Immunohistochemical characterization of endometriosis-associated smooth muscle cells in human peritoneal endometriotic lesions

Maria L. Barcena de Arellano, Jessica Gericke, Uta Reichelt, Ali Fuat Okuducu, Andreas D. Ebert, Vito Chiantera, Achim Schneider, and Sylvia Mechsner

Background: Smooth muscle cells (SMC) are common components of endometriotic lesions. SMC have been characterized previously in peritoneal, ovarian and deep infiltrating endometriotic lesions and adenomyosis. The aim of this retrospective study was to investigate the extent of differentiation in endometriosis-associated SMC (EMaSMC) in peritoneal endometriotic lesions.

Methods: We obtained biopsies from peritoneal endometriotic lesions (n = 60) and peritoneal sites distant from the endometriotic lesion (n = 60), as well as healthy peritoneum from patients without endometriosis (control tissue, n = 10). These controls were hysterectomy specimens from patients without endometriosis or adenomyosis. Histopathological examination of peritoneal specimens using antibodies against oxytocin receptor (OTR), vasopressin receptor (VPR), smooth muscle myosin heavy chain (SM-MHC), estrogen receptor (ER) or progesterone receptor (PR) was performed. To identify SMC and their level of differentiation, antibodies for smooth muscle actin desmin and caldesmon were used.

Results: SMC were detected in all endometriotic lesions. SMC were more abundant in unaffected peritoneum of women with endometriosis (38%) compared with women without endometriosis (6%; P < 0.0001). Depending on the level of differentiation, SMC stained for SM-MHC, OTR, VPR, ER and PR. OTR was only detected in fully differentiated SMC.

Conclusions: Identification of OTR, VPR, ER and PR leads to the hypothesis that the EMaSMC might be functionally active and possibly involved in the generation of pain associated with endometriosis.

Key words: oxytocin receptor / vasopressin receptor / endometriosis / smooth muscle metaplasia / smooth muscle actin

Introduction

Endometriosis is defined as a benign, chronic, estrogen-dependent gynaecologic disease affecting millions of women of reproductive age (Bulun, 2009). Most of the patients suffer from cycle-dependent symptoms, such as dysmenorrhoea and pelvic pain, whereby 50% of those need long-term treatment with recurrent surgery and/or persistent hormonal treatment (Gao et al., 2006). Owing to the complex pathogenesis of endometriosis, therapeutic options are currently available for the treatment of symptoms but not for healing (Leyendecker et al., 2009). In spite of surgical excision of the lesions and adjuvant hormonal treatment, the recurrence rate and persistence of symptoms associated with endometriosis is up to 40% (Catenacci et al., 2009). As long as the pathogenesis of endometriosis, especially the pathogenesis of pain generation, is not fully understood, the development of specific, successful treatment options will be hindered.

Currently, the histological criteria to define endometriosis are the detection of ectopic endometrial glandular cells with surrounding stromal cells and/or macrophages. In addition, smooth muscle cells (SMC) are frequent components of endometriotic lesions (Khare et al., 1996; Anaf et al., 2000; Leyendecker et al., 2002; Mechsner et al., 2005). SMC have been detected in peritoneal (Anaf et al.,...
2000; Mechsner et al., 2005), ovarian (Fukunaga, 2000; Mechsner et al., 2005) and deep infiltrating (van Kaam et al., 2008) endometriotic lesions, and also in adenomyosis uteri (Mechsner et al., 2010) and in extragenital bowel wall endometriosis (Kazakov et al., 2007; Mechsner et al., 2009b).

However, to date, the origin of these endometriosis-associated SMC (EMaSMC) is still unclear. There is some evidence that EMaSMC are of metaplastic origin (Anaf et al., 2000; Mechsner et al., 2005; Odagiri et al., 2009). Furthermore, the biological functionality of EMaSMC has not been clarified.

