed a high level of *FMO3* and a low level of *DMBT1* expression. However, 14 (44%) endometriosis cases showed a reversed expression pattern with these two markers. Results were similarly seen in the methods of western blot and immunohistochemistry. The findings suggest that approximately 60% of the ovarian endometriosis we studied may be derived from the fallopian tube, whereas about 40% of the cases may be of endometrial origin. The fallopian tube epithelia may represent one of the tissue sources contributing to ovarian endometriosis. Such novel findings, which require confirmation, may have a significant clinical impact in searching for alternative ways of prevention and treatment of endometriosis.

Introduction

Endometriosis is defined by the presence of endometrial tissue outside the uterus. It is one of the most common benign gynecologic disorders associated with pelvic pain and infertility. Endometriosis, most commonly involving the ovary, affects approximately 10% of women in their reproductive age and up to 50% of women suffering infertility

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and abdominal pain.[1–5] The pathogenesis of endometriosis remains unclear and elusive since it was first described by Von Rokitansky over 100 years ago.[6] Sampson's retrograde menstruation theory[7,8] has been widely accepted since 1920s, but remains controversial as retrograde menstruation occurs in up to 90% of women in reproductive age[9] but only 6–10% of those women have endometriosis. Retrograde menstruation may explain occurrence of endometriosis within the pelvis or abdominal cavity but fails to explain the presence of endometriosis in remote sites outside the peritoneal cavity. These controversial points led Iwanoff and Meyer to propose the coelomic metaplasia theory,[8] which explained that endometriosis may derive from mesothelial cells through metaplasia. The presence of endometriosis in remote areas and the rare endometriosis in males support the metaplasia

theory.[10] However, metaplastic theory also lacks a cellular or molecular basis.[11,12] Lymphatic and vascular transportation of the endometrium has also been proposed as a complimentary theory to explain rare cases of endometriosis occurring in unusual locations far from the pelvis,[13] but this is unlikely to be the primary mechanism of disease spread.[14–16] Another compelling proposal suggests that bone marrow-derived stem cells may differentiate into endometriotic tissue within and outside of endometrial cavity.[17] But the stem cell theory is primitive and not completely understood, although it is interesting.[18] Overall, no single theory perfectly accounts for the pathogenesis of all cases of endometriosis.

The fallopian tube was previously recognized only as a carrier for the menstrual endometrium to pass into the peritoneal cavity or onto the ovarian surface. The fallopian tube has never been examined as a possible tissue or cellular source of endometriosis. However, we thought the fallopian tube likely contributes to ovarian endometriosis formation based on our clinicopathological observations. Tubal mucosa is known to be able to form endometrial-like tissue. For instance, endometrialization is commonly seen within the tubal lumen after tubal ligation.[8,19] It is also known that tubal epithelia shed viable cells onto the ovarian surface forming endosalpingiosis or ovarian epithelial inclusions, a common finding seen within the ovary in approximately 30% of the cases.[20,21] In a recent study on the cell origin of ovarian low-grade serous carcinoma, we demonstrated that the majority of the ovarian epithelial inclusions are derived from tubal epithelia[20] and the ovarian epithelial inclusions could be transformed into ovarian endometriosis through a probable metaplastic process.[22] Therefore, we hypothesize that tubal epithelium contributes to the formation of ovarian endometriosis. In this study, we identified a set of novel genes, which are either highly expressed in the fallopian tube or in the endometrium through a gene differential array study. We validated these unique genes and their corresponding protein expression in ovarian endometriosis by comparing their expression levels to paired specimens of fallopian tube and endometrium within individual patients.

Materials and methods

Tissue Specimens

Tissue samples including human tubal fimbria, paired endometrium and ovarian endometriosis were obtained from surgical pathology specimens within 30 min of resection at the Department of Gynecology, Qilu Hospital of Shandong University, Shandong, China. The human subject research protocols were approved by the institutional review board. A total of 147 specimens derived from 56 patients were studied. Among them, each of 35 patients with ovarian endometriosis generated a set of samples including fallopian tube, endometrium and ovarian endometriosis. The remaining non-endometriosis patients generated 21 paired fallopian tube and endometrial samples. All these patients underwent total hysterectomy and bilateral salpingo-oophorectomy for either ovarian endometriosis or benign gynecologic disease without endometriosis. The patients' age ranged from 35 to 51 years with a mean age of 42. No patient studied received hormonal treatment within the 6 months before surgical resection. Representative portions of the same tissue specimen were either snap frozen and stored in liquid nitrogen until use or fixed in 10% neutral formaldehyde overnight and embedded in paraffin for routine histological examination. Tissue identification and histologic diagnosis of ovarian endometriosis were confirmed by gynecologic pathologists. Tissue sections containing areas of endometriosis, tubal mucosa and endometrium with both glandular epithelia and stroma were confirmed under microscope and hand microdissected for either real-time PCR or western analysis.

