Time to Redefine Endometriosis Including Its Pro-fibrotic Nature

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Abstract and Introduction

Abstract

Endometriosis is currently defined as presence of endometrial epithelial and stromal cells at ectopic sites. This simple and straightforward definition has served us well since its original introduction. However, with advances in disease knowledge, endometrial stromal and glands have been shown to represent only a minor component of endometriotic lesions and they are often absent in some disease forms. In rectovaginal nodules, the glandular epithelium is often not surrounded by stroma and frequently no epithelium can be identified in the wall of ovarian endometriomas. On the other hand, a smooth muscle component and fibrosis represent consistent features of all disease forms. Based on these observations, we believe that the definition of endometriosis should be reconsidered and reworded as 'A fibrotic condition in which endometrial stroma and epithelium can be identified'. The main reasons for this change are: (1) to foster the evaluation of fibrosis in studies on endometriosis pathogenesis using animal models; (2) to limit potential false negative diagnoses if pathologists stick stringently to the current definition of endometriosis requiring the demonstration of endometrial stromal and glands; (3) to consider fibrosis as a potential target for treatment in endometriosis. This opinion article is aimed at boosting the attention paid to a largely neglected aspect of the disease. We hope that targeting the fibrotic process might increase success in developing new therapeutic approaches.

Introduction

With advances in knowledge, borders of diseases may change. Occasionally, these changes are large enough to require a redefinition of the disease. In endometriosis, since the original description by Sampson (1927), there have been radical changes in our vision of the disease, starting with a better description of the various manifestations and more specific pathologic findings. Moreover, our understanding continues to expand with increasing knowledge of genetics and risk factors and progresses in biological mechanisms and animal models of the disease. With these advances, clinicians and specialists in genetics, epidemiology, pathology and basic science have developed their own conceptualizations of endometriosis which are much more than the current simplistic definition that is based on the mere presence of endometrial epithelial and stromal cells in ectopic sites.

Several important issues challenge this obsolete definition. Although the presence of endometrial stromal and glands in ectopic sites may be the starting point in the pathogenesis of endometriosis, it is unquestionable that endometrial stromal and glands represent only a minor component of endometriotic lesions. Indeed, this classic pathologic evidence may even be lacking. In rectovaginal nodules, the glandular epithelium can often be observed deeply in the fibromuscular tissue without any surrounding stroma (Donnez et al., 1996), and in 40% of ovarian endometriomas, no endometrial epithelium can be identified and the inner surface of the cyst is covered only by fibrotic tissue (Muzi et al., 2007). Finally, pelvic adhesions are typically free of endometrial components despite being an essential pathologic characteristic of the disease (Somigliana et al., 2012). It is noteworthy that pelvic adhesions may contribute to the origin of some classical endometriosis-related symptoms, such as deep dyspareunia, chronic pelvic pain and infertility, and may play a role in the formation of endometriomas or deep nodules (Somigliana et al., 2012). This opinion paper is intended as an introductory discussion article, an opening of dialog in order to consider some changes in the general definition of endometriosis. The need for a modification is also supported by previous attempts in this context (Holt and Weiss, 2000). We will emphasize the consistent presence of fibrosis and myofibroblasts in endometriotic lesions and their crucial role in the pathogenesis of the disease (Anaf et al., 2000; Barcena de Arellano et al., 2011; Zhang et al., 2016). Highlighting these features is aimed at boosting up the attention of the scientific community to a largely neglected but essential disease aspect. Ultimately, an enhanced sensitivity to fibrosis may orient the focus of researchers towards a more modern and realistic vision of endometriosis, direct animal models to the real nature of the disease, open new and more fruitful avenues of pharmacological research and increase the success of new therapeutic approaches to endometriosis, which have shown a high attrition rate during the last decades (Vercellini et al., 2011).

