Fibromuscular differentiation in deeply infiltrating endometriosis is a reaction of resident fibroblasts to the presence of ectopic endometrium

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BACKGROUND: In this study, we characterized the fibromuscular (FM) tissue, typical of deeply infiltrating endometriosis, investigated which cells are responsible for the FM reaction and evaluated whether transforming growth factor-β (TGF-β) signaling is involved in this process. METHODS: FM differentiation and TGF-β signaling were assessed in deeply infiltrating endometriosis lesions (n = 20) and a nude mouse model of endometriosis 1, 2, 3 and 4 weeks post-transplantation. The FM reaction was evaluated by immunohistochemistry using different markers of FM and smooth muscle cell differentiation (vimentin, desmin, alpha-smooth muscle actin, smooth muscle myosin heavy chain). TGF-β signaling was assessed by immunostaining for its receptors and phosphorylated Smad. RESULTS: Deeply infiltrating endometriosis lesions contain myofibroblast-like cells that express multiple markers of FM differentiation. Expression of TGF-β receptors and phospho-Smad was more pronounced in the endometrial component of the lesions than in the FM component. In the nude mouse model, alpha-smooth muscle actin expression was observed in murine fibroblasts surrounding the lesion, but not in human endometrial stroma. CONCLUSIONS: FM differentiation in deeply infiltrating endometriosis is the result of a reaction of the local environment to the presence of ectopic endometrium. It shares characteristics with pathological wound healing, but cannot be explained by TGF-β signaling alone.

Keywords: transforming growth factor-β; endometriosis; ectopic endometrium; smooth muscle metaplasia; fibromuscular differentiation

Introduction

Endometriosis is a common benign gynecological condition characterized by the presence of endometrial glands and stroma at ectopic locations outside the uterine cavity. Deeply infiltrating endometriosis is defined as the presence of endometriosis >5 mm under the peritoneal surface (Cornillie et al., 1990) and is often associated with symptoms such as dysmenorrhea, dyspareunia and pelvic pain. Deeply infiltrating lesions are nodular in appearance and are histologically characterized by dense tissue composed of smooth muscles and fibrosis with islands or strands of glands and stroma. In contrast to other lesion types, the major component of these nodular lesions is fibromuscular (FM) tissue rather than endometrial tissue (Itoga et al., 2003). For this reason this type of lesion is often referred to as adenomyosis, and is considered by some to be a specific disease entity, distinct from peritoneal or ovarian endometriosis (Nisolle and Donnez, 1997). However, it cannot be excluded that these lesions have developed from superficial peritoneal implants in the pouch of Douglas (Vercellini et al., 2000). Smooth muscles are frequent components of peritoneal, ovarian, uterosacral and rectovaginal lesions but are absent in their respective unaffected sites and in eutopic endometrium of women with and without endometriosis (Anaf et al., 2000a). Nerve fibers trapped in these FM lesions are a significant contributor to the induction of pain symptoms in patients (Anaf et al., 2000b).

There is no unequivocal explanation for the presence of smooth muscle-like cells in endometriosis, and in particular deeply infiltrating endometriosis lesions. Several explanations can account for this phenomenon. First, the FM cells may result from smooth muscle metaplasia of endometrial stromal fibroblasts. It has been shown that endometrial stromal cells decidualized by progesterone in vitro express alpha-smooth muscle actin, a contractile microfilament that is expressed solely by smooth muscle cells, myofibroblasts and related cells (Kim et al., 2005). Smooth muscle metaplasia of endometrial stromal cells has also been described in ovarian endometriosis (Fukunaga et al., 2000). Second, smooth
muscle-like cells in deeply infiltrating endometriosis lesions originate from transdifferentiation of local tissue fibroblasts into a more contractile phenotype bearing features of smooth muscle, a phenomenon that has been extensively described within the context of tissue injury and wound healing (Gabbiani, 2003). Third, the cells may have developed from remnants of the Müllerian duct system. The latter explanation is less likely, however, as smooth muscle differentiation was not restricted to deep infiltrating lesions but was observed in all lesion types (Anaf et al., 2000a).