Microarray and Data Analysis

In order to identify tissue-specific biomarkers, we compared the gene expression between the fallopian tube and the endometrium from patients without evidence of endometriosis by gene array analysis. Three pairs of fresh human endometrium (two proliferative phase and one secretory phase) and corresponding tubal fimbria specimens were selected from the pool of the 21 paired samples mentioned above, labeled, and sent to Kang Chen Bio-Tech (Shanghai, China) to perform whole-genome expression microarray analysis using the Agilent array platform. All the endometrial samples were histologically examined under routine microscope and no tubal metaplasia was found in any of the samples we analyzed. Total RNA from three pairs of hand microdissected epithelial samples were prepared using TRIzol (Invitrogen, Gaithersburg, MD, USA), further quantified by the NanoDrop 1000 and RNA integrity was confirmed by standard denaturing agarose gel electrophoresis. The Human Gene Expression Array was manufactured by Agilent with 41 000 + genes and transcripts represented, all with public domain annotations.

Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies, Palo Alto, CA, USA) and described elsewhere.[23] Median normalization and subsequent data processing were performed by using the GeneSpring GX v11.0 software package. After median normalization of the raw data, genes that at least six out of six samples have flags in

present ('all targets value') were chosen for further data analysis. Differentially expressed genes were identified through fold change filtering. Hierarchical clustering was performed using the Agilent GeneSpring GX software (version 11). Gene ontology analysis and pathway studies were performed in the standard enrichment computation method.

The criteria for selection of differentially expressed genes were as follows: (1) the cutoff value differentially expressed level between the tubal fimbria and the endometrium is more than twofold; and (2) $P < 0.05$ in fold change filtering. Genes that fit these criteria were considered significant for discrimination.

Validation of Microarray Data by Real-Time PCR

To verify the gene expression data obtained from the microarray, real-time PCR was performed on two selected genes, using total RNA from 21 paired tubal fimbria and corresponding endometrial samples including those three paired specimens for the gene array analysis. Among the 21 paired specimens, 17 were in proliferative phase and the remaining 4 were in secretory phase within the menstrual cycle. The target genes for this study were selected based on the level of expression after comparing with tubal expression: *FMO3* (55.72-fold) and *DMBT1* (− 44.57-fold). *FMO3*, flavin-containing monooxygenase 3 belongs to the *FMO* family, encoding microsomal flavin-containing monooxygenase, which was involved in the oxidative metabolism of a variety of xenobiotics. In human beings, *FMO3* is predominantly expressed in the adult liver, but it appears not sex dependent. The gene *DMBT1* codes for a protein belonging to the superfamily of scavenger receptor cysteine-rich proteins. *DMBT1* is involved in the immune defense and may partially have role in epithelial differentiation. *GAPDH* expression was used as the internal control. Thirty-five cases with ovarian endometriosis and paired tubal and endometrial samples were analyzed by real-time PCR. Primers were designed using Primer 3 software and the sequences were as follows: *FMO3*: F5′-AATTCGGGCTGTGATATTGC-3′ and R5′-TTGAGGAAGGTTCCAAATCG-3′; *DMBT1* F5′-TGCTCTGTCTGCCAAATCAC-3′ and R5′-GTCATTGTCTGCCTGCTTGA-3′.

The protocol of real-time PCR is described elsewhere.[24] Data analysis was performed with StepOnePlus™ Real-Time PCR System software, version 2.2 (Applied Biosystem, Hercules, CA, USA). For relative quantification of gene expression, the comparative Ct method (ΔΔCt) was used. To calculate the quantification, amplification products were normalized to *GAPDH* (ΔCt). Statistical analysis was performed using paired two-tailed *t*-test to compare relative mRNA expression levels in the fallopian tube and the corresponding endometrium,

or ovarian endometriosis lesions. Statistical significance was defined as a P-value < 0.05.

