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Special Article

Cellular Components Contributing to Fibrosis in Endometriosis: A Literature Review

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ABSTRACT

Endometriosis-related fibrosis represents a complex phenomenon with underlying mechanisms still to be clarified. Fibrosis is consistently present in all disease forms and contributes to classic endometriosis-related symptoms of pain and infertility. The purpose of this literature review was to examine the role of various cellular populations and biological mechanisms/signaling pathways in inducing fibrogenesis of endometriotic lesions. A search was performed through PUBMED and Medline for animal and human studies published in the English language in the last 23 years examining fibrosis in superficial, ovarian, and deep infiltrating endometriosis. The main cell types found to be involved in the development of fibrosis were platelets, macrophages, ectopic endometrial cells, and sensory nerve fibers. Interactions between each of the cell types contribute to the production of fibrosis through the production of soluble factors, mostly transforming growth factor-β but also other cytokines and neuropeptides. Cell types known to be critical to the pathophysiology of endometriosis also contribute to fibrogenesis, thus supporting the theory that fibrosis is an inherent part of endometriosis.

Keywords Endometrium; Macrophages; Peritoneum; Platelets

INTRODUCTION

The development of fibrosis in endometriotic lesions represents a complex phenomenon with underlying mechanisms yet to be fully clarified. Fibrosis is consistently present in all endometriosis forms (peritoneal, ovarian, deep infiltrating endometriosis, endometriosis-related adhesions) leading to classic endometriosis-related symptoms of pain and infertility [1].

The American Society of Reproductive Medicine Classification scoring system is highly influenced by endometriosis pelvic adhesions [2,3], although does not consider pathology-based staging according to the natural history of fibrosis and endometriosis (ie, epithelial-to-mesenchymal transition [EMT], fibroblast-to-myofibroblast transdifferentiation [FMT]); consequently, some patients with endometriosis-related adhesions and fibrotic components may remain without correct diagnosis [1]. Further, histologic analyses show that, especially in deep lesions, endometrial-like tissue is minimal. The major component of nodular lesions is not endometrial tissue but fibromuscular tissue with sparse, fingerlike extensions of glandular and stroma tissue [3].

In general, the biological process of fibrosis in endometriosis resembles that of other fibrotic conditions involving myofibroblasts and smooth muscle cell activity as well as production of high levels of transforming growth factor (TGF)-β, epithelial transition to mesenchyme, and collagen deposit [1].

The aim of this review was to gain insight into the mechanisms underlying the development of fibrosis in endometriosis with a particular focus on the responsible cell types in an effort to better understand for future developments in this field.

LITERATURE SEARCH AND REVIEW

An advanced search of PubMed and Medline was limited to English language and peer-reviewed manuscripts published between 1996 and March 2019 using key words: “endometriosis” or “endometrioma” or “endometriotic” or “ectopic endometrium” in combination with either “fibrosis” or “fibrogenesis”.

Selection was made based on titles and abstracts, and then full-text manucripts were evaluted. All authors reviewed a reference list from the selected studies to identify additional articles for

inclusion. In this phase, specific topics were assigned by the corresponding author (PV) to five authors (JO, LB, MS, RV, GB) who reviewed the manuscripts independently. Studies in animals, mouse and non-mouse models, and in humans were included. Case series, case reports, RCT and articles investigating fibrosis in adenomyosis were excluded.

Studies conducted in animal models highlight some of the pathogenetic mechanisms underlying fibrosis development [4-25]. However, it remains unclear whether results obtained in animal studies are applicable to humans as animal models may not parallel exactly the natural course of the disease toward fibrosis in humans. Table 1 [4-25] shows the animal studies to date and the endometriotic lesions and mechanisms of development.

To present the role of various cellular populations contributing to fibrogenesis in endometriotic lesions in humans, the results of this literature search are noted according to the different forms of disease and characterization of the respective cellular environment.

Cellular components involved in fibrosis development

Platelets

Activated platelets have been suggested to be involved in fibrosis development both in mice and humans. According to Zhang et al, endometriotic lesions are essentially wounds undergoing repeated tissue injury and repair (ReTIAR), ultimately leading to fibrosis [4,5], implying the development of 'leaky' blood vessels with the consequent extravasation and aggregation of platelets that induce fibrosis in endometriosis lesions through TGF-β1 release and induction of the TGF-β1/Smad3 signaling pathway [7]. Studies in mice showed that the STAT3 signaling pathway is a potent inducer of EMT, FMT, and smooth muscle metaplasia (SMM) in endometriotic epithelial and stromal cells, resulting in increased contractility, collagen deposit, and fibrosis [8,9]. In turn, endometriotic stromal cells produce potent platelet-activating molecules such as thrombin and thromboxane A2, that coupled with increased angiogenesis and vascular permeability, results in further platelet extravasation/aggregation [4,10,11] (Figure 1). Confirmation of ReTIAR occurring in endometriotic lesions comes from studies in baboons showing that platelets aggregate in the stroma [4]. Levels of TGF-β1, activation of Smad3 signaling pathway and formation of α-smooth

muscle actin (SMA)-positive myofibroblasts increase progressively as lesion develops.

A similar role of activated platelets that promote EMT, FMT, and differentiation to smooth muscle cells has been suggested for human ovarian endometriosis and deep infiltrating endometriosis (DIE) [26,27]. However, recent immunohistochemistry analysis revealed, albeit not consistently, that DIE is characterized by a higher fibrotic content, higher expression of vimentin, and production of TGF-β1, but lower E-cadherin, less vascularity, and less platelet aggregation when compared to ovarian endometriosis [28, 29]. The accelerated fibrosis observed in DIE might involve factors other than platelets [28].