Peritoneal EMaSMC (pEMaSMC) express oxytocin receptors (OTRs), estrogen receptors (ERs) and progesterone receptors (PRs), which are essential components characterizing uterine myometrial cells (Mechsner et al., 2005). In contrast, the ability of pEMaSMC to show contractions has not been demonstrated. It seems possible that peritoneal SM contractions could stimulate peritoneal nociceptors leading to the generation of endometriosis-associated pain (Mechsner et al., 2009). To characterize EMaSMC in peritoneal endometriotic lesions, we performed immunohistochemical analysis of well-known uterine markers and markers of smooth muscle differentiation which are expressed in human peritoneal endometriotic lesions.

**Materials and Methods**

**Tissue samples**

Biopsies were obtained from 60 patients who underwent diagnostic laparoscopy/laparotomy for symptomatic endometriosis (pelvic pain, dysmenorrhea, dyspareunia, dysuria or dyschezia). The biopsy specimens were obtained by performing a wide excision, without using coagulating diathermy. All women were premenopausal with a mean age of 33 ± 6.5 years (range: 19–47). Thirty-eight women had regular menstrual cycles without hormonal treatment, whereas 22 patients were under hormonal treatment. The respective phases of the cycle were defined by correlating the date of the last menstrual period and the date of surgery (secretory phase n = 20, proliferative phase n = 18). The staging of endometriosis was performed according to the revised classification of the American Society of Reproductive Medicine (rASRM; stage I, n = 23; II, n = 19, III, n = 13; IV, n = 5; Medicine, 1997). Peritoneal endometriotic lesions were classified into black or white fibrotic implants (n = 40) and red lesions (n = 3), and 17 could not be assessed. The lesions were excised from peritoneum of the lateral pelvic wall (n = 4), the bladder (n = 16), pouch of Douglas (n = 11), the fossa ovarica (n = 10), the uterosacral ligament (n = 14) and peritoneum (n = 5).

In addition, peritoneal biopsies, distant from the endometriotic lesion, from patients with endometriosis (n = 60) and peritoneal biopsies of healthy peritoneum from patients without endometriosis (control tissue: n = 10) were used. All peritoneal biopsies had subperitoneal fat at the lower border. All excised lesions were fixed immediately in 4% buffered formalin (Hedinger, Stuttgart, Germany) for 12 h and then embedded in paraffin. Serial sections (1 μm thick) were cut and stained with haematoxylin and eosin (H&E), or immunostained.

All patients gave their written informed consent and the study was approved by the Institutional Review Board.

**Immunohistochemistry**

Negative control sections were processed by omitting the primary antibody and by competition test with a specific blocking peptide (Santa Cruz Biotechnology Inc., CA, USA). Endometrium was used as positive control for CD10, ER and PR staining; myometrium from a pregnant uterus was used for the OTR and vasopressin receptor (VPR) staining; and leiomyoma tissue was the control for the staining of smooth muscle actin (smActin), caldesmon, desmin and smooth muscle myosin heavy chain (SM-MHC). The quality of the staining was supervised and confirmed by our pathologist and also confirmed by the controls.

**CD10 staining**

Immunohistochemical analysis was performed as described previously (Mechsner et al., 2009b). Briefly, the sections were incubated for 1 h at room temperature with a monoclonal mouse anti-CD10 antibody (a specific marker for endometrial stromal cells; McCluggage et al., 2001; dilution 1:50, Sigma, Germany). After rinsing with tris-buffered saline (TBS), the Labelled-Strept-Avidin-Biotin-Kit (LSAB; Dako, Hamburg, Germany) was applied to visualize the specific immunoreactive CD10 staining.

**smActin, caldesmon, desmin and SM-MHC staining**

The sections were processed as described above and then incubated for 1 h at room temperature with a monoclonal mouse anti-smActin ([specific marker for SMC and myofibroblast (Hasegawa et al., 2003)]), mouse anti-caldesmon [marker for differentiated SMC (Hasegawa et al., 2003)], mouse anti-desmin [marker for differentiated SMC (Hasegawa et al., 2003)] or a mouse anti-SM-MHC ([a marker molecule restricted for the SMC (Manabe and Owens, 2001)] antibody (dilution 1:50, Dako, Hamburg, Germany). After rinsing with TBS, the sections were treated as described above.