All hypotheses involve differentiation of fibroblasts to myofibroblasts and, possibly, differentiated smooth muscle cells. Myofibroblasts are a unique group of smooth muscle-like fibroblasts that have acquired the capacity to neoeexpress alpha-smooth muscle actin, the actin isoform typical of vascular smooth muscle cells, and to synthesize important amounts of collagen and other extracellular matrix components (Darby et al., 1990; Schurch et al., 2006). The fibroblast/myofibroblast transition is accepted as the key event in the formation of granulation tissue during wound healing and fibrotic changes. It has been shown that the cytokine transforming growth factor-β1 (TGF-β1) is responsible for inducing the synthesis of alpha-smooth muscle actin in fibroblastic cells and for stimulating the production of collagen type I (Desmoulière et al., 1993). In this respect, TGF-β1 is the key cytokine in the evolution of lesions characterized by myofibroblast formation. This is further supported by the clinical observation that overproduction of TGF-β1 has been implicated in the pathogenesis of several fibrocontractive diseases at various sites throughout the body, such as pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, skin scarring and peritoneal adhesion formation (Okuda et al., 1990; Broekelmann et al., 1991; Border and Noble, 1994; Roberts, 1995; Chegini, 1997).

From animal studies it has become clear that transient overexpression of active TGF-β in the lung induces a chronic fibrotic response (Sime et al., 1997). Conversely, blocking TGF-β inhibits experimentally induced fibrosis in the lung, skin and liver (Giri et al., 1993; McCormick et al., 1999; Nakamura et al., 2000). Given the fact that smooth muscle metaplasia and more or less extensive fibrosis can be observed in and around deeply infiltrating endometriosis lesions, we hypothesize that active TGF-β1 signaling may be a key feature in the development of this type of endometriosis.

In this study we aim (i) to characterize the FM component of deeply infiltrating endometriotic lesions using immunohistochemical markers of smooth muscle differentiation, (ii) to investigate the origin of smooth muscle-like cells in endometriosis lesions in a nude mouse model and (iii) to assess a possible causative role for TGF-β1 in this process by using immunohistochemical markers of active TGF-β signaling.

Materials and Methods

Patients and tissue specimens

Twenty patients with a surgical and histological diagnosis of deep infiltrating endometriosis who were operated between 1998 and 2004 in the University Hospital of Maastricht were included in the study. Deeply infiltrating endometriosis was defined as the presence of one or more deeply infiltrating lesions in the rectovaginal septum, bowel wall, vaginal wall and/or bladder wall. After evaluation of histology by a gynecopathologist, serial sections (5 μm) were cut from paraffin-embedded deeply infiltrating endometriosis lesions.

Normal endometrium for the nude mouse experiment was collected during laparoscopy in two women who had normal ovulatory cycles. Tissue was collected by transvaginal biopsy using a sampling device (Gynotec, Malden, The Netherlands) on cycle Days 7 and 9 of the menstrual cycle. No gynecological pathology was found in the endometrium biopsies. The use of human endometrium was approved by the institutional ethical review committee of University Hospital Maastricht. All women gave written informed consent.

Nude mouse model

Eight female mice (Swiss v/v, Charles River, Maastricht, The Netherlands) were individually housed in autoclaved cages and bedding, in laminar flow filtered hoods. The animal room was maintained at 26°C with a 12-h light, 12-h dark cycle, and mice were fed ad libitum with autoclaved laboratory rodent chow and acidified water. All handling was performed in laminar flow filtered hoods. A mixture of ketamine/xylazine (100 mg/kg ketamine and 10 mg/kg xylazine; Eurovet, Bladel, the Netherlands), injected s.c. in a volume of 0.1 ml/10 g bodyweight, was used to anesthetize mice before invasive procedures, using sterile instruments. The Maastricht University ethical review committee for animal experiments approved the use of mice for this study.

At the age of 5 weeks, sterile 60-d release capsules containing 18 mg 17β-estradiol (Innovative Research of America, Sarasota, FL, USA) were placed s.c. in the neck of each animal. According to the manufacturer’s information, capsules provide continuous release of hormone to give serum concentrations of 150–250 pmol/l in the range of physiological levels in mice during the estrous cycle (Bronson et al., 1974). This stable physiological level of estrogen promotes the growth of transplanted human endometrium and eliminates intermouse differences related to various stages of the estrous cycle.