Macrophages

Macrophages are main cellular components playing a key role in fibrosis development [12,13,15,30]. In mice models, a transition from classical M1 macrophage activity with pro-inflammatory function to an alternate M2 profile with reparative, tissue remodeling function has been observed. M2a pro-fibrotic macrophages can produce extracellular matrix (ECM) products [14]. Once activated by interleukin (IL)-4, they can induce EMT and FMT through production of TGF-β1 and activation of TGF-β1/Smad3 signaling pathway in endometriotic cells, resulting in increased cellular contractility and collagen production *in vitro* [31].

In humans, macrophages produce some mediators critical to profibrotic phenomena such as TGF-β1, vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF)-α, IL-1, IL-6, IL-8, and lysyl oxidases (LOXs) [32]. TNF-α levels seem to vary considerably and inversely with the degree of pelvic adhesions while IL-1 concentration is higher in severe adhesions [33, 34]. The presence of adhesions also correlated with increased levels of IL-6 whereas incidence and score were negatively associated with increased levels of VEGF-A. The negative association between VEGF-A and adhesions may be, at least partially, explained by the fibrinolytic effects that may inhibit the profibrotic activity of TGF-β1 and activate tissue plasminogen activator (tPA) [35, 36]. Other effects cannot be excluded as some benefits were derived from the block of the angiogenic factor [37]. Contrary to VEGF, inflammatory cytokines such as IL-1 and IL-6 can lead to adhesion formation via inhibition of fibrinolysis and activation of plasminogen activator inhibitor-1 (PAI-1) [35, 38].

Ectopic endometrium

Increased levels of TGF-β1 have been found in peritoneal fluid and serum of patients with adhesions and/or endometriosis and the molecule expression was up-regulated in ectopic endometrium and peritoneum [39]. A substantial increase in TGF-β mRNA expression has been observed in peritoneal cells adjacent to endometriotic lesions compared to sites distant to lesions. The possible roles of TGF-β1 in the pathophysiology of peritoneal endometriosis include the regulation of EMT and cell metabolism (Warburg effect) [39]. A substantial positive correlation between concentrations of lactate and TGF-β1 in ectopic lesions has been reported, suggesting that TGF-β1 may regulate changes in cell metabolism able to fuel ectopic cell survival [40, 41]. Levels of adiponectin that suppress the gene expression of TGF-β were conversely decreased in endometriosis [42], and other pathways and molecules have been found to have a profibrotic effect in endometrotic tissue [22,23,43].

Sensory nerve fibers

In a mouse model of nodular lesions, the intraperitoneal injection of uterine fragments, along with the infusion of the neuropeptide substance P (SP) and/or calcitonin gene-related peptide (CGRP), or the rat dorsal root ganglia (DRG) supernatant induced EMT and FMT in endometriotic lesions, resulting in production of collagen and fibrosis [17] (Figure 1) implying that sensory nerve fibers play a facilitatory role in fibrogenesis of endometriotic lesions [17]. Furthermore, SP and CGRP are vasodilators that induce M2 polarization and thus can activate the signaling resulting in platelet extravasation/aggregation [4, 31]. TGF-β1 protein levels are considerably increased in nerve fibers of peritoneal endometriosis lesions compared to peritoneum in patients without endometriosis [44]. Infiltrating endometriotic lesions are hyperinnervated with sensory nerves and are in close proximity to several nerve plexuses [45, 46] explaining why DIE lesions have more fibrosis than other lesions [29, 47].

Cellular components involved in fibrosis in peritoneal endometriosis

Peritoneal lesions tend to evolve from red lesions, the most active and vascularized lesions to black lesions because of fibrotic components. The subsequent fibrosis leads to areas of white opacification and yellow-brown lesions, considered latent stages of endometriosis [48].

Mesothelial and submesothelial cells

In mice, mesothelial cells may lose their apico-basal polarity and gain a fibroblast-like phenotype with increased tendency to invade the submesothelial zone. Mesothelial cells converted into myofibroblasts tend to up-regulate α-SMA and the transcription factor Snail and acquire the ability to synthesize ECM components [8,9,49].

Smooth muscle cells of the subcoelomic mesenchyme in peritoneal biopsies of human endometriotic lesions have been identified predominantly from the region of the uterosacral ligaments and pelvic side wall, whereas biopsies without a smooth muscle layer were from the pararectal area or rectal serosa [50]. Where present, the smooth muscle varied in prominence, depth, thickness, and organization of muscle bundles. Neovascularization was observed in some areas with prominent smooth muscle development.

Anaf et al detected smooth muscles in 54 endometriotic lesions originating from four different pelvic locations (peritoneum, ovary, rectovaginal septum, uterosacral ligaments) [51]. Smooth muscle cells were found to be more frequent in endometriotic lesions compared with healthy tissue or eutopic endometrium. The smooth muscle content in black lesions was higher than in red lesions supporting, at least partially, the occurrence of a metaplastic phenomenon (SMM) in the pathogenesis of endometriotic lesions [51].

Ectopic endometrium

During embryological development, the endometrium is formed from intermediate mesoderm via mesenchymal to epithelial transition (MET). As in animal models of endometriosis, it may be assumed that also in humans, retaining some imprint of their mesenchymal origin, endometriotic

epithelial cells may be prone to return to this state via EMT with a following transdifferentiation of stromal cells via FMT and SMM in smooth muscle cells. Matzusaki et al investigated the expression of molecular markers for EMT in different forms of endometriosis [29] and found that epithelial cells of red peritoneal lesions and ovarian endometriosis showed less epithelial marker (cytokeratin) expression and more mesenchymal marker (vimentin) expression than menstrual endometrium, suggesting that endometrial epithelial cells might undergo an EMT-like process after attachment of endometrium to peritoneum, resulting in red lesions with a more invasive nature. In contrast, epithelial cells of black peritoneal lesions and DIE showed more epithelial marker (E-Cadherin) expression than those of menstrual endometrium and red lesions. These findings suggest that MET-like processes may occur during the evolution of DIE and peritoneal endometriotic implants resulting in black lesions. In addition, dephosphorylated beta-catenin protein expression was considerably higher in epithelial cells of DIE than red and black lesions and ovarian endometriosis, suggesting that a more epithelial cell-like phenotype along with Wnt/beta-catenin pathway activation might characterize DIE evolution. FMT-like processes may then occur during the evolution of peritoneal implants, resulting in black, yellow-brown, and white peritoneal endometriosis. An increased accumulation of type I collagen has also been observed in black compared with red peritoneal lesions [52, 53].

