Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis†

Sachiko Matsuzaki1,2,* and Claude Darcha3

1CHU Clermont-Ferrand, CHU Estang, Chirurgie Gynécologique, 1, Place Lucie Aubrac, 63003 Clermont-Ferrand, France 2University of Auvergne Clermont I, CENTI, 2ETG, Bâtiment 3C, 28, Place Henri Dunant, 63000 Clermont-Ferrand, France 3CHU Clermont-Ferrand, Service d’Anatomie et Cytologie Pathologiques, Clermont-Ferrand, France

*Correspondence address. Tel: +33-4-73-75-01-38; E-mail: sachikoma@aol.com

Submitted on September 10, 2011; resubmitted on November 10, 2011; accepted on November 28, 2011

BACKGROUND: Endometrium is derived from intermediate mesoderm via mesenchymal to epithelial transition (MET) during development of the urogenital system. By retaining some imprint of their mesenchymal origin, endometrial epithelial cells may be particularly prone to return to this state, via epithelial to mesenchymal transition (EMT). We hypothesized that pelvic endometriosis originates from retrograde menstruation of endometrial tissue and that EMT-like and MET-like processes might be involved in the pathogenesis of pelvic endometriosis.

METHODS: We investigated commonly used molecular markers for EMT, including cytokeratin, E-cadherin, N-cadherin, vimentin, S100A4 and dephosphorylated beta-catenin by immunohistochemistry in different forms of pelvic endometriosis: deep infiltrating endometriosis, ovarian endometriosis and superficial peritoneal endometriosis (red and black lesions), as well as samples of menstrual endometrium, other benign ovarian cysts (mucinous and serous cyst adenoma), and abdominal scar endometriosis for comparison.

RESULTS: Epithelial cells of red peritoneal lesions and ovarian endometriosis showed less epithelial marker (cytokeratin, \( P < 0.0001 \)) expression and more mesenchymal marker (vimentin and/or S100A4, \( P < 0.0001 \)) expression than those of menstrual endometrium. In contrast, epithelial cells of black peritoneal lesions and deep infiltrating endometriosis showed more epithelial marker (E-cadherin) expression than those of menstrual endometrium (\( P < 0.03 \)), red peritoneal lesions (\( P < 0.0001 \)) and ovarian endometriosis (\( P < 0.0001 \)), but maintained expression of some mesenchymal markers (vimentin, S100A4). In addition, dephosphorylated beta-catenin protein expression was significantly higher in epithelial cells of deep infiltrating endometriosis (\( P < 0.0001 \)) than in epithelial cells of red and black peritoneal lesions and ovarian endometriosis.

CONCLUSIONS: EMT-like and MET-like processes might be involved in the pathogenesis of pelvic endometriosis.

Key words: epithelial to mesenchymal transition / endometrium / endometriosis / mesenchymal to epithelial transition / pathogenesis

Introduction
Endometriosis, a common cause of infertility and pelvic pain, is defined as the presence of endometrial glands and stroma within extra-uterine sites (Clemeny, 1994). This condition affects ~10% of women of reproductive age (Eskenazi and Warner, 1997). However, the pathogenesis of endometriosis remains unclear. Knowledge of the origin of endometriotic cells is indispensable for the development of strategies for prevention and targeted treatment of endometriosis. The most widely accepted theory about the pathogenesis of endometriosis is implantation theory (Giudice and Kao, 2004). Endometriosis originates from retrograde menstruation of endometrial tissue that passes through patent Fallopian tubes into the peritoneal cavity. According to implantation theory, two processes appear to be critical for the establishment of endometriosis: migration and invasion (Giudice and Kao, 2004; Hull et al., 2008). Although endometriosis is a benign condition, it can lead to severe pain, infertility, and other complications.
disease, studies suggest that the invasive phenotype shares aspects with tumor metastasis (Gaetje et al., 1997; Zeitvogel et al., 2001). Conversely, growing evidence suggests that epithelial to mesenchymal transition (EMT)-like processes might be a key mechanism underlying the induction of invasion and metastasis of tumors (Thiery, 2002).