Western Blot Analysis

Monoclonal antibodies for FMO3 and DMBT1 were obtained from Abcam, USA, FMO3 for 1:8000 dilution, DMBT1 for 1:1000 dilution. All samples mentioned above were subsequently evaluated for protein expression by western blot and the detailed method is described elsewhere.[25] GAPDH antibody was used as the loading control.

Immunohistochemistry

Immunohistochemistry with antibodies to FMO3 and DMBT1 was performed as described previously.[20] The fallopian tubal mucosa and proliferative endometrial sections served as positive controls for FMO3 and DMBT1, respectively. Negative controls were carried out by replacing primary antibodies with class-matched mouse and rabbit IgGs on parallel sections. The subcellular staining localization for FMO3 was cytoplasmic, whereas for DMBT1 it was both cytoplasmic and membranous. More than 25% positively stained epithelial cells were arbitrarily defined as positive, whereas 25% or less stained were negative.

The potential stromal cell contamination from those microdissected endometrial or tubal epithelia was examined by using immunohistochemical staining with CD10 (positive for endometrial stromal cells) and vimentin (positive for tubal stromal cells). The purity of the microdissected epithelia reached > 99% as there were barely any CD10 or vimentin-positive cells observed in representative samples.

Statistical Analysis

A *t*-test was used for analyzing the differences of mRNA and protein expression between the tubal fimbria and the paired endometrium. ANOVA was used to analyze the different expression levels among the tubal fimbria, the paired endometrium and the foci of ovarian endometriosis. Multiple comparisons by LSD *t*-test were carried out among groups of ovarian endometriosis, fallopian tube and endometrium. Analyses were performed using the SPSS statistical software program version 13.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant.

Results

In order to examine if the fallopian tube truly has a role in the pathogenesis of ovarian endometriosis, we have to examine the possibility of the endometrial origin in the same setting. On the basis of this understanding, we have used total mRNA

microarray analysis to identify unique genes and corresponding proteins from the tubal and endometrial samples of patients without endometriosis. Identified unique products were then studied in the lesions of ovarian endometriosis as well as in paired tubal and endometrial tissues.

Multiple Unique Genes Identified in the Fallopian Tube Over the Endometrium

A total of 4114 and 3451 genes were identified in the fallopian tube and endometrium, respectively. The gene expression profile of the fallopian tube and paired endometrium was compared by using a Volcano Plot. The threshold for the gene expression level comparison between the fallopian tube and the endometrium was ≥ 2.0 fold change. There were 1796 genes identified with more than twofold differential expression between human fallopian tube and endometrial tissues. All these differentially expressed genes were further scrutinized and the highly differentially expressed genes were summarized. Compared with the endometrium, the fallopian tube showed a total 911 upregulated genes. These included 50-fold or more ($n=8$), 20-fold or more ($n=28$) and 2-fold or more ($n=875$). Compared with the fallopian tube, the endometrium showed a total of 885 upregulated genes including 20-fold or more ($n=7$) and 2-fold or more ($n=878$). There were no genes with >50-fold upregulation found in the endometrial tissue. The representative up- or downregulated genes are listed in Table 1 as Supplementary Data.

FMO3 was Highly Expressed in the Fallopian Tube, whereas DMBT1 was in the Endometrium

There were many upregulated genes either in the fallopian tube or in the endometrium identified through the gene array analysis. We sought target genes, which were uniquely stable, conservative, not easily regulated by menstrual cycle hormones or cell proliferative status as the endometrial and tubal tissue are more or less regulated by menstrual hormones and such hormone-regulated genes may not be representative for current study. Meanwhile, in a practical level, the corresponding protein products should have antibodies that are known to work both in immunohistochemistry and in western blot assays. After screening, we identified two genes, one was highly expressed in the fallopian tube (*FMO3*) and the other in the endometrium (*DMBT1*), which matched our specified conditions. We validated these two genes with real-time PCR, western blot and immunohistochemistry.