The Biological Basis of Fibrosis Development: The Crucial Role of Myofibroblasts

Myofibroblasts are contractile non-muscle cells that are usually activated in response to injury with the intent to repair damaged extracellular matrix (ECM). These cells can differentiate from different cellular lineages including tissue resident fibroblasts, endothelial cells undergoing endothelial-to-mesenchymal transition, vascular smooth muscle cells and epithelial cells after epithelial-to-mesenchymal transition. A myofibroblast is activated when the α-smooth muscle isoform of actin (α-SMA) is neo-expressed and incorporated in stress fiber-like bundles which are pivotal to promote the specific myofibroblast function of contracting the ECM. Two factors seem critical to activate myofibroblasts from various precursor cells in the vast majority of organs studied: Transforming Growth Factor (TGF)-β and the stiffness of the tissue. Indeed, TGF-β1 is able to induce neo-
expression of α-SMA by fibroblasts in vivo and in vitro and in cultures on stiff substrates (e.g. a fibrotic scar) can activate a variety of different progenitors to become myofibroblasts (Richter et al., 2015; Hinz, 2016a).

When activated, myofibroblasts display increased proliferation, migratory ability, production of cytokines and interstitial matrix with the consequence of disrupting the function of intact residual tissues and altering the biochemical and biophysical microenvironment. A persistent myofibroblast activity causes accumulation and contraction of collagenous ECM, a condition called fibrosis. Macroscopically, due to accumulation of ECM, contraction of myofibroblasts and reduced vasculature, fibrotic organs usually display an uneven surface, are pale and not elastic. This process ultimately results in disruption of the normal anatomical structure (Bochaton-Piallat et al., 2016). Myofibroblasts are present in all fibrotic diseases, such as scleroderma, as well as liver, kidney, and lung fibrosis and are prominent in heart failure and repair after myocardial infarction (Rockey et al., 2015; Chistiakov et al., 2016). Myofibroblast-produced tissue contractures can become life-threatening when fibrosis affects vital organs (Rockey et al., 2015).

Discriminating between myofibroblasts and smooth muscle cells may be demanding and is a matter of controversy (Hinz, 2016a). Neo-expression of α-SMA in stress fibers is the most commonly used molecular marker for myofibroblasts that also express mesenchymal marker proteins such as N-cadherin, vimentin and S100A4. However, these latter markers are also expressed in smooth muscle cells, at least during tissue repair. Smooth muscle cells conversely express a number of late differentiation markers, such as smooth muscle myosin heavy chain, h-caldesmon, smoothelin and the muscle intermediate filament protein desmin, that are absent from myofibroblasts in most organs. However, discriminating smooth muscle cells from myofibroblasts is quite difficult in pathological conditions, so their distinction is usually a rather semantic issue (Hinz, 2016a). It is noteworthy that a metaplastic transformation from stromal cells to smooth muscle cells via differentiation from fibroblasts to myofibroblasts has been suggested (Zhang et al., 2016).

Not surprisingly, the interest of researchers in various fields of medicine has recently focused on anti-fibrotic therapeutic strategies aimed at blocking cytokines and factors that directly control myofibroblast activation (Yang et al., 2014). The complex presentation and activation mechanisms of TGF-β1 have led to the development of various anti-TGF-β1 approaches to prevent myofibroblast formation and fibrosis development. Some initial findings were disappointing in terms of both efficacy and safety. However, clinical trials using different anti-TGF-β1 treatments are ongoing in various diseases. Interestingly, since all the ov integrins have been shown to be able to activate TGF-β1 and are expressed in a tissue- and cell-distinctive manner, inhibiting their TGF-β1 activating function may be biologically more specific compared to the global inhibition of TGF-β1 itself. Some anti-integrin molecules are currently under investigation in clinical trials to treat patients with lung fibrosis and initial findings seem promising (Hinz, 2016b).