Endometrium is derived from intermediate mesoderm via mesenchymal to epithelial transition (MET) during the development of the urogenital system. By retaining some imprint of their mesenchymal origin, endometrial epithelial cells may be particularly prone to urogenital system. By retaining some imprint of their mesenchymal origin, endometrial epithelial cells may be particularly prone to epithelial to mesenchymal transition (EMT)-like processes might be a key mechanism underlying invasion and metastasis of tumors (Thiery, 2002).

Conversely, growing evidence suggests that epithelial to mesenchymal transition (MET) during the development of the urogenital system. By retaining some imprint of their mesenchymal origin, endometrial epithelial cells may be particularly prone to invasion and metastasis of tumors (Thiery, 2002).

55 ovarian endometriosis tissues, 76 superficial peritoneal endometriosis tissues and 18 menstrual endometrial tissues of patients with pelvic endometriosis were used for the present analysis. Endometrial tissue biopsies were performed just prior to surgery using an endometrial suction catheter (Pipelle, Laboratoire CCD, Paris, France). Deep infiltrating endometriosis was defined as endometriosis located 5 mm under the peritoneal surface. Deep infiltrating endometriosis was localized in the rectovaginal septum (n = 32), uterosacral ligament (n = 16), bladder wall (n = 8) or bowel wall (n = 12). We also included patients with endometriotic ovarian cysts >3 cm in diameter. Superficial peritoneal endometriosis was defined as endometriosis located on the peritoneal surface. Superficial peritoneal endometriotic lesions were further categorized as red, black or white according to the latest version of the American Society for Reproductive Medicine (rASRM) classification (American Society for Reproductive Medicine, 1997). Overall, 30 samples were categorized as red lesions and the remaining 46 samples were classified as black lesions.

In addition, menstrual endometrial tissues from women without endometriosis (n = 14), other benign ovarian cysts (serous cyst adenoma: n = 23; mucinous cyst adenoma: n = 20), and abdominal scar endometriosis tissues resulting from Caesarean section (n = 13) were obtained. Clinical characteristics of the patients and tissues used in the present study are shown in Table I.

If metaplasia theory is correct, ovarian endometriosis is derived from ovarian surface epithelial cells and peritoneal endometriosis is derived from peritoneal mesothelial cells (Giudice and Kao, 2004). In addition, serous cyst adenoma is thought to resemble Fallopian tube epithelium and mucinous cyst adenoma to resemble normal intestinal mucosa (Kurman and Shih, 2010, 2011). To investigate expression of epithelial and mesenchymal markers in normal peritoneal mesothelial cells, ovarian surface epithelial cells, Fallopian tube epithelial cells, and colon epithelial cells, macroscopically normal peritoneal tissues from patients undergoing hysterectomy without endometriosis (n = 5), aneexectomy samples of benign dermoid ovarian cysts (n = 3) and samples of colon from patients with deep infiltrating endometriosis who underwent colon resection (n = 5) were obtained.

None of the women had received hormonal treatments, such as gonadotropin-releasing hormone agonists (GnRHa) or sex steroids, and none of them had used intrauterine contraception for at least 6 months prior to surgery.

All samples were fixed in 10% formalin-acetic acid and embedded in paraffin for immunohistochemical analysis. Endometrial dating was performed independently by C.D. and an independent pathologist.

All tissue samples were obtained with full and informed patient consent. The research protocol was approved by the Consultative Committee for Protection of Persons in Biomedical Research (CCPPRB) of the Auvergne (France) region.