Both the *FMO3* and *DMBT1* genes follow the trend differences of the microarray results. In the 21 pairs of the tubal and corresponding endometrial samples in real-time PCR analysis, *FMO3* was highly expressed in the fallopian tube compared with the

paired endometrium, with fold increment ranging from 4 to 324 (average fold change $= 44.38$, $P<0.001$). In contrast, *DMBT1* was highly expressed in endometrium compared with the fallopian tube with fold change ranging from 5 to 456 (average fold change $= 22.11$, $P<0.001$). The data pool of the 21 paired cases is shown in Figures 1a and b. Among the 21 paired tubal and endometrial samples, there were 17 pairs in proliferative phase and 4 pairs in secretory phase of the menstrual cycle. In either fallopian tube or endometrial tissue, the gene expression level of *FMO3* and *DMBT1* were indifferent between the proliferative or secretory phases ($P>0.10$). In addition, none of the 21 pairs of samples showed discordance with the trend toward co-expression of *FMO3* and *DMBT1* in fallopian tube *vs* endometrial tissue.

Among the 21 pairs of tubal and endometrial samples, 14 pairs were adequate for western blot analysis. FMO3 protein expression was significantly higher in the fallopian tube samples than that in the endometrium, with an average fold of increment 11.05 ($P=0.006$). In contrast, the DMBT1 protein level was significantly lower in the fallopian tube (average decreasing fold $= 32.08$) compared with the expression in the endometrium ($P=0.001$). These results were compatible with the findings from real-time PCR validation, indicating *FMO3* and *DMBT1* do not change significantly at the transcriptional and post-transcriptional levels. Representative protein expression level is shown in Figures 1c and d.

All 21 pairs of the tissue samples were studied for the cellular location of both FMO3 and DMBT1 by immunohistochemistry. Both biomarkers were mainly cytoplasmic. No nuclear stainings were identified for these two genes. Of the 21 paired samples, 19 (91%) showed moderately to strongly positive staining of FMO3 in the majority epithelial cells of the fallopian tube, whereas the remaining two tubal samples were weakly stained. In contrast, FMO3 was only weakly and focally expressed in the three (14%) endometrial samples, mainly within the endometrial glands. There were some stromal and endothelial cell stainings identified with no specific pattern. DMBT1 cellular localization was totally different from FMO3. DMBT1 was strongly and diffusely positive in the majority of glandular cells in all 21 endometrial samples, but not in the tubal sections we studied. These results will be further presented in the next section when ovarian endometriosis was compared.

FMO3 and DMBT1 Expression in Ovarian Endometriosis

After validation of differentially expressed *FMO3* and *DMBT1* in paired tubal and endometrial samples, we further examined these genes and corresponding protein expression in patients with ovarian endometriosis as well as its corresponding fallopian tubal
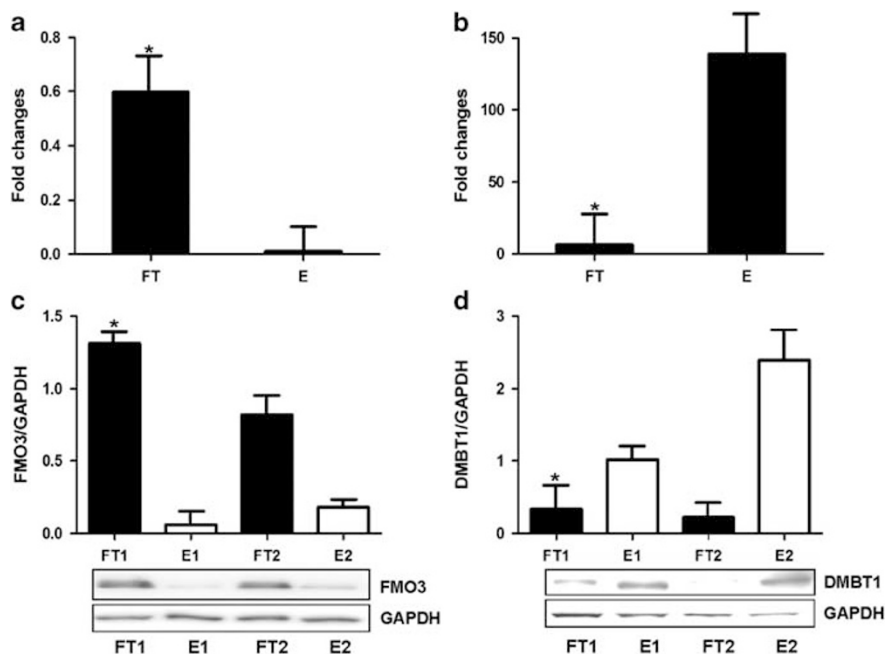
Figure 1 *FMO3/DMBT1* expression level in paired tubal and endometrial tissue samples. Comparisons of mRNA expression levels in the fallopian tube and the endometrium by quantitative PCR in 21 paired samples (pooled data): *FMO3* was significantly higher in the fallopian tube than that in the endometrium (**a**, $P < 0.001$); whereas *DMBT1* showed a complete reverse pattern (**b**, $P < 0.001$). Comparisons of protein level of expression in the fallopian tube and the endometrium by western blots in two representative paired cases (**c**, **d**). Similarly, FMO3 was higher in the fallopian tube than the endometrium (**c**, $P = 0.006$), whereas DMBT1 was higher in the endometrium (**d**, $P = 0.001$). The western bands were in the lower panel, whereas the bar graphs reflected the bands density after balanced with the GAPDH control. Each experiment was conducted three times, and the data were expressed as mean ± s.e.m. *Statistically significant by comparing with the paired cases. E, endometrium; FT, fallopian tube.