Four days after insertion of the estrogen pellet, an entrance was made to the peritoneal cavity in the midline of the lower abdomen with an 18-gauge needle, and with the help of a pipette, 10 fragments of fresh human endometrium in 200 μl sterile phosphate-buffered saline (PBS) (pH 7.2) were inoculated i.p. to mimic the situation after retrograde menstruation in women. Another entrance was made s.c. through the skin in the flank, and 10 fragments of fresh human endometrium were pipetted s.c. to increase the probability of recovery. Endometrium collected on cycle Days 7 and 9 was pooled and was transplanted in all 8 mice. Two mice at a time were killed by cervical dislocation 1, 2, 3, and 4 weeks after implantation of the endometrium fragments to study the development of endometriosis lesions in time.

Analysis of endometriosis lesions in nude mice

To evaluate endometriosis lesions the abdominal skin was opened, and the abdominal s.c. region, the peritoneum and visceral organs were examined under a binocular microscope. Organs and areas suspect of endometriosis were removed, fixed in 10% buffered formalin and embedded in paraffin wax. Paraffin sections (4 μm) were cut from the entire specimen (150–200 sections) and sections were stained with hematoxylin and eosin or used for immunohistochemistry. Histology of endometriosis lesions was evaluated by a gynecopathologist and a laboratory animal pathologist.

Characterization of FM tissue and detection of TGF-β signaling

Myofibroblasts were distinguished by antibody reaction to vimentin and desmin (intermediate filaments), alpha-smooth muscle actin in
Table I. Primary antibodies and conditions used for immunohistochemistry of human and mouse endometriosis lesions.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Species</th>
<th>Catalog number</th>
<th>IgG class</th>
<th>Dilution</th>
<th>Manufacturer</th>
<th>Antigen retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin</td>
<td>Mouse</td>
<td>M0725</td>
<td>IgG1k</td>
<td>1:100</td>
<td>DAKO, Copenhagen, Denmark</td>
<td>Citrate (pH 6.0)</td>
</tr>
<tr>
<td>α-smooth muscle actin</td>
<td>Mouse</td>
<td>M0851</td>
<td>IgG2ak</td>
<td>1:500</td>
<td>DAKO, Copenhagen, Denmark</td>
<td>Citrate (pH 6.0)</td>
</tr>
<tr>
<td>Desmin</td>
<td>Mouse</td>
<td>MUB0400</td>
<td>IgG2b</td>
<td>1:700</td>
<td>MUBio products BV, Maastricht, the Netherlands</td>
<td>Citrate (pH 6.0)</td>
</tr>
<tr>
<td>SM-MHC</td>
<td>Mouse</td>
<td>26980-1</td>
<td>IgG1k</td>
<td>1:3000</td>
<td>Northstar Bioproducts, East Falmouth, MA, USA</td>
<td>Citrate (pH 6.0)</td>
</tr>
<tr>
<td>TGF-β receptor type I</td>
<td>Rabbit</td>
<td>sc-398</td>
<td>Not specified</td>
<td>1:2000</td>
<td>Santa Cruz Biotechnology, Santa Cruz, CA, USA</td>
<td>Citrate (pH 6.0)</td>
</tr>
<tr>
<td>TGF-β receptor type II</td>
<td>Rabbit</td>
<td>sc-220</td>
<td>Not specified</td>
<td>1:3000</td>
<td>Santa Cruz Biotechnology</td>
<td>Citrate (pH 6.0)</td>
</tr>
<tr>
<td>Phosphorylated Smad2</td>
<td>Rabbit</td>
<td>3101</td>
<td>Not specified</td>
<td>1:1000</td>
<td>Cell Signaling, Danvers, MA, USA</td>
<td>Citrate (pH 6.0)</td>
</tr>
</tbody>
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Ig, immunoglobulin; SM, smooth muscle; TGF-β, transforming growth factor-β.

results

Human deeply infiltrating endometriosis lesions

All 20 paraffin-embedded deeply infiltrating endometriosis tissue specimens contained connective tissue, endometriosis lesions consisting of endometrial epithelium and endometrial stroma and FM tissue surrounding endometriosis lesions (Fig. 1). Seventeen out of 20 paraffin-embedded tissue samples contained visceral smooth muscle. Immunohistochemistry results from patient tissue did not differ between lesions obtained from rectovaginal septum, bowel wall, vaginal wall or bladder wall. To investigate the immunophenotype of the lesions and surrounding tissues, sections were stained for vimentin, alpha-smooth muscle actin, desmin and smooth muscle myosin (Figs 1 and 2). Representative photographs of the negative controls for the various immunohistochemical staining procedures are presented in Fig. 3.