**Immunohistochemistry**

Immunohistochemical staining was performed on paraffin sections with mouse monoclonal antibodies directed against cytokeratin, E-cadherin, N-cadherin, vimentin or N-terminally dephosphorylated beta-catenin and rabbit polyclonal antibodies against S100A4 or PAX8 (Table I). Sections were deparaffinized and antigen retrieval was performed in 0.01 M sodium citrate (pH 6.0) for 3 min at full pressure using a pressure cooker. Sections were then treated with 3% hydrogen peroxide solution for 5 min to inhibit endogenous peroxidase activity. After rinsing in 0.01 M phosphate-buffered saline (PBS, pH 7.2), sections were incubated overnight at 4°C with primary antibody in PBS with 3% bovine serum albumin. Appropriate positive and negative controls were used with each antibody during each staining run. After rinsing in PBS, sections were incubated with peroxidase-labeled anti-mouse IgG (DAKO EnVision,
DAKO) or anti-rabbit IgG (DAKO EnVision, DAKO) for 30 min. Sections were then washed with PBS, colored with aminoethylcarbazole substrate, counterstained with Mayer’s haematoxylin, and mounted.

Quantification of immunostained epithelial cells

To quantify immunostained epithelial cells objectively, we utilized a computerized image analysis system consisting of a light microscope (Leica, Lyon, France) (×106 objective, ×10 ocular) and a color charge coupling device camera (Sony, Paris, France) connected to a SAMBA 2005 computer analysis system (Alcatel-TITN, Grenoble, France). Several parameters per sample for EMT markers in epithelial cells were computed: the percentage of immunostained surface (compared with the counterstained surface), the mean staining intensity, and an immunostaining score (percentage of immunostained surface × mean staining intensity) (Charpin et al., 1993; Matsuzaki et al., 2009, 2010a,b). The entire field of each section was analyzed for all EMT markers.

Statistical analysis

The Statview 4.5 program (Abacus Concepts, Inc., Berkeley, CA, USA) was used for statistical analysis. Comparisons were made using one-way analysis of variance (ANOVA) following Scheffe’s method, Mann–Whitney U-test or Fisher exact test. Statistical significance was defined as P < 0.05.

Results

In the present analysis, we detected no significant difference in expression levels of epithelial or mesenchymal markers in menstrual endometrium between patients with and without endometriosis. Thus, we included all of the data from both patients with and without endometriosis as examples of menstrual endometrium for the present analysis. In addition, we detected no significant difference in expression levels of epithelial and mesenchymal markers between the proliferative and secretory phases in pelvic endometriosis, other benign cysts, and abdominal scar endometriosis after Caesarean section. Thus, we analyzed all of the data irrespective of menstrual phase.

Pelvic endometriosis versus menstrual endometrium

The immunostaining score for cytokeratin in epithelial cells was significantly lower in red peritoneal lesions, black peritoneal lesions, ovarian endometriosis and deep infiltrating endometriosis compared with that of menstrual endometrium (Table III, Figs 1 and 2). Immunostaining scores were lower in all categories of pelvic endometriosis compared with menstrual endometrium.
scores for E-cadherin in epithelial cells were significantly higher in deep infiltrating endometriosis and black peritoneal lesions compared with those of ovarian endometriosis, red peritoneal lesions and menstrual endometrium (Table III, Figs 1 and 2).

No N-cadherin expression was detected in epithelial cells of menstrual endometrium (Supplementary data, Table SI). The immunostaining score for N-cadherin in epithelial cells of red peritoneal lesions was significantly higher in deep infiltrating endometriosis, ovarian endometriosis and mucinous cystadenoma compared with those of ovarian endometriosis (Table IV, Figs 2 and 3).

The immunostaining scores for vimentin and N-cadherin in epithelial cells were significantly higher in red peritoneal lesions and ovarian cysts (serous cyst adenoma and mucinous cyst adenoma) compared with those in ovarian endometriosis (Table IV, Figs 2 and 3).