Cellular components involved in fibrosis in ovarian endometriosis

Cells lining the surface of the endometriotic cysts

Endometrioma cells are prominently involved in fibrosis formation. Positive immunostaining for α-SMA antibody has been demonstrated in all ovarian cysts by Khare et al and Anaf et al [51, 54]. According to Mechsner et al, smooth muscle content was present in 87% of ovarian lesions in contrast to the 17.5% reported by Fukunaga and coworkers [55,56]. Reduced protein expression in the stromal cells of ovarian lesions with an increased α-SMA proportion were found by Konrad et al [57]. Ovarian samples analyzed by Liu et al consistently showed markers of FMT that stained positively for fibrosis [28].

Several factors have been suggested to be involved in this process that leads to fibrosis in the

ovarian endometriotic lesion and are shown in Figure 2 [29,32,58,59,60]

Endometriotic mesenchymal stem cells

Li et al demonstrated that treatment with endometriotic mesenchymal stem cells (Ecto-MSCs) conditioned medium (CM) promoted proliferation, migration, invasion and collagen gel contraction of endometriotic stromal cells derived from ovarian endometrioma increasing the expression of genes involved in fibrogenesis [61].

Fibroblasts outside endometriomas

Fibroblast cells in interstitial spaces around the endometriotic cyst seem to be involved in the fibrosis process. Kitajima et al compared the histologic features in apparently normal ovarian cortical tissue from ovaries with small endometriomas and from the contralateral healthy ovaries and found fibrosis to be more frequent in the cortex from ovaries with endometriomas (80%) than in those without (27%) [62]. Cells in interstitial spaces also stained positively for nerve growth factor (NGF) suggesting that the interstitial components include smooth muscle cells and nerve cells with vascularization [63].

Cellular components involved in fibrosis in deep infiltrating endometriosis (DIE)

Deep infiltrating nodules such as the rectovaginal nodules proliferate smooth muscle cells with active glandular epithelium and scanty stroma (Figure 3).

Ectopic endometrium

Liu et al found positivity for α-SMA even in the glandular epithelium in DIE, noting that ectopic endometrial stromal cells of DIE are involved in fibrosis [28]. Recently, oxidative stress present in ectopic lesions has been associated with the activation of A Disintegrin and Metalloproteases (ADAM17)/Notch signaling pathway supposed to promote endometriosis development and fibrosis [21]. Levels of advanced oxidation protein products, increased in peritoneal fluid of DIE, correlate with ADAM17 activity. Binding to the Notch complex expressed on the surface of ectopic

endometrial cells, ADAM17 induces the release and transport of the Notch intracellular domain (NICD) protein and transcription of fibrosis-related genes [64].

Proliferation of normal fibroblasts is typically regulated by the presence of type I collagen. In endometriosis, deep endometriotic stromal cells are not inhibited in their growth by the surrounding fibrotic envoirnment. This uncontrolled growth seems to be owing to aberrant activation of AKT and ERK pathways [65].

DISCUSSION

The indepth study of endometriosis-associated fibrosis represents a relatively novel area of research and has long been considered a critical aspect of endometriosis although mechanisms underlying its development have received little attention. With better understanding of the cellular and molecular pathways at the basis of fibrosis in other fields [66], this has become the focus of some studies in endometriosis. This review indicates that several cell types contribute to fibrosis in all manifestations of endometriosis. Activated platelets, macrophages, ectopic endometrial cells and sensory nerve fibers all facilitate fibrogenesis inducing the release of factors that allow EMT, FMT, collagen deposit, and fibrosis. Based on these observations, some potential therapeutics have been tested in animal models. Supporting the role of activated platelets, Guo et al [9] found that P-selectin notably slowed the development of endometriosis and fibrosis in a mouse model. Soluble P-selectin treatment or a tromboxane 2 synthase inhibitor markedly reduced lesion size and fibrotic content through decreased platelet aggregation, angiogenesis, and macrophage infiltration [7-9]. The same group investigated tashinone IIA (TAN), another antiplatelet molecule, in treatment of endometriosis in mouse models resulting in the limitation of EMT, FMT, SMM, and fibrogenesis in ectopic lesions as well as reduced lesion weight [5]. Finally, the administration of scutellarin (a potent flavonoid-isolated antiplatelets agent) has been tested in untreated versus low- and high-dose groups in a mouse model, also reducing lesion weight [15]. Some drugs have been tested for their ability to block factors released by macrophages. Treatment with tocilizumab, an IL-6 blocker, in a mouse model, reduced implant volumes and histologic and fibrosis scores of the disease [67]. Medication with Sunitinib (an oral broad spectrum receptor-tyrosine kinase

inhibitor, able to inhibit VEGF actions) reduced volume and extent of implants, disease severity, and total score of adhesions [37]. Based on a potential involvement of the ADAM17/Notch signaling pathway in fibrosis in DIE, Notch Clivage inhibitors have been used to treat endometrial and endometriotic stromal cells with a consequent reduction of fibrosis markers [21]. Based on a potential involvement of the EZH2 molecule, an EZH2 inhibitor was used in a mouse model resulting in reduction of lesion growth, EMT, and fibrosis [22]. Several other potential therapeutics have been proposed with promising results in terms of reduction of lesion size and degree of fibrosis [68-70].