**ER and PR staining**

Immunohistochemistry analysis was performed as described above. Briefly, the sections were incubated with a monoclonal mouse anti-ER antibody (clone 1D5, dilution 1:35, DacoCytomation, Denmark) or with a monoclonal mouse anti-PR (clone 1A6, 1:50 DacoCytomation, Denmark) for 1 h at room temperature. The secondary biotinylated rabbit anti-mouse immunoglobulin (Ig) G antibody (dilution 1:400, Dianova, Germany) was applied to visualize the specific immunoreactive CD10 staining.

**OTR and VPR staining**

The sections were incubated for 1 h at room temperature with a polyclonal goat anti-OTR antibody (dilution 1:35, DacoCytomation, Denmark) or polyclonal goat anti-VPR antibody (dilution 1:100, Santa Cruz Biotechnology Inc.) for 1 h at room temperature. The secondary biotinylated rabbit anti-mouse immunoglobulin (Ig) G antibody (dilution 1:400, Dianova, Germany) was applied for 40 min. Streptavidin (dilution 1:400, Roche, Germany) was applied for 40 min. Fuchsin-substrate (Dako, Hamburg, Germany) was used to visualize the specific immunoreactive staining.

**Evaluation of the immunohistochemistry**

The staining was evaluated using a Carl Zeiss (Gottingen, Germany) Axiophot microscope. Pictures were taken with different magnifications (×25, ×40 or ×100).

The number of SMC in peritoneal endometriotic lesions was estimated in different locations according to their relationship to the endometriotic lesions [intrastromal SMC (ISMC), surrounding SMC (SSMC) and peripheral SMC (PSMC)] and in control tissue (i.e. healthy peritoneum from women without endometriosis) by defining the percentage of the total...
area studied, occupied by SMC, unassociated with blood vessels, as described (Mechsner et al., 2005). Briefly, the relative area (percentage, mean ± SD) occupied by SMC was evaluated in at least 10 non-overlapping randomly selected high power fields (×400) on each slide for endometriotic lesions and in each biopsy for healthy peritoneum with the same pelvic location. Sections were evaluated by two independent observers who were blinded to the nature of the samples.

The OTR, ER and PR staining was scored as described previously (Mechsner et al., 2005).

**Statistical analysis**

The data were evaluated using the non-parametric test (Mann–Whitney test, to test two independent groups), the non-parametric one-way analysis of variance (ANOVA) (Kruskal–Wallis test, to test three or more groups) or the two-way ANOVA (to test two independent variables). The Dunn’s multiple comparison and the Bonferroni post-test were used as post hoc tests. Statistical significance was defined as *P* < 0.05. Statistical analysis was performed using Prism 4 for Windows (GraphPad Software, 2003, San Diego, CA, USA).

**Results**

In all samples studied, H&E staining did confirm the presence of endometriotic glands with epithelial cells and surrounding stromal cells.

---

**Figure 1** Smooth muscle in peritoneum of patients with endometriotic lesions. (a) SMC in the peritoneum of patients with and without endometriosis (data are mean and SD; Mann–Whitney test; control: *n* = 10, EM: *n* = 60); ***P* < 0.0001 for the percentage of SMC in the endometriosis group versus the control group. (b) SmActin staining of healthy peritoneum (arrows show vascular SMC, magnification 25×). (c) SmActin staining of peritoneum from patients with endometriosis (magnification 25×). (d) CD10 staining of peritoneum with endometriotic lesion (magnification 25×). (e) Smooth muscle in peritoneum from endometriosis patients and the control group; data are mean and SD (Mann–Whitney test; control: *n* = 10, EM: *n* = 60); ***P* < 0.0001 for the percentage of SMC in the endometriosis group versus the control group. Control = healthy peritoneum from women without endometriosis; EM = peritoneum of women with endometriosis.
Detection of SMC in peritoneal endometriotic lesions

Using anti-smActin antibody, SMC could be detected in all peritoneal specimens from women with endometriosis, while in healthy peritoneum from women without endometriosis (control group), only 30% of the specimens did contain SMC (Mann–Whitney test; \( P < 0.0001 \); Fig. 1a–c). CD10 staining was strong in the stromal region of peritoneal endometriotic lesions (Fig. 1d). The tissue surrounding endometriotic lesions is smActin positive but not in healthy peritoneum from the control group (Fig. 1b and c). The total number of smActin-positive cells was higher in the peritoneum of women with endometriosis (38%) compared with healthy peritoneum of patients without endometriosis (control group; 6%; Mann–Whitney test; \( P < 0.0001 \); Fig 1e).