The immunostaining scores for vimentin and N-cadherin in epithelial cells were significantly higher in red peritoneal lesions and ovarian cysts (serous cyst adenoma and mucinous cyst adenoma) compared with those in ovarian endometriosis (Table IV, Figs 2 and 3).

No dephosphorylated beta-catenin expression was detected in epithelial cells of menstrual endometrium (Supplementary data, Table SI). The immunostaining score for dephosphorylated beta-catenin in epithelial cells was significantly higher in deep infiltrating endometriosis compared with those of ovarian endometriosis, red and black peritoneal lesions (Table III, Figs 1 and 2).

PAX8 expression was detected in epithelial cells of all pelvic endometriosis and menstrual endometrium samples.

**Table III** Immunostaining score for EMT markers: Pelvic endometriosis versus menstrual endometrium.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Menstrual endometrium (n = 32)</th>
<th>Endometriosis Deep infiltrating (n = 68)</th>
<th>Ovarian (n = 55)</th>
<th>Superficial peritoneal Red (n = 30)</th>
<th>Black (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin</td>
<td>10.5 ± 3.1*</td>
<td>2.2 ± 0.5</td>
<td>2.4 ± 0.7</td>
<td>0.03 ± 0.02</td>
<td>0.2 ± 0.08</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>11.2 ± 1.3</td>
<td>20.3 ± 1.6*</td>
<td>3.3 ± 0.8</td>
<td>2.9 ± 0.6</td>
<td>19.4 ± 1.4*</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>0.0 ± 0.0</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>3.9 ± 0.80*</td>
<td>1.7 ± 0.7</td>
</tr>
<tr>
<td>Vimentin</td>
<td>10.5 ± 0.5</td>
<td>20.5 ± 2.3*</td>
<td>1.5 ± 0.6</td>
<td>34.3 ± 2.5*</td>
<td>63.4 ± 5.7*</td>
</tr>
<tr>
<td>S100A4</td>
<td>0.0 ± 0.0</td>
<td>2.4 ± 0.7</td>
<td>16.1 ± 2.3*</td>
<td>13.0 ± 2.4*</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Dephosphorylated beta-catenin</td>
<td>0.0 ± 0.0</td>
<td>18.5 ± 1.4*</td>
<td>5.3 ± 1.5</td>
<td>10.8 ± 2.2</td>
<td>9.7 ± 2.1</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SEM.
Statistical significance (One way ANOVA plus Scheffe test)
*P < 0.0001 versus all endometriosis.
$P < 0.0001$ versus menstrual endometrium, $P < 0.001$ versus ovarian and red peritoneal.
$P < 0.001$ versus menstrual endometrium, $P < 0.0001$ versus ovarian and red peritoneal.
$P < 0.0001$ versus deep infiltrating and ovarian, $P < 0.0006$ versus black peritoneal.
*P < 0.0001 versus menstrual endometrium and ovarian.
$P < 0.001$ versus red peritoneal, $P < 0.006$ versus black peritoneal.
$P < 0.0001$ versus menstrual endometrium, deep infiltrating and black peritoneal.
$P < 0.0001$ versus ovarian and red peritoneal.
$P < 0.0001$ versus ovarian and red peritoneal.

Summarising these observations by cell type, epithelial cells of red peritoneal lesions expressed significantly lower levels of cytokeratin and significantly higher levels of S100A4 and vimentin compared with epithelial cells of menstrual endometrium. Epithelial cells of black peritoneal lesion expressed significantly higher levels of E-cadherin and significantly lower levels of S100A4 compared with epithelial cells of red peritoneal lesions. E-cadherin expression was significantly higher than that of menstrual endometrium.

Ovarian endometriosis versus other benign ovarian cysts (serous cyst adenoma and mucinous cyst adenoma)

Immunostaining scores for cytokeratin in epithelial cells were significantly higher in mucinous cystadenoma and serous cystadenoma compared with that in ovarian endometriosis (Table IV, Figs 2 and 3).