and endometrial samples. There were a total of 35 patients with the requisite three samples for the study. These gene expression levels were compared in patients with ovarian endometriosis and those without ovarian endometriosis from the data presented above. Both *FMO3* and *DMBT1* expression levels in the fallopian tubal and endometrial samples showed no statistical difference between the patients with ovarian endometriosis and those without ovarian endometriosis. In other words, *FMO3* remained high in the fallopian tube, whereas *DMBT1* was high in the endometrium (data not shown).

Among the 35 cases with ovarian endometriosis, 32 paired samples were adequate for real-time PCR analysis. *FMO3* was highly expressed in 18 (56%) of the 32 samples studied, with the fold increment ranging from 4.9 to 123 (average fold change = 7.21, $P = 0.016$) compared with the endometrium. However, *FMO3* expression in the remaining 14 ovarian endometriosis samples was significantly lower than that in FT ($P < 0.01$), but similar to the level of expression in the corresponding endometrium ($P = 0.184$). In contrast, the 18 patients with high level of *FMO3* expression showed a significantly low expression of *DMBT1* in the ovarian endometriosis lesions compared with the paired endometrial samples (average decreasing fold = 6.94,

$P = 0.022$). Meanwhile, *DMBT1* expression showed no statistical differences between the ovarian endometriosis and fallopian tube samples ($P = 0.144$). The data are summarized in Figures 2a and b. There were no statistical difference of age between the 18 cases with high *FMO3* expression and the remaining 14 samples.