Therefore, we defined those SMC in peritoneal specimens of patients with endometriosis as EMaSMC.

Localization of the EMaSMC

EMaSMC spread could be differentiated according to their relationship with the endometriotic lesion. ISMC, surrounding SSMC and PSMC could be distinguished (Fig 2a). These forms of SMC were present in all lesions investigated, and 25% of the stromal region, 65% of the surrounding tissue and 31% of the peripheral tissue were positive for smActin (Fig. 2b). The amount of SMC in the tissue surrounding the lesion is increased when compared with stromal or peripheral tissue (Kruskal–Wallis test; \( P < 0.0001 \); Fig 2c).

Further, the localization of EMaSMC in different macro-anatomical regions of the peritoneum from women with endometriosis was analysed. There were no significant differences in the amount of the EMaSMC between the anatomical regions (ISMС, SSMС or PSMС; two-way ANOVA; \( P > 0.05 \); Fig. 2d).

There was no association between the SM-amount in the peritoneal endometriotic lesion and the current endocrine treatment or phase of

---

**Figure 2**  Differential localization of SMC in peritoneum of women with endometriosis. (a and b) SmActin staining in the intrastromal SMC (ISMС), surrounding SMC (SSМС) and peripheral SMC (PSМС) (magnification 25× and 200×). (c) Muscle level in the EMаSMС, data are mean and SD (Kruskal–Wallis test; \( n = 60 \)); ***(P < 0.001 for the percentage muscle content in the SSMС in comparison with ISМС and PSMС. (d) EMаSMС in different anatomical regions of the peritoneum from women with endometriosis; data are mean and SD (two-way ANOVA; \( n = 60 \)).
Detection of SM-MHC, OTR, VPR and ER/PR in EMaSMC

SM-MHC, OTR and VPR immunostaining was studied in ISMC, SSMC and PSMC. Minimal to moderate staining of the SM-MHC was detected in 100% of the ISMC, SSMC and PSMC in investigated specimens (Fig. 4a). Forty-seven percent of the ISMC smActin cells were positive for SM-MHC together with 62% of the SSMC and 88% of the PSMC (Fig. 4b). The percentage of SM-MHC-positive cells was higher in PSMC compared with the ISMC or SSMC (two-way ANOVA; P < 0.001 and P < 0.01, respectively; Fig. 4b).

Weak to moderate OTR staining was found in 97% of the SSMC and PSMC, while OTR was only expressed in 61% of the endometriotic tissue in the ISMC (P < 0.001). Only 40% of the smActin-positive cells in the ISMC and SSMC expressed the OTR, while 83% of the SMC in PSMC were stained for OTR (Fig. 4a and b).

In addition, the functionality of the EMaSMC was also analysed with the VPR. The VPR was weakly stained in ISMC (10%), but stained in 90% of the SSMC and PSMC (P < 0.0001). Eight percent of smActin-positive cells positive for the VPR could be determined in the ISMC, 28% in the SSMC and 62% in the PSMC (Fig. 4a and b).

Additionally, moderate staining for ER and PR was detected in 98% of EMaSMC in the peritoneal endometriotic lesion (Fig. 5). Eighty-eight per cent of epithelial cells and 66% of stromal cells showed the presence of ER, while 95% of epithelial cells and 82% of stromal cells stained for PR (Fig. 5a). The staining for both receptors was higher in the epithelial cells compared with stromal cells (two-way ANOVA; P < 0.001). Only 40% of the smActin-positive cells in the ISMC and SSMC expressed the OTR, while 63% of the SM-MHC were detected in 100% of the ISMC, SSMC and PSMC in investigated specimens (Fig. 3b). The number of differentiated cells was increased in the PSMC when compared with the ISMC or SSMC (two-way ANOVA; P < 0.001; Fig 3b).

