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

K.J.A.F. van Kaam¹,², J.P. Schouten¹,², A.W. Nap¹,², G.A.J. Dunselman¹,² and P.G. Groothuis³

¹Research Institute GROW, University of Maastricht/University Hospital Maastricht, Maastricht, The Netherlands; ²Department of Obstetrics and Gynaecology, University of Maastricht/University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, The Netherlands; ³Department of Pharmacology, Organon Biosciences, PO Box 20, 5340 BH Oss, The Netherlands

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 deep 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 neoexpress 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 iv/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 290 μl sterile phosphate-buffered saline (PBS) (pH 7.2) were inoculated i.p. to mimic the situation made to the peritoneal cavity in the midline of the lower abdomen. After evaluation of histology by a gynecopathologist, serial sections (5 μm) were cut from the entire specimen (150–200 sections) and sections were stained with hematoxylin and eosin or used for immunohistochemistry. Histology by a gynecopathologist and a laboratory animal pathologist.

Analysis of endometriosis lesions in nude mice

To evaluate endometriosis lesions the abdominal skin was opened, and with the help of a pipette, 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 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...
(cytoskeletal element) and smooth muscle myosin heavy chain (SM-MHC) (microfilament). A classification system of myofibroblasts is based on immunohistochemical staining of cytoskeletal filaments. Myofibroblasts that express vimentin are called V type, those that express vimentin and alpha-smooth muscle actin are called VA type, those that express vimentin and desmin are called VD type, those that express vimentin, alpha smooth muscle actin and desmin are called VAD type, and those that express vimentin, alpha-smooth muscle actin and SM-MHCs with and without desmin are called VA/D/M type. Active TGF-β signaling was detected by using antibodies directed against phosphorylated Smad2. In addition we stained for TGF-β receptors type I and II to identify the target cells in deep infiltrating endometriosis lesions and surrounding tissues.

**Immunohistochemistry**

A summary of the primary antibodies used and conditions applied is given in Table I.

Sections of human and mouse endometriosis lesions were fixed on Starfrost adhesive slides (Klinipath, Duiven, The Netherlands). Sections were deparaffinized in xylene and rehydrated in alcohol series prior to blocking endogenous peroxidase activity by incubation with 3% hydrogen peroxide/methanol for 20 min. After rinsing three times with PBS (pH 7.2), antigen retrieval was performed (see Table I). Sections were cooled down to room temperature and washed again three times in PBS followed by incubation with the primary antibody (room temperature, 2 h). After three PBS rinses, sections were exposed to the secondary antibody (Envision rabbit anti-mouse, ChemMate™ detection kit, DAKO, Copenhagen, Denmark) for 30 min at room temperature. Antibody binding was visualized using 3, 3′-diaminobenzidine. Sections were counterstained mildly with hematoxylin, dehydrated and mounted in Entellan for light microscopy. Negative control slides for the monoclonal antibodies were incubated with mouse immunoglobulin (Ig)Gs of the same class and same dilution as the primary antibodies. Negative control slides for the rabbit polyclonal antibodies (pAbs) were incubated with rabbit IgG at the same dilution as the pAbs.

**Evaluation of immunostaining in human endometriotic tissues**

The percentage of stained cells (0, 0–10, 10–50, >50%) and the intensity of staining (absent, weak, moderate, strong) were determined (0, 1, 2 or 3 for each variable) for the entire lesion with respect to (i) visceral smooth muscle (if present), (ii) connective tissue not comprising part of FM reaction, (iii) FM tissue surrounding endometriosis lesions, (iv) endometrial stromal cells and (v) endometrial epithelial cells. A staining index (SI, ranging from 0 to 9) was calculated by multiplying categorized parameters. Two different observers (P.G.G. and K.J.A.F.K) performed evaluation of immunostaining in a blinded fashion. Both observers scored the sections once. The mean of these two observations was used for analyses. The level of inter-observer agreement was determined by calculating kappa statistics for ordinal variables and showed that concordance between the two observers was adequate with respect to the percentage of stained cells (κ 0.73), intensity of the staining (κ 0.86) and SI (κ 0.76).

**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.](https://academic.oup.com/humrep/article-abstract/23/12/2692/610347/10347)
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 $8.7 \pm 0.8$, Fig. 2). In some tissue samples, connective tissue fibroblasts showed weak desmin expression (mean SI $2.0 \pm 2.6$), whereas expression of myosin heavy chain and alpha-smooth muscle actin was completely absent in connective tissue fibroblasts of all specimens.

Figure 2: Staining indices for vimentin, ASMA, desmin and SM-myosin in the different tissue components of endometriotic lesions and host environment. Gray square, visceral smooth muscle; striped square, connective tissue; open square, fibromuscular tissue; dark gray square, endometrial stroma; dotted square, endometrial epithelium.