The 18 ovarian endometriosis samples with high *FMO3* and low *DMBT1* expression also showed concordant level of the protein expression by western blot. The FMO3 expression increased ranging from 3.3 to 58.5-fold with an average fold increment of 7.2 ($P = 0.007$) compared with the expression in the endometrium. Among the remaining 17 ovarian endometriosis samples, 8 showed a similar level of FMO3 expression to the endometrium but lower than the tube, whereas 9 showed no statistical difference compared with either the endometrium or the fallopian tube. Among the 35 paired patients, 19 cases showed that DMBT1 protein expression in ovarian endometriosis samples was similarly low in the fallopian tube, whereas significantly high in the endometrium. DMBT1 in the remaining 16 cases showed high expression in areas of ovarian endometriosis and the endometrium ($n = 12$) and no difference ($n = 4$) between ovarian endometriosis and paired endometrium. Representative western data are presented in Figures 2c and d.
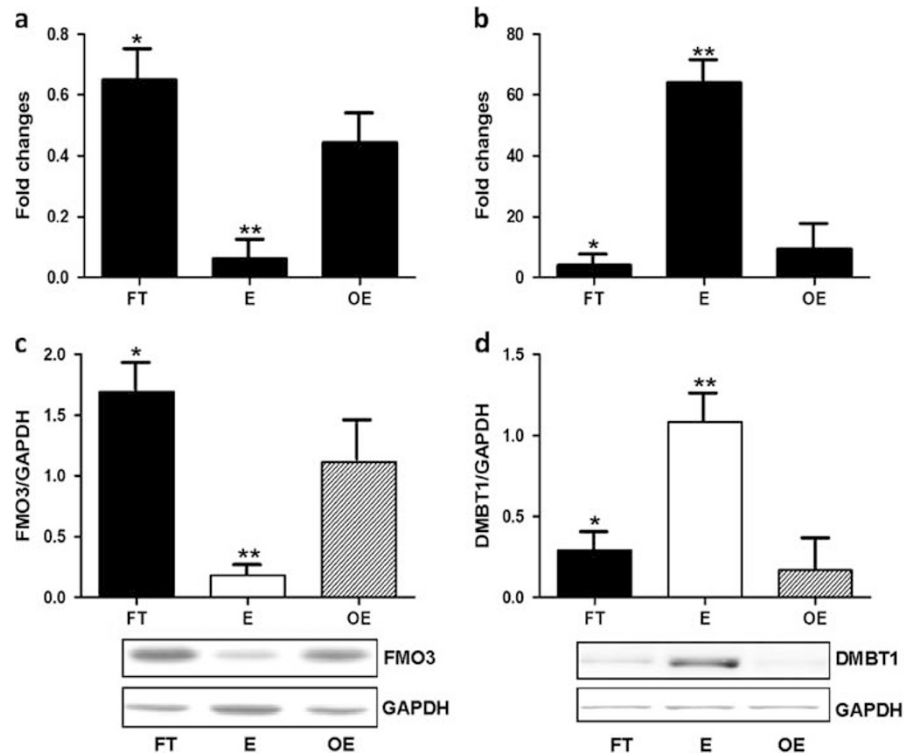
Figure 2 *FMO3/DMBT1* expression level in paired tubal, endometrial and ovarian endometriosis samples. Comparisons of mRNA expression levels in the fallopian tube, the endometrium and the foci of endometriosis by quantitative PCR in 18 paired cases (pooled data): *FMO3* level in fallopian tube was similar to the level of endometriosis, but both were higher than that in the endometrium (**a**, $*P = 0.184$ (fallopian tube *vs* ovarian endometriosis) and $**P = 0.016$ (endometrium *vs* ovarian endometriosis)); *DMBT1* level in fallopian tube was similar to the level of endometriosis, but both were lower than that in the endometrium (**b**, $*P = 0.144$ (fallopian tube *vs* ovarian endometriosis) and $**P = 0.022$ (endometrium *vs* ovarian endometriosis)). Comparisons of protein level of expression in the tubal, endometrial and ovarian endometriosis by western blot in one representative patient (**c**, **d**). FMO3 showed a strong band in both tubal and ovarian endometriosis samples, whereas barely detected in the corresponding endometrium (**c**, $*P = 0.102$ (fallopian tube *vs* ovarian endometriosis) and $**P = 0.007$ (endometrium *vs* ovarian endometriosis)); whereas DMBT1 showed a strong band only in the endometrial sample, not in the samples of the fallopian tube and endometriosis (**d**, $*P = 0.221$ (fallopian tube *vs* ovarian endometriosis) and $**P = 0.006$ (endometrium *vs* ovarian endometriosis)). The western bands were in the lower panel, whereas the bar graphs reflected the bands density after balanced with the GAPDH control. Each experiment was conducted three times, and the data were expressed as mean ± s.e.m. E, endometrium; FT, fallopian tube.

Cellular localization of these gene products was examined by immunohistochemistry. Among the 35 paired samples, 19 (54%) showed that FMO3 was positively staining in the cytoplasm of ovarian endometriosis epithelium, whereas the remaining 16 ovarian endometriosis samples showed either low expression in focal areas ($n = 10$) or inadequate expression for analysis due to loss of glandular epithelia within the sections ($n = 6$). In terms of DMBT1, 17 (49%) ovarian endometriosis samples were negative, whereas the remaining 18 ovarian endometriosis samples showed positive staining ($n = 10$) or were inadequate ($n = 8$). Representative pictures for the FMO3 and DMBT1 immunostainings in the fallopian tube, endometrium and ovarian endometriosis samples are illustrated in Figure 3.

Discussion

Endometriosis is a worldwide problem affecting a significant number of women in their reproductive age. The histogenesis of endometriosis has perplexed investigators for decades. Despite various hypotheses, no single explanation fits all observed clinical manifestations.