The immunostaining score for E-cadherin in epithelial cells was significantly higher in mucinous cystadenoma compared with those in serous cyst adenoma and ovarian endometriosis (Table IV, Figs 2 and 3).

The immunostaining scores for vimentin and N-cadherin in epithelial cells were significantly higher in serous cyst adenoma compared with those in mucinous cyst adenoma and ovarian endometriosis (Table IV, Figs 2 and 3).

Immunostaining scores for S100A4 in epithelial cells were significantly higher in serous cyst adeno and ovarian endometriosis compared with those in mucinous cyst adenoma (Table IV, Figs 2 and 3).

The immunostaining score for dephosphorylated beta-catenin in epithelial cells was significantly higher in mucinous cystadenoma compared with those in serous cyst adenoma and ovarian endometriosis (Table IV, Figs 2 and 3).
Abdominal scar endometriosis after Caesarean section versus menstrual endometrium

The immunostaining score for E-cadherin in epithelial cells was significantly higher for abdominal scar endometriosis tissue \((P < 0.0001)\) than that of menstrual endometrium (Table V, Fig. 2). No significant difference in immunostaining score was observed in epithelial cells for cytokeratin and vimentin between abdominal scar endometriosis and menstrual endometrium (Table V, Fig. 2). PAX8 expression was detected in epithelial cells of all abdominal scar endometriosis samples.

Peritoneal mesothelial cells, ovarian surface epithelial cells, Fallopian tube epithelial cells and colon epithelial cells

Results were shown in Table VI and Fig. 3. Cytokeratin expression was detected in all cell types examined (peritoneal mesothelial cells, ovarian surface epithelial cells, Fallopian tube epithelial cells and colon epithelial cells). While E-cadherin expression was detected in colon epithelial cells and epithelial cells of the distal Fallopian tubes, none was detected in peritoneal mesothelial cells, ovarian surface epithelial cells or epithelial cells of the proximal Fallopian tubes. N-cadherin and vimentin expression was detected in peritoneal mesothelial cells, ovarian surface epithelial cells and Fallopian tube epithelial cells, whereas none was detected in colon epithelial cells. No S100A4 expression was detected in any cells. Dephosphorylated beta-catenin expression was detected in Fallopian tube epithelial cells and colon epithelial cells, whereas none was detected in peritoneal mesothelial cells or ovarian surface epithelial cells. PAX8 expression was detected in Fallopian tube epithelial cells, but not in peritoneal mesothelial cells, ovarian surface epithelial cells or colon epithelial cells.

Discussion

Pelvic endometriosis

Superficial peritoneal lesions

The absent or sparse expression of N-cadherin in the majority of pelvic endometriosis samples is in agreement with the results of a previous study that used the same primary antibody (Van Patten et al., 2010). However, the frequency of N-cadherin-positive samples...
Supplementary data, Table SI) as well as the immunostaining score were significantly higher in red peritoneal lesions compared with black peritoneal lesions. The present findings suggest that red peritoneal endometriosis might have a more invasive nature (Gaetje et al., 1997; Zeitvogel et al., 2001), along with increased expression of mesenchymal markers, compared with black peritoneal lesions. Growing evidence suggests that black and red peritoneal lesions may represent different stages of the spontaneous evolution of endometriotic implants, with red lesions being the first stage (Nisolle and Donnez, 1997; Fazleabas et al., 2002). In the present study, expression of the N-terminally dephosphorylated form of beta-catenin was detected in peritoneal endometriosis, suggesting activation of the Wnt/beta-
peritoneal endometriosis, a recent study demonstrated that EMT/molecular mechanisms underlying EMT and MET processes in superficial metastatic deposits are responsible for the discordance between migratory and invasive carcinoma cells with mesenchymal features, as well as the carcinomatous, epithelioid macrometastatic deposits that present clinically, although functional evidence supporting this hypothesis has not yet been forthcoming (Lee et al., 2009). These findings suggest that endometrial epithelial cells might undergo an EMT-like process after attachment of endometrium to peritoneum, resulting in red peritoneal endometriosis. MET-like processes may then occur during the evolution of peritoneal endometriotic implants, leading to expression levels of epithelial and mesenchymal markers in epithelial cells of ovarian endometriosis compared with peritoneal and mesenchymal cells, thereby suggesting different underlying pathogenic mechanisms.