Figure 3: Representative photographs of the negative controls for the vimentin, ASMA, desmin, SM-myosin, transforming growth factor-β (TGF-β) receptor 1 and 2 and phosphorylated Smad immunostainings. Scale bar = 100 μm.

Figure 4: Immunostaining of vimentin, ASMA, desmin and SM-myosin in submucosal connective tissue of the large bowel. Scale bar = 100 μm.
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...
et al. demonstrated 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’ (Desmoulière 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 endometriosis 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 (Desmoulière 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, phospho- 
ylated 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 recep-
tors 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 differen-
tiation and maintenance of the myofibroblast phenotype 
could be mechanical strain (Serini et al., 1998). Deeply infiltr-
ating 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 induced tension induced 
alpha-smooth muscle actin expression in fibroblasts in the 
wound; relief of the mechanical pressure resulted in disappear-
ance 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 
increased signs of inflammation, including increased concen-
tration of white blood cells and macrophages, and increased 
activation status of these macrophages (Oral et al., 1996; 
Dunselman et al., 1988). In association with the activated 
state of these macrophages, an increase in the release of 
macrophage-derived cytokines such as TGF-β1 has been 
demonstrated in the peritoneal fluid of women with endome-
triosis (Oosterlynck et al., 1994; Pizzo et al., 2002; Kyama 
et al., 2006). Overproduction of TGF-β1 by activated macro-
phages in peritoneal fluid may therefore contribute to a micro-
environment that favors the formation of FM tissue 
surrounding endometriosis lesions.

In conclusion, in this study we show that the FM cells sur-
rounding deep infiltrating endometriosis lesions express mul-
tiple 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 endo-
metriosis lesions. In this respect, the presence of mechanical 
tension and increased inflammatory activity in peritoneal 
fluid of women with endometriosis may be important contribut-
ing factors in the establishment of myofibroblast-containing 
deep infiltrating endometriosis lesions.

References

Anaf V, Simon P, Fayt I, Noel J. Smooth muscles are frequent components of 

Anaf V, Simon P, El Nakadi A, Fayt I, Buxant F, Simonart T, Peny MO, Noel 
JC. Relationship between endometriotic foci and nerves in rectovaginal 

Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. N 

Brekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth 
factor beta 1 is present at sites of extracellular matrix gene expression in 

Bronson FH, Desjardins C. Circulating concentrations of FSH, LH, estradiol, 
and progesterone associated with acute, male-induced puberty in female 

Bruner KL, Eisenberg E, Gorstein F, Osteen KG. Progesterone and 
transforming growth factor-beta coordinately regulate suppression of 
endometrial matrix metalloproteinases in a model of experimental 

Chegini N. The role of growth factors in peritoneal healing: transforming 

Cornillie FJ, Oosterlynck D, Lauweryns PM, Koninckx PR. Deeply infiltrating 
pelvic endometriosis: histology and clinical significance. Fertil Steril 

Darby I, Skalli O, Gabbiani G. Alpha-Smooth muscle actin is transiently expressed 
by myofibroblasts in experimental wound healing. Lab Invest 1990;63: 
21–29.

Desmoulière A, Chaponnier C, Gabbiani G. Tissue repair, contraction, and the 

Desmoulière A, Geinoz A, Gabbiani G, Fabbri B. Transforming growth 
factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue 
myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell 

Desmoulière A, Guyot C, Gabbiani G. The stroma reaction myofibroblast: a 
key player in the control of tumor cell behavior. Int J Dev Biol 

Dunselman GA, Hendrix MG, Bouckaert PX, Evers JL. Deeply infiltrating 

Fukunaga M. Smooth muscle metaplasia in ovarian endometriosis. 

Gabbiani G. The myofibroblast in wound healing and fibrocontractive 

Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth 
factor beta on bleomycin induced accumulation of lung collagen in mice. 

Groothuis PG, Nap AW, Winterhager E, Grummer R. Vascular development in 

Hinz B, Mastrandelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical 
tension controls granulation tissue contractile activity and myofibroblast 

Itoga T, Matsumoto T, Takeuchi H, Yamasaki S, Sasahara N, Hoshi T, 
Kinosita K. Fibrosis and smooth muscle metaplasia in rectovaginal 

Kim MR, Park DW, Lee JH, Choi DS, Hwang KJ, Ryu HS, Min CK. 
Progesterone-dependent release of transforming growth factor betal from 
epithelial cells enhances the endometrial decidualization by turning on the 

Kyama CM, Overbergh L, Debrock S, Valkx D, Vander Perre S, Meuleman C, 
Mihalyi A, Mwenda JM, Mathieu C, D’Hooghe TM. Increased peritoneal 
and endometrial gene expression of biologically relevant cytokines and


Submitted on August 15, 2008; resubmitted on January 22, 2008; accepted on February 22, 2008