Figure 1: Representative photographs of deep-invasive endometriotic lesions stained with antibodies against vimentin, alpha-smooth muscle actin (ASMA), desmin and smooth muscle myosin heavy chain (SM-myosin). Vimentin staining is strong in the stroma (Str) of the ectopic endometrium, whereas the cells surrounding the lesion (FM) are positive for ASMA. Some areas are also positive for desmin and SM-myosin; most likely resident smooth muscle cells (SM). Scale bar = 100 μm.
**Connective tissue fibroblasts**

As a reference to assess the extent of FM differentiation close to the lesions, we evaluated the immunophenotype of connective tissue fibroblasts of the submucosal connective tissue of the large bowel in patients with deep infiltrating endometriosis lesions. An example is presented in Fig. 4. The intermediate filament vimentin was strongly expressed in connective tissue fibroblasts (mean SI 8.7 ± 0.8, Fig. 2). In some tissue samples, connective tissue fibroblasts showed weak desmin expression (mean SI 2.0 ± 2.6), whereas expression of myosin heavy chain and alpha-smooth muscle actin was completely absent in connective tissue fibroblasts of all specimens.
Endometrial stroma

Endometrial stromal cells in deeply infiltrating endometriosis lesions strongly express vimentin (mean SI 7.2 ± 2.3). Weak expression of alpha-smooth muscle actin (mean SI 1.3 ± 0.8) and desmin (mean SI 2.0 ± 1.2) could be observed, mostly localized in endothelial cells of blood vessels within the lesions (Figs 2 and 5). SM-MHC expression (mean SI 0.3 ± 0.6) was very weak and could only be observed in a small minority of lesions (Figs 2 and 5).

FM tissue

Cells comprising part of the FM reaction around endometriosis lesions also strongly express vimentin (mean SI 7.3 ± 1.9). As opposed to fibroblasts in connective tissue, these cells abundantly express alpha-smooth muscle actin (Figs 2 and 6; mean SI 7.0 ± 1.7), thereby demonstrating their myofibroblastic nature. Within the regions that stain positive for alpha-smooth muscle actin, focal areas showing moderate to strong expression of smooth muscle differentiation markers desmin and myosin heavy chain (mean SI 3.3 ± 1.6 and 3.4 ± 1.6, respectively) can be observed (Fig. 6).

Visceral smooth muscle

Visceral smooth muscle shows weak vimentin expression (mean SI 1.3 ± 1.6) and abundant generalized expression of desmin (mean SI 7.9 ± 0.7), myosin heavy chain (mean SI 7.1 ± 1.0) and alpha-smooth muscle actin (mean SI 6.3 ± 0.6), all markers of smooth muscle differentiation (Figs 2 and 7). Sometimes endometriotic lesions are present in the visceral muscle layer (Fig. 7).

TGF-β signaling

Smad2 phosphorylation was closely associated with the presence of TGF-β receptors type I and II. Expression of TGF-β receptor I, TGF-β receptor II and phosphorylated Smad2 was most pronounced in endometrial epithelium (mean SI 6.5 ± 1.2/6.8 ± 1.6/7.9 ± 1.3, respectively) followed by endometrial stroma (mean SI 4.0 ± 1.0/3.7 ± 1.0/6.8 ± 1.4, respectively), visceral smooth muscle (mean SI 4.5 ± 1.2/3.4 ± 0.9/6.0 ± 2.1, respectively) and connective tissue (mean SI 3.8 ± 1.1/2.3 ± 1.6/5.0 ± 2.0, respectively) and was least pronounced in cells comprising part of the FM reaction (mean SI 1.9 ± 0.7/1.5 ± 0.8/4.6 ± 2.2, respectively) (Figs 8 and 9).

Mouse endometriosis lesions

Endometriosis lesions were identified in all except one mouse that was sacrificed after 2 weeks. Most lesions were found at the s.c. injection sites, some lesions were present on the peritoneum near the umbilical region. All lesions consisted of
endometrial glands and stroma. For immunohistochemical analysis the s.c. lesions were used. Cells of human origin could be distinguished from mouse cells by positive staining for the human-specific vimentin antibody (Fig. 10).