Discussion

The present study provides first evidence for a possible metaplastic origin of EMaSMC with various grades of differentiation, and expression of uterine marker molecules (OTR, VPR and ER/PR) in fully developed SMC.

Owing to the low visibility of SMC in H&E staining, the SMC could not be distinguished easily from the fibroadipocyte connective tissue in peritoneum. However, De Snoo already described in his morphological-histological studies of 1942 that EMaSMC are frequent components in endometriosis (De Snoo, 1942). Using modern techniques of immunohistochemistry, SMC were detectable in all types of endometriotic lesions (Anaf et al., 2000; Fukunaga, 2000; Mechsner et al., 2005; Kazakov et al., 2007; van Kaam et al., 2008; Mechsner et al., 2009b, 2010). The origin and functionality of SMA cells in endometriosis is still not completely known.

In the first step of our analysis, we calculated the SM-amount in peritoneal endometriotic specimens in comparison with healthy unaffected peritoneum and we could demonstrate that the SM-amount in peritoneal endometriotic lesions was significantly higher than in healthy peritoneum. In all endometriotic specimens SMC have been

Table I Quantification of peritoneal SMC in three locations, defined according to the spatial relationship to the endometriotic lesions.

<table>
<thead>
<tr>
<th></th>
<th>ISMC</th>
<th>SSMC</th>
<th>PSMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone therapy</td>
<td>25 ± 1.01</td>
<td>62 ± 2.22</td>
<td>29 ± 2.07</td>
</tr>
<tr>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>No hormone therapy</td>
<td>26 ± 0.86</td>
<td>62 ± 3.13</td>
<td>32 ± 2.43</td>
</tr>
<tr>
<td>(n = 38)</td>
<td>(n = 38)</td>
<td>(n = 38)</td>
<td></td>
</tr>
<tr>
<td>Secretory phase</td>
<td>26 ± 0.06</td>
<td>69 ± 2.81</td>
<td>34 ± 2.17</td>
</tr>
<tr>
<td>(n = 20)</td>
<td>(n = 20)</td>
<td>(n = 20)</td>
<td></td>
</tr>
<tr>
<td>Proliferative phase</td>
<td>24 ± 0.81</td>
<td>58 ± 3.48</td>
<td>29 ± 2.66</td>
</tr>
<tr>
<td>(n = 18)</td>
<td>(n = 18)</td>
<td>(n = 18)</td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>25 ± 0.95</td>
<td>61 ± 2.88</td>
<td>26 ± 2.06</td>
</tr>
<tr>
<td>(n = 38)</td>
<td>(n = 38)</td>
<td>(n = 38)</td>
<td></td>
</tr>
<tr>
<td>New case</td>
<td>25 ± 0.86</td>
<td>65 ± 2.63</td>
<td>40 ± 2.57</td>
</tr>
<tr>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td>(n = 22)*</td>
<td></td>
</tr>
</tbody>
</table>

Table II Quantification of peritoneal SMC from patients with endometriosis, according to the stage of disease.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISMC</td>
<td>26 ± 0.65</td>
<td>26 ± 0.83</td>
<td>23 ± 1.18</td>
<td>24 ± 1.34</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 19)</td>
<td>(n = 13)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>SSMC</td>
<td>66 ± 2.46</td>
<td>67 ± 2.40</td>
<td>52 ± 3.54</td>
<td>51 ± 2.68</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 19)</td>
<td>(n = 13)</td>
<td>(n = 5)</td>
<td></td>
</tr>
<tr>
<td>PSMC</td>
<td>34 ± 2.31</td>
<td>25 ± 2.11</td>
<td>24 ± 2.02</td>
<td>48 ± 2.48</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 19)</td>
<td>(n = 13)</td>
<td>(n = 5)</td>
<td></td>
</tr>
</tbody>
</table>

rASRM, revised classification of the American Society of Reproductive Medicine. P > 0.05; Mann–Whitney test.
detected and the SM-content was up to 40%. The SMC were present independent of the anatomical localization of the peritoneal biopsy, the stage of disease, the phase of the cycle or hormonal treatment. Only in cases of recurrent disease was the SM-amount lower than in first-diagnosed cases. SMC close to the endometriotic lesion (ISMC/SSMC) seem to be immature, whereby the PSMC are fully differentiated. These findings could be demonstrated by immunohistological staining of the SM-differentiation markers, caldesmon and desmin. The staining for both OTR and VPR was also significantly higher in the mature than in immature SMC.