This review has strengths and limitations. As the mechanisms underlying fibrosis development in endometriosis have only recently indepth investigated, a thorough literature search is challenging. Many older studies considered fibrosis secondary to endometriosis development and study of fibrosis has been minimal. After a thorough literature search based on specific MESH terms, several additional papers were identified only from the references. This challenge makes this review up-to-date and timely to generate a thorough overview and understanding from past evidence that can result in potential cellular and molecular targets for therapy.

The inclusion of experimental studies in animals, consideration of the different cellular components of fibrosis in the various forms of the disease, and the collection of all findings are considered strengths of this review that allowed for a general overview and understanding of fibrosis.

Conclusions

Cell types known to be critical to the pathophysiology of endometriosis also contribute to fibrogenesis thus supporting the theory that fibrosis is an inherent part of endometriosis. The elucidation of this phenomenon is only beginning but, based on the number of therapeutics already tested in mouse models, promises to open novel treatments for endometriosis.

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REFERENCES

1. Vigano P, Candiani M, Monno A, Giacomini E, Vercellini P, Somigliana E. Time to redefine endometriosis including its pro-fibrotic nature. *Hum Reprod*. 2018;33:347-352.
2. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67:817-21.
3. Somigliana E, Vigano P, Benaglia L, Busnelli A, Vercellini P, Fedele L. Adhesion prevention in endometriosis: a neglected critical challenge. J Minim Invasive Gynecol. 2012;19:415-21.
4. Zhang Q, Duan J, Olson M, Fazleabas A, Guo SW. Cellular Changes Consistent With Epithelial-Mesenchymal Transition and Fibroblast-to-Myofibroblast Transdifferentiation in the Progression of Experimental Endometriosis in Baboons. *Reprod Sci.* 2016;23:1409-21.
5. Zhang Q, Liu X, Guo SW. Progressive development of endometriosis and its hindrance by anti-platelet treatment in mice with induced endometriosis. Reprod Biomed Online. 2017;34:124-136.
6. Lin YJ, Lai, Lei, HY, Wing LY. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. Endocrinology. 2006;147:1278-1286.
7. Guo SW, Ding D, Liu X. Anti-platelet therapy is efficacious in treating endometriosis induced in mouse. *Reprod Biomed Online*. 2016;33:484-499.
8. Ding D, Liu X, Duan J et al. Platelets are an unindicted culprit in the development of endometriosis: clinical and experimental evidence. *Hum Reprod.* 2015;30:812-832.
9. Guo SW, Ding D, Geng JG, et al. P-selectin as a potential therapeutic target for endometriosis. *Fertil Steril.* 2015;103:990-1000.
10. Guo SW, Du Y, Liu, X. Endometriosis-derived stromal cells secrete thrombin and thromboxane A2, inducing platelet activation. *Reprod. Sci.* 2016;23:1044-1052.

11. Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. Vascular endothelial growth factor (VEGF) in endometriosis. *Hum. Reprod.* 1998;13:686-1690.

12. Johan MZ, Ingman WV, Robertson SA, Hull ML. Macrophages infiltrating endometriosis-like lesions exhibit progressive phenotype changes in a heterologous mouse model. *J Reprod Immunol*. 2019;132:1-8.

13. Bacci M, Capobianco A, Monno A et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. *Am J Pathol.* 2009;175:547-56.

14. Capobianco A, Monno A, Cottone L, et al. Proangiogenic Tie2+ macrophages infiltrate human and murine endometriotic lesions and dictate their growth in a mouse model of the disease. Am J Pathol. 2011;179:2651-2659.

15. Ding D, Cai X, Zheng H, Guo SW, Liu X. Scutellarin Suppresses Platelet Aggregation and Stalls Lesional Progression in Mouse With Induced Endometriosis. *Reprod Sci*. 2018; 17:1933719118817661.

16. Lu Z, Zhang W, Jiang S, Zou J, Li Y. Effect of lesion location on endometriotic adhesion and angiogenesis in SCID mice. *Arch Gynecol Obstet.* 2014;289:823-30.

17. Liu X, Yan D, Guo SW. Sensory nerve-derived neuropeptides accelerate the development and fibrogenesis of endometriosis. *Hum Reprod.* 2019; 34:452-468.

18. Zheng Y, Khan Z, Zanfagnin V, Correa LF, Delaney AA, Daftary GS. Epigenetic Modulation of Collagen 1A1: Therapeutic Implications in Fibrosis and Endometriosis. *Biol Reprod*. 2016; 94:87.

19. Daftary GS, Zheng Y, Tabbaa ZM et al. . A novel role of the Sp/KLF transcription factor KLF11 in arresting progression of endometriosis. *PLoS One*. 2013;8:e60165.

20. Richards EG, Zheng Y, Shenoy CC, et al. KLF11 is an Epigenetic Mediator of DRD2/Dopaminergic Signaling in Endometriosis. *Reprod Sci*. 2017;24:1129-1138.

21. González-Foruria I, Santulli P, Chouzenoux S, Carmona F, Chapron C, Batteux F. Dysregulation of the ADAM17/Notch signalling pathways in endometriosis: from oxidative stress to fibrosis. *Mol Hum Reprod*. 2017;23:488-499.