The tubal fimbriated (distal) end, which 'floats' in the pelvis over the ovarian surface and the peritoneum of the pouch of Douglas, has a unique and delicate role in capturing the egg released from the ovary and provides a conduit for transport and fertilization of the egg. In the past, the attention to the fallopian tube has been primarily in younger women with infertility or the potentially life-threatening situation of ectopic pregnancy.[26] Recently, the distal fallopian tube has attracted considerable attention not only as site of origin for serous ovarian cancer in women with *BRCA* mutations, but also as a distinct anatomical entity where the majority of pelvic serous carcinomas apparently develop. Awareness is growing that the fallopian tube requires attention by both pathologists and gynecological surgeons. Through recent understanding of the tubal origin of ovarian 'high-grade' and
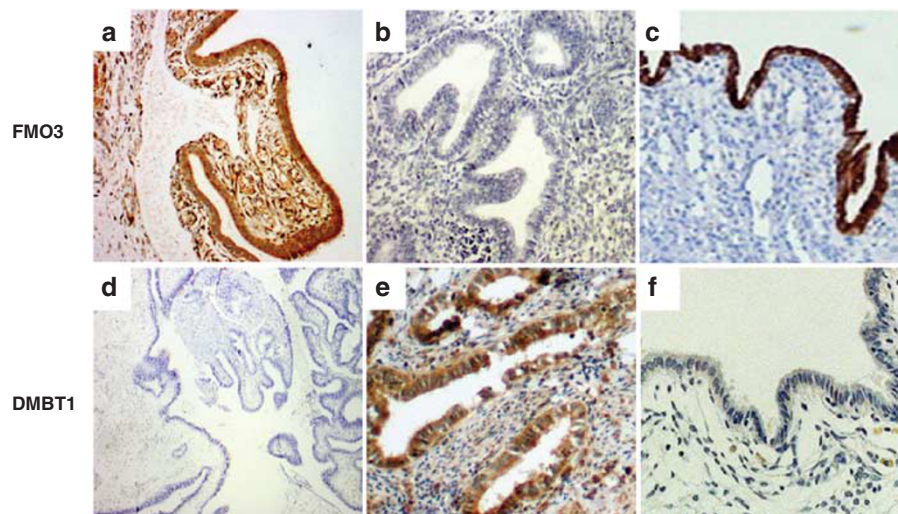
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Figure 3 FMO3 and DMBT1 protein detection by immunohistochemistry in the tubal, endometrial and ovarian endometriosis samples. Both FMO3 and DMBT1 expression were mainly cytoplasmic. Moderately to strongly FMO3 immunoreaction was observed in the tubal (**a**) and ovarian endometriosis (**c**) sections, but it was negative in the endometrium (**b**). However, DMBT1 was only strongly expressed in the endometrium (**e**), not in the fallopian tube (**d**) and the ovarian endometriosis (**f**). Original magnification: ×40 for **d**, the remaining were ×100.

'low-grade' serous carcinoma,[20,27,28] we have learned many biologic properties of the fallopian tube, which were previously unrecognized. Within women's pelvis, the fallopian tube has a close contact with the ovary,[20,27,29–31] the tubal epithelial cells are easily detached from the tubal mucosa,[27,32] and the majority of the ovarian epithelial inclusions are derived from the fallopian tube.[20] We proposed that ovarian endometriosis, at least partially, may be derived from the fallopian tube. This hypothesis of tubal origin of ovarian endometriosis is novel, which has not been proposed elsewhere in the past.

The main obstacle for us to verify this novel hypothesis is to challenge the most popular retrograde menstruation theory. That means we need to study both the fallopian tube and the endometrium as potential sources of ovarian endometriosis. With this understanding, we identified differentially expressed genes between the endometrium and the fallopian tubes by using whole-genome expression microarray. Among many differentially expressed genes, *FMO3* and *DMBT1* turned out to be two good biomarkers to test the hypothesis. *FMO3* was highly expressed in the tubal epithelia while low in the paired endometrium. In contrast, *DMBT1* was high in the endometrium but low in tubal mucosa in those 21 patients without endometriosis. These two markers were therefore highly specific and therefore appropriate for examining our hypothesis as *FMO3* appears to be a marker of tubal epithelia and *DMBT1* of the endometrial glandular cells. We tested the tubal and endometrial samples with these two markers on 32 patients with ovarian endometriosis. It was found that *FMO3* and *DMBT1* were similarly differentially expressed in the fallopian tube and in the endometrium as seen in the 21 patients without