Table IV Immunostaining score for EMT markers: Ovarian endometriosis versus other benign ovarian cysts.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cyst adenoma (n = 20)</th>
<th>Serous (n = 23)</th>
<th>Ovarian endometriosis (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>19.9 ± 1.8⁸</td>
<td>12.8 ± 1.0⁸</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>6.9 ± 1.0⁶</td>
<td>2.1 ± 0.8</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>0.0 ± 0.0</td>
<td>4.6 ± 0.4⁵</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Vimentin</td>
<td>0.0 ± 0.0</td>
<td>5.2 ± 1.5⁴</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>S100A4</td>
<td>1.4 ± 1.0⁸</td>
<td>16.1 ± 2.4</td>
<td>16.1 ± 2.3</td>
</tr>
<tr>
<td>Dephosphorylated beta-catenin</td>
<td>11.4 ± 1.4⁷</td>
<td>0.9 ± 0.3</td>
<td>5.3 ± 1.5</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SEM. Immunostaining score (percentage of immunostained surface x mean staining intensity). Statistical significance (One way ANOVA plus Scheffe test).

Table V Immunostaining score for EMT markers: Abdominal scar endometriosis after Caesarean section versus menstrual endometrium.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Menstrual Endometrium (n = 32)</th>
<th>Abdominal scar endometriosis* (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>10.5 ± 3.1</td>
<td>13.4 ± 2.9</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>11.2 ± 1.3</td>
<td>21.8 ± 5.5⁴</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>0.0 ± 0.0</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>Vimentin</td>
<td>10.5 ± 0.5</td>
<td>12.1 ± 6.8</td>
</tr>
<tr>
<td>S100A4</td>
<td>0.0 ± 0.0</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Dephosphorylated beta-catenin</td>
<td>0.0 ± 0.5</td>
<td>0.2 ± 0.2</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SEM. *After Caesarean section Immunostaining score (percentage of immunostained surface x mean staining intensity). Statistical significance (Mann-Whitney U test).

MET processes depend on environmental triggers (Burk et al., 2008). The transcriptional repressor zinc-finger E-box binding homeobox 1 (ZEB1) is a crucial inducer of EMT in various human tumors (Burk et al., 2008). ZEBI triggers a microRNA-mediated feed forward loop that stabilizes EMT and promotes invasion of cancer cells (Burk et al., 2008). Alternatively, depending on the environmental trigger, this loop might switch and induce epithelial differentiation, i.e. MET (Burk et al., 2008).

However, it is possible that the phenomenon observed may not be a transition of epithelial cells but rather an invasion of the lesions from the underlying peritoneal mesenchymal cells. Further studies are needed to exclude this possibility.

Ovarian endometriosis

The present study demonstrated distinct characteristics with respect to expression levels of epithelial and mesenchymal markers in epithelial cells among ovarian endometriosis tissues and serous and mucinous ovarian benign cysts, thereby suggesting different underlying pathogenic mechanisms.