The question now is: where do these SMC come from? Are they developed by a remodelling pathway of current peritoneal fibroblasts, by metaplastic changes of endometriotic stromal cells or by reactivation of coelomic epithelial cells?

The common part of all these theories is the differentiation of fibroblasts to myofibroblasts, which leads to the expression of smActin in differentiated SMC (Desmouliere et al., 1993). The basic mechanism which induces this process is still unclear. The close morphological connection of immature SMC formations with stromal cells of endometriotic lesions could suggest smooth muscle metaplasia (SMM) out of stromal cells. The expression of the OTR/VPR, markers for uterine SMC (Fuchs et al., 1985, 1998), indicates that these cells may originate from a metaplastic transformation of uterine stromal cells outside the uterus. In addition, the reported expression of CD10, a marker for endometrial stromal cells, suggests the metaplastic origin of the components in endometriotic lesions from uterine cells (Sumathi and McCluggage, 2002).

However, whether these EMaSMC cells derive from basal stem cells or reactivated coelomic epithelial cells is still unclear (Signorile et al., 2009). There are several arguments encouraging both hypotheses. De Snoo discussed the origin of these EMaSMC as reactivation of residua out of the paramesonephric ducts, like the embryological

**Figure 3** Staining of differentiation markers of the SMC in peritoneum from women with endometriosis. (a) SmActin, caldesmon (Cal) and desmin (Des) staining of ISMC, SSMC and PSMC (magnification 200 ×). (b) Extent of EMaSMC differentiation in the peritoneum from patients with endometriosis; data are mean and SD (two-way ANOVA; \( n = 60 \)); *\( P < 0.05 \) for the percentage of differentiation grade in the PSMC; **\( P < 0.001 \) for the percentage of differentiation grade in the ISMC and SSMC.
development of the uterus and Fallopian tubes out of omnipotent cells, and described ectopic endometriotic cells as ‘miniature uteri’ (De Snoo, 1942). The observation of differentiated and undifferentiated SMC in primary umbilical endometriotic lesions affirmed the hypothesis that epithelial and stromal cells might develop from persistent coelomic epithelial cells by metaplasia (Mechsner et al., 2009b). Coelomic epithelial cells arise during the embryonic period in the umbilicus and regress after closing the physiological omphalocele at 12 weeks of gestation. However, persistent umbilical hernia after birth could be one factor related to the persistence of such multipotent coelomic epithelial cells (Meyer, 1930): in addition, the recent analysis of dislocated primitive endometrial tissue outside the uterine cavity during organogenesis supported this hypothesis (Signorile et al., 2009).

On the other hand, SMC were observed in secondary, abdominal wall endometriosis, which have developed in scars following the transplantation of active endometrial tissue during surgery, for example during Caesarean section (Kazakov et al., 2007). In primary and secondary abdominal wall endometriosis, SMC were localized between endometriotic stromal cells, suggesting that, in both cases, SMC originate from endometriotic cells as a result of epithelial–mesenchymal transition of the cells (Kazakov et al., 2007; Hayashida et al., 2010). In this respect, physiological SMM in the junctional zone of the uterus has previously been reported (Konishi et al., 1984; Fujii et al., 1989). It seems to be possible that cells from the basal endometrial layer, with stem cell characteristics, were catapulted out of the junctional zone due to pathological contractility patterns (Leyendecker et al., 2009). These stem cell-like fragments may be able to adhere