endometriosis. The results were quite consistent among the three validation methods we used in this study. However, when we analyzed *FMO3* and *DMBT1* expression in the samples of ovarian endometriosis, two different outcomes emerged. In all, 18 (56%) of the 32 ovarian endometriosis lesions showed a high level of *FMO3* expression but low with *DMBT1*. In contrast, 14 (44%) endometriosis samples showed a reversed expression pattern with these two markers. Again, the results were similarly seen among the methods of real-time PCR, western blot and immunohistochemistry. Our findings indicate that approximately 60% of the ovarian endometriosis we studied is likely to be derived from the fallopian tubal epithelia, whereas about 40% of the cases may be of endometrial origin. Therefore, this study supports our hypothesis that ovarian endometriosis may be at least partially derived from the fallopian tube.

The finding of possible tubal origin of ovarian endometriosis is interesting and may have many clinical impacts, although the study result is preliminary and the underlying mechanism is unclear. However, evidence that tubal epithelia are potentially able to form endometriosis exists. Endometrialization of the fallopian tube representing endometrium-like tissue within the proximal end of the tubal segment is commonly observed in patients who underwent tubal ligation for undesired fertility.[33–35] Although we cannot exclude the possibility of endometrial colonization for those tubal ligation cases, many investigators believe that metaplasia from tubal epithelia seems more likely as a transitional area from normal looking tubal epithelia to minimally formed endometriosis or endometrial-like tissue are commonly present.[27,36] In order to

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form ovarian endometriosis, tubal cells have to find a reasonable route to get onto the ovary. The easy detachment of tubal epithelia makes it practical. This is supported by the findings that the tubal epithelial cells are easily obtained for culture by flushing the fallopian tube.[27,32] The process is further facilitated by close spatial anatomic relationship between the tubal fimbria and the ovarian surface and their synergistic role of ovulation and egg capture and subsequent transportation.[26,29] Furthermore, ovulation induced wound formation on the ovarian surface,[37–39] pelvic inflammation,[40–42] inflammation induced ovarian surface adhesion[43,44] and ovarian stromal proliferation under the influence of gonadotropins[39,45–47] provide all necessary conditions for those tubal epithelia to enter the ovarian cortex. Actually, this common process from morphologic perspective has long been described as 'endosalpingiosis'.[48–50] Such epithelial inclusions found in the ovary are also called as ovarian cortical or epithelial inclusions.[51,52]

The question remains how endosalpingiosis or ovarian epithelial inclusions or the tubal type epithelia are transformed into endometriosis (endometrial type cells in morphology). One of our recent studies regarding the cell origin of ovarian serous cancers have demonstrated that ovarian epithelial inclusions are mainly derived from fallopian tube,[20] supporting the terminology of endosalpingiosis, a simple but direct morphologically described entity. In addition, the transformation from endosalpingiosis or ovarian epithelial inclusions can be explained by metaplasia, a process commonly seen in müllerian system.[53] This interpretation is supported by our previous observation of ovarian initial endometriosis.[22] Initial endometriosis within the ovary defines the morphologic transition of ovarian epithelial inclusions with half of the glandular epithelia showing the earliest morphologic changes of endometriosis.[22] Such a morphologic transition is certainly not interpretable by retrograde menstruation theory, whereas a metaplasia from tubal epithelia instead of from mesothelia via unidentified factors is more likely applicable in this situation. It is interesting to note that tubal stromal cells were positive for FMO3 staining (Figure 3a) but negative in the stroma of ovarian endometriosis (Figure 3c). The reasonable interpretation for this observation is that the endometriotic epithelial cells were likely derived from tubal epithelia, whereas the associated stromal cells may be derived from ovarian stroma in a *de novo* process, albeit the phenomenon remains clarified in near future.

The novel findings of this study may provide another angle to think the histogenesis of ovarian endometriosis and such different view may ultimately shed light of our understanding the pathogenesis of endometriosis and lead us to develop a practical way of endometriosis prevention. However, again the findings remain primitive and descriptive. Additional studies from different perspectives are necessary to strengthen our understanding of fallopian tubal origin of ovarian endometriosis.

Acknowledgments

Disclosure/conflict of interest

The authors declare no conflict of interest.

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