Cytokeratin expression was significantly lower in epithelial cells of ovarian endometriosis compared with those of menstrual endometrium. During the development of ovarian endometriosis from endometrial epithelial cells, EMT-like processes might occur and expression of the mesenchymal marker S100A4 might be acquired. However, the present study revealed significantly lower expression of vimentin in ovarian endometriosis compared with peritoneal and deep infiltrating lesions, in agreement with the results of a previous analysis by Donnez et al. (1996). In the present study, a total of 47 samples (85.4%) were vimentin-negative (Supplementary data, Table S4). Scar endometriosis samples from patients with a previous history of Cesarean section were included in the present study; although the pathogenesis of this condition remains to be clarified, scar endometriosis most likely originates from eutopic endometrium. In the present study, a total of 9 (69.2%) cases of scar endometriosis were vimentin-negative (Supplementary data, Table SIII). These findings suggest that endometrial epithelial cells might lose some imprinting signaling pathway, which is a well-known EMT–MET regulator during organ development (Clevers, 2006; Thiery et al., 2009). These findings suggest that endometrial epithelial cells might undergo an EMT-like process after attachment of endometrium to peritoneum, resulting in red peritoneal endometriosis. MET-like processes may then occur during the evolution of peritoneal endometriotic implants, resulting in black peritoneal endometriosis. MET has been suggested to be responsible for the discordance between migratory and invasive carcinoma cells with mesenchymal features, as well as the carcinomatous, epithelioid macrometastatic deposits that present clinically, although functional evidence supporting this hypothesis has not yet been forthcoming (Lee et al., 2006; Hugo et al., 2007; Wells et al., 2008). Although further studies are required to investigate the molecular mechanisms underlying EMT and MET processes in superficial peritoneal endometriosis, a recent study demonstrated that EMT/
of their mesenchymal origin after implantation, depending on their microenvironment. Additional studies designed to investigate the molecular mechanisms underlying loss of vimentin expression in ovarian endometriosis might provide further insight into the pathogenesis of endometriosis.

Deep infringing pelvic endometriosis

The present study demonstrated that E-cadherin expression was significantly higher in epithelial cells of deep infiltrating endometriosis compared with those of menstrual endometrium. These findings suggest that a MET-like process might occur in deep infiltrating endometriosis.

The present results suggest that both black peritoneal lesions and deep infiltrating endometriosis may undergo a MET-like process. However, black peritoneal lesions are generally much smaller in size than deep infiltrating endometriosis. In the present study, expression levels of dephosphorylated beta-catenin were significantly higher in deep infiltrating endometriosis compared with black peritoneal lesions. The Wnt/beta-catenin pathway is involved in cell proliferation, migration and invasion (Moon et al., 2004). A more epithelial cell-like phenotype along with Wnt/beta-catenin pathway activation might facilitate growth and infiltration in deep infiltrating endometriosis.

Metaplasia theory

The present study demonstrated cytokeratin (epithelial cell marker) and N-cadherin (mesenchymal cell marker) expression in peritoneal mesothelial cells and ovarian surface epithelial cells. However, E-cadherin (epithelial cell marker), S100A4 (mesenchymal cell marker) and PAX8 expression was not detected in peritoneal mesothelial cells and ovarian surface epithelial cells. If ovarian endometriosis is derived from ovarian surface epithelial cells and peritoneal endometriosis is derived from peritoneal mesothelial cells (metaplasia theory), E-cadherin (epithelial cell marker) and S100A4 (mesenchymal cell marker) expression might be acquired, whereas cytokeratin (epithelial cell marker) expression and N-cadherin (mesenchymal cell marker) expression might be markedly decreased during metaplasia. In addition, PAX8 expression was observed in both peritoneal and ovarian endometriosis, suggesting Müllerian duct origin (Ozcan et al., 2011). These findings suggest that superficial peritoneal endometriosis and ovarian endometriosis are unlikely to be derived from ovarian surface epithelial cells and peritoneal mesothelial cells, respectively.