Fibrosis and Myofibroblasts in Endometriosis Lesions

Peritoneal Lesions

The first study on peritoneal endometriosis with a monoclonal antibody against α-SMA was published back in 1996 by Khare et al. (1996) who used immunoperoxidase and Masson’s trichrome stains to determine, respectively, the presence of myofibroblasts and collagen in 10 pelvic wall samples. Well-formed smooth muscle bundles and dense type I collagen were found in these lesions. In 2000, Anaf and coworkers (2000) demonstrated by immunohistochemistry that all the 21 peritoneal lesions considered were consistently positive for α-SMA staining, although variable in intensity, whereas unaffected peritoneum and eutopic endometrial biopsies were negative. Leyendecker et al. (2002) analyzed 35 endometriotic lesions with a specific α-SMA antibody by immunohistochemistry and all of them stained positively for the marker. Although the authors did not formally discriminate between various disease forms, they clearly showed representative sections of peritoneal endometriotic lesions stained for α-SMA. The group of Sylvia Mechsner similarly evaluated peritoneal endometriosis specimens in two different studies. In the first one, 76% of 120 lesions showed α-SMA expression (Mechsner et al., 2005) while in the second one, all 60 lesions showed positivity (Barcena de Arellano et al., 2011). Therefore, smooth muscle content seems to represent an important and consistent feature of peritoneal endometriosis lesions.

Interestingly, TGF-β1 levels were found to be significantly increased in the peritoneal fluid of women with peritoneal lesions compared to women without the disease. Exposure of mesothelial cells to TGF-β1 increased the production of lactate, with reduction in the local pH. This increase in the amount of lactate resulted in acid activation of the TGF-β ligand with secondary induction of myofibroblast differentiation (Young et al., 2014).

Ovarian Cysts

It is well known that fibrosis is present in the ovarian cyst wall. Indeed, the cyst’s pseudocapsule is mostly constituted of fibrotic tissue. Of note, the inner surface of the cyst is usually not entirely covered by an endometrial lining and where the endometrial lining is missing, only fibrotic tissue is identifiable. Positive immunostaining for α-SMA antibody was demonstrated in all of 10 and 13 ovarian cysts by Khare et al. (1996) and Anaf et al. (2000), respectively. According to Mechsner et al. (2005) smooth muscle content was present in 87% of the 40 ovarian lesions they evaluated. Liu et al. (2017) investigated the histologic features of deep and ovarian endometriotic lesions and observed a higher fibrotic content in the former compared with the latter lesion type. Nevertheless, the 25 ovarian samples consistently showed markers of fibroblast-to-myofibroblast transdifferentiation and stained positively for fibrosis at Masson’s trichrome technique. Fibrosis was also identified in ovarian cortex surrounding the endometrioma. Indeed, follicular density was found to be lower in the ovarian cortex adjacent to the endometriotic cyst and this phenomenon is thought to be associated with tissue alterations, such as formation of fibrosis and vascular deficiency, and does not seem to be related to mere mechanical stretching. Kitajima et al. (2014) compared the histologic features in apparently normal
ovarian cortical tissue from ovaries with small endometriomas and from the contralateral healthy ovaries. Fibrosis, as determined by Masson's trichrome staining with methyl green, was significantly more frequent in cortex from ovaries with endometriomas (80%) than in those without (27%) and the presence of fibrosis with concomitant loss of cortex-specific stroma was observed in 55% of cortical samples from ovaries with endometriomas but in none of those from contralateral healthy ovaries.

Interestingly, Sun-Wei Guo's group has recently shown that, in cells derived from ovarian endometriosis, activated platelets promoted epithelial to mesenchymal transition, fibroblast-to-myofibroblast transdifferentiation and differentiation to smooth muscle cells, resulting in increased cell contractility, collagen production and ultimately to fibrosis, via the release of TGF-β1 and the induction of TGF-β/Smad signaling pathway. TGF-β1 blockade could reverse these phenomena (Zhang et al., 2016).