22. Zhang Q, Dong P, Liu X, Sakuragi N, Guo SW. Enhancer of Zeste homolog 2 (EZH2) induces epithelial-mesenchymal transition in endometriosis. *Sci Rep*. 2017;7:6804.
23. Marcellin L, Santulli P, Chouzenoux S et al. Alteration of Nrf2 and Glutamate Cysteine Ligase expression contribute to lesions growth and fibrogenesis in ectopic endometriosis. Free *Radic Biol Med*. 2017;110:1-10.
24. Walter I, Handler J, Reifinger M, Aurich C. Association of endometrosis in horses with differentiation of periglandular myofibroblasts and changes of extracellular matrix proteins. *Reproduction*. 2001;121:581-586.
25. Nishimoto-Kakiuchi A, Netsu S, Matsuo S, et al. Characteristics of histologically confirmed endometriosis in cynomolgus monkeys. *Hum Reprod*. 2016;31:2352-9.
26. Zhang Q, Duan J, Liu X, Guo SW. Platelets drive smooth muscle mesenchymal transition and fibroblast-to-myofibroblast transdifferentiation. *Mol Cell Endocrinol*. 2016;428:1-16.
27. Shi LB, Zhou F, Zhu HY, et al. Transforming growth factor beta1 from endometriomas promotes fibrosis in surrounding ovarian tissues via Smad2/3 signaling. *Biol Reprod*. 2017; 97:873-882.
28. Liu X, Zhang Q, Guo SW. Histological and Immunohistochemical Characterization of the Similarity and Difference Between Ovarian Endometriomas and Deep Infiltrating Endometriosis. *Reprod Sci.* 2018;25:329-340.
29. Matsuzaki S, Darcha C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. *Hum Reprod*. 2012;27:712-21.
30. Capobianco A, Rovere-Querini P. Endometriosis, a disease of macrophage. *Front Immunol.* 2013;28:4-9.
31. Duan J, Liu X, Wang H, Guo SW. The M2a macrophage subset may be critically involved in the fibrogenesis of endometriosis in mice. *Reprod Biomed Online*. 2018;37:254-268.
32. Ruiz LA, Báez-Vega PM, Ruiz A, et al. Dysregulation of Lysyl Oxidase Expression in Lesions and Endometrium of Women With Endometriosis. *Reprod Sci.* 2015;22:1496-508.

33. Cheong YC, Shelton JB, Laird SM, et al. IL-1, IL-6 and TNF-alpha concentrations in the peritoneal fluid of women with pelvic adhesions. *Hum Reprod.* 2002;17:69-75.
34. Wieser F, Tempfer C, Schneeberger C, van Trotsenburg M, Huber J, Wenzl R. Interleukin-1 receptor antagonist polymorphism in women with peritoneal adhesions. *BJOG.* 2002; 109:1298-300.
35. Barcz E, Milewski Ł, Dziunycz P, Kamiński P, Płoski R, Malejczyk J. Peritoneal cytokines and adhesion formation in endometriosis: an inverse association with vascular endothelial growth factor concentration. *Fertil Steril.* 2012;97:1380-6.
36. Rapkin A, Morgan M, Bonpane C, Martinez-Maza O. Peritoneal fluid interleukin-6 in women with chronic pelvic pain. *Fertil Steril.* 2000;74:325-8.
37. Pala HG, Erbas O, Pala EE et al. The effects of sunitinib on endometriosis. *J Obstet Gynaecol*. 2015;35:183-7.
38. Hellebrekers BW, Emeis JJ, Kooistra T, et al. A role for the fibrinolytic system in postsurgical adhesion formation. *Fertil Steril*. 2005;83:122-9.
39. Young VJ, Ahmad SF, Duncan WC, Horne AW. The role of TGF-β in the pathophysiology of peritoneal endometriosis. *Hum Reprod Update.* 2017;23:548-559.
40. Young VJ, Ahmad SF, Brown JK, Duncan WC, Horne AW. Transforming growth factor-β induced Warburg-like metabolic reprogramming may underpin the development of peritoneal endometriosis. *J Clin Endocrinol Metab.* 2014;99:3450-3459.
41. Young VJ, Ahmad SF, Brown JK, Duncan WC, Horne AW. ID2 mediates the transforming growth factor-β1-induced Warburg-like effect seen in the peritonuem of women with endometriosis. *Mol Hum Reprod.* 2016;22:648-654.
42. Takemura Y, Osuga Y, Harada M, et al. Concentration of adiponectin in peritoneal fluid is decreased in women with endometriosis. *Am J Reprod Immunol.* 2005;54:217-21.
43. Shao X, Wei X. FOXP1 enhances fibrosis via activating Wnt/β-catenin signaling pathway in endometriosis. *Am J Transl Res*. 2018;10:3610-3618.
44. Tamburro S, Canis M, Albuisson E, Dechelotte P, Darcha C, Mage G. Expression of transforming growth factor beta 1 in nerve fibers is related to dysmenorrhea and

laparoscopic appearance of endometriotic implants. *Fertil Steril.* 2003;80:1131-1136.

45. Wang G, Tokushige N, Markham R, Fraser IS. Rich innervation of deep infiltrating endometriosis. *Hum Reprod.* 2009;24:827-34.
46. Arnold J, Barcena de Arellano ML, Rüster C et *al*. Imbalance between sympathetic and sensory innervation in peritoneal endometriosis. *Brain Behav Immun.* 2012;26:132-41.
47. Van Kaam, KJ, Schouten JP, Nap AW, Dunselman GA, Groothuis PG. Fibromuscular differentiation in deeply infiltrating endometriosis is a reaction of resident fibroblasts to the presence of ectopic endometrium. *Hum Reprod.* 2008;23:2692-700.
48. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril*. 1997;68:585-96.
49. Yanez-Mo M, Lara-Pezzi E, Selgas R, et al. Peritoneal dialysis and epithelial to mesenchymal transition of mesothelial cells. N Engl J Med. 2003;348:2004-201
50. Fredericks S, Russell P, Cooper M, Varol N. Smooth muscle in the female pelvic peritoneum: a clinicopathological analysis of 31 women. *Pathology*. 2005;37:14-21.
51. Anaf V, Simon P, Fayt I, Noel J. Smooth muscles are frequent components of endometriotic lesions. *Hum Reprod.* 2000;15:767-71.
52. Matsuzaki S, Canis M, Darcha C, Fukaya T, Yajima A, Bruhat MA. Increased mast cell density in peritoneal endometriosis compared with eutopic endometrium with endometriosis. *Am J Reprod Immunol.* 1998;40:291-4.
53. Matsuzaki S, Canis M, Darcha C, Dechelotte P, Pouly JL, Bruhat MA. Fibrogenesis in peritoneal endometriosis. A semi-quantitative analysis of type-I collagen. Gynecol Obstet Invest. 1999;47:197-9.
54. Khare VK, Martin DC, Eltorky M. A comparative study of ovarian and pelvic wall-infiltrating endometriosis. *J Am Assoc Gynecol Laparosc*. 1996;3:235-9.
55. Mechsner S, Bartley J, Loddenkemper C, Salomon DS, Starzinski-Powitz A, Ebert AD. Oxytocin receptor expression in smooth muscle cells of peritoneal endometriotic lesions and ovarian endometriotic cysts. *Fertil Steril*. 2005;83:1220-31.