One week after tissue inoculation, alpha-smooth muscle actin was highly expressed in the mouse cells directly surrounding the lesion (Fig. 10) but not in the human endometrial cells or in connective tissue fibroblasts remote from the lesion. Two weeks after inoculation, alpha-smooth muscle actin expression in mouse cells slightly decreased. After three and four weeks alpha-smooth muscle actin expression progressively declined but remained visible as a thin sheath directly adjacent to and surrounding the endometrial glandular epithelium. The number of endometrial stromal cells appeared to decrease at the same time that collagen deposition became apparent in a circular pattern surrounding the lesion after three weeks.

Discussion

Deeply infiltrating endometriosis is characterized by the existence of nodular lesions largely composed of FM tissue. In this study we show that FM tissue surrounding endometriosis lesions contains myofibroblastic cells that, in addition to alpha-smooth muscle actin, express multiple markers of smooth muscle differentiation such as desmin and SM-MHC.

Our findings support the contention that the formation of deeply infiltrating endometriosis lesions shares characteristics with pathological wound healing. During the initial phase of normal wound healing, alpha-smooth muscle actin is highly expressed by myofibroblasts in granulation tissue, thereby effectuating the necessary wound contraction. The contractile activity of myofibroblasts is terminated when the tissue is repaired: alpha-smooth muscle actin expression decreases.
and myofibroblasts disappear through massive apoptosis (Desmoulie`re et al., 2005). In pathological fibrotic phenomena, however, this wave of apoptosis is lacking and alpha-smooth muscle actin expression persists in the tissue myofibroblasts in addition to the expression of other markers of smooth muscle differentiation such as desmin and SM-MHC (Skalli et al., 1989).

The presence of smooth muscles in endometriosis lesions is not a new finding: Anaf et al. (2000a) demonstrated alpha-smooth muscle actin positivity in areas surrounding peritoneal, ovarian, uterosacral and rectovaginal endometriosis lesions. Smooth muscles were absent in the unaffected peritoneum and in the eutopic endometrium of women with and without pelvic endometriosis. In line with the induction theory (Levander and Normann, 1955), these authors speculated that the smooth muscle component of endometriosis

might result from the capacity of the secondary Müllerian system to differentiate into both smooth muscle cells and endometrial glands and stroma. Alternatively, endometrial cells arriving through retrograde transplantation at ectopic sites could undergo smooth muscle metaplasia, or induce the surrounding tissue to undergo smooth muscle metaplasia. With the aid of a nude mouse model we were able to demonstrate that as soon as one week after inoculation with human endometrium, alpha-smooth muscle actin expression is induced in the surrounding murine fibroblasts, whereas no expression was observed in the human cells. These findings strongly suggest that the presence of smooth muscle-like tissue in deeply infiltrating endometriosis lesions is accounted for by a reaction of the local environment to the presence of ectopic endometrium rather than smooth muscle metaplasia of the ectopic endometrium itself.

This phenomenon mimics what is frequently observed in malignancies. Many epithelial tumors are also characterized by the presence of an ‘activated’ stroma consisting of fibroblastic and myofibroblastic cells that produce collagen and extracellular matrix components, a phenomenon that is referred to as the stroma reaction or ‘desmoplastic reaction’ (Desmoulie`re et al., 2004). Desmoplasia is considered a response of the resident stromal fibroblasts of the host environment to inductive stimuli exerted by tumor cells, such as diffusible factors, extracellular matrix and/or direct cell-to-cell contacts. The reciprocal interactions between the tumor cells and resident fibroblasts potentiate tumor growth, stimulate angiogenesis and induce fibroblasts to undergo differentiation into myofibroblasts. Although endometriosis cannot be regarded as a bona fide neoplasm, it displays certain important characteristics of malignant tumor growth such as invasion of the extracellular matrix (Spuijbroek et al., 1992) and the acquisition of its own blood supply (Groothuis et al., 2005). Therefore, it is conceivable that a similar process may take place in the evolution of smooth muscle-containing endometriotic lesions. However, a limitation of the present study is constituted by the fact that the endometrial tissue used for the animal study was sampled from only two women and the experiment was carried out only once in a relatively small number of animals. The possibility that these results occurred by chance can therefore not be ruled out completely.