Deep Infiltrating Endometriosis

Donnez and coworkers (1996) demonstrated for the first time that deep endometriotic nodules were histologically composed of scanty stroma and glandular epithelium disseminated in extensive fibromuscular tissue. Gomori trichrome stain was used to detect muscle tissue. They speculated that this smooth muscle content pre-existed in the correspondent normal area and was invaded by the ectopic endometrium. Subsequently, Anaf et al. (2000) evaluated 12 rectovaginal nodules and eight uterosacral lesions and found them to be consistently positive for an anti-α-SMA antibody; they disputed the pre-existence of smooth muscle tissue in the rectovaginal nodules and conversely supported a transdifferentiation of endometrial stromal cells. Itoa et al. (2003) examined 90 rectovaginal nodules for the presence of fibrosis by elastic-van Gieson staining of collagen and for positivity to anti-α-SMA and anti-desmin antibodies. Fibrosis was observed in all but one of the samples, and immunoreactivity for smooth muscle actin and desmin was observed in 89% of the specimens. In deep nodules (n = 20), staining levels for α-SMA, desmin, collagen I and extent of fibrosis were shown to be higher than those of ovarian disease (Liu et al., 2017). van Kaam et al. (2008) not only showed that all the 20 deep infiltrating endometriotic lesions studied comprised fibromuscular tissue containing α-SMA-, desmin- and myosin-positive myofibroblastic cells, but again raised reasonable doubts on the origin of this muscle content. Indeed, they demonstrated that the inoculation of human endometrium into a nude mouse could induce α-SMA expression in the surrounding murine tissue. This would suggest that a reaction of the local environment to the presence of ectopic endometrium, rather than the stromal differentiation toward smooth muscle cells, could be the basis of fibrosis development.

Despite the identification of a fibrotic component in deep infiltrating disease, Matsuzaki et al. (2017) showed that the TGF-β1 signaling may be absent when culturing endometriotic cells taken from this type of lesions. They suggested that endometrial stromal cells from patients affected might differentiate into myofibroblasts without TGF-β1 treatment and produce collagen type I. Increased stiffness through increased myofibroblast collagen production may then further increase matrix stiffness resulting in a fibrotic environment in deep disease over time.

Summary of the Literature Overview

Regardless of the different hypotheses provided to explain the origin of myofibroblasts and fibrosis in endometriotic lesions (summarized in Figure 1) (Young et al., 2014; Zhang et al., 2016; Matsuzaki et al., 2017; Albertsen and Ward, 2017), all investigators agree on the importance of this component. One may argue that fibrosis represents a secondary event triggered by an insult (the presence of ectopic cells) in an affected tissue (Walton et al., 2017). However, fibrosis appears as the phenomenon underpinning endometriosis-associated morbidity and some manifestations of the disease (i.e. adhesions). Thus, in line with what is recognized for other conditions of unknown etiology such as scleroderma (Tsou and Sawalha, 2017), fibrosis seems to represent a self-amplifying event of endometriosis.
Main pathogenetic models proposed to explain the presence of myofibroblasts and the development of fibrosis in endometriosis. Epithelial to mesenchymal transition, fibroblast-to-myofibroblast transdifferentiation, increased collagen production and ultimately fibrosis have been suggested to be triggered in endometriotic cells by the presence of stimulating factors (e.g. Tranforming Growth Factor (TGF) β1 [B and C], platelets [B] or a stiff tissue matrix [D]). Similar phenomena in other tissues (A, surrounding connective tissue or C, mesothelial barrier) have been also proposed.

**Why Changing the Definition**

There are essentially three reasons for including the term 'fibrosis' in the definition of endometriosis:

1. Myofibroblasts and fibrosis may receive more attention as potential targets of medical treatments for endometriosis. Shifting the focus on fibrosis with a new definition may re-orient current research efforts towards more effective therapies. When considering the challenges of treating a fibrotic disease such as endometriosis, there is a pressing need to identify effective pharmacological agents to block fibrosis, in addition to seek for agents acting on ectopic endometrium (Somigliana et al., 2012). Moreover, the scientific community should pay utmost attention to the progress on the management of fibrosis in other areas of medicine. If in the future some effective and safe antifibrotic drugs will be developed for other disorders, endometriosis might benefit as well.