56. Fukunaga M. Smooth muscle metaplasia in ovarian endometriosis. *Histopathology*. 2000; 36:348-52.

57. Konrad L, Kortum J, Nabham R, et al. Composition of the Stroma in the Human Endometrium and Endometriosis. *Reprod Sci*. 2018;25:1106-1115.

58. Yan D, Liu X, Guo SW. Neuropeptides Substance P and Calcitonin Gene Related Peptide Accelerate the Development and Fibrogenesis of Endometriosis. *Sci Rep*. 2019;9:2698.

59. Cai X, Shen M, Liu X, Guo SW. Reduced Expression of Eukaryotic Translation Initiation Factor 3 Subunit e and Its Possible Involvement in the Epithelial-Mesenchymal Transition in Endometriosis. *Reprod Sci*. 2018;25:102-109.

60. Zeng X, Yue Z, Gao Y, et al. NR4A1 is Involved in Fibrogenesis in Ovarian Endometriosis. *Cell Physiol Biochem*. 2018; 46:1078-1090.

61. Li J, Dai Y, Zhu H, Jiang Y, Zhang S. Endometriotic mesenchymal stem cells significantly promote fibrogenesis in ovarian endometrioma through the Wnt/β-catenin pathway by paracrine production of TGF-β1 and Wnt1. *Hum Reprod*. 2016;31:1224-35.

62. Kitajima M, Defrère S, Dolmans MM, et al. Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. *Fertil Steril*. 2011;96:685-91.

63. Odagiri K, Konno R, Fujiwara H, Netsu S, Yang C, Suzuki M. Smooth muscle metaplasia and innervation in interstitium of endometriotic lesions related to pain. *Fertil Steril.* 2009;92:1525-31.

64. Kavian N, Servettaz A, Weill B, Batteux F.New Insights into the Mechanism of Notch Signalling in Fibrosis. *Open Rheumatol J.* 2012;6:96-102.

65. Matsuzaki S, Darcha C. Co-operation between the AKT and ERK signaling pathways may support growth of deep endometriosis in a fibrotic microenvironment in vitro. Hum Reprod. 2015;30:1606-16.

66. Hinz B. The role of myofibroblasts in wound healing. *Curr Res Transl Med*. 2016;64:171-177.

67. Taskin MI, Gungor AC, Adali E, Yay A, Onder GO, Inceboz U. A Humanized Anti-Interleukin 6 Receptor Monoclonal Antibody, Tocilizumab, for the Treatment of Endometriosis in a Rat Model. *Reprod Sci.* 2016;23:662-9.

68. Itil IM, Cirpan T, Akercan F, et al. Effect of BCG vaccine on peritoneal endometriotic implants in a rat model of endometriosis. *Aus NZ J Obstet Gynecol.* 2006;46:38-41.

69. Szymanowski K, Chmaj-Wierzchowska K, Yantczenko A, et al. Endometriosis prophylaxis and treatment with the newly developed xenogenic immunomodulator RESAN in an animal model. *Eur J Obstet Gynecol Reprod Biol.* 2009;142:145-8.

70. Wu RF, Huang ZX, Ran J, Dai SJ, Lin DC, Ng TW, Chen QX, Chen QH. Lipoxin A(4) Suppresses Estrogen-Induced Epithelial-Mesenchymal Transition via ALXR-Dependent Manner in Endometriosis. *Reprod Sci*. 2018;25:566-578.

Figure legends

Figure 1: Main cellular types and molecules involved in the development of endometriosis-related fibrosis.

Figure 2: Main cellular types and molecules involved in the development of ovarian endometriosis-related fibrosis.