We hypothesized that prolonged TGF-β signaling is involved in the development of FM tissue in deep infiltrating endometriotic lesions. It has been shown that the cytokine TGF-β1 is responsible for inducing the synthesis of alpha-smooth muscle actin in fibroblastic cells and for stimulating the production of collagen type I (Desmoulie`re et al., 1993), and overproduction of TGF-β1 has been implicated in the pathogenesis of disorders characterized by fibrosis (Okuda et al., 1990; Broekelmann et al., 1991; Border and Noble, 1994; Roberts, 1995; Chegini, 1997). In this respect, TGF-β1 is a key cytokine in the evolution of lesions characterized by myofibroblast formation. TGF-β1 is expressed in the human endometrium throughout the menstrual cycle, it is regulated by ovarian steroids (Brunet et al., 1999; Luo et al., 2003), and could be responsible for the induction of the FM reaction in the fibroblasts of the host environment. Surprisingly however,
the expression of the marker of active TGF-β signaling, phosphorylated Smad 2, was consistently lower in myofibroblasts of the FM tissue compared with surrounding tissues. Consistent with these findings, we found that the expression of the receptors for TGF-β was most pronounced in the endometrial tissue of the lesion, and not in the surrounding FM tissue. Most TGF-β signaling apparently occurs in the endometrial tissue and not in the local host cells.

An important factor which may contribute to the differentiation and maintenance of the myofibroblast phenotype could be mechanical strain (Serini et al., 1998). Deeply infiltrating endometriosis lesions are often found in areas subjected to continuous or intermittent mechanical tension: the bowel wall and rectovaginal septum repeatedly stretch and relax as a result of peristalsis and the passage of feces. Frequent mechanical stress could constitute an important determinant in the development of deep infiltrating endometriosis lesions. In rat models for wound healing in the skin, Hinz et al. (2001) showed that mechanically inflicted tension induced alpha-smooth muscle actin expression in fibroblasts in the wound; relief of the mechanical pressure resulted in disappearance of the actin filaments which preceded a decrease in TGF-β1 levels. These results indicate that TGF-β1 alone may not be sufficient to maintain myofibroblast differentiation, and that a mechanical stimulus is equally important. Shi et al. (1996) showed in injured porcine arteries that after 14 days of coexpression, TGF-β1 expression disappeared, whereas alpha-smooth muscle actin expression in the neointima of injured porcine arteries remained high up to 90 days. As arteries are also subject to mechanical strain due to the high intravascular pressure, it is plausible that this is responsible for the continued alpha-smooth muscle actin expression.

Alternatively, the excessive FM reaction could be explained by the fact that ectopic endometrium arriving through repeated retrograde menstruation induces a chronic inflammatory response in the peritoneal cavity. It has been shown that the peritoneal fluid of women with endometriosis is marked by increases in the release of inflammatory cytokines such as TNF-α and IL-1β and that a mechanical stimulus is equally important. Shi et al. (1996) showed that mechanically inflicted tension induced alpha-smooth muscle actin expression in fibroblasts in the wound; relief of the mechanical pressure resulted in disappearance of the actin filaments which preceded a decrease in TGF-β1 levels. These results indicate that TGF-β1 alone may not be sufficient to maintain myofibroblast differentiation, and that a mechanical stimulus is equally important. Shi et al. (1996) showed in injured porcine arteries that after 14 days of coexpression, TGF-β1 expression disappeared, whereas alpha-smooth muscle actin expression in the neointima of injured porcine arteries remained high up to 90 days. As arteries are also subject to mechanical strain due to the high intravascular pressure, it is plausible that this is responsible for the continued alpha-smooth muscle actin expression.

In conclusion, in this study we show that the FM cells surrounding deep infiltrating endometriosis lesions express multiple markers of FM differentiation, resembling the situation in pathologic wound healing and fibrocontractive diseases. In a nude mouse model, we showed that alpha-smooth muscle actin expression is induced in the host tissue after implantation of human endometrial fragments, suggesting that the presence of smooth muscle-like tissue in endometriosis lesions is the result of a reaction of the local environment to the presence of ectopic endometrium rather than smooth muscle metaplasia of the ectopic endometrium itself. Based on our observations it is not likely that TGF-β1 signaling alone is sufficient to account for the excessive FM tissue in deep infiltrating endometriosis lesions. In this respect, the presence of mechanical tension and increased inflammatory activity in peritoneal fluid of women with endometriosis may be important contributing factors in the establishment of myofibroblast-containing deep infiltrating endometriosis lesions.

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