**Figure 4** Molecules associated with smooth muscle contraction in peritoneal SMC from women with endometriosis. (a) SM-MHC, OTR and VPR staining in ISMC, SSMC and PSMC (magnification 400×). (b) Quantification of contraction-associated molecules in pEMaSMC from patients with endometriosis; data are mean and SD (two-way ANOVA; n = 60); ***p < 0.01 for the percentage of SM-MCH-positive cells in the SSMC versus PSMC; ****p < 0.001 for the percentage of SM-MCH-positive cells in the ISMC versus PSMC and for the percentage of OTR- or VPR-positive cells in the ISMC or SSMC versus PSMC.
to the peritoneum and differentiate into a ‘miniature-uterus’ by a metaplastic process (De Snoo, 1942; Leyendecker et al., 2002; Mechsner et al., 2005; Leyendecker et al., 2009). The recent characterization of stem cells in the endometrial basal layer encourages this discussion (Gotte et al., Epub2010). Furthermore, the expression and release of transforming growth factor (TGF) β1 in endometrial epithelial cells leads to decidualization of endometrial stromal cells, followed by the expression of smActin (Kim et al., 2005).

Contrary to the hypothesis of SMM, the development of SMC as a reaction of the surrounding tissue has to be discussed. In an ovarian endometriotic cyst, SMC were identified (Fukunaga, 2000; Mechsner et al., 2005). In contrast to peritoneal and abdominal wall endometriosis, these SMC were arranged in a circular manner around the ovarian endometriotic cyst, with no mixing of stromal cells and SMC (Fukunaga, 2000). The observation of SMC in the ovary is not just an endometriosis-associated phenomenon as the occurrence of SMC could be detected in other benign masses (Doss et al., 1999), suggesting that the development of SMC is a reaction of the ovarian stromal cells.

SMC are also well described in deep infiltrating endometriosis (DIE) (Nisolle and Donnez, 1997; Anaf et al., 2000; Itoga et al., 2003). Markers of SM differentiation, such as vimentin, desmin, α-smActin and SM-MHC, have been studied in DIE (van Kaam et al., 2008). In agreement with our data, different levels of SM maturation in the endometriosis-associated fibro-connective tissue were found (van Kaam et al., 2008). Furthermore, TGF-β expression in endometriotic epithelial cells was demonstrated, suggesting the influence of TGF-β-positive cells on the induction of SMM. The transplantation of human endometrial cells in a mouse model induced SMM; however, these SMC seem to result from the reaction of the fibro-connective tissue of the mouse (van Kaam et al., 2008), suggesting an environment similar to wound healing (van Kaam et al., 2008). In addition, Hull et al. (2008) studied the endometrial–peritoneal interaction during endometriotic lesion establishment in a nude mouse model: they demonstrated a host reaction against human tissue, with a detection of murine macrophages and smooth muscle actin-positive cells within the human ectopic endometrial lesion. Owing to the fact that endometrial tissue from the functional layer (collected using pipelle biopsy) was transplanted into the peritoneal cavity of the nude mice, a failure of smooth muscle actin-positive cells seems to be plausible. This model demonstrates the host reaction with the ability of myofibroblastic transdifferentiation. In fact, peritoneal myofibroblastic transdifferentiation/conversion of mesothelial cells in women without endometriosis is well documented, especially in patients with peritoneal fibrosis (Yang et al., 2003; De Vriese et al., 2006; Witkowicz, 2008), and seems to be another important mechanism in the development of endometriotic lesions. Khare et al. investigated the myofibroblast proliferation and changes in collagen deposition in ovarian and pelvic wall-infiltrating endometriosis and demonstrated that in ovarian endometriosis the endometriotic lesions are lying on the inside of the chocolate cyst and are not freely intermixed with native ovarian collagen, whereas pelvic wall-infiltrating endometriosis showed endometriotic lesions mixing freely with the native pelvic wall collagen (Khare et al., 1996). However, both kinds of SMM are possible and do not exclude each other, and further investigations are needed to clarify the origin of these EMaSMC.