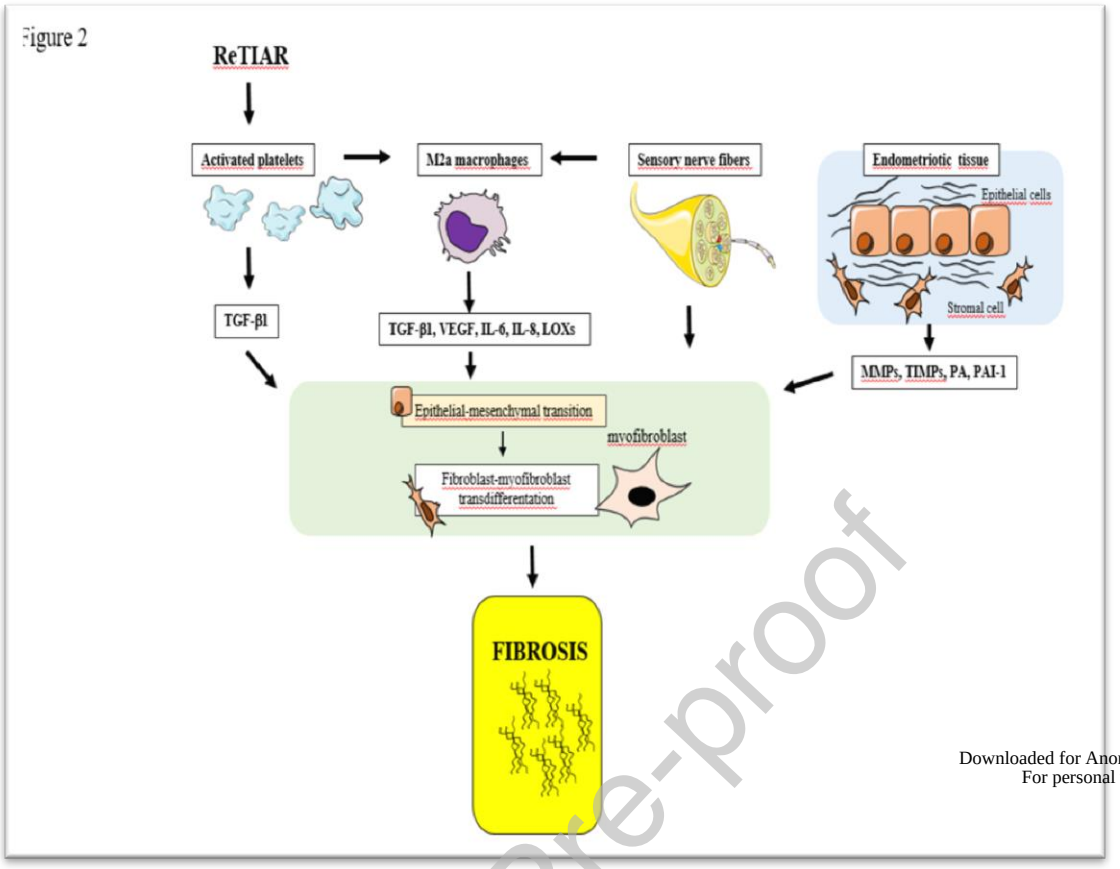
Figure 3: Main cellular types and molecules involved in the development of deep infitrating endometriosis-related fibrosis.

Table 1. Animals models of endometriosis and fibrotic components.

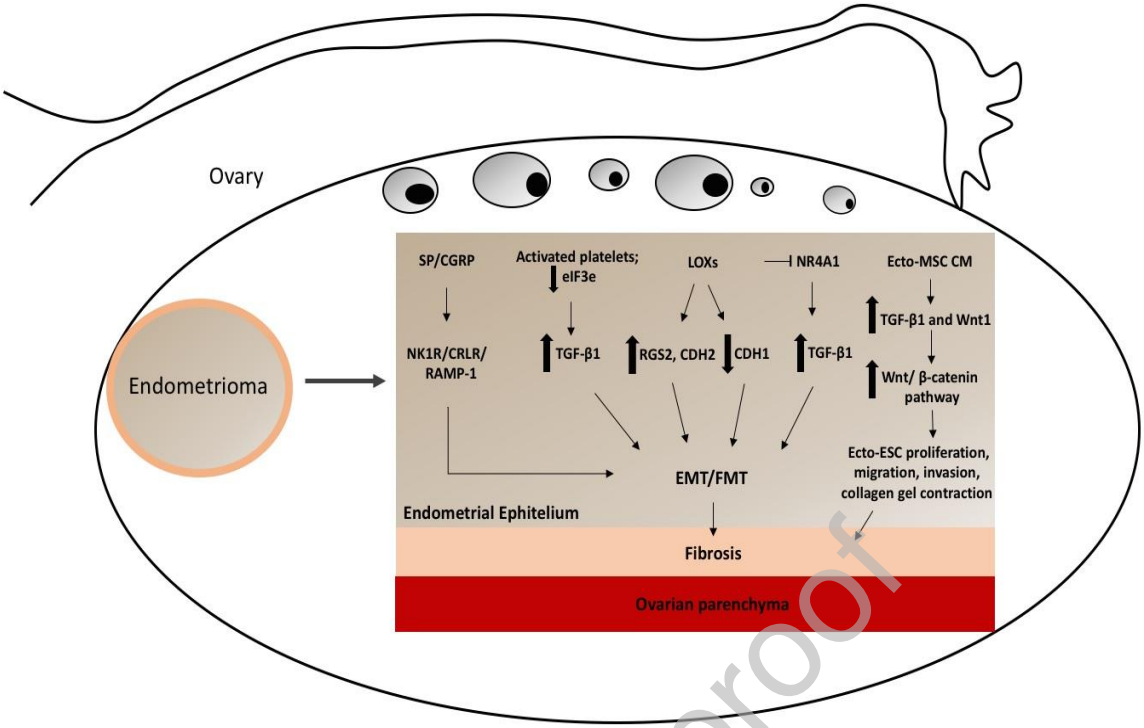
Models	Histology	Cellular components involved in fibrosis and molecular factors involved	References
Mouse	Endometrial epithelial and stromal cells	Ectopic endometrium → Thrombin and thromboxane A2, KLF11, DRD2, EZH2, NRF2, ADAM17/Notch	[4-23]
	Inflammatory cells Macrophages Fibroblast, myofibroblast	Activated platelets → Thrombin, thromboxane A2, TGF-β1, VEGF, SP	
	Smooth muscle cells	Neutrophils and M2 macrophages → IL-6, IL-1, VEGF, IL-10, TGF-β1, TNF α	
	Fibrosis	Peritoneal mesothelial cells → TGF-β1	
		Sensory nerve fibers → SP and CGRP	
Equines	Endometrial epithelial and stromal cells	Activated platelets → TGF-β1	[21]
	Fibroblast, myofibroblast		[24]
	Smooth muscle cells		
	Periglandular fibrosis		
Monkeys	Endometrial epithelial and stromal cells	Activated platelets → TGF-β1	[4,25]