2. Given the consistent presence of myofibroblasts and fibrosis in all disease forms, animal models of endometriosis should also present this feature. The current definition could lead researchers astray in this regard, as they tend to consider an animal model reliable merely because endometrium is placed at ectopic sites. However, endometriosis is much more than that and fibrosis represents a crucial histological aspect. Mouse, hamster or rat models have been developed so far by intraperitoneal or subcutaneous transplantation of autologous endometrial tissue from the same or syngeneic donors, or from humans in nude mice. 'Endometriosis' is often induced surgically by suturing fragments of uterine tissue onto the peritoneum or, in mice, an alternative procedure is to simply inject fragments of minced uterine horns from donor mice into the peritoneum of recipient animals (Mariani et al., 2012; Greaves et al., 2017). A great variety of compounds with different functional activities have been used in these models, and many of them have shown various degrees of inhibition of lesion
growth (Bedaiwy et al., 2017). Unfortunately, to date, translation of these findings to the clinic has been limited, with some paradoxical but enlightening results.Raloxifene, for instance, was repeatedly demonstrated to be effective in rodent models (Altintas et al., 2010) but, when tested in women in a RCT, it even accelerated pelvic pain recurrence after surgery when compared to placebo (Stratton et al., 2008). A possible explanation could be the poor alignment of the outcome measures evaluated in the current animal models to the real nature of the disease. Disease features in an animal model should also include the evaluation of fibrosis presence that can be done in several ways (Kushiyama et al., 2011; Dong et al., 2017; Rittié, 2017) (Figure 2).

3. A modification in the definition of endometriosis would not only aim at driving research towards more successful therapies, but may also have some immediate clinical implications. Indeed, from a diagnostic standpoint, based on histologic findings, endometriotic lesions can sometimes be misjudged. Some cases of endometriosis-related extensive pelvic adhesions may paradoxically remain without a definite diagnosis or erroneously considered long-term consequence of pelvic inflammatory disease (PID). This may be particularly true in the absence of endometriomas or deep peritoneal lesions and/or when surgical access to pelvic organs at surgery is impeded by the severity of the adhesions. In fact, extending the definition of endometriosis beyond the mere presence of ectopic endometrial tissue would allow clinicians to classify women with extensive pelvic adhesions and without evidence of past pelvic insults (such as for instance a damage of the tubal mucosa) as affected even in the absence of the two classic components of the histologic diagnosis, i.e. endometrial stroma and glands. It is noteworthy that even when available, surgical specimens are rarely serially sectioned in standard practice, and lesions with no or only small areas with endometrial lining can be missed by pathologists (Nisenblat et al., 2016). False negative diagnoses can occur if pathologists stick stringently to the current definition of endometriosis requiring the concurrent demonstration of both endometrial stroma and glands. With a cautious approach in order not to increase false positive cases, the definitive recognition of fibrosis as an essential component of endometriosis may overcome these uncertainties.

Of note, the debate on the reliability of non-invasive diagnosis of endometriosis may also be influenced by a change in the definition of endometriosis. For instance, one cannot exclude that the current high accuracy of transvaginal ultrasound for the diagnosis of endometriomas (sensitivity of 93% and specificity of 94%) (Nisenblat et al., 2016) may improve further if the definition of endometriosis was modified. Sensitivity in particular may increase and transvaginal ultrasound could reach the requirements to become a replacement test (sensitivity ≥94% and specificity ≥79%) and thus definitively replace laparoscopy for the diagnosis of these lesions. It is noteworthy that for some fibrosis-based conditions such as retroperitoneal fibrosis, the diagnosis relies more upon the typical imaging features on CT or MRI, than on percutaneous biopsy (Cohan et al., 2017).
Sirius red staining of an ectopic endometrial tissue in the mouse to visualize the area occupied by fibrous collagen. This stain is often used to assess a fibrotic phenomenon (Kushiyama et al., 2011; Rittié, 2017). Left panels, magnification 2.5×; right panels, magnification 16×.

Conclusions

The present definition of endometriosis based on the histologic feature of the concomitant presence of endometrial stroma and epithelium in ectopic sites has been developed in order to guarantee a uniform identification of the condition. However, with increasing knowledge of the disease mechanisms and the improvement of the diagnostic tools, nowadays this definition appears too simplistic to represent the different histologic forms and clinical manifestations of this complex disease. Therefore, whilst on the one hand the identification of the specific histopathologic characteristics remains extremely important to diagnose endometriosis, on the other hand other aspects need to be taken into account from both a diagnostic and a therapeutic point of view. In our view, the endometriosis definition should be reconsidered. 'A fibrotic condition in which endometrial stroma and epithelium can be identified' could represent a realistic starting point.

References

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**Authors’ roles**

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