Another point of interest in this study was the possible contractility of EMaSMC. We previously reported that EMaSMC express OTR and ER/PR (Mechsner et al., 2005), which may be involved in endometriosis-associated pain induction. It seems possible that the chaotic/irregular contractions of peritoneal SMC mediated by OTR could lead to peritoneal irritations and to an activation of nociceptors. Furthermore, contractions may lead to local tissue injury with induction of

![Figure 5 ER- and PR expression in EMaSMC in women with endometriosis. (a) Quantification of the ER and PR in stroma and epithelial cells of peritoneal lesions; data are mean and SD (two-way ANOVA; n = 60). ***p < 0.001 for percentage of ER-positive cells in stroma cells versus epithelial cells; **p < 0.01 for percentage of PR-positive cells in stroma cells in comparison with epithelial cells; *p < 0.05 for ER-positive cells versus PR-positive cells in epithelial cells; ***p < 0.001 for ER-positive cells versus PR-positive cells in stroma cells. (b) ER and PR staining of SSMC and PSMC (magnification 400×). (c) ER and PR in EMaSMC, mean and SD (two-way ANOVA; n = 60). ***p < 0.01 for percentage of the expression of ER smActin-positive cells in comparison with the PR smActin-positive cells in SSMC and in PSMC.](https://academic.oup.com/humrep/article-abstract/26/10/2721/610614)
repair mechanisms associated with P450 aromatase overexpression and the consequences on local estrogen synthesis (Leyendecker et al., 2009). Recently, the OT-dependent uterine contractions of the junctional zone in the non-pregnant uterus were described (Kunz et al., 1998). Our results demonstrate a comparable grade of muscle level in peritoneal endometriotic lesions independent of the activity of the lesions (red active lesions produce all kinds of bioactive pro-inflammatory substances and estrogen, whereas black or white inactive lesions lack this excretion) and rASRM classification. The SM level is low in healthy peritoneum (Mechsner et al., 2005) and significantly increased in areas of peritoneal endometriotic lesions. These findings could explain the stage-independent presence of endometriosis-associated symptoms (Fedele et al., 1990; Vercellini et al., 1996).

OT seems to promote the release of prostaglandin (PG)F2α from endometrial cells (Wilson et al., 1988; Burns et al., 2001), and the release of PGF2α and endothelin mediates myometrial contractility in a paracrine and/or autocrine fashion (Bacon et al., 1995). Therefore, we believe that OT could mediate a similar effect in epithelial cells of peritoneal endometriosis and the adjacent SMC. Here, a further uterotonic marker, VPR, was detected in pEMaSMC. In agreement with Odagiri et al. (2009), these data showed a biological explanation for SM contraction, which might be an important mechanism in the generation of endometriosis-related pelvic pain. Furthermore, the expression of myosin heavy-chain—a marker molecule restricted to the SMC (Manabe and Owens, 2001)—suggests the possibility of contractile activity in EMaSMC.

In addition to the other known pain mechanisms of endometriotic implants, such as the release of pain mediators or nerve growth factor and PGs produced by endometriotic lesions (Vernon et al., 1986; Muzii et al., 1997; Anaf et al., 2002), and the close topographical relationship to nerve fibres (Anaf et al., 2002; Mechsner et al., 2009a) with perineural invasion, EmaSMC seems to be a further factor in the complex process of pain generation.

In summary, this is the first study which suggests metaplastic changes in pEMaSMC in different grades of SM-differentiation, with the possibility of contraction, although a dynamic in vitro or in vivo study is required to fully demonstrate metaplasia of cells. Our data suggest that the specific inhibition of OTR might be a promising additional new approach for the treatment of pain associated with endometriosis.

**Authors’ roles**

M.L.B.A. performed analysis of the data, contributed towards the preparation of the paper and supervised the statistical analysis. J.G. performed the immunohistochemical staining and analysis of the data. U.R. prepared the specimens and supervised the immunohistochemical analysis of the sections. A.D.E., V.C. and A.S. performed the surgery of the patients. S.M. Conceived the scientific idea, supervised of the project and contributed towards the preparation of the paper.

**References**


Kazakov DV, Ondic O, Zamecnik M,榭lekhova KV, Mukensnabl P, Hes O, Dvořák V, Michal M. Morphological variations of