	Infllammatory cells Macrophages Fibroblast, myofibroblast		
	Smooth muscle cells		
	Fibrosis		

Note: VEGF= vascular endothelial growth factor; SP= Substance P; IL-6= interleukin 6; IL-1= interleukin 1; IL-10= interleukin 10; CGRP= calcitonin gene related peptide; KLF11= Krüppel-like Factor; DRD2= Dopamine D2 Receptor; TGF-β1= Transforming growth factor beta 1; NRF2= Nuclear Factor Erythroid-derived 2-like 2; EZH2= Enhancer of Zeste Homolog 2; ADAM17= disintegrin and metalloproteinase domain 17; TNF α= Tumor necrosis factor alpha;

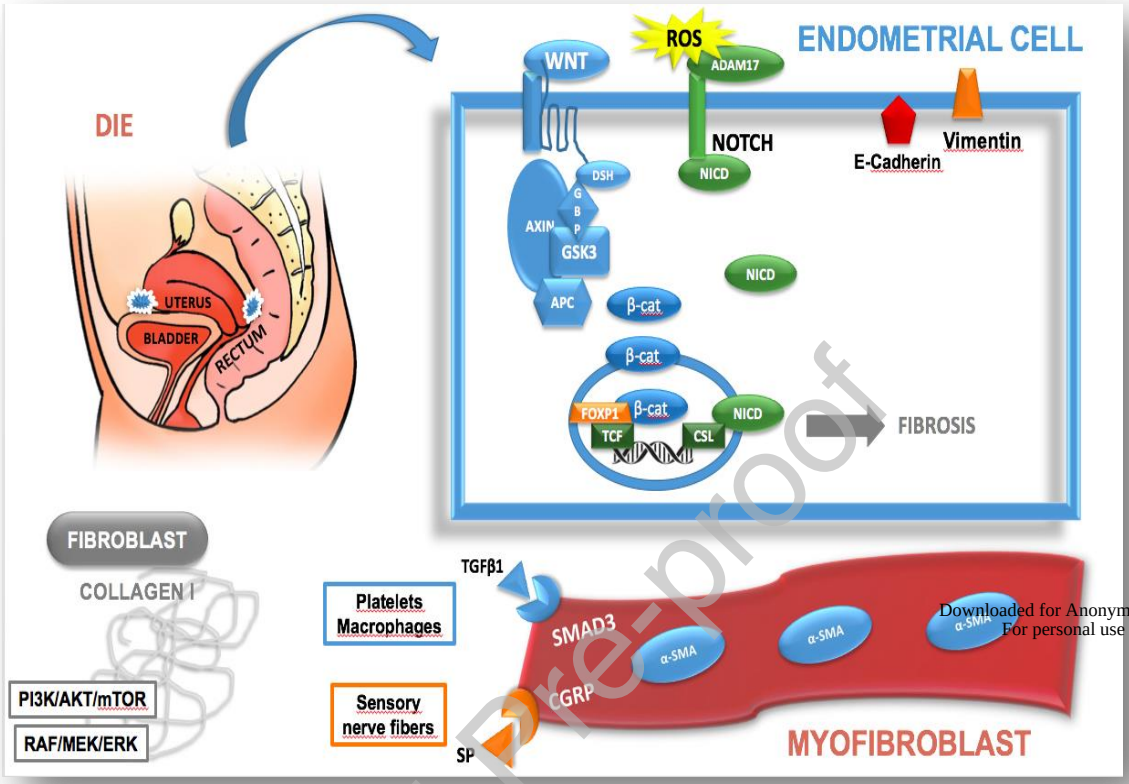


Note: ReTIAR= Recurrent Tissue Injury And Repair; TGF- β1= Transforming Growth Factor beta 1; VEGF= Vascular-Endothelial Growth Factor; IL-6= Interleukin 6; IL-8= Interleukin 8; LOXs= Lysyl Oxidase; MMPs= Matrix Metalloproteinases; TIMPs= Tissue Inhibitors Of Metalloproteinases; PA= Plasminogen Activator; PAI-1= Plasminogen Activator Inhibitor-1.



Note:

SP= neuropeptides substance P; CGRP= Calcitonin Gene Related peptide; NK1R= neurokinin receptor 1; CRLR= calcitonin receptor like receptor; RAMP-1= receptor activity modifying protein 1; eIF3e= eukaryotic translation initiation factors; TGF-β1 = Transforming Growth Factor beta 1; LOXs= Lysyl Oxidase; RGS2= regulator of G-protein signalling 2; CDH2= N-cadherin; CDH1= E-cadherin; Ecto-MSC CM= endometriotic mesenchymal stem cells conditioned medium; EMT= epithelial–mesenchymal transition; FMT= fibroblast to-myofibroblast transdifferentiation.



Note: SP= neuropeptides substance P; CGRP= Calcitonin Gene Related peptide; TGF-β1 = Transforming Growth Factor beta 1; α-SMA= alfa smooth muscle actin; ROS= Reactive Oxygen Species; ADAM17= ADAM Metallopeptidase Domain 17; NICD= Notch intracellular domain; β-cat= β-catenin; DSH= Dishevelled protein; APC= Adenomatous Polyposis coli gene product; GSK3= glycogen synthase kinase 3; GBP= G-coupled binding protein; TCF= T-cell factor; LEF= Lymphoid enhancer factor; FOXP1= Forkhead box protein P1; PIK3= Phosphatidylinositol 3-kinase; mTOR= Mammalian target of rapamycin; RAF= Rapidly accelerated fibrosarcoma; MEK= Mitogen-activated proteine kinase; ERK= Extracellular regulated kinase.