A previous study demonstrated expression of WNT7A and PAX8 in normal-appearing peritoneum, suggesting that peritoneal endometriosis can arise through metaplasia by engaging those developmental steps that are involved in the ontogenesis of the female genital tract (Gaete et al., 2007). The present study demonstrated that expression of both S100A4 and dephosphorylated beta-catenin was positive in superficial peritoneal endometriosis. However, no S100A4 expression was observed in eutopic endometrial epithelium (Xie et al., 2007) or tubal epithelium; thus, S100A4 is not likely to be expressed during female genital tract development. In addition, a mouse study demonstrated a unique connection between WNT7a and beta-catenin in establishment of oviduct coiling in the anterior Müllerian duct (Deutscher and Hung-Chang Yao, 2007). However, beta-catenin is probably not responsible for transducing a WNT7a signal during the formation of uterine glands in the middle Müllerian duct (Deutscher and Hung-Chang Yao, 2007). These findings suggested that the Wnt/beta-catenin signaling pathway might not be activated in the middle Müllerian duct during development (Deutscher and Hung-Chang Yao, 2007). Thus, it is unlikely that peritoneal endometriosis arises through metaplasia by engaging those developmental steps that are involved in the ontogenesis of the female genital tract.

Embryologic origin of endometriosis development of embryonic remnants

Donnez and Nisolle (1997) proposed that the pathogenesis of rectovaginal endometriotic nodules involves metaplasia of Müllerian remnants located in the rectovaginal septum. The present findings do not necessarily exclude the possibility of this type of pathogenesis for rectovaginal deep infiltrating nodules. Interestingly, recent studies demonstrated the presence of such ectopic endometrium in the rectovaginal septum, in the Douglas pouch, in the rectum of human female fetuses (Signorile et al., 2010, 2011). These investigators hypothesized that ectopic endometrium might be misplaced outside the uterine cavity during the earlier steps of organogenesis (Signorile

<table>
<thead>
<tr>
<th>Marker</th>
<th>Peritoneal mesothelial cells (n = 5)</th>
<th>Ovarian surface epithelial cells (n = 3)</th>
<th>Fallopian tube epithelial cells (n = 3)</th>
<th>Colon epithelial cells (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>–</td>
<td>+</td>
<td>+ distal tubes +; proximal tubes –</td>
<td>–</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>S100A4</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dephosphorylated beta-catenin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>PAX8</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: +, positive staining; –, negative staining.
Multiple sites might share the same pathogenic mechanisms. The Wnt/ beta-catenin signaling pathway may possibly be activated by hormonal inputs in the ectopic endometrium (Kouzmenko et al., 2004; Wang et al., 2009). However, the distribution of pelvic endometriosis is not similar to that of embryonic duct remnants; thus, it is unlikely that all deep infiltrating endometrium could arise from Müllerian duct remnants. In addition, in the present study, all deeply infiltrating endometriosis findings were consistent, irrespective of anatomic sites. These findings suggested that deep infiltrating endometriosis in different sites might share the same pathogenic mechanisms.

**Conclusion**

We propose that the origin of endometriotic epithelial cells might be endometrial epithelial cells. Endometrial epithelial cells might be adapted to specific microenvironments after implantation, resulting in different types of pelvic endometriosis: superficial peritoneal endometriosis, ovarian endometriosis, and deep infiltrating endometriosis. Further in vivo and in vitro studies that investigate the molecular regulation of EMT- and MET-like processes in epithelial cells of pelvic endometriosis could provide more information about the pathogenesis of endometriosis.

In addition, further studies should be necessary to investigate the origin of endometriotic stromal cells.

**Supplementary data**

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

**Acknowledgements**

We are most grateful to all of the patients who participated in the present study. We thank Elodie Maleysson and Christelle Picard for excellent technical assistance. We also thank the staff at Department of Gynecology and operating room, CHU Estaing, CHU Clermont-Ferrand. We are also indebted to the staff in the Department of Pathology, CHU Estaing, CHU Clermont-Ferrand.

**Authors’ roles**

S.M. and C.D. were responsible for the experimental design, sample collection, carrying out the experiments, analyzing and interpreting the data, and involved in drafting the manuscript.

**Funding**

This study was supported in part by Karl Storz Endoscopy & GmbH (Tuttlingen, Germany).

**Conflict of interest**

None